Role of Endoplasmic Reticulum Stress and Autophagy in Tumor Drug Resistance

Zhenwang Zhang

Abstract: Chemotherapy is widely used to treat tumors, either systemically or locally, and plays an important role in restricting the development of tumors. In clinic treatment for tumors, a satisfying response can be achieved when they firstly expose to the chemotherapeutic drugs. However, with anticancer drugs frequently exposing to the tumors, chemotherapy gradually becomes insensitive. The reasons for tumor drug resistance have been extensively investigated. For example, it may result from the change of its molecular target structure, apoptosis inhibition or some increased enzymes activity. With so many studies on the mechanisms of tumor drug resistance, recent researches mainly focus on the role of endoplasmic reticulum (ER) stress and autophagy in tumor drug resistance. ER stress is a kind of cellular stress condition caused by disturbance in the folding capacity because of various endogenous and exogenous insults. Autophagy, as a survival-promoting pathway, is a process of capturing, degrading, and recycling intracellular proteins and organelles in lysosomes. Accumulating evidences show that ER stress and autophagy are involved in the progression of tumors. Recently, it has been found that ER stress and autophagy play an important part in tumor drug resistance, which may provide another mechanism for drug resistance. Therefore, it is of significance to find a new way of overcoming tumor drug resistance through good knowledge of ER stress and autophagy.

Keywords: endoplasmic reticulum stress; autophagy; tumor; drug resistance; chemotherapy

1. Introduction

Cancer is one of the major causes for death in the world. It is reported to have more than 7.6 million deaths and more than 12.4 million new cases of cancers every year according to World Health Organization [1]. Chemotherapy remains one of the leading approaches to treat tumors at all stages [2]. Significant progress has made in chemotherapy and its efficacy has improved over time, but it is not curative and has various side effects such as treatment-related toxic effects [3]. For example, acute and long-run toxicities of chemotherapy may result in multi-drug resistance of cancer cells [4]. Chemotherapy is a method of treating cancer, and its mechanism of action mainly involves affecting the ability of cancer cells to divide and reproduce [5]. Chemotherapy drug weakens cancer cells and destroys them by acting directly on the cancer site or through the blood. The first stage of the development of chemotherapy started with the clinical application of folic acid antagonists and nitrogen mustard. The second stage in the history of chemistry, starting with Watson and Crick ’s description of the DNA helix structure [6], found the main family of anti-tumor drugs such as anti-mitotic drugs (vinca alkaloids), antibiotics (such as actin Bacteriocin D, bleomycin, anthracycline, etc.), nucleoside analogs and nucleobases (6-mercaptopurine, cytarabine and fluoropyrimidine) or cisplatin etc [7–16]. At this stage, the three main pillars of current chemotherapy are developed: (1) Intermittent administration of different drugs; (2) Combination of drugs with different mechanisms of action; (3) Adjuvant chemotherapy, chemotherapy can be used as a supplement to surgery and radiation therapy. Since the late 1990s, chemotherapy has transitioned to the so-called “targeted therapy” era. With the discovery of different oncogenes, tumor suppressor genes, and signaling pathways related to the carcinogenesis process and angiogenesis, it was found that guided chemotherapy drugs have developed targeted drugs such as Imatinib, Dasatinib, Nilotinib, Sorafenib, Erlotinib, Gefitinib, and Vemurafenib. At present, there are about 50 different kinds of chemotherapeutic drugs for the treatment of approximately 200 different kinds of cancers [1]. However, the efficacy is suboptimal and the major impediment to effective chemotherapy is drug resistance [17]. There are two types of tumor drug resistance including inherent resistance, where resistance exists before therapy and acquired resistance, which appears after treatment [18]. It is believed that the causes of tumor drug resistance are associated with the random drug-induced mutational events and the drug-induced karyotypic alterations [2]. Miserably, the decisive factor for this phenomenon is still unknown.

Currently, increasing studies show that endoplasmic reticulum (ER) stress and autophagy are closely related to...
tumors. ER, as an essential organelle in eukaryotic cells, is important to the synthesis, folding and secretion of protein and has to keep in a folding environment which is rich in Ca\(^{2+}\) and has strictly regulated oxidation [19]. ER stress refers to protein misfolding and accumulation of misfolded proteins which results from the disruption of the ER protein-folding environment because of environmental, physiological and pathological factors, as well as nutrient fluctuations [20]. Impairment of proper protein folding leads to accumulation of misfolded proteins and activates a specific cellular process known as the unfolded protein response (UPR) which is helpful to restore ER functionality and homeostasis [21]. Moderate ER stress can promote the growth and proliferation of cells, while severe ER stress may cause cell death [22]. In addition, another cellular process similar to ER stress is called autophagy, which also plays an important role in cancers. Autophagy is a cellular process where cells capture intracellular proteins, lipids and organelles, and send them to the lysosomal compartment for degradation [23]. Autophagy is primarily a mechanism of cell survival. However, excessive autophagy can lead to depletion of cellular organelles and self-destruction which are caused by some factors like DNA damage, oxidative stress, and starvation [22]. In recent years, a large number of studies have correlated autophagy with cancer and cancer theory [24]. This review gives a brief introduction to the biogenesis of ER stress and autophagy, their mode of action and their roles in tumor, especially tumor drug resistance.

