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Review

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Autophagy and cancer treatment: four functional forms of autophagy and their therapeutic applications

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Abstract: Cancer is the leading cause of death worldwide. Drugs play a pivotal role in cancer treatment, but the complex biological processes of cancer cells seriously limit the efficacy of various anticancer drugs. Autophagy, a self-degradative system that maintains cellular homeostasis, universally operates under normal and stress conditions in cancer cells. The roles of autophagy in cancer treatment are still controversial because both stimulation and inhibition of autophagy have been reported to enhance the effects of anticancer drugs. Thus, the important question arises as to whether we should try to strengthen or suppress autophagy during cancer therapy. Currently, autophagy can be divided into four main forms according to its different functions during cancer treatment: cytoprotective (cell survival), cytotoxic (cell death), cytostatic (growth arrest), and nonprotective (no contribution to cell death or survival). In addition, various cell death modes, such as apoptosis, necrosis, ferroptosis, senescence, and mitotic catastrophe, all contribute to the anticancer effects of drugs. The interaction between autophagy and these cell death modes is complex and can lead to anticancer drugs having different or even completely opposite effects on treatment. Therefore, it is important to understand the underlying contexts in which autophagy inhibition or activation will be beneficial or detrimental. That is, appropriate therapeutic strategies should be adopted in light of the different functions of autophagy. This review provides an overview of recent insights into the evolving relationship between autophagy and cancer treatment.

Key words: Autophagy; Cancer treatment; Precision treatment; Cell death mode

1 Introduction

Cancer is a major public health problem and is the leading cause of death worldwide. In 2020, there were 19.3 million new cancer cases and 10 million deaths (Sung et al., 2021). It is a disease caused by the loss of normal regulation and excessive cell proliferation. Therefore, inhibiting cell proliferation and killing cells using anticancer drugs are the main ways to treat cancer (van der Velden et al., 2019). Unfortunately, the complex biological processes of cancer cells and some uncertain biological effects of drugs on cancer cells seriously limit the efficacy of various anticancer drugs.

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Autophagy, as a crucial regulator in cellular physiology, has attracted extensive attention from researchers over the past decade. The awarding of the 2016 Nobel Prize for Medicine to Yoshinori OHSUMI for the discovery of the molecular mechanisms of autophagy further highlighted the importance of autophagy in health and disease (Tooze and Dikic, 2016; Galluzzi and Green, 2019). The role of autophagy in cancer is of particular importance: it can not only promote tumorigenesis, but also inhibit the proliferation of cancer cells (Yamamoto et al., 2020; Lu et al., 2021). Autophagy also plays other roles in the process of anticancer drug treatment (Gewirtz, 2014). Thus, whether we should enhance or inhibit autophagy has become an important question for cancer therapy. Furthermore, drugs exert their anticancer efficacy by triggering different cell death modes, such as apoptosis, senescence, mitotic catastrophe (MC), ferroptosis, necroptosis, and pyroptosis (Galluzzi et al., 2018). In this review, we focus on the different roles of autophagy

in cancer treatment, highlighting recent insights linking autophagy and apoptosis and other cell death pathways.

2 Autophagy

Autophagy, an evolutionarily ancient and highly conserved cycle, is a process in which surplus or damaged cytoplasmic material is decomposed through a lysosomal mechanism (Tooze and Dikic, 2016). There are three types of autophagy: macroautophagy, microautophagy, and chaperone-mediated autophagy (Gewirtz, 2014; Tooze and Dikic, 2016; Galluzzi and Green, 2019). The main mechanism operating in eukaryotic cells is macroautophagy (referred to hereafter simply as autophagy).

Autophagy is controlled by a highly regulated set of signaling events, occurs at a basal level in all cells, and can be induced by diverse signals and cellular stresses, such as oxidative stress, pathogen infection, hypoxia, and energy or nutrient shortages (Levy et al., 2017; Chaeichi-Tehrani et al., 2021). There are more than 20 core autophagy-related (ATG) proteins involved in the process of autophagy. These control autophagy by regulating autophagy initiation, autophagosome nucleation, autophagosome membrane deformation, autolysosome assembly, and intravesicular product degradation (Tooze and Dikic, 2016; Wen et al., 2020; Zhao et al., 2020).

