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Autophagy and Cell Death: Antitumor Drugs Targeting Autophagy

Hai Zhang and Zhinan Chen

Abstract

Autophagy, a degradation mechanism conserved among eukaryotes, plays an important role in cellular homeostasis by maintaining nutrients and energy balance. It is not surprising that autophagy has been associated with various pathological conditions such as neurodegeneration, aging, infection, and cancer. Its roles in cancer are complex and context-dependent. In this chapter, we will give an overview of regulation of autophagy with an emphasis in cancer and summarize the recent efforts in developing cancer therapeutics targeting autophagy.

Keywords: autophagy, autophagic cell death (ACD), autophagy-related genes (ATGs), signaling transduction pathway, antitumor drugs

1. Introduction

Autophagy derives from the Greek word "auto" and "phagein," auto means "self," and phagein means "eating," so autophagy refers to a "self-eating" physiological phenomenon.

The concept was first proposed in the 1960s, when de Duve observed that intracellular components are encased in membranes to form cystic structures and transported to a small compartment, the lysosome, which is responsible for recycling to degrade these ingredients [1]. Further studies show cell components, even organelles, are transported to the lysosome for degradation by cytoplasmic vesicles. Thus, the term autophagy was used for the first time to describe this process. But not until the early 1990s, the seminal work by Yoshinori Ohsumi on *Saccharomyces cerevisiae* resolved its molecular mechanism [2]. Now it is well-known that autophagy is the mechanism which maintains cellular homeostasis; damaged organelles or misfolded proteins are digested in lysosomes through autophagy for cellular energy recycling. However, autophagy is involved in the occurrence of cancer, neurodegenerative diseases, aging, and infection under pathological status. In this chapter, we focus on the formation and biological function of autophagy in cancer and discuss the application of antitumor drugs that target autophagy-related molecules or regulate autophagic activities.

2. Molecular mechanisms and biological functions of autophagy

2.1 Molecular mechanisms of autophagy

Autophagy is a lysosomal-mediated self-degradation process of intracellular components. Under normal physiological conditions, autophagy degrades damaged organelles and nonfunctional proteins in cells to provide energy and nutrients to maintain intracellular homeostasis. However, autophagy may be induced to participate in disease processes during fasting, drug interactions, nutritional deficiencies, hypoxia, or other stress reactions. Autophagy is divided into three stages: initiation, phagophore membrane formation and extension, and maturation. When the cellular sensation is subjected to external pressure, the signal-transduction molecules are activated, which regulates autophagy-related genes (ATGs) and promotes autophagy. The molecular mechanisms of the autophagy process are summarized in **Figure 1** [3].

The energy receptor, AMP-activated protein kinase (AMPK), is activated to rapidly induce the upregulation of autophagy levels when cells are under nutrient and energy deficiency, protein accumulation, and stress. Autophagy induction is mainly achieved by the interaction between the Unc-51 like autophagy activating kinase 1 (ULK1) complex (including Atg1/ULK1, Atg17/FIP200, and Atg13) and mammalian target of rapamycin complex 1 (mTORC1) [4]. First, mTORC1 is activated when cellular energy is sufficient, which then phosphorylates ULK1 and Atg13 to inhibit autophagy. In contrast, mTORC1 activity is inhibited when cellular energy is deficient, and the resultant dephosphorylated Atg13 forms a complex with ULK1 and interacts with FIP200 to initiate autophagy [5]. The initiation of autophagy is closely related to the Class III PI3K (Vps34)-Atg6/Beclin1 complex [6, 7]. The Beclin1-PI3K complex recruits Atg12-Atg5 and Atg16L multimers and Atg8/microtubule-associated protein light chain 3 (LC3), which promotes the stretching and extension of autophagosome membranes [8]. The expansion of autophagosomes mainly depends on two ubiquitin-like conjugation systems: Atg12
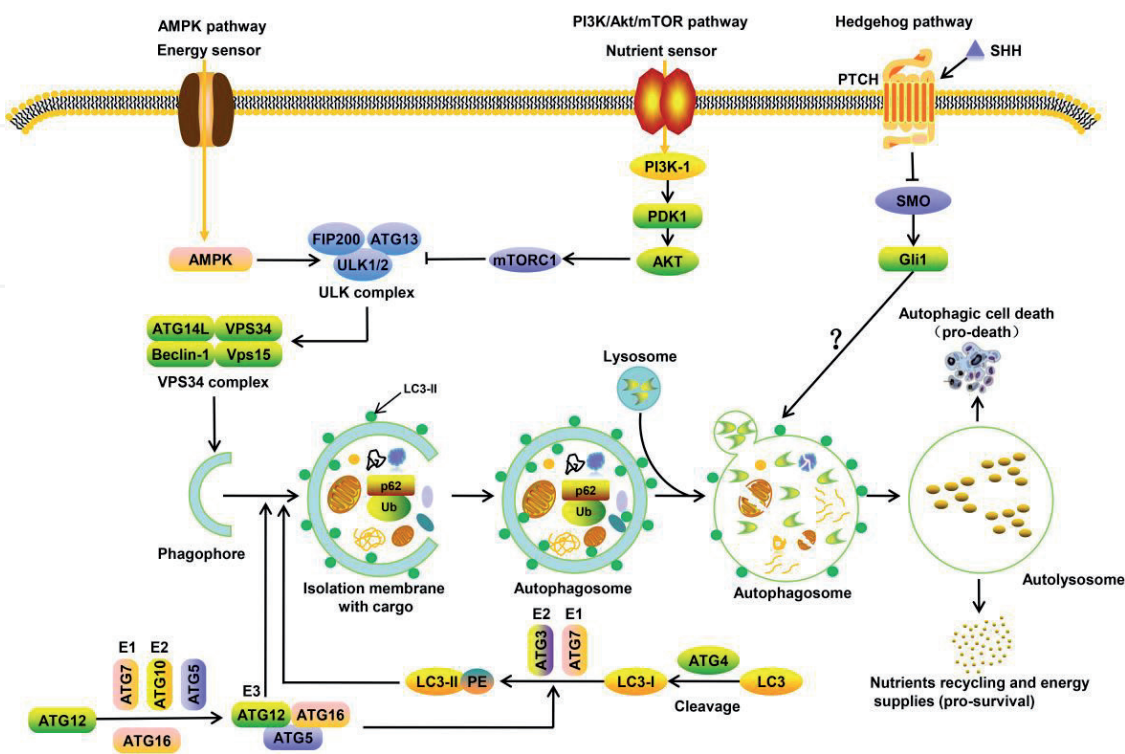