2. The Occurrence of ER Stress

Endoplasmic reticulum (ER) is a very important organelle in eukaryotic cells. It is an important site for protein synthesis, folding and secretion. ER has a very strong homeostatic system, but many physiological and pathological factors can lead to an imbalance of homeostasis in ER, which is known as ER stress. These factors include malnutrition, ischemia and reperfusion, changes of glycosylation levels, DNA damage, oxidative stress and so on. In order to deal with ER stress, cells have formed an adaptive signaling pathway known as the unfolded protein response (UPR). UPR can re-establish homeostasis and alleviate ER stress through two mechanisms which are improving folding ability through expression of protein folding chaperones and downregulation of ER protein client load through suppressing general protein translation and enhancing the degradation of misfolded proteins [25]. In tumor cells, adaptive UPR can correct ER stress in a timely manner by addressing unfolded or misfolded proteins to maintain tumor growth and proliferation. In the process of tumorigenesis, the rapid proliferation of tumor cells requires the synthesis, modification and processing of a large number of proteins and this may cause physiological ER stress which is beneficial to the growth of tumor cells. UPR is mainly composed of three signaling pathways including inositol requiring enzyme 1 (IRE1α), activating transcription factor 6 (ATF6) and Protein kinase R (PKR)-like endoplasmic reticulum kinase (PERK) [26]. Under normal physiological conditions, glucose regulated proteins 78 (GRP78), a marker protein of ER stress, can combine with IRE1, ATF6 and PERK to inhibit the activity of these three receptors. When ER stress occurs in the cell, the three receptors are dissociated from GRP78, and at this time, ER receptor is activated [27]. Proper ER stress may play a protective role in cells. However, when strong or persistent ER stress occurs, a large number of misfolded proteins accumulated in the ER cannot be corrected and degraded in time. At this moment, the apoptotic signaling pathway is predominant.

ER is also an important site for storing Ca\(^{2+}\). Ca\(^{2+}\), as the second messenger in cells, plays a crucial role in the maintenance of cell division, movement, survival and apoptosis [28]. The concentration of Ca\(^{2+}\) in ER is 100–1000 μM, which is 1000 times the concentration of Ca\(^{2+}\) in the nucleus and cytoplasm. Many ER resident proteins are Ca\(^{2+}\) dependent. The Ca\(^{2+}\) concentration in ER is regulated by three pathways including inositol 1, 4, 5-trisphosphate receptor (IP3R), ryanodine receptor (RyR) and sarco/endoplasmic reticulum calcium adenosine triphosphatases (SERCA) [29]. When ER stress occurs, Ca\(^{2+}\) in ER will be released to mitochondria and cytoplasm. Intracellular disorder of Ca\(^{2+}\) and increased Ca\(^{2+}\) in mitochondria and cytoplasm play an important role in cell apoptosis [30]. ER regulates the homeostasis of Ca\(^{2+}\) through many Ca\(^{2+}\) binding proteins which include calreticulin (CRT), calnexin (CNX), and GRP78, GRP94 and protein disulfide isomerase (PDI) [31]. At the same time, the combination of these proteins with Ca\(^{2+}\) also enhances their functions and activity. Alteration of Ca\(^{2+}\) levels in ER can disrupt protein folding ability and cause mass accumulation of unfolded or misfolded proteins in ER, thereby activating UPR signaling pathway. GRP78, as a polypeptide dependent adenosine triphosphatase (ATP) enzyme, can use ATP to avoid folding of concomitant protein and accumulation in the process of folding. GRP78 can also stimulate the release of Ca\(^{2+}\) through hydrolysis of ATP and depletion of ATP in the ER cavity, so that the ATP/ADP ratio is maintained by promoting oxidative phosphorylation. The combination of CRT/CNX and aspartic acid sugar chains can promote the formation of the correct disulfide bond by activating the activity of ERp57. During the formation of disulfide bond, PDI is reduced and re-oxidized by thiol oxidase Ero1. Finally, electrons pass to oxygen. In addition to the chaperone proteins, troponin and chromogranin can also regulate Ca\(^{2+}\) level in ER.

Sarcoplasmic/endoplasmic reticulum Ca\(^{2+}\) ATPase (SERCA) is the main channel for the uptake of Ca\(^{2+}\) in ER and it can pump Ca\(^{2+}\) from the cytoplasm into ER [32]. Ca\(^{2+}\) pumping into ER requires 1 ATP hydrolysis. SERCA is mainly
composed of three homologous genes including SERCA1, SERCA2 and SERCA3. Increased Ca\(^{2+}\) level in in the cytoplasm can stimulate the activity of SERCA. However, resident proteins in ER such as CNX and CRT can inhibit the activity of SERCA and reduce the intake of Ca\(^{2+}\). Therefore, the activity of SERCA is the key factor to maintain the stability of Ca\(^{2+}\) in ER. When ER stress occurs, Ca\(^{2+}\) in ER decreases. At this time, the released Ca\(^{2+}\) can stimulate the activity of Ca\(^{2+}\) influx factor (CIF) and CIF will transfer to the cell membrane and activate store-operated Ca\(^{2+}\) entry channels (SOCs), which is known as Ca\(^{2+}\) internal flow induced by ER calcium pool depletion. The release pathway of Ca\(^{2+}\) in ER mainly consists of IP3R and RyR [33]. Increasing evidence showed that IP3R played a crucial role in the development, metastasis, drug resistance and metabolism of malignant tumors. RyR is mainly localized on the sarcoplasmic reticulum of cardiomyocytes and plays an important role in myocardial excitation-contraction coupling induced by the release of Ca\(^{2+}\).

The chemotherapy induced ER stress which contributes to drug resistance in cancer. Drug-resistant tumor cells are resistant to cell death triggered by endoplasmic reticulum stress [34]. In rapidly proliferating tumor tissues, tumor cells metabolize sugar rapidly, and solid tumors grow faster than their blood supply. Therefore, tumors are generally in short supply Sugar, acidosis and severe hypoxia [35]. In the face of changes in external environmental factors such as hypoxia, nutrient depletion, acidosis, unauthorized stromal cell and extracellular matrix exchange, the endoplasmic reticulum usually cannot express properly folded proteins, which leads to unfolded misfolded proteins. Accumulation in the reticulum cavity triggers ERS and activates UPR [36]. The activation of UPR protects cell survival during mild ERS and promotes the development of tumor cells in a more malignant direction. Therefore, ERS may also be one of the important mechanisms that regulate the development of drug resistance in tumor cells.