For autophagy initiation, the inactivation of mammalian target of rapamycin complex 1 (mTORC1) and activation of adenosine monophosphate (AMP)-activated protein kinase (AMPK) are the best-characterized triggers (Ma et al., 2021; Wang et al., 2021). These two kinases regulate autophagy initiation by regulating the activation of the Unc-51-like kinase (ULK) complex (involving ULK1, ULK2, ATG13, focal adhesion kinase (FAK) family-interacting protein of 200 kD (FIP200)), which activates downstream class III phosphoinositide-3-kinase (PI3K) complex by directly phosphorylating vacuolar protein sorting 34 (VPS34) and Beclin-1 (Tooze and Dikic, 2016). Notably, Beclin-1 is one of the key proteins involved in membrane nucleation and usually interacts with B-cell leukemia/lymphoma 2 (Bcl-2) to inhibit autophagy. However, in some cases, disruption of this interaction allows Beclin-1 to bind with the lipid kinase VPS34, thus promoting membrane nucleation (Xu and Qin, 2019; Zhou et al., 2021). In the expansion stage, two ubiquitin-like protein conjugation systems control elongation of the isolation membrane. ATG12-ATG5 conjugation is mediated by the E1-like enzyme ATG7 and E2-like enzyme ATG10, which can bind to ATG16L to form the ATG12-ATG5-ATG16L complex (Lin et al., 2020). This complex serves as an E3-like enzyme in coordination with ATG7 and the E2-like enzyme ATG3 to conjugate phosphatidylethanolamine (PE) to the GABA type A receptor-associated protein (GABARAP)/light chain 3 (LC3) family of proteins, and is then recruited to the autophagosome membrane (Lystad et al., 2019). ATG4 cleaves LC3 family members to create LC3-I (diffuse form) and then conjugates PE to form LC3-II (also known as microtubule-associated protein 1 light chain 3β (MAP1LC3B)) (Xu and Qin, 2019). This lipid-conjugated form of LC3 is required for phagophore expansion and closure (Chang et al., 2021; Vujić et al., 2021). Furthermore, the typical autophagosome marker LC3-II can be recognized by adaptor proteins, including Bcl2-interacting protein 3-like (BNIP3L, also known as Nix), Bcl-2-interacting protein 3 (BNIP3), Tax1-binding protein 1 (TAX1BP1), optineurin, and sequestosome-1 (SQSTM1)/p62, which transport the specifically labeled substrates into autophagosomes (Chang et al., 2021). Finally, autophagosomes can fuse with lysosomes to form autolysosomes, and their macromolecule contents can then be degraded and recycled as new metabolic substrates (Tooze and Dikic, 2016; Levy et al., 2017) (Fig. 1).

3 Roles of autophagy in cancer treatment

Autophagy plays a complex role in cancer development, progression, and treatment. According to its different functions in cancer treatment, autophagy can be divided into four main forms: cytoprotective (cell survival), cytotoxic (cell death), cytostatic (growth arrest), and nonprotective (no contribution to cell death or survival) (Gewirtz, 2014; Bai et al., 2018; Tyutyunyk-Massey and Gewirtz, 2020). The different functional forms of autophagy are distinguished by determining the impact of autophagy inhibition on drug sensitivity, but are not completely distinguishable through morphology and biochemistry (Gewirtz, 2014; Xu et al., 2020). Considering the increased interest in autophagy in clinical cancer research as a