Figure 1.
Schematic diagram of the molecular mechanisms of autophagy.

binding and LC3-modification processes [9]. Atg12 binding is a ubiquitin-like process that requires the participation of ubiquitin-activating enzymes, E1 and E2. Atg12 is firstly activated by Atg7 (E1-like enzyme) and is then transported to Atg5 through Atg10 (E2-like enzyme), which subsequently binds to Atg16 to form a multibody complex and participates in the expansion of the autophagosome [10–12]. The LC3-modification process also requires the participation of ubiquitin-activating enzymes, E1 and E2. After the formation of the LC3 precursor, it is processed into cytosolic soluble LC3-I by Atg4 and is then covalently linked to phosphatidylethanolamine (PE) by Atg7 (E1-like enzyme) and Atg3 (E2-like enzyme) to form a fat-soluble LC3-II-PE that participates in the extension of the autophagosome membrane. LC3-II binds to the newly formed autophagosome membrane until the formation of the autolysosome. Therefore, LC3-II is often used as a marker for autophagy [11, 13]. After formation, autophagosomes fuse with lysosomes through the microtubule cytoskeleton under the action of the endosomal-sorting complex required for transport (ESCRT), as well as through monomeric GTPase (RabS), to form autolysosomes. The autophagy-receptor protein, P62/SQSTM1, recognizes and binds to autophagy-substrate proteins by binding to the ubiquitin-associated (UBA) domain, either dependently or independently. P62/SQSTM1 also anchors to the autophagosome membrane through the LC3/Atg8 interaction region (LIR); ultimately fusion between autophagosomes and lysosomes leads to P62/SQSTM1 entering into autolysosomes, together with the corresponding substrates and their degradation [14].

In addition to the direct involvement of the Atg gene in autophagy, some important signaling pathways also regulate autophagy. Among these pathways, the PI3k/Akt/mTOR pathway is a classical signal-transduction pathway associated with autophagy. Under nutrient-rich conditions, PI3k/Akt signaling activates the downstream mammalian target of rapamycin (mTOR) to form the mTOR complex consisting of ULK1/2, mATG13, FIP200, and Atg101. Once the mTOR complex is formed, phosphorylation of ULK1 and Atg13 results in ULK1 inactivation, which suppresses autophagy. In the absence of nutrients, the LKB/AMPK pathway is activated, and the binding of mTORC1 to ULK is blocked, thereby suppressing mTOR activity. Subsequently, ULK1 phosphorylates Atg13 and FIP200 to form autophagosomes, ultimately inducing autophagy [15]. The AMPK pathway is a cellular energy sensor, and it regulates the occurrence of autophagy. In the absence of nutrients, AMPK senses the changes in AMP levels and is activated, thereby phosphorylating tuberous sclerosis 2 (TSC2) and aggravating the inhibition of Rheb GTPase by TSC1/2, which ultimately inhibits mTOR activity and induces autophagy. In addition, AMPK activates ULK1 through phosphorylation, thereby promoting autophagy. Under nutrient-rich conditions, mTOR phosphorylates ULK1 to prevent AMPK from activating ULK1, which ultimately suppresses ULK1 activity and thereby inhibits autophagy [16]. The Hedgehog (Hh) signaling pathway also regulates autophagy. After Sonic hedgehog (SHh) in the Hh signaling pathway senses signaling stimulation, it transfers and binds to PATCHED-1 (PTCH) receptor, thereby releasing inhibition of the G-protein-coupled receptor (GPCR)-like protein Smoothened (Smo). The activation of Smo ultimately leads to the activation of the downstream Gli transcription factor. Gli has three homologues, of which Gli1 is involved in autophagy and inhibition of Gli1 induces autophagy. However, the mechanism by which Gli1 induces autophagy has not been well clarified [17, 18].

2.2 Autophagic cell death (ACD)

Programmed cell death is an orderly process, and, once this process is initiated, it proceeds automatically. Programmed cell death can be classified into at least three

types: type I (apoptosis), type II (ACD), and type III (necrosis or lysosomal death). Type I programmed cell death is accompanied with typical morphological changes of the cells—such as cell shrinkage, chromatin condensation, and apoptotic-body formation—following the initiation of apoptosis (**Figure 2a**). The morphological features of cells undergoing type II programmed cell death are not as obvious, and only autophagic vacuoles can be observed under electron microscopy (**Figure 2b**). Necrosis is a form of cell death characterized by organelle swelling, cytoplasmic-membrane rupture, and leakage of cellular contents (**Figure 2c**). Therefore, ACD should be defined according to the following three features: (1) apoptosis is not triggered; (2) there is an increase in autophagic flux before or during cell death, rather than a mere elevation in autophagy marker; and (3) application of pharmaceutical inhibitor, like 3-MA, chloroquine, etc., or small interfering RNA (siRNA) against autophagy-related genes (Beclin1 or Atg5) inhibits cell death [19–21]. In short, ACD is a type of cell death caused by autophagy. Autophagy may also be involved in other types of cell death. Therefore, it is necessary to distinguish the difference between cell death that occurs with autophagy and cell death that is caused by autophagy. If inhibition of autophagy only changes cell morphologies but not the fate of cell death, then the death in question is cell death with autophagy. If inhibition of autophagy leads to alleviation or inhibition of cell death, then the death in question is cell death by autophagy [22].