3. ER Stress-Related Anti-Tumor Drugs

It was reported that ER stress was closely related to the initiation and progression of tumors. GRPs are stress proteins produced by cells to assist in proper synthesis and folding of proteins. Compared with normal cells, GRP78 increased in tumor cells. High expression of GRP78 in tumor cells contributes to processing the unfolded or misfolded proteins for ER, thereby slowing down ER stress and maintaining cell survival [37]. Similarly, IRE1α and PERK also promote the survival and proliferation of tumor cells [38]. In embryonic fibroblasts, the deficiency of PERK may increase apoptosis induced by drugs. Besides, growing evidence suggested that UPR targeting compounds may be potential candidates for clinical cancer resistance medicine. Since UPR plays a dual role in cell survival and death, UPR targeting drugs are mainly divided into the following two types: drugs inducing severe ER stress and drugs hindering UPR that plays a protective role in cell survival. In current studies, this compound can be used as an independent therapeutic drug and can also combine with traditional anticancer drugs. Over the past few years, the development of compounds targeting UPR has become a research hotspot. There are several typical UPR targeting compounds.

3.1. Proteasome Inhibitors

To alleviate ER stress, cells can rely on ERAD pathway to degrade unfolded or misfolded proteins [39]. The proteins that need degradation in ER are transferred to the cytoplasm at first, and then they need to be modified by ubiquitination. Finally they are degraded by the 26S proteasome pathway. Proteasome with multiple catalytic functions regulates the expression of many kinds of proteins in cells. Inhibiting proteasome pathway can induce apoptosis by increasing the accumulation of proteins in ER and the degree of ER stress. Bortezomib is a 26S proteasome inhibitor and currently it has successfully entered the critical clinical trial phase. The main mechanism of bortezomib is that it can promote the mass accumulation of unfolded or misfolded proteins in ER and cytoplasm by inhibiting the activity of proteasome pathway and thus aggravate the degree of ER stress as well as activate apoptosis related to ER stress [40]. Bortezomib can act as a therapeutic agent for myeloma and hematologic malignancies. At the same time, studies have shown that bortezomib can increase the sensitivity of cancer to chemotherapeutic drugs.

3.2. Brefeldin A

Since proteasome inhibitors achieved good results in the treatment of cancers, many studies began to investigate the possibility of using unfolded or misfolded proteins transport process as therapeutic targets. Brefeldin A (BFA) is a naturally occurring macrolide antibiotic protein transport inhibitor. It can inhibit the transport of secretory proteins from ER to the Golgi apparatus by blocking the formation of coat protein II (COPII) vesicles, which promotes the accumulation of secretory proteins in ER and induces ER stress related apoptosis [41]. BFA has achieved good results in inducing apoptosis of tumor cells in vitro, but the effect of it in inhibiting solid tumors is not very good, which may be directly related to the poor solubility and stability of BFA in vivo. Therefore, Breflate, as a new protein transport inhibitor, emerged
under these conditions and it is water-soluble and more stable. Breflale has been shown to have a good therapeutic effect in mouse tumor models.

3.3. Hsp90 Inhibitors

Heat shock protein 90 (Hsp90) is an important molecular chaperone. It can regulate many kinds of intracellular proteins such as BCR-ABL, c-RAF, BRAF, AKT and VEGFR. Since Hsp90 plays an important role in signal transmission, compounds targeting Hsp90 may be potential therapeutic targets [42]. Hsp90 can also interact with the intracellular domains of PERK and IRE1 to influence the UPR process [43]. Hsp90 inhibitor 17-AAG and monorden can induce ER stress in myeloma cells and then activate three signal pathways of UPR as well as induce apoptosis. However, another Hsp90 inhibitor IPI-504 can induce apoptosis by inhibiting UPR not activating UPR. These conflicting results illustrate that different Hsp90 inhibitors have different specific substrates [44]. GRP94 is an important member of Hsp90 family. The previous study showed that inhibiting GRP94 could induce the expression of GRP78, while another Hsp90 inhibitor 514 could not induce the expression of GRP78.

3.4. Drugs Targeting GRP78

GRP78 and Hsp70 are highly homologous and GRP78 is considered as a member of the Hsp70 family. GRP78 is the most representative protein in ER, which can promote protein folding and maturation, and play a role in promoting cell survival. At present, GRP78 has been widely used in the treatment of cancers. In addition to being able to predict the sensitivity of cancer patients to chemotherapeutic drugs, GRP78 can also be a potential target in cancer treatment [45]. The low expression of GRP78 can increase the sensitivity of malignant glioma to anticancer drugs like temozolomide, etoposide and cisplatin. Macrocyclic compound versipelostatin, as a specific inhibitor of GRP78, can effectively block certain components of UPR, inhibit glucose starvation, and eventually lead to GRP78 transcription. Versipelostatin and cisplatin have a good synergistic effect on the treatment of solid tumors. Besides, GRP78 can also combine with melanoma differentiation-associated gene-7/IL-24 (mda-7/IL-24), and inhibit the mda-7/IL-24 induced apoptosis [46]. GRP78 is also involved in the folding and synthesis of secretory proteins, and in addition, it can also play a constructive role in the biological signaling pathway.