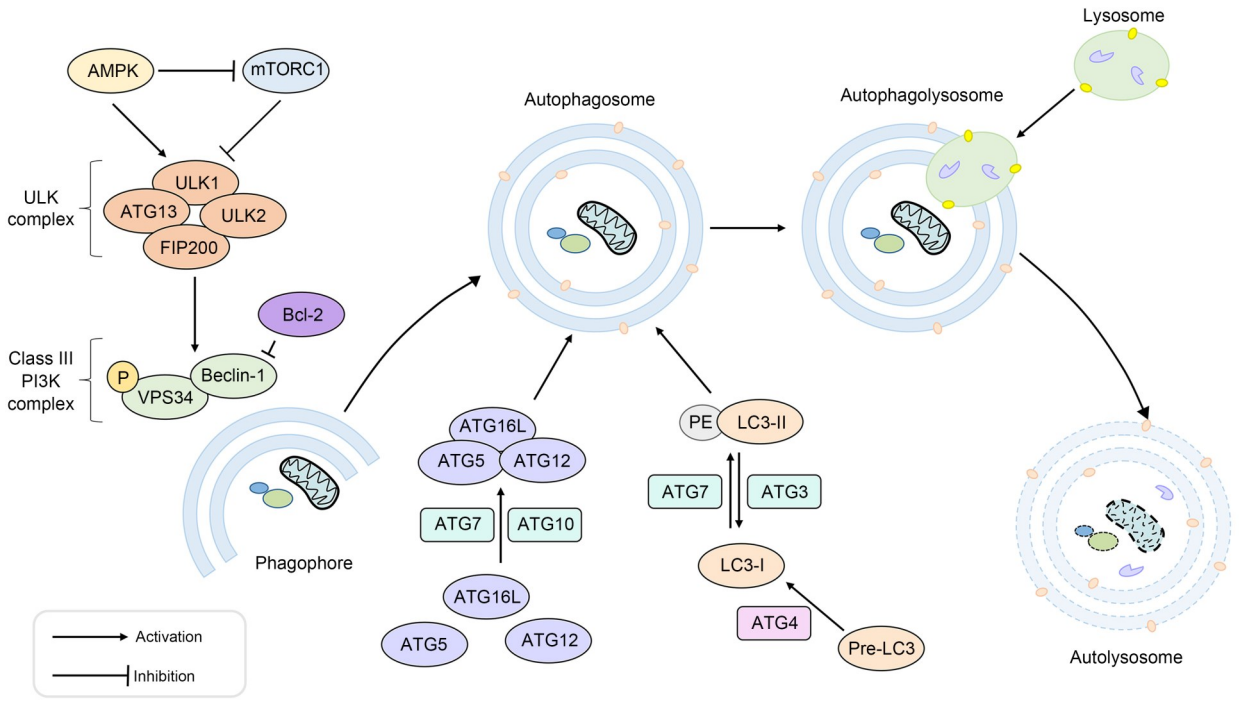

Fig. 1 Molecular mechanism of autophagy regulation in mammals. The autophagic process consists of several phases including initiation, nucleation, maturation, fusion, and degradation. AMPK: adenosine monophosphate (AMP)-activated protein kinase; mTORC1: mammalian target of rapamycin complex 1; ULK: Unc-51-like kinase; ATG: autophagy-related gene; FIP200: focal adhesion kinase (FAK) family-interacting protein of 200 kD; Bcl-2: B-cell leukemia/lymphoma 2; VPS34: vacuolar protein sorting 34; PI3K: phosphoinositide-3-kinase; LC3: light chain 3; PE: phosphatidylethanolamine; P: phosphorylation.

promising treatment strategy, it is necessary to illustrate the complex effects of autophagy in cancer cells and evaluate the application potential.

3.1 Cytoprotective autophagy

Autophagy literally means "self-eating." It can provide cytoprotection by selectively eliminating potential cytotoxic materials and preventing the accumulation of damaged proteins and organelles (Tooze and Dikic, 2016; Yamamoto et al., 2020). Autophagy can maintain cellular homeostasis in the event of energy or nutrient shortages, and can respond to various cytotoxic insults. Basal levels of autophagy are essential for normal tissue homeostasis. Unfortunately, glucose deprivation and hypoxia are common in the tumor microenvironment and can activate autophagy to maintain tumor tissue homeostasis and support cancer cell survival (Call and Nichenko, 2020). Elevated levels of autophagy have often been observed in many cancers, while the survival of some cancer cells depends on autophagic activity. Beclin-1, an important mediator of autophagy, is upregulated in colorectal cancer, gastric cancer, liver cancer, and various other cancers, and contributes to autophagy enhancement and tumorigenesis (Giatromanolaki et al., 2018; Zheng et al., 2020). Furthermore, the deletion of essential autophagy genes in cancer cells causes a dramatic reduction in tumor growth and a corresponding prolongation of the survival of model animals (Levy et al., 2017).

Drugs also often induce cytoprotective autophagy in cancer cells when they exert anticancer effects. Autophagy inhibition has been shown to increase the effects of anticancer drugs in cancer treatment. For example, paclitaxel, which has been identified as an effective mitotic inhibitor to treat various aggressive malignancies, promotes apoptotic cell death, accompanied by the induction of autophagy in cancer cells. Meanwhile, pretreatment with autophagy inhibitor 3-methyladenine (3-MA) or small interfering RNA (siRNA) against the autophagic gene *BECN1* can enhance the chemotherapeutic effect of paclitaxel (Xi et al., 2011). Cisplatin induces protective autophagy through the induction of Beclin-1 in human bladder cancer cells, but its combination with autophagy inhibitors can enhance its anticancer effects (Lin et al.,

2017). A randomized phase II preoperative clinical study demonstrated that the addition of the autophagy inhibitor hydroxychloroquine (HCQ) to preoperative gemcitabine and nab-paclitaxel improved the pathologic response in potentially resectable pancreatic cancer (Zeh et al., 2020). In another phase I/II clinical trial, the combination of everolimus and HCQ was well-tolerated, with only everolimus-related toxicities. Further, the median progression-free survival (PFS) of this regimen was 6.3 months, which was longer than that (4.0 months) of everolimus for RECORD-1 (Haas et al., 2019).