The discovery of ACD has provided new ideas and strategies for anticancer therapy. It is well-known that autophagy plays a role as a "double-edged sword" in tumorigenesis (i.e., as a promoter and a suppressor of tumors). In terms of promoting tumorigenesis, autophagy is a protective mechanism that protects tumor cells from being killed. However, ACD breaks the protective mechanism of autophagy. Drug and hypoxia-mediated ACD converts the autophagy that originally benefits tumor-cell survival to become harmful to tumor cells and inhibits tumor-cell proliferation. In addition, there is a relationship between ACD and apoptosis, and autophagy has been considered to be essential for the occurrence of apoptosis. Autophagy usually occurs before apoptosis, with apoptosis being initiated after autophagy, which leads to accelerated cell death. Some researchers believe that the occurrence of autophagy inhibits apoptosis and, thus, protects tumor cells. In addition, apoptosis can coexist with autophagy to collectively promote cell death [23]. Therefore, regardless of the single role of ACD or the combined effect of ACD and apoptosis, the above studies provide an important theoretical basis for the development of antitumor drugs. In fact, many antitumor drugs, either newly developed ones or old drugs with a new target, share the same mechanism in anticancer therapy by targeting programmed cell death.

Since the discovery of autophagy, especially its role in tumorigenesis and tumor development, the use of autophagy to regulate the programmed cell death of tumor cells has become a useful tool for anticancer therapy. Drug-induced ACD is also a
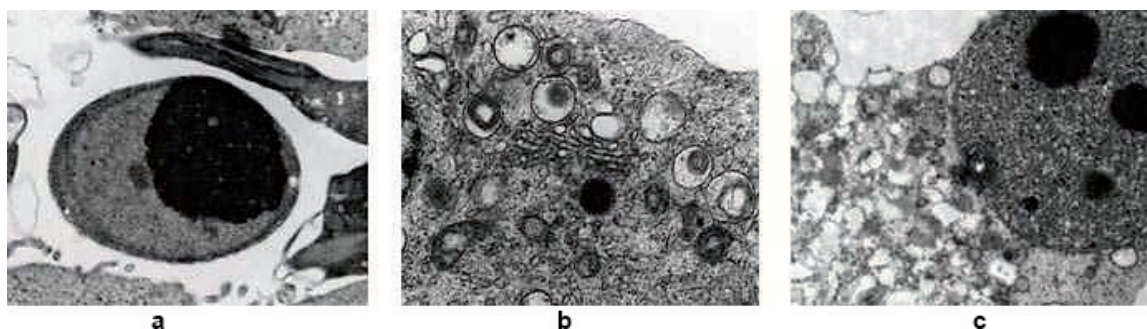

Figure 2.
Types of programmed cell death. a) apoptosis, b) autophagy and c) necrosis [22].

primary mechanism for antitumor drugs. Therefore, autophagy has been widely studied as a drug target in antitumor research. First, this new research strategy of old drugs provides ideas for autophagy-targeted drug discovery. Some repurposing drugs—such as the antimalarial drug chloroquine, the antifungal drug bafilomycin A1, and the immunosuppressant rapamycin—had not had their autophagy functions revealed when they were discovered. However, subsequent studies have revealed their other functions, which do not only induce autophagy but also inhibit tumor-cell proliferation through regulating autophagic activity. These old drugs are good candidates for being repurposed into antitumor drugs. Additionally, in the past few years with the development of omics technologies, a number of small-molecule compounds targeting autophagy molecules or autophagy pathways have been discovered by high-throughput screening methods. These drugs also inhibit tumor-cell proliferation through the ACD pathway. The following section summarizes drugs that control tumor proliferation by regulating autophagy activity in cancer stem cells (CSCs) and tumor cells, as well as their mechanisms of action.

3. Antitumor drugs that regulate autophagy to target cancer stem cells

Cancer stem cells (CSCs) are a type of cell with stem cell characteristics in tumors that exhibit strong self-renewal and proliferative ability and play an important role in tumor survival, proliferation, metastasis, and recurrence. Taken together, CSCs maintain the vitality of tumor-cell populations through self-renewal and infinite proliferation [24]. CSCs in tumor tissues in microenvironments with insufficient nutrients increase the recycling of intracellular substances by autophagy to continuously survive. Apoptosis, proliferation, and differentiation of CSCs are regulated by many factors, and autophagy and its signaling pathways play an important role in the apoptosis and differentiation of CSCs [25, 26]. A previous study has shown that the autophagy activity regulated by autophagy-related genes or autophagy pathways promotes the survival of CSCs. High Beclin1 expression promotes the survival and tumorigenicity of breast CSCs [27]. Chloroquine inhibits autophagy via the Janus kinase 2 (JAK2) and Hh pathways and kills breast and pancreatic CSCs [28, 29]. Nevertheless, another study has shown that autophagy also promotes the death of CSCs. CSCs from malignant gliomas cause cell death due to the accumulation of autophagy-related proteins and autophagosomes [30]. In addition, CSCs adapt to the changes of the microenvironment simultaneously through autophagy, and a large number of autophagosomes are found in damaged blood vessels and hypoxic parts of tumors. Autophagy induced by hypoxia-inducible factor (HIF) promotes metastasis of CD133+ pancreatic CSCs. Inhibition of autophagy reduces the viabilities of pancreatic cancer cells with stem cell characteristics and CD133+ liver CSCs under oxygen/nutrition deprivation [31]. Multi-regulation of autophagy on CSCs has prompted researchers to explore the feasibility of autophagy as a drug target for anticancer therapy. Numerous studies have demonstrated that some chemical synthetic drugs or natural extracts have a clear therapeutic effect on CSCs through regulating autophagy activity and may be used as novel antitumor treatments.