4. Roles of ER Stress in Cancer Chemotherapy

Although we have achieved great progress in the treatment of many kinds of tumors, drug resistance remains to be a major problem and may lead to poor clinical outcomes for patients [2]. Therefore, it is necessary to get a better understanding of the mechanism of drug resistance. Recently, increasing studies have showed that the UPR response is also one of the main mechanisms by which tumor cells can tolerate chemotherapeutic drugs. The rapid proliferation of tumors can lead to ischemia, hypoxia and malnutrition. At this time, a large number of unfolded or misfolded proteins accumulate in the ER, thereby activating UPR. In mild ER stress, the activation of UPR can promote the proliferation of tumors and increase its tolerance to chemotherapeutic drugs. However, in severe or prolonged ER stress, the pro-survival response of the UPR turns to a pro-death response, finally activating intrinsic apoptosis [19]. The relationship between ER stress and tumor drug resistance is being demonstrated by increasing reports.

4.1. Breast Cancer

Breast cancer, as one of the most common cancers among women, is a serious threat to women's health and life and is highly drug resistant. Cisplatin chemotherapy is the principal option for the treatment of malignant breast cancer and can prolong the patient's life. The main mechanism of cisplatin for the treatment of breast cancer is to inhibit the DNA replication and damage the cell membrane structure of cancer cells [47]. In addition, studies have shown that cisplatin can induce apoptosis of breast cancer cell line MCF-7 by disrupting the Ca^{2+} balance of cells. Long-term use of cisplatin can lead to drug resistance and adverse effects in patients such as gastrointestinal reactions, nephrotoxicity, and neurotoxicity. Cisplatin combined with other drugs has become a major trend in the clinical treatment of breast cancer. Steroid hormone can increase the sensitivity of breast cancer cells to cisplatin by increasing the expression of high mobility group protein 1 (HMG1). It has suggested that nelfinavir (NFV) should be regarded as an anticancer drug. ER stress induction and AKT inhibition seem to be related to the anticancer activity NFV. Angiogenesis plays an important role in tumor growth in vivo and ER stress is believed to inhibit the functions of endothelial cells and angiogenesis [48].
4.2. Ovarian Cancer

Ovarian cancer is the most common and lethal cancer in gynecologic malignancies. For lack of early screening methods, early detection and diagnosis are rare, and most patients with ovarian cancer are found to be in advanced stage. The traditional treatment for advanced ovarian cancer is surgical resection and adjuvant cisplatin chemotherapy. Cisplatin, a first-line anticancer drug, has been shown to be effective in the treatment of ovarian cancer. But with the long-term use of cisplatin, the patient is prone to be drug resistant, which largely affects the treatment effect. Although cisplatin has some side effects and resistance in the treatment of ovarian cancer, its derivatives and itself are still the first-line drugs for the treatment of ovarian cancer. It was found that S1 inhibited the survival of SKOV3 ovarian cancer cells and their related cisplatin resistant SKOV-3/DDP cells, while it induced apoptosis in drug-resistant tumor cells through the caspase-4 pathway mediated by ER stress [49].

4.3. Lung Cancer

Lung cancer is one of the most clinically common malignant tumors, which seriously threatens people's health and life span. Small cell lung cancer (SCLC) accounts for about 15% of lung cancer. At present, platinum is widely used in the treatment of SCLC. Cisplatin is the most commonly used platinum drug in the treatment of SCLC. Cisplatin has strong activity against SCLC cells, but its long-term use can cause adverse reactions in patients, including nephrotoxicity, nausea and vomiting. Besides, carboplatin, as the second generation of platinum compound with good therapeutic effect and small side effect, has also been widely used in the treatment of lung cancer. However, lung cancer cells may eventually be resistant to cisplatin though they may have sensitivity to this drug in the initial stage. Recent studies suggest that the ER serves as a cytosolic target of cisplatin and cisplatin can induce cell death through ER stress pathway [50]. In a previous study of human lung cancer, it was demonstrated that cisplatin could induce ER stress via the expression of GRP78, IRE1 and PERK [51].

4.4. Pancreatic Cancer

Pancreatic cancer, as a highly deadly malignancy, is expected to outstrip breast and colorectal cancers to become the second most common reason for deaths caused by cancers by 2020 [52]. Over the past few decades, although great efforts have been made to alleviate symptoms and to increase the survival of patients with pancreatic cancer through chemotherapy, this cancer still has a bad prognosis, because of the drug resistance including gemcitabine which is generally seen as the first-line chemotherapy regimen [2]. Increased evidence shows that this drug resistance is associated with specific metabolic aberrations of pancreatic cancer cells, which helps to regulate apoptosis, angiogenesis and drug targets, and ER stress is believed to inhibit angiogenesis [48,52]. In addition, ER stress often occurs in pancreatic cancer cells, which is caused by the accumulation of misfolded proteins in the ER because of various factors like protein overexpression, oxidative stress, hypoxia, and ER stress can also cause cellular inflammation and be associated with the induction of chronic pancreatitis [53].

5. Key Regulators of ER Stress in Chemoresistance

UPR signaling pathway is mainly composed of three receptors including IRE1α, ATF6 and PERK. GRP78 is the first discovered ER stress chaperone that participates in a variety of cellular processes, including tumor distant metastasis, drug resistance, and the correct folding of new synthetic proteins [54]. Apart from being a chaperone, GRP78 is an important regulator of ER stress sensors. Under normal physiological conditions, GRP78 will combine with these three sensors, thus inhibiting their activity. When ER stress occurs, GRP78 combines with new unfolded or misfolded proteins, leading to the separation of these three receptors from GRP78 [55]. At this point, the activity of the three receptors is activated.