Since autophagy plays a protective role in cancer treatment, cytoprotective autophagy is also considered to be an important factor in mediating multidrug resistance (MDR) in cancer cells. Recent mechanistic investigations have demonstrated that autophagic pathways are associated with the development of MDR in cancer cells. Shang et al. (2019) found that circular RNA PAN3 (circPAN3) contributes to drug resistance in acute myeloid leukemia by regulating autophagy. Li et al. (2019) proved that autophagy weakens osimertinib cytotoxicity through the regulation of stem cell-like properties in lung cancer. Adenosine triphosphate (ATP)-binding cassette (ABC) transporters are closely related to the MDR of cancer cells. Recent studies of tumor samples from colorectal cancer patients have found that the expression of ABC subfamily B member 1 (ABCB1) is positively associated with the expression of LC3 and Beclin-1 (Wu et al., 2015). Autophagy can also sustain cell proliferation, promote epithelial-mesenchymal transition (EMT), and reprogram tumor metabolism, which may regulate MDR during cancer treatment. For instance, EMT impairs breast carcinoma cell susceptibility to cytotoxic T lymphocyte (CTL)-mediated lysis through induction of autophagy (Akalay et al., 2013). In antiangiogenic therapy, autophagy acts as an adaptive response that contributes to regulating the tumor microenvironment and mediates MDR in cancer cells (Hu et al., 2012). Moreover, in a single-arm phase Ib/II study, in patients with metastatic non-small-cell lung cancer (NSCLC), the addition of HCQ to chemotherapy (carboplatin and paclitaxel) was safe and resulted in a modest improvement in response rate for selected patients with newly diagnosed metastatic NSCLC. Further, autophagy inhibition induced by HCQ may overcome chemotherapy resistance in advanced NSCLC (Malhotra et al., 2019).

Cytoprotective autophagy can also protect normal cells or tissues from damage caused by anticancer drugs and reduce the side effects of these drugs. Doxorubicin, an anticancer drug, can cause dose-dependent cardiotoxicity and heart failure after long-term use. Feliz-Mosquea et al. (2018) found that in mice treated with doxorubicin, the systemic suppression of cluster of differentiation 47 (CD47) protected cardiac tissue viability and function through the upregulation of autophagic flux. As mentioned above, basal levels of autophagy are essential for normal tissue homeostasis. While the suppression of bone marrow is the main and most common side effect of anticancer drugs, it seems likely that autophagy inhibitors would have the capacity to collaterally increase drug toxicity in sensitive normal tissues such as bone marrow (Liu et al., 2018).

Thus, is it a good idea to suppress autophagy in cancer therapy? To answer this question, we need to understand the underlying contexts in which autophagy inhibition will be beneficial and those in which it could be detrimental.

3.2 Cytotoxic autophagy

Autophagy as a means of killing cells was first advanced by Clark's phenotypic description of "Type II autophagic cell death" in 1990 (Bialik et al., 2018). Currently, autophagy-dependent cell death is defined by the Nomenclature Committee on Cell Death as regulated cell death that depends on the autophagy machinery, with extensive cytoplasmic vacuolization, phagocytic uptake, and lysosomal degradation without extensive condensation of the nucleus or caspase activation (Galluzzi et al., 2018; Geng et al., 2020). This is consistent with definition of autophagic cell death, which is also commonly referred to as cytotoxic autophagy (Gewirtz, 2014; Zhang et al., 2021). Functionally, the reduced number of viable cells and/or clonogenic survival after treatment is attributed to cytotoxic autophagy. When autophagy is suppressed by both pharmacological inhibitors and genetic approaches, cancer cells become less sensitive to the treatment modality, and their survival rate increases. Currently, there are multiple examples in the literature where drugs exert anticancer effects by promoting cytotoxic autophagy. Artesunate, a derivative of artemisinin, exhibits potent anticancer activity by activating AMPK-mTOR-ULK1 pathway-dependent

autophagy in human bladder cancer cells (Zhou et al., 2020). Coptisine induces autophagic cell death through the downregulation of the PI3K-protein kinase B (Akt)-mTOR signaling pathway in Hep3B hepatocellular carcinoma cells (Kim et al., 2021). In addition, Kong et al. (2018) found that patients with chronic lymphocytic leukemia and high expression of ATG5 messenger RNA (mRNA) had a longer treatment-free survival. ABTL0812 is an autophagy inducer that promotes cancer cell death by the selective activation of cytotoxic autophagy in tumor cells. The safety, acceptable tolerability profile, and preliminary anticancer efficacy of ABTL0812 were demonstrated in a first-in-human phase I/Ib dose-escalation clinical trial (Vidal et al., 2021). Rapamycin is a representative mTOR inhibitor and autophagy inducer. However, its poor solubility and pharmacokinetics resulted in the development of several rapamycin analogs. Temsirolimus, a classical rapamycin analog, was approved by the US Food and Drug Administration (FDA) in 2007 for treating advanced renal cancer carcinoma (RCC) (Miricescu et al., 2021). Everolimus, another rapamycin analog, was also approved as a therapeutic agent for various cancers, including RCC, astrocytoma, breast cancer, angiomyolipoma, and neuroendocrine cancer (Mo et al., 2021).

Additionally, apoptosis induction has been a prevalent model used to develop anticancer drugs, but apoptosis evasion makes cancer cells resistant to drugs (Galluzzi et al., 2018). Autophagy, acting as a type II cell death mode, can avoid the apoptosis tolerance of cancer cells and exhibit strong anticancer effects. For example, cannabidiol overcomes oxaliplatin resistance by enhancing nitric oxide synthase 3 (NOS3)- and superoxide dismutase 2 (SOD2)-induced autophagic cell death in human colorectal cancer cells (Jeong et al., 2019). Neferine induces autophagy-dependent cell death in apoptosis-resistant cervical, breast, prostate, liver, and lung cancer cells (Law et al., 2019).