Small-molecule compounds induce programmed cell death in tumor cells through apoptosis and autophagy as their main antitumor mechanisms. After the concept of CSCs was proposed, researchers began to continuously explore small-molecule compounds and their effective targeting of CSCs. In 2009, Gupta et al. screened more than 16,000 compounds to discover that salinomycin has strong killing activity against breast CSCs and that the killing activity is >100-fold higher than that of paclitaxel [32]. Further studies have shown that salinomycin

has an inhibitory effect on a variety of CSCs. Salinomycin does not only induce apoptosis in CSCs but also inhibits DNA repair of CSCs. Interestingly, the proliferation of CSCs is inhibited by the increase or decrease of autophagic activity after the action of salinomycin [33]. On the one hand, after the action of salinomycin, the increase of reactive oxygen species (ROS) activates c-Jun N-terminal kinase (JNK) and AMPK signaling pathways to induce an increase of autophagy activity; on the other hand, salinomycin also affects the process of autophagosome and lysosome fusion and reduces the level of autophagic flux. These factors are the mechanisms by which salinomycin inhibits the proliferation of CSCs [34–36]. PI3k/Akt/mTOR is a key pathway for autophagy regulation. When cells sense signs of starvation and hypoxia, they inhibit the activity of the PI3k/Akt/mTOR pathway and promote autophagy by phosphorylating Akt at amino-acid position 473 and mTOR at amino-acid position 2448. NVP-BEZ235 is a dual ATP-competitive PI3K and mTOR inhibitor, and its induced autophagy and apoptosis enhance the radiosensitivity of glioma stem cells [37]. Rottlerin, a small-molecule compound that acts as a protein kinase C-δ (PKC-δ) inhibitor, regulates the PI3k/Akt/mTOR pathway to activate autophagy. Rottlerin promotes the death of pancreatic CSCs through an endogenous apoptotic pathway after the activation of autophagy [38]. The same phenomenon is also observed in Rottlerin-treated prostate CSCs [39]. Nearly all malignant tumors have epigenetic abnormalities. Antitumor drugs targeting histone deacetylase (HDAC) are a primary focus in epigenetic research. Small-molecule inhibitors that target HDAC have a clear inhibitory effect on the proliferation of CSCs. Givinostat (ITF2357) and suberoylanilide hydroxamic acid (SAHA) are currently the most studied HDAC small-molecule inhibitors. Studies have shown that givinostat and SAHA can induce autophagy in lung and glioma CSCs, respectively, leading to cell death [40–42].

Autophagy is associated with drug resistance and metastasis of CSCs. Autophagy inhibitors or silencing of autophagy-related genes also affect the self-renewal and differentiation of CSCs or enhance the sensitivity of CSCs to chemotherapeutic drugs, thereby killing CSCs. The autophagy inhibitor, chloroquine, not only does inhibit autophagy activity of breast CSCs but also reduces the cell number and metastasis of breast CD44+/CD24−/low CSCs; this effect is damaging to the mitochondrial membrane structure, which leads to decreased cytochrome-c activity and increases oxidative stress. This process also leads to increased expression of the double-stranded DNA damage marker, γ-H2AX, which reduces the repair capacity of double-stranded DNA breaks, thereby inhibiting the proliferation of breast CSCs [43]. Drug resistance is a challenge for antitumor therapy. The small-molecule compound, baicalein competitively binds to the GTPase SAR1B protein, guanosine triphosphate, which is required for autophagy and inhibits autophagy activity, thereby selectively enhancing the sensitivity of mice liver CD133+ tumor-initiating stem cell-like cells (TICs) to mTORC1 inhibitors [44]. The autophagy inhibitor, quinacrine, which can pass through the blood-brain barrier, also increases the sensitivity of glioblastoma stem-like cells (GSCs) to the chemotherapeutic drug, temozolomide (TMZ). The combination of quinacrine and TMZ promotes iron-dependent cell death (ferroptosis) in GSCs [45]. The bromodomain and extra-terminal domain (BET) inhibitor, JQ1, is ineffective for drug-resistant CD34+ CD8- leukemia stem cells (LSCs) and hardly induces apoptosis in drug-resistant LSCs. However, JQ1 increases the expression of autophagy-related molecules, such as Beclin1 and LC3-II, through the AMPK-ULK1 pathway to promote autophagosome formation. The AMPK inhibitor, compound C, and AMPKα siRNA inhibit autophagy to further promote the apoptosis of drug-resistant CD34+CD8− LSCs [46].

In addition to small-molecule compounds, plant extracts also have an inhibitory effect on CSCs. Curcumin is a chemical component extracted from the roots