5.1. IRE1α

IRE1α, also known as ERN1 (endoplasmic reticulum to nucleus signaling 1), is a type I transmembrane protein and is highly conservative [56]. Its cytoplasmic end has the activity of endonuclease and serine/threonine kinase. Mammalian IRE1 has 2 homologous isoforms, which are IRE1α and IRE1β. IRE1α is widely expressed and highly expressed in placenta, while IRE1β is mainly expressed in respiratory epithelial cells. Once dissociated from GRP78, IRE1α undergoes autophosphorylation and dimerization, thereby activating its own activity and RNase activity. The endonuclease domain of the activated endonuclease Ire1α can specifically cut out 26 introns of bases from the mRNA of X box-binding protein-1 (XBP-1). Sheared XBP-1 is an active transcription factor and it can promote the gene transcription of endoplasmic
reticulum stress responsive element (ERSE) and UPR target molecules, thus promoting the protein folding, phospholipid biosynthesis and ER-associated degradation (ERAD) in the ER [56]. In addition to cutting mRNA of XBP-1, endoribonuclease IRE1 can also cut other mRNA localized in the ER, which is called IRE1-dependent decay of mRNA (RIDD). Recent studies have reported that IRE1 can also cut microRNAs, which may activate inflammatory reaction and apoptotic signals. Besides, the Ser/Thr protein kinase domain of IRE1 can be combined with tumor necrosis factor alpha (TNFa) receptor associated factor 2, thereby activating the signaling pathways of nuclear factor-kappa B (NF-k B) and c-Jun N-terminal kinase (JNK) [57]. At the same time, IRE1a can also activate other kinases, such as extracellular signal-regulated kinases (ERKs). Besides, IRE1a-XBP1 signaling pathway plays an important role in the growth of mouse embryos, cell differentiation and survival.

5.2. PERK

Similar to IRE1α, PERK is also a type I transmembrane protein localized in the ER. Its N end is localized in the ER and C end is localized in the cytoplasm. Its C end is the main functional area, which has the activity of eukaryotic initiation factor 2α (eIF2α) protein kinase. When ER stress occurs, PERK dissociates from the GRP78 compound, and subsequently, the dissociated PERK can undergo oligomerization and autophosphorylation in its C end domain. At this moment, PERK can inhibit protein synthesis through the phosphorylation of fifty-first serine on eIF2α, and ultimately alleviate the stress level of ER. The phosphorylation of fifty-first site on eIF2α is important for the activation of the PERK signaling pathway. At the same time, it has been showed that the phosphorylation of eIF2α is of significance in maintaining normal processes of islet cells [58]. Besides, there are other three eIF2α kinases in mammals, including protein kinase double-stranded RNA-dependent (PKR), heme-regulated inhibitor (HRI) and general control non-derepressible-2 (GCN2). Each kinase receives a different stimulus signal: dsRNA can activate PKR kinase; the lack of amino acids can activate GCN2 kinase; the deficiency of hemoglobin activates HRI kinase. PERK signaling pathway can be activated in the early stage of ER stress [59]. At this point, PERK promotes cell survival by inhibiting protein synthesis and maintaining the ER homeostasis. However, when ER stress is prolonged, the activating transcription factor 4 (ATF4) in the downstream of PERK/eIF2α signaling pathway binds to growth arrest and DNA damage inducible 153 (GADD153/CHOP) and induces its expression. CHOP protein is a marker protein of ER stress related apoptosis as well as a key protein for the change of ER stress from a survival signal into an apoptotic signal [60]. Therefore, when ER stress occurs, PERK can not only inhibit the synthesis of proteins, but also play a role in promoting apoptosis.

5.3. ATF6

ATF6 is a type II transmembrane protein of c AMP-response element binding protein/basic leucine zipper (CREB/bZIP) transcription factor domain in the N-terminal part [61]. It is divided into two types: ATF6α and ATF6β. It has three domains: cytoplasm, transmembrane region and ER cavity. The C terminus, located in the ER cavity, has two binding Golgi-localization signals (GLS) and multiple GRP78 binding sites. There are also other bZIPs with similar structures and functions with ATF6 in vivo, including CREB, Tisp40, ATF1, Jun and so on. When ER stress occurs in vivo, ATF6α dissociates from the GRP78 compound and then shifts to the Golgi apparatus in a vesicular manner. In Golgi apparatus, inactive ATF6 is successively cleaved by site-1 protease (S1P) and site-2 protease (S2P), finally releasing the ATF6 with transcriptional activity [62]. Active ATF6 is successively released into the cytoplasm and the nucleus, and then starts the transcription of ER stress response element (ERSE) gene promoter by binding to the spliced XBP1 through ISO dimerization, which leads to increased expression of ER stress related proteins like GRP78, PDI and ERP72. These proteins promote the proper folding and transport of misfolded or unfolded proteins, thereby alleviating ER stress and maintaining the normal functions of ER. However, when ER stress is excessive or prolonged, unfolded or misfolded proteins cannot be properly folded or degraded in time. At this moment, cells will start the apoptotic signal. In recent years, the role of ATF6 in tumorigenesis has become a hot topic. Previous studies showed that ATF6 could not only promote the survival of cancer cells by up-regulating the expressions of GRP78 and PDI, but also induce apoptosis of cancer cells by activating caspase12 and CHOP and inhibiting the expression of Mcl-1 [63]. This distinct result may be related to the intensity and duration of ER stress.

6. What is Autophagy?

Autophagy is a life phenomenon that widely exists in eukaryotic cells [64]. It is a process in which cells wrap the damaged proteins and organelles through their monolayer or bilayer structures, forming autophagy through the growth of membranes and forming an autolysosome in lysosomes, and finally degrading their inclusions. Autophagy originates from what is known as the autophagic membrane and the source of the autophagic membrane has become the forefront and
hottest point in the field of autophagy. As for the origin of the autophagic membrane, there are two main viewpoints in the academic field. One is that intracellular organelles (including plasma membranes, ER, Golgi apparatus, ER–Golgi intermediates and mitochondria) are potential sources of the autophagic membrane. Another is that the autophagic membrane is assembled and synthesized at the site of the assembly of autophagy. Inclusions of autophagy degradation include sugars, nucleosides/nucleotides, amino acids and fatty acids, which can be released into the cytoplasm again for cell reuse. It should be noted that the molecular mechanisms regulating autophagy are highly conserved because the regulatory mechanisms are highly consistent in cells of yeast, nematodes, and higher vertebrates.