Extensive and prolonged autophagy seems to result in cytotoxicity, but in view of the protective functions of autophagy, theoretically, cytotoxic autophagy cannot be sustained.

3.3 Cytostatic autophagy

In addition to killing cells, inhibition of cell proliferation is an effective anticancer strategy (Galluzzi et al., 2018). Recent studies have identified an additional form of autophagy termed "cytostatic autophagy." The therapeutic implications of cytostatic autophagy would seem to be similar to those for cytotoxic autophagy. A novel orally available selenopurine molecule suppresses triple-negative breast cancer cell proliferation and progression to metastasis by inducing cytostatic autophagy (Chang et al., 2019). Berberine, an isoquinoline alkaloid from *Coptidis Rhizoma*, has been characterized as a potential anticancer drug that induces cytostatic autophagy via the inhibition of mitogen-activated protein kinase (MAPK)/mTOR/p70S6K and Akt signaling pathways (Zhang Q et al., 2020). Ivermectin can also markedly inhibit the growth of breast cancer cells by stimulating p21 (RAC1)-activated kinase 1 (PAK1)-mediated cytostatic autophagy in vitro and in vivo (Wang et al., 2016). Similar to the impact on cytotoxic autophagy in cancer treatment, pharmacological inhibition of cytostatic autophagy could also reduce the sensitivity of cancer cells to drugs. Furthermore, drug-induced cytostatic autophagy represents an alternative strategy for the treatment of apoptosis-deficient cancer cells. Notably, what distinguishes this form of autophagy from cytotoxic autophagy is that evidence of cell killing during cancer treatment is not detected.

Theoretically, autophagy is an effective strategy to maintain cell homeostasis under nutrient deprivation and stress conditions (Towers et al., 2020). In addition, some researchers have suggested that cytostatic autophagy is an early state of cytotoxic autophagy and that the intensity and duration of autophagy are not enough to induce cell death (Sharma et al., 2014). However, others believe that cytostatic autophagy is a precursor of apoptosis, and could induce apoptosis when the effects of cytostatic autophagy accumulate to a certain extent. Dou et al. (2016) found that ivermectin-induced autophagy inhibited the growth of breast cancer cells. No significant apoptosis was observed until 48 h after ivermectin treatment, suggesting that short-term treatment with ivermectin induces cytostatic autophagy in breast cancer cells. Therefore, accumulating evidence is revealing the great therapeutic potential of autophagy inducers in cancer with promising clinical benefits and controlled toxicity.

3.4 Nonprotective autophagy

In recent years, Andrew THORBURN and David A. GEWIRTZ discovered another form of autophagy

termed "nonprotective autophagy" (Gewirtz, 2014; Thorburn, 2020). The inhibition of nonprotective autophagy fails to sensitize cancer cells to drugs during cancer treatment. Radiation-induced autophagy has been proven to be essentially nonprotective in H460 cells because the autophagy inhibitors 3-MA and bafilomycin A1 both fail to alter radiation sensitivity or promote apoptosis in H460 cells (Xu et al., 2018). Moreover, the inhibition of autophagy had no observable impact on the anticancer effects of cisplatin in p53 wild-type NSCLC, which is consistent with the functional definition of nonprotective autophagy (Patel et al., 2020). In a phase II trial, high-dose pantoprazole inhibited docetaxel-induced autophagy in metastatic castration-resistant prostate cancer. Further, the combination of docetaxel and pantoprazole was well-tolerated, but the resultant clinical activity was not sufficient to meet the ambitious predefined target (Hansen et al., 2019). In addition, our previous study proved that 5-(3,4,5-trimethoxybenzoyl)-4-methyl-2-(*p*-tolyl) imidazol (BZML), a novel colchicine-binding site inhibitor, exhibited desirable anticancer activity against various cancer cells, including A549, HCT116, SW480, and Caco-2 cells, where autophagy acted as a nonprotective type of autophagy during cancer cell apoptosis (Bai et al., 2017, 2018, 2020). However, little is known about the roles or mechanisms of nonprotective autophagy during cancer treatment. Notably, nonprotective autophagy is not a meaningless function. Most researchers ignore the potential homeostatic advantages provided by nonprotective autophagy, and accumulation of these homeostatic advantages could have substantial effects on cells in the future. Therefore, nonprotective autophagy reflects our limited understanding of the functions and mechanisms of autophagy.