Drugs	Cancer stem cells	Autophagy activity	Mechanism of autophagy regulation	References
Chloroquine	Breast	Autophagy inhibition	Jak2, DNMT1	[27, 42]
	Pancreatic	Autophagy unchanged	CXCR4	[28]
Salinomycin	Breast	Autophagy increasing	ROS	[33]
	Breast	Autophagy inhibition	Apoptotic pathway	[35]
	Colon	Autophagy induction	ROS	[33]
NVP-BEZ235	Gliomas	Autophagy induction	PI3k/Akt/mTOR	[36]
Rettlerin	Pancreatic	Autophagy induction	Apoptotic pathway	[37]
	Prostate	Autophagy induction	Apoptotic pathway	[38]
Givinostat	Lung	Autophagy induction	HDAC	[39]
	Glioblastoma	Autophagy inhibition	HDAC	[40]
SAHA	Glioblastoma	Autophagy induction	HDAC	[41]
Baicalein	Mice liver tumor	Autophagy inhibition	GTPase SAR1B protein	[43]
Quinacrine	Glioblastoma	Autophagy inhibition	Lipid peroxides	[44]
JQ1	Leukemia	Autophagy induction	AMPK-ULK1 pathway	[45]
Curcumin	Colon	Autophagy induction	DCLK1	[46]
Resveratrol	Breast	Autophagy induction	Wnt/β-catenin pathway	[47]
	Colon adenocarcinoma	Autophagy induction	Wnt pathway	[48]
Bitter-melon fruit extracts	Colon and progenitor cells	Autophagy induction	AMPK pathway	[49]

Abbreviations: Jak2: Janus kinase 2; DNMT1: DNA (cytosine-5)-methyltransferase 1; CXCR4: C-X-C chemokine receptor type 4; ROS: reactive oxygen species; HDAC: histone deacetylases; DCLK1: Doublecortin like kinase 1; ULK1: Unc-51 like autophagy activating kinase 1

Table 1.
Antitumor drugs that target cancer stem cells by regulating autophagy.

of some plants in the Zingiberaceae and Araceae families. Among the numerous biological activities of curcumin, its antitumor activity has aroused the attention of tumor biologists. Studies have shown that curcumin has an inhibitory effect on liver and ovarian CSCs. Autophagy induced by curcumin treatment in the DCLK1+ colon CSCs inhibits cell proliferation via apoptosis [47]. Resveratrol not only does inhibit the proliferation of breast CSCs by autophagy [48] but also increases the trans-differentiation of colon CSCs into endothelial cells [49]. Bitter-melon whole

fruit (BMW) and skin (BMSk) extracts significantly inhibit the proliferation and colonization of colon CSCs, and the BMW-treated colon CSCs also cause the upregulated expression of LC3-II, Beclin1, Atg7, and Atg12 to promote autophagy [50]. **Table 1** summarizes the recent progress of mechanism, targets, and references of drugs in anticancer stem cells.

4. Antitumor drugs that regulate autophagy to target tumor cells

In the processes of tumorigenesis and tumor progression, autophagy has dual roles, and inhibition or promotion of autophagy is related to tumorigenesis. Some important signaling pathways—such as PI3K/Akt/mTOR, MAPK, JNK, and Hh pathways—do not only regulate tumor-cell growth and proliferation but also affect tumorigenesis by regulating autophagic activity. In addition, autophagy-related molecules—such as Atg5, Atg7, and Beclin1—are associated with tumorigenesis. Therefore, research in recent years regarding autophagy-targeted antitumor drugs includes not only autophagy-related signaling pathways but also autophagy-related molecules. In this section, antitumor drugs, like compounds and nature extracts, which target the signaling pathway (**Table 2**) or autophagy-related molecules (**Table 3**), are introduced, and their antitumor mechanisms are discussed.

Drugs	Cancer cell	Autophagy activity	Mechanism of autophagy regulation	References
Metformin	Myeloma	Autophagy induction	AMPK	[50]
	Rat liver carcinoma	Autophagy inhibition	AMPK, p38/MAPK	[51]
Cannabinoids	Pancreatic cancer	Autophagy induction	AMPK	[52]
Safingol	Colon carcinoma	Autophagy induction	PI3K/Akt/mTOR	[54]
Compound 6	Lung carcinoma	Autophagy induction	PI3K/Akt/mTOR	[55]
Cabazitaxel	Lung carcinoma	Autophagy induction	PI3K/Akt/mTOR	[56]
Ophiopogon japonicus	Lung carcinoma	Autophagic cell death	PI3K/Akt/mTOR	[57]
Capsaicin	Nasopharyngeal cancer cell	Autophagy induction	PI3K/Akt/mTOR	[58]
NVP-BEZ235	T-cell acute lymphoblastic leukemia	Autophagy induction	PI3K/Akt/mTOR	[59]
CYT-Rx20	Breast cancer	Autophagy induction	MAPK	[60]
AJ-5	Melanoma	Autophagic cell death	MAPK	[61]
Arsenic-trioxide	Glioma	Autophagic cell death	PI3K/Akt, ERK1/2	[62]
Fangchinoline	Liver cancer	Autophagic cell death	p53/sestrin2/AMPK	[63]

Drugs	Cancer cell	Autophagy activity	Mechanism of autophagy regulation	References
PEITC	Liver cancer	Autophagy induction	p53	[64]
Bafilomycin A1	B-cell acute lymphoblastic leukemia cell	Autophagy inhibition	Autophagosome-lysosome fusion inhibition	[66]
DQ661	Melanoma	Autophagy inhibition	Autophagosome-lysosome fusion inhibition	[67]
lys05	Glioblastoma	Autophagy inhibition	Autophagosome-lysosome fusion inhibition	[68]
Itraconazole	Breast and liver cancer	Autophagic cell death	Hh	[17]
GANT61	Breast and liver cancer	Autophagic cell death	Hh	[17]
HhAntag	Embryonal rhabdomyosarcoma	Autophagy induction	Hh	[69]
Sonidegib	Mantle cell lymphoma	Autophagy inhibition	Hh	[70]
SAHA	Colon and liver cancer	Autophagy induction	HDAC inhibitor	[71]
Panobinostat	Colon cancer	Autophagy induction	HDAC inhibitor	[72, 73]
MGCD0103	B-cell chronic lymphocytic leukemia	Autophagy inhibition	HDAC inhibitor	[74]

Abbreviations: AMPK: 5' AMP-activated protein kinase; MAPK: mitogen-activated protein kinase; PEITC: phenethyl isothiocyanate; SAHA: Suberoylanilide hydroxamic acid; HDAC: histone deacetylases; Hh: Hedgehog

Table 2.
Antitumor drugs that target cancer cells by regulating autophagy.