The process of autophagy mainly consists of the following four stages: the formation of autophagic membranes, the fusion of autophagosomes and lysosomes and the degradation of inclusions by autolysosomes [65]. There are three main types of autophagy including chaperone-mediated autophagy (CMA), microautophagy and macroautophagy. CMA means that the lysosomal chaperone Hsc70 and lysosome-associated membrane protein 2A (LAMP-2A) can help unfolded or misfolded proteins enter into autolysosomes and then these proteins are degraded and recycled. CMA is characterized by high specificity and it can only selectively degrade proteins with KFERQ-sequences, and cannot degrade damaged organelles at the same time. CMA plays an important role in the maintenance of intracellular homeostasis and protection of oxidative damage. Microautophagy is a process in which the protein or organelle in the cytoplasm is encapsulated and degraded directly by lysosomes [66]. It is a form of lysosomal direct and active phagocytosis of cytoplasmic components. Microautophagy is not specific for substrate selection, and its role in higher eukaryotes is not yet clear. In general, autophagy refers to macroautophagy, which is a process of intracellular organelles and proteins being transported to lysosomes and being degraded. Its substrate selection can be specific or nonspecific.

7. Role of Autophagy in Tumor

Autophagy is closely related to the initiation and development of tumors [67]. At present, it is a hot spot in tumor research. The occurrence and development of tumor is a long and complicated biological process and autophagy plays different roles in different stages of tumors. In the early stages of tumorigenesis, autophagy can maintain genomic stability and normal cell growth by degrading damaged proteins and organelles. At this point, autophagy plays a role in inhibiting tumorigenesis. Actually, autophagy plays a dual role in the development of tumors. Autophagy can promote the survival of cancer cells by resisting a range of stress conditions such as malnutrition and hypoxia. Autophagy can also induce apoptosis of cancer cells, which is known as autophagic cell death [68].

Unlike apoptosis, autophagy is a type II programmed cell death. In normal tissues, basal levels of autophagy play a "stewardship" role and maintain the growth of cells. Autophagy and ubiquitination of proteasome pathways in cells can clear unfolded or misfolded proteins to maintain intracellular environmental stability [69]. Therefore, dysfunction of autophagy can lead to the development of diseases and tumors. The deletion of autophagy related Atg5 and Atg7 genes can result in impaired functions of normal cells [70]. Therefore, autophagy plays a role in clearing up the accumulation of aberrant proteins and maintaining the stability of the internal environment. Studies have shown that autophagy plays a role in inhibiting tumor growth. As early as 1999, it was demonstrated that Beclin1, as a potential tumor suppressor, was downregulated in breast cancer compared with normal breast tissues. Therefore, it was inferred that low expression of autophagy associated proteins could contribute to the development and progression of breast cancer. Similarly, the deletion of Beclin1 could accelerate the progression of HBV hepatitis into liver cancer. These results indicate that Beclin1 is a tumor suppressor.

Besides Beclin1, some other autophagy related proteins can also inhibit the growth of tumors [71]. The deletion of Atg4C can accelerate the process of carcinogenesis induced fibrosarcoma. Bax-interactive factor-1 (Bif-1) is a positive regulator of autophagy and apoptosis and it plays an important role in the development of tumors. The deletion of Bif can inhibit programmed cell death and promote tumor growth. Ultraviolet radiation resistance-associated gene (UVRAG) is another autophagy related protein. It can bind with Beclin1 and thus induce autophagy. The deletion of UVRAG can decrease the activity of autophagy and induce the development of colon cancer and gastric cancer. Autophagy can inhibit the development of tumors, which does not depend on only one autophagy factor, but the interaction of all autophagy related factors.

Although it has been demonstrated that autophagy can inhibit the development of tumors, increasing evidence shows that autophagy also plays a role in promoting tumor survival [72]. In the process of tumor development, cancer cells need large amounts of oxygen and nutrients to maintain the rapid proliferation of tumor cells. However, many tumors are in a microenvironment of hypoxia and undernutrition during the course of their occurrence and development. The reason why these cells can survive in the environment of hypoxia and undernutrition may lie in autophagy which can maintain and promote tumor cell survival. Inhibition of autophagy can increase the sensitivity of cancer cells to stress conditions by
blocking autophagy dependent survival signals. Cancer cells can occur, develop and migrate under various stress conditions, which include energy deficiency, hypoxia, growth factor deletion, and cytotoxic effects mediated by chemotherapeutic drugs. Autophagy can degrade and reuse intracellular damaged proteins and organelles, thereby providing energy for cancer cells to maintain their survival [73]. Besides, some hold the view that autophagy is an early response to apoptosis, but when the degree of cell damage exceeds the protective load of autophagy, the cell executes apoptotic signals. Therefore, increased autophagy levels in cells can attenuate death induced by various stress conditions.