4 Interaction between autophagy and other cell death modes

4.1 Autophagy and apoptosis

Apoptosis, also known as type I programmed cell death, is a method of orderly cell death controlled by genes to maintain cell homeostasis (Galluzzi et al., 2018; Sun et al., 2021). Most drugs exert anticancer effects by inducing apoptosis, and this process is often accompanied by autophagy. In other words, common upstream signals may contribute to the triggering of autophagy and apoptosis, leading to combined autophagy and apoptosis in cancer treatment. Undoubtedly, there are multiple connections between the apoptotic and autophagic processes, and these two phenomena jointly seal the fate of cells. Therefore, the relationship between autophagy and apoptosis is more complex than the relationship between autophagy and other modes of cell death.

Four different functions of autophagy have been found in the process of apoptosis induced by anticancer drugs (Gewirtz, 2014; Bai et al., 2018; Xu et al., 2018; Patel et al., 2020). Beclin-1 can enhance cisplatin-induced apoptosis in Hep-2 laryngeal carcinoma cells via Bcl-2-modulated autophagy (Yang et al., 2018). In contrast, in A549 human lung cancer cells, the disruption of autophagy via Beclin-1 inhibition may promote cisplatin-induced apoptotic cell death (Chen et al., 2018). It is incredible that the same drug can have opposite effects in cancer treatment. In addition, cisplatin has been shown to induce apoptosis accompanied not only by nonprotective autophagy in p53 wild-type H460 cells, but also by protective autophagy in isogenic p53 null H460 cells (Patel et al., 2020). These results indicate that autophagy regulation is a controversial strategy in cancer therapy, and that the effect of autophagy on cancer cell fates, especially cell apoptosis, is unpredictable. Therefore, the regulation of autophagy during the process of apoptosis induced by anticancer drugs should be further analyzed, and we should not choose to inhibit autophagy in all circumstances.

4.2 Autophagy and cellular senescence

Besides apoptosis, cellular senescence is an important anticancer effect induced by drugs (Liu et al., 2020). It is an irreversible cell cycle arrest in response to different damaging stimuli including DNA damage, oxidative stress, and oncogenic stress, acting as a potent tumor suppressive mechanism (Galluzzi et al., 2018; Liu et al., 2020). It was originally thought that to suppress cellular senescence, the damaged macromolecules or organelles occurring in this process should be removed by autophagy. Interestingly, some recent studies claim that autophagy can promote cellular senescence by facilitating the synthesis of senescence-associated secretory proteins (McKay and White, 2021; Zhang et al., 2021).

Inhibition of cyclin-dependent kinase 4/6 (CDK4/6) by palbociclib leads to cellular senescence accompanied by autophagy induction in gastric cancer cells. Furthermore, the simultaneous blockade of CDK4/6 and autophagy in these cells exacerbates the senescence phenotype, suggesting that autophagy represents an adaptive mechanism that promotes cell survival rather than an effector mechanism of senescence (Valenzuela et al., 2017). In addition, Valenzuela et al. (2017) reported that CDK4/6 and autophagy inhibitors synergistically induced senescence in Rb-positive cytoplasmic cyclin E-negative cancers. In contrast, Huang et al. (2014) showed that autophagy promoted radiation-induced senescence in human breast cancer cells. Moreover, potent and persistent activation of autophagy induced by inhibitors targeting mTOR leads cancer cells to arrest senescence and cellular proliferation (Nam et al., 2013). This suggests that the effects of cytostatic autophagy may be realized by causing cellular senescence during cancer treatment. Current research indicates that autophagy is an important mechanism in cell senescence regulation, but the seemingly opposite effects of autophagy in cell senescence indicate its complex regulatory functions in the cellular senescence process. Therefore, how to make full use of the anticancer effects of autophagy and senescence remains to be determined.

4.3 Autophagy and mitotic catastrophe

MC is a newly identified type of anticancer mechanism in cancer treatment and MDR prevention that has received more attention in recent years. It differs from other cell death modes in which it is characterized by unique nuclear alterations, such as multi- or micro-nucleation (Bai et al., 2017). This suggests that we should pay attention to the interaction between autophagy and MC.

In colorectal carcinoma cell lines, MC induction by the DNA-damaging drug doxorubicin and the antimitotic drug colcemid ultimately led to autophagy followed by apoptosis, demonstrating that apoptosis and autophagy are consequences of MC induction (Sorokina et al., 2017). In addition, in non-small-cell lung carcinoma cells with *ATG13* knockdown, autophagy suppression led to a dramatic decrease in the MC rate, which demonstrates that autophagy is a necessary step for cell death induction after MC provocation (Sorokina et al., 2017). However, pharmacological inhibition of autophagy does not prevent DNA-damaging agent (etoposide and cisplatin)-induced ATG5-dependent MC, but shifts the balance to early caspase-dependent cell death (Maskey et al., 2013). Interestingly, our previous studies (Bai et al., 2017, 2018) proved that autophagy acted in its nonprotective role during BZML-induced apoptosis in A549 cells, whereas it acted in a cytoprotective manner against BZML-induced MC in A549/Taxol cells (an MDR A549 cell line). In detail, autophagy can block the occurrence and development of MC in BZML-treated A549/Taxol cells (Bai et al., 2017, 2018). This may explain why the cell death time of MC is longer than that of other cell death modes during cancer treatment.