4.1 Autophagy pathways

4.1.1 Drugs that target AMPK pathway

AMPK is a metabolism and energy receptor of cells. The intracellular balance of energy and metabolism in tumor cells is often chaotic, leading to changes in AMPK activity to further cause activity changes of a series of downstream signaling pathways, which affect the autophagy process. In addition to its therapeutic effect on type II diabetes, metformin has also been found to have a therapeutic effect on tumors. Wang et al. has shown that metformin targets the molecules of AMPK/mTORC1 and mTORC2 pathways in the tumor cells of multiple myeloma. Activation of AMPK signaling inhibits mTORC1 and mTORC2 to further induce autophagy and inhibit myeloma cell proliferation [51]. In contrast, metformin also inhibits autophagy and promotes apoptosis through AMPK and p38/MAPK signaling pathways in the glucose-deprived H4IIE rat hepatocellular-carcinoma cell line [52]. Cannabinoids have a variety of biological activities, of which tumor inhibition has aroused attention from researchers. AMPK pathways activate autophagy after ROS-dependent activation in cannabinoid-treated pancreatic cancer cells, while ROS promotes GAPDH nuclear translation, leading to reduced glycolysis and NADH accumulation to block the Krebs cycle after blocking the respiratory-chain function. Simultaneously with the activation of the AMPK pathway, cannabinoids

also inhibit the Akt/c-Myc pathway, resulting in the reduction of pyruvate kinase isoform M2 (PKM2) activity, glycolysis, and glutamine uptake. Therefore, cannabinoids inhibit the proliferation of pancreatic cancer cells through autophagy and cellular-metabolic pathways regulated by the AMPK pathway [53].

4.1.2 Drugs that target PI3K/Akt/mTOR pathway

The PI3K/Akt/mTOR pathway is directly involved in tumor-cell proliferation, and small-molecule drugs or natural extracts act on the PI3K/Akt/mTOR pathway to regulate tumor cell proliferation through autophagy. Specifically, 3-MA, LY294002, and wortmannin are classic inhibitors of the PI3K/Akt/mTOR pathway to suppress autophagy, which have killing effects on various tumor cells [54]. In addition, many compounds that regulate autophagy and inhibit tumor cells via the PI3K/Akt/mTOR pathway have been discovered in recent years. Protein kinase C and the sphingosine kinase inhibitor, Salingol, inhibit the phosphorylation level of key molecules of the PI3K/Akt/mTOR pathway—such as Akt, p70S6k, and rS6—to induce ACD in colon cancer cells [55]. A novel hybrid of the 3-benzyl coumarin seco-B-ring derivative and phenylsulfonylfuroxan (compound 6) reduces the phosphorylation levels of mTOR-S2448, Akt-S373, and the downstream p70s6K-S371 and 4EBP1-pT45 proteins in a concentration-/time-dependent manner to induce autophagy and apoptosis of A549 cells [56]. The antiprostate-cancer drug, cabazitaxel, also inhibits proliferation of A549 cells through autophagy which is regulated by the PI3K/Akt/mTOR pathway [57]. *Ophiopogon japonicus* is a traditional medicinal plant, and its main components are flavonoids and steroidal saponins. A previous study has shown that *O. japonicus* inhibits PI3K/Akt/mTOR signaling to induce ACD in A549 lung cancer cells [58]. Similarly, the inhibitory effect of capsaicin in the nasopharyngeal carcinoma cell line, NPC-TW01, is also achieved by autophagy regulated by the PI3K/Akt/mTOR pathway [59]. Specifically, mTORC1 is a key signaling molecule in the PI3K/Akt/mTOR pathway. Some small-molecule drugs, such as rapamycin and NVP-BEZ235, inhibit mTORC1 activity and induce autophagy and have a killing effect on T-cell acute lymphoblastic leukemia cells [60].

4.1.3 Drugs that target the MAPK pathway

The MAPK pathway plays an important role in the process of tumor-cell proliferation, growth, apoptosis, and cell-to-cell functional synchronization. The MAPK family members, ERK, p38 MAPK, JNK, and ERK5 are also involved in the regulation of autophagy. CYT-Rx20, a derivative of β-nitrostyrene, inhibits proliferation in the breast cancer cell lines, MDA-MB-231 and MCF-7 [61]. A new binuclear palladacycle complex, AJ-5, does not only induce apoptosis in melanoma cells but also inhibit Akt/mTOR activity and activate p38 and ERK1/2 MAPK signaling to induce ACD, with dual roles in apoptosis and ACD to inhibit the proliferation of melanoma cells [62]. Similar to AJ-5, arsenic-trioxide and ionizing-radiation combination treatment on glioma cells also inhibits Akt/mTOR activity and activates ERK1/2 MAPK signaling to promote ACD [63].

4.1.4 Drugs that target the p53 pathway

p53 is a tumor-suppressor protein that regulates the expression of multiple genes involved in apoptosis, growth inhibition, and DNA repair. Studies have shown that the regulation of autophagy by p53 is bidirectional depending on its localization. Cytoplasmic p53 inhibits autophagy in a transcriptional-independent manner, while nuclear p53 enhances autophagy by transactivation target gene.