8. Autophagy Related Chemotherapy in Cancer

Chemotherapy drugs are the most commonly used adjuvant therapy for cancers and they have achieved good results in the treatment of advanced cancers [74]. However, with the long-term use of chemotherapeutic drugs, patients are resistant to these drugs, which limits the clinical use of chemotherapeutic drugs. Autophagy may be one of the major mechanisms for drug resistance of human tumors. Autophagy can promote tumor cells to survive better in adverse environment. Autophagy is more likely to be induced in the hypoxic environment of tumor cells. This protective effect of autophagy can lead to drug resistance, which makes the treatment of tumors more difficult. Autophagy maintains normal cellular metabolism through autocytoysis and avoids access to apoptotic pathway. Increasing evidence indicates that inhibition of autophagy increases the sensitivity of cancer cells to a range of treatments, including chemotherapy and radiotherapy. At present, the major methods of inhibiting autophagy include autophagy inhibitors and ShRNA methods [75]. Targeting autophagy has become a hot topic in cancer research. Inhibition of autophagy increases the sensitivity of breast cancer cells to trastuzumab. Interestingly, ShRNA can reverse the resistance of breast cancer to trastuzumab by decreasing the expression of LC3 [76].

Since autophagy is closely related to tumors, autophagy has become a potential target in the study of cancers. At present, drugs targeting autophagy at different stages are under study and application. For example, autophagy inhibitors 3-methyladenine (3-MA), LY294002 and wortmannin can suppress the early stage of autophagy by inhibiting the activity of PI3K [77]. Chloroquine (CQ) inhibits the late stage of autophagy by neutralizing the acidic environment of lysosomes. These inhibitors have already achieved good results in medical research. Because of the dual relationship between autophagy and tumors, the inhibition of autophagy may not necessarily reverse the tolerance of chemotherapy drugs. Therefore, autophagy inhibitors should not be blindly put into clinical use. The key problems are how to eliminate the protective effects of autophagy on tumor cells and how to induce autophagic cell death.

8.1. Cervical Cancer

Cervical cancer is one of the most common gynaecologic cancers in the world and paclitaxel combined with platinum chemotherapy is a recommended way for the treatment of advanced cervical cancer with only 30–60% the 5-year survival rate [78]. Besides, cisplatin alone or combining other anticancer drugs can be used to treat solid tumors including cervical cancer and it has been demonstrated that cisplatin can induce apoptosis in human cervical cancer HeLa cells, via the traditional Bax/Bcl-2, caspase-3, and mitochondrial apoptotic pathways [79]. Considerable anticancer drugs can activate autophagy and targeting autophagy may serve as a potential approach for the treatment of tumors and drug resistance [80]. After treatment with antitumor agents, some cancer cells experience autophagy, which can be regarded as a temporary survival mechanism, and the inhibition of autophagy results in apoptosis, thereby promoting antitumor effects [81].

8.2. Gastric Cancer

Gastric cancer (GC), as a very common malignant tumor, has unfavorable prognosis and high mortality rates around the world [82]. Therefore, it is very necessary to find an effective therapeutic way without side effects to treat gastric cancer. Cisplatin is a crucial drug used in chemotherapy for resectable and advanced GC and it can induce apoptosis by DNA damage via crosslinking of the DNA [2]. However, oncotherapy with anticancer drugs can lead to tumor drug resistance, which makes the prospect for patients with GC little optimistic. Apoptosis is a response to numerous cytotoxic stimuli like anticancer drugs and the key points of apoptosis are caspases which are regulated by two main pathways including an extrinsic pathway and an intrinsic mitochondrial pathway [83]. Recent studies have shown that apoptosis induction is related to increased autophagy and the two pathways share key molecular regulators. Autophagy, as an evolutionarily conserved stress response mechanism, usually occurs in apoptosis-defective cancer cells and can protect against cell death [82].

8.3. Bladder Cancer

Bladder cancer, the eleventh most common cancer around the world, is a malignant urinary tract cancer and it can
progress to metastatic disease with a poor prognosis [84]. Various standard treatments for this cancer like surgery, chemotherapy, and radiotherapy, have not obviously prolonged patient’s life span and patients with muscle invasive bladder cancer, one kind of bladder cancer, can be treated with chemotherapy, which is a rational alternative to cystectomy [2]. At present, cisplatin based combination therapies are standard treatment for advanced or metastatic bladder cancer [84]. However, intrinsic or acquired resistance to cisplatin in bladder cancer cells apparently obstructs the successful treatment [85].

A previous study has shown that in bladder cancer, autophagy is central to regulate trained immunity induced by BCG (Bacillus Calmette-Guerin), which is a widely used vaccine in the world [86]. Autophagy is a morphological process that can maintain cellular homeostasis. It has been demonstrated that baicalin, a new anticancer drug, can induce autophagy via Akt signaling pathway in T24 human bladder cancer cells [87].

9. Pathways of Autophagy in Tumor Drug Resistance

The mammalian target of rapamycin (mTOR) is the major signal transduction pathway regulating autophagy in normal cells. Under normal physiological conditions, the organism can inhibit autophagy by activating the PI3K/AKT/mTOR signaling pathway, thereby maintaining the growth and differentiation of normal cells [88]. However, factors such as malnutrition, growth factor restriction and hypoxia can activate autophagy by inhibiting PI3K/AKT/mTOR signaling pathway, thereby inhibiting the growth and differentiation of normal cells [89]. The regulation of autophagy is more complex in cancer cells than in normal cells because of the abnormal expression of PI3K/AKT/mTOR signaling pathway and the activation of other signaling pathways regulating autophagy [90]. There are some classic signaling pathways regulating autophagy in tumor cells.