The molecular mechanisms underlying the occurrence and development of MC are still lacking. Further studies on the molecular mechanism of autophagy and its interaction with MC may provide new insights for understanding the occurrence and development of MC. Collectively, autophagy and MC are tightly bound at the molecular level. This means that genotoxic stress may cause a complicated series of molecular events regulating both of these processes rather than only one.

4.4 Autophagy and ferroptosis

Ferroptosis, an iron-dependent process of regulated cell death, was discovered by Dixon et al. (2012). It lacks the morphological and biochemical characteristics of apoptosis, necrosis, and autophagy, but the keys for its identification are iron dependence, lipid peroxide accumulation, and a decrease in glutathione content. Genetically, cyclooxygenase-2 (COX2), acyl-CoA synthetase long chain family member 4 (ACSl4), and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase 1 (Nox1) are upregulated, while glutathione peroxidase 4 (GPx4), solute carrier family 7 member 11 (SLC7A11), ferritin, and ferritin light chain are downregulated in ferroptotic cells (Dixon et al., 2012; Galluzzi et al., 2018).

Initially, Dixon et al. (2012) found that erastin-induced ferroptosis was not modulated by inhibitors of lysosomal function/autophagy (bafilomycin A1, 3-MA, and chloroquine (CQ)). Some researchers also claimed that autophagy and ferroptosis often occur in parallel and independently in cancer treatment. For example, Chen et al. (2020) suggested that dihydroartemisinin induced lysosomal degradation of ferritin in an autophagy-independent manner, and Ma et al. (2017)

reported that ferroptosis and autophagy-induced cell death occur independently in breast cancer cells.

However, increasing evidence confirming that autophagy contributes to ferroptosis by the degradation of ferritin in cancer cells has challenged these early observations. Autophagy is needed for the occurrence of ferroptosis, and when autophagy is inhibited, cells cannot undergo ferroptosis. For example, the knockout or knockdown of *ATG5* and *ATG7* limits erastin-induced ferroptosis with decreased intracellular ferrous iron levels and lipid peroxidation (Hou et al., 2016). In addition, lysosomal activity is involved in lipid reactive oxygen species (ROS)-mediated ferroptotic cell death through the regulation of cellular iron equilibria and ROS generation. However, pharmacological inhibition of autophagy or lysosomes attenuates drug-induced ferroptosis in cancer cells. This may be attributed to the blockade of autophagy-induced ferroptosis feedback loop activation resulting from prolonged iron-mediated ROS production (Torii et al., 2016). Furthermore, many ferroptosis inducers have been reported to trigger excessive activation of autophagy, thereby favoring the induction of cell death (Gao et al., 2016). Thus, an increasing number of researchers have considered ferroptosis to be a type of autophagy-dependent cell death. That is, autophagy can induce cancer cell death by promoting ferroptosis and can reverse apoptosis defect-mediated MDR in cancer cells. In total, these results suggest that the inhibition of autophagy is not a wise approach during anticancer drug-induced ferroptosis in cancer cells.

4.5 Autophagy and necroptosis

Necroptosis, an important form of programmed cell death, is a highly regulated death-receptor-mediated caspase-independent cell death. It is mediated mainly by receptor-interacting serine/threonine-protein kinase 1 (RIPK1), RIPK3, and mixed lineage kinase domain-like protein (MLKL) (Najafov et al., 2017; Galluzzi et al., 2018). Inhibition of necroptosis by necrostatin-1 is an important marker to identify in cancer treatment (Zhuang and Chen, 2020). Increased clinical research has revealed the important functions of necroptosis in cancer prognosis, progression, and metastasis, which affect cancer immunosurveillance and cancer subtypes (Najafov et al., 2017). In addition, as evasion and resistance to apoptosis in cancer cells are increasingly popular research topics, necroptosis has emerged as a novel target against apoptosis resistance for cancer therapy. Therefore, it is necessary and urgent to discuss the relationship between autophagy and necroptosis.

It has been reported that shikonin, a natural naphthoquinone pigment purified from *Lithospermum erythrorhizon*, exhibits a potent anticancer effect by inducing necroptosis in NSCLC. Interestingly, inhibition of autophagy can further enhance necroptosis in combination with shikonin treatment (Najafov et al., 2017). Thus, the modulation of the anti-necrotic function of autophagy might be a novel preventive or therapeutic approach for NSCLC. Necroptosis has also been considered to be a highly immunogenic activity, often mediated via the release of damage-associated molecular patterns. Lin et al. (2018) found that shikonin can cause necroptosis accompanied by enhanced autophagy in mouse stage IV mammary carcinoma 4T1-luc2 cells. Interestingly, the enhanced immunogenicity and vaccine efficacy obtained via shikonin and CQ co-treatment of cancer cells suggested that autophagy may result in immunosurveillance (Lin et al., 2018). Moreover, autophagy inhibition enhances artepillin C-induced necroptosis in prostate cancer cells (Endo et al., 2018). Graphene oxide-CQ nanoconjugates induce necroptotic death in A549 cancer cells by blocking autophagic flux (Arya et al., 2018).