Drugs	Cancer cell	Autophagy activity	Mechanism of autophagy regulation	Refs.
SBI-0206965	Lung cancer cell	Autophagy inhibition	ULK1 inhibitor	[75]
MRT67307	Mouse embryonic fibroblast	Autophagy inhibition	ULK1 inhibitor	[76]
MRT68921	Mouse embryonic fibroblast	Autophagy inhibition	ULK1 inhibitor	[76]
LYN-1604	Breast cancer cell	Autophagy induction	ULK1 inhibitor	[77]
SAR405	Renal tumor	Autophagy inhibition	Vps34 inhibitor	[78]
Spautin-1	Cervical cancer	Autophagy inhibition	Vps34 inhibitor	[80]
S130	Colon cancer	Autophagy inhibition	Atg4B inhibitor	[81]
NSC185058	Saos-2 osteosarcoma cell	Autophagy inhibition	Atg4B inhibitor	[82]
Obatoclax	Lymphoblastic leukemia	Autophagic cell death	Bcl-2 inhibitor	[87]
ABT-737	Cervical cancer	Autophagy induction	Bcl-2 inhibitor	[86]
ABT-737	Colon cancer	Autophagy inhibition	Bcl-2 inhibitor	[87]
gossypol	Glioma	Autophagic cell death	Bcl-2 inhibitor	[88, 89]
Verteporfin	Prostate cancer cell	Autophagy inhibition	p62/SQSTM1 inhibitor	[90]

Table 3.
Small molecular inhibitors of autophagy-related molecules.

P53 in human hepatocellular-carcinoma cells treated with fangchinoline is transported from the cytoplasm to the nucleus through nuclear translocation and selectively activates sestrin2 to initiate autophagy and promote ACD [64]. Numerous studies have shown that cruciferous-vegetable-derived phenethyl isothiocyanate (PEITC) reactivates the normal activity of mutant p53 molecules in tumor cells and has an antitumor effect. PEITC has growth-inhibitory activity on tumor cells expressing p53R175H and restores the wide-type conformation and transcriptional activity of p53. In addition, PEITC enables p53R175H tumor cells to be sensitive to proteasome and autophagy-mediated degradation processes. Analysis of the mechanism showed that PEITC activates the classical p53 downstream target gene, causing tumor cells to arrest in the S phase and G2/M phase of the cell cycle and to undergo apoptosis [65].

4.1.5 Drugs that target the autophagosome-lysosome fusion pathway

The fusion of autophagosomes and lysosomes forms autolysosomes, which degrade the encapsulated materials. The degradation activity can be evaluated by autophagic flux. A previous study has shown that antimalarial drugs—such as quinine, chloroquine, and its derivatives—block the fusion processes of autophagosomes and lysosomes to reduce the autophagic flux and kill tumor cells by inhibiting

autophagy activity [66]. Bafilomycin A1 is a vesicular H^+-ATPase proton-pump inhibitor that inhibits autolysosome activity to promote tumor cell killing by inhibiting the activity of vacuolar V-ATPases [67]. In addition to the above drugs, studies in recent years have shown that some small-molecule drugs, such as lys05 and DQ661, share similar antitumor mechanisms [68, 69].

4.1.6 Drugs that target the Hh pathway

The Hh pathway plays an important role in embryonic development and tissue regeneration. Abnormal expression of SMO, PTCH, and Gli1 molecules in the Hh pathways is associated with tumorigenesis. The Gli1 small-molecule inhibitor, GANT61, does not only inhibit the proliferation of human hepatocellular-carcinoma cells but also synergize with itraconazole to kill breast cancer cells through the ACD pathway [18]. HhAntag, a small-molecule inhibitor of downstream element of Hh pathway, inhibits embryonal rhabdomyosarcoma (ERMS) proliferation through autophagy induction [70]. SMO antagonist, Sonidegib, selectively targets cell migration and adhesion of mantle cell lymphoma (MCL) and suppresses the proliferation of MCL via autophagy inhibition [71].

4.1.7 Drugs that target the epigenetic pathways

Epigenetic modification can regulate the occurrence of autophagy, including histone acetylation and DNA methylation, which play an important role in regulating the biological function of autophagy. Inhibition of HDAC activity to induce cell death has been the research strategy of antitumor drugs. Development of small-molecule inhibitors targeting HDAC has become a primary focus in this field. The small-molecule inhibitor, SAHA, does not only induce a killing effect on CSCs but also promote colon cancer and liver cancer cell death by FoxO1-dependent autophagy [72]. Panobinostat (LBH589) induces autophagy in a tumor-suppressor death-associated protein kinase (DAPK)-dependent manner and inhibits colon cancer cell proliferation. The combination of panobinostat and sorafenib significantly improves the therapeutic outcomes of liver cancer treatment [73, 74]. MGCD0103 not only induces apoptosis in B-cell chronic lymphocytic leukemia (CLL) but also inhibits tumor-cell protective autophagy and promotes CCL cell death through the activation of the PI3K/AKT/mTOR signaling molecules and caspase pathways [75].

4.2 Small molecular inhibitors of autophagy-related molecules

4.2.1 Atg1/ULK1

The autophagy-related gene, Atg1 homologue, ULK1, is an unc-51-like serine/threonine kinase that plays an important role in initiating autophagy. When cells are under nutrient deficiency, hypoxia, and other stresses, the upstream molecules of ULK1, mTORC1, and AMPK are activated, and the phosphorylation of ULK1 and Atg13 molecules regulates autophagy. In recent years, ULK1 has been a popular molecule in the research of autophagy-targeted drugs. ULK1 not only is a promoter of autophagy but is also an important kinase, which is more favorable for the study of small-molecule inhibitors. The high-throughput screening of Egan et al. [76] has shown that the small-molecule compound, SBI-0206965, inhibits ULK1 activity through the mTOR pathway, leading to a decrease in autophagy and inhibition of A549 cell proliferation. Unlike SBI-0206965, MRT67307 and MRT68921 inhibit both ULK1 and ULK2 activities and block autophagy [77]. The small-molecule compound, LYN-1604, and ULK1

reactive proteins—ATF3, RAD21, and caspase 3—activate autophagy to inhibit the proliferation of MDA-MB-231 triple-negative breast cancer cells [78].