In general, autophagy plays a negative role against necroptosis. That is, the inhibition of autophagy may be an effective approach to enhance necroptosis in cancer treatment.

4.6 Autophagy and pyroptosis

Pyroptosis, a newly discovered caspase-dependent programmed cell death mode that plays a key role in sepsis, immune defense, and cancer treatment, is characterized by cell disruption induced by unruptured cells with continuous expansion. The released cell content finally causes an inflammatory response (Wang et al., 2017). Pyroptosis is triggered by canonical caspase-1 inflammasomes or noncanonical caspase-4/5/11. For cancer cells, the activation of pyroptosis may promote cell death and exert anticancer properties (Galluzzi et al., 2018).

Recently, some relationships between autophagy and pyroptosis have been reported. Yu et al. (2019) found that doxorubicin induced pyroptosis in melanoma SK-MEL-5, SK-MEL-28, and A375 cells. Interestingly,

doxorubicin also induced melanoma cell autophagy. Autophagy inhibition resulting from siRNA-Beclin-1 transfection or CQ pretreatment enhances pyroptosis significantly, suggesting that autophagy plays a protect role against pyroptosis (Yu et al., 2019). Further, paclitaxel treatment caused pyroptotic cell death, along with the activation of caspase-1 and maturation of interleukin (IL)-1β, as well as cleavage of gasdermin D (GSDMD) in advanced nasopharyngeal carcinoma cells. It is disappointing that autophagy could negatively regulate pyroptosis by inhibiting caspase-1/GSDMD activation (Wang et al., 2020). In contrast, nobiletin (a well-known polymethoxyflavonoid extracted from citrus fruits) induced ROS-mediated pyroptosis through regulating autophagy in ovarian cancer cells, but 3-MA treatment decreased nobiletin-induced cleavage levels of GSDMD and gasdermin E (GSDME) (Zhang RJ et al., 2020). These data suggest that the relationship between autophagy and pyroptosis is complex and uncertain in cancer treatment. Therefore, the use of autophagy to modulate the anticancer effects of pyroptosis should be approached with caution.

5 Conclusions

In summary, autophagy plays a very important role in cellular physiological processes and exhibits complex and diverse biological regulation in normal and cancer cells. Induction and altered autophagy are unavoidable in cancer treatment because most drugs can affect autophagy-related pathways. In addition, nutrient deprivation, hypoxia, and other physiological stimuli, which are important factors known to induce autophagy, are common in cancer cells.

Given this situation, it may be considered that the best strategy is to simply inhibit autophagy in cancer treatment. However, many clinical trials testing the enhancement of the anticancer activity of drugs by CQ and HCQ inhibition of autophagy are underway. Unfortunately, the clinical effects have not been fully satisfactory, and the roles of autophagy in cancer treatment are still debated (Levy et al., 2017). Depending on the type of cancer and the treatment strategy, autophagy fulfills different functions, including pro-survival, pro-death, and other properties. In addition, different drugs may exhibit potent anticancer effects by inducing different cell death modes in different cancer cells. This causes the interaction between autophagy and cell death modes to be more complex in cancer treatment. Therefore, this is a reminder that a "one size fits all" approach with interventions designed to inhibit or enhance autophagy in cancer therapy will not be successful.

To solve this problem, how autophagic functions are differentially regulated in different cancer cells and which factors determine the tissue-specific inhibition and/or activation of autophagy need to be established. Currently, there is no uniformly accepted methodology for assessing autophagy in clinical samples, let alone defining the function of autophagy should it be occurring. Fortunately, tumors and blood samples before and after treatment are easy to obtain, and gene detection technology has also been widely used in the clinic. This may aid the development of better biomarkers to identify beneficial or harmful autophagy during cancer treatment (Ben-Amar and Mliki, 2021; Cardozo et al., 2021). That is, we must try to tailor interventions according to the particular situation.

Together, these results imply that factors such as the type of cancer, drug therapy, and function of autophagy should be evaluated before targeting autophagy for cancer treatment.

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Author contributions

Zhaoshi BAI and Lingman MA designed the review. Zhaoshi BAI, Yaling PENG, Xinyue YE, Zhixian LIU, and Yupeng LI searched references. Zhaoshi BAI, Yaling PENG, and Lingman MA collated and summarized references. Zhaoshi BAI, Yaling PENG, and Lingman MA wrote the manuscript. All authors approved the final manuscript.

Compliance with ethics guidelines

Zhaoshi BAI, Yaling PENG, Xinyue YE, Zhixian LIU, Yupeng LI, and Lingman MA declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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