4.2.2 Vps34

Vps34 is a Class III phosphoinositide-3 kinase (PI3K-III), and its 3-phos-phophosphatidylinositol (PI3P), which catalyzes the formation of the substrate phosphatidylinositol (PI), is necessary for autophagosome formation. The small-molecule inhibitor, SAR405, inhibits the catalytic activity of PIK3C3/Vps34, blocks the transport of vesicles from late endosomes to lysosomes, and inhibits autophagy, which suppresses tumor-cell proliferation [79]. In addition, PIK-III and Vps34-IN1 are also Vps34 inhibitors, which inhibit autophagy and have killing effects on tumor cells [80]. Spautin-1 is a potent and specific small-molecule inhibitor. It inhibits two ubiquitin-specific peptidases, USP10 and USP13, and promotes ubiquitination and degradation of the Vps34-PI3K complex, thereby inhibiting autophagy [81].

4.2.3 Atg4B

Atg4 is a key molecule in autophagy that cleaves the C-terminal arginine of Atg8/LC3 to expose the C-terminal glycine residue for covalent attachment to phosphatidylethanolamine (PE). This process induces Atg8/LC3-PE to be anchored to the membrane of autophagosomes to regulate autophagy. Fu et al. [82] identified a novel ATG4B small-molecule inhibitor, S130, through in silico screening and in vitro high-throughput screening system for fluorescence resonance-energy transfer and showed that S130 did not affect the autophagosome function and autophagosome fusion with lysosomes. Instead, S130 inhibited the delipidation process of LC3-PE and ultimately blocked autophagy. An antitumor study has shown that S130 effectively inhibits the growth of colon cancer cells, and nutrient deficiency further enhances the antitumor effect of S130; however, Atg4 overex-pression partially counteracts the tumor-cell death process caused by S130. Similar to S130, NSC185058 is also a newly discovered small-molecule inhibitor that acts on the Atg4B target and inhibits autophagy after LC3B lipidation, leading to the death of the Saos-2 osteosarcoma cells [83].

4.2.4 B-cell lymphoma/leukemia-2 (Bcl-2)

Bcl-2 is an antiapoptotic protein containing multiple Bcl-2 homology (BH) domains. Under starvation conditions, activation of JNK1 phosphorylates Bcl-2 protein and causes the separation between Bcl-2 and Beclin1, thereby inducing autophagy [84]. In addition, multiple Bcl-2 homology 3 (BH3) mimetics can disrupt the interaction of Bcl-2/xl with Beclin1 to induce autophagy by competing for the BH3 domains [7]. Obatoclax (GX15-070) is a pan-Bcl2 inhibitor that induces apop-tosis in various tumor cells. It also induces ACD in acute lymphoblastic leukemia cells in an Atg-dependent manner [85]. ABT-737, as a BH analogue, binds to the BH3 binding groove of Bcl-2 and Bcl-xl. A study by Maiuri et al. [86] has shown that the degree of polymerization of Beclin1, Bcl-2, and Bcl-xl is significantly reduced in ABT-737-treated cells, which enhances the autophagy level. However, ABT-737 also enhances the sensitivity of colon cancer cells to the chemotherapeutic drug, ixazo-mib, through downregulation of Mcl-1 and autophagy inhibition [87]. Similarly, gossypol is a small-molecule inhibitor of Bcl-2. Under natural conditions, gossypol is a mixture of (+)-gossypol and (−)-gossypol, and compared with (+)-gossypol, (−)-gossypol has higher antitumor activity. Gossypol and (−)-gossypol induce autophagy by inhibiting the response of Beclin1 and Bcl-2 in different tumor cells.

They can inactivate cytoprotective autophagy to cause tumor cells to escape [88], promote pro-survival autophagy, and initiate ACD [89].

4.2.5 p62

p62, also known as SQSTM1 protein, plays an autophagy and apoptotic role in tumor cells. The LIR domain of four domains of the p62 protein is responsible for binding to the autophagy-receptor protein, Atg8/LC3, while the UBA domain can recruit ubiquitinated proteins to mediate autophagic degradation. Verteporfin is an inhibitor of p62 and has no effect on cell growth under normal conditions. However, verteporfin reduces the survival of MCF-7 cells during nutrient deprivation. In vivo experiments have shown that verteporfin also inhibits tumor formation frequency of PC-3 prostate cancer cells in a xenograft model [90].

In addition to small-molecule drugs and natural extracts, some macromolecular-antibody drugs and noncoding miRNAs (miR)—such as anti-EGFR monoclonal antibody panitumumab, anti-20 monoclonal antibody rituximab, miR-22, and miR-101—have been reported to inhibit tumors in recent years through regulating autophagy activity [91, 92]. Although the emergence of autophagy provides a new idea for the development of antitumor drugs, anticancer treatments still encounter many challenges. Screening for drugs that target autophagy activity in cells may be the solution to some of these problems in the future.

5. Conclusion

The molecular mechanism and biological function of autophagy are now basically clear, and Janus role of autophagy determines that it plays an important role in the antitumor process. Some compounds, plant extracts killing tumor cells through the regulation of autophagy activity, especially induced autophagic cell death has also become an important strategy against tumor. However, there are still many obstacles to overcome in order to develop autophagic drugs; for example, there is a lack of specific biomarkers to distinguish autophagic cell death from other types of cell death. There is also a need to clarify which type of autophagy, cytoprotective, or cytotoxic should be targeted. Moreover it is difficult but important to determine to what extent autophagy should be induced before the cells reach the point of no return and undergo autophagic cell death.

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