9.1. PI3K-AKT-mTOR Signaling Pathway

Phosphatidylinositol 3-kinase (PI3K) is an important signaling factor involved in extracellular signals and cellular responses in cancer cells. It takes part in the proliferation, differentiation, migration, survival and glucose transport of cancer cells. PI3K can be divided into class I PI3K, class II PI3K and class III PI3K [91]. Among them, class I PI3K is most often studied, which is a heterogeneous dipolymer composed of a catalytic subunit and a regulatory subunit. Class I PI3K itself has serine/threonine (Ser/Thr) kinase activity and it can activate AKT by phosphorylation of the Ser3308 and Thr308 sites of the AKT protein. In contrast to class I PI3K, phosphatase and tensin homology deleted on chromosome 10 (PTEN) can inhibit the activity of AKT, thereby preventing the reverse signals regulated by AKT [92]. AKT can prevent the negative regulation of small G protein Rheb (Ras homology enriched in brain) through the phosphorylation of TSC1/TSC2 (tuberous sclerosis complex), finally activating the activity of mTOR [93]. Therefore, class I PI3K is a negative regulator of autophagy, while class III PI3K can induce autophagy by binding to autophagy related gene Beclin1. Therefore, PI3K-AKT-mTOR signaling pathway plays an important role in the regulation of autophagy. However, it is still necessary to have more studies about the specific role of autophagy regulated by this signaling pathway in the development and progression of tumors.

9.2. LKB1-AMPK-mTOR signaling pathway

The LKB1-AMPK-mTOR signaling pathway plays an important role in the regulation of lipid and carbohydrate synthesis in metabolically active tissues, including liver, muscle, and adipose tissue [94]. Interestingly, AMPK is also involved in the metabolism and development of tumor cells. Serine/threonine kinase LKB1 is the upstream kinase of AMPK and activates AMP by phosphorylation of threonine 172α ring site on the AMPKα subunit. Nutritional deficiency and energy metabolism stress can down regulate intracellular ATP levels, and also increase the proportion of intracellular AMP/ATP. Increased AMP/ATP can activate LKB1 and further activate AMPK, thereby inhibiting mTOR and inducing autophagy. In addition to regulating intracellular energy metabolism, AMPK also directly phosphorylates TSC2 and mTOR related regulatory protein Raptor, inhibiting the activity of mTOR. In hypothalamic neurons, T cells and endothelial cells, calmodulin-dependent protein kinase kinase β (Ca MKK β) is also involved in the activation of AMPK. In addition to regulating autophagy, the LKB1-AMPK-mTOR signaling pathway increases its stability by inducing p27kip phosphorylation. P27kip is a cyclin dependent kinase inhibitor and it can induce cell cycle arrest [95]. Besides, transforming growth factor-β activated kinase-1 (TAK1) is also an upstream activator of AMPK. Therefore, further research on the LKB1-AMPK-mTOR signaling pathway will help to understand the different regulatory mechanisms of autophagy, and also contribute to the research and development of anti-tumor drugs targeting autophagy.
9.3. P53

In nearly half of tumor cells, the expression of tumor suppressor protein p53 is down regulated or absent. Under various stress conditions, p53 is activated, which further leads to cell cycle arrest, aging and apoptosis. According to its different subcellular localization, p53 plays a dual role in the regulation of autophagy. P53 located in the nucleus can inhibit the activity of mTOR by activating the activity of AMPK and TSC1/TSC2, thus activating autophagy [96]. P53 can also activate autophagy by activating other downstream targets such as damage-regulated autophagy modulator (DRAM) [97]. Autophagy is activated by genes or drugs inhibiting p53 in the cytoplasm, which shows that non intranuclear p53 is an autophagy inhibitor. Under the conditions of hypoxia or nutritional depletion, the deletion of p53 activates autophagy by maintaining intracellular levels of ATP. Although p53 in the cytoplasm inhibits autophagy, the mutant form of p53 in the nucleus does not stop autophagy. Therefore, it is necessary to further investigate the mechanisms involved in the dual role of p53 in autophagy regulation. The regulation of autophagy by p53 may depend on the stress microenvironment in which cells are located [98]. In the early stages of tumorigenesis, p53 dependent autophagy acts as a "gatekeeper" that removes damaged cells and inhibits tumorigenesis. In advanced tumors, low expression or absence of p53 can maintain the growth of tumor cells by increasing the reuse of energy.

9.4. Beclin-2

Beclin1 plays a crucial role in the initial stage of autophagy. The function of Beclin1 is evolutionarily conservative. Under normal physiological conditions, the anti-apoptotic protein Becl-2 can bind to Beclin1, thereby inhibiting its activity [99]. The formation of Beclin1 and Bcl-2 compound depends on the BH3 domain of Beclin1 and the BH3 receptor of Bcl-2 [100]. Under stress condition, c-JNK can promote the phosphorylation of Bcl-2, which promotes dissociation of Bcl-2 from the BH3 domain of Beclin1, eventually activating autophagy. In the early stage of tumors, high expression of Beclin1 inhibits tumor progression by clearing damaged organelles. In the advanced stage of tumors, high expression of Beclin1 can restore autophagy and inhibit tumor development [101]. These findings show that Beclin1 inhibits the growth of tumors in the early and advanced stages of tumors.

10. The Interplay of ER Stress and Autophagy in Cancer

ER, as one of the most important organs in eukaryotic cells, plays a significant role in protein synthesis, intracellular Ca\(^{2+}\) homeostasis and lipid synthesis [102]. Intracellular stress conditions such as hypoxia, malnutrition, and intracellular Ca\(^{2+}\) imbalance can result in a large accumulation of misfolded proteins in the ER and eventually induce ER stress. As a protective mechanism, ER stress can activate autophagy, thereby alleviating ER stress, while inhibiting autophagy can enhance apoptosis mediated by ER stress. When ER stress occurs, Ca\(^{2+}\) is released from the ER to the cytoplasm and further activates the Ca\(^{2+}\) dependent signaling pathway. The activation of Ca\(^{2+}\) and CAMKK\(\beta\) dependent AMPK can activate autophagy [103]. Besides, it was found that PERK and IRE1 could also induce autophagy [104].

Autophagy and UPR are two newly discovered metabolic pathways and they are important for the survival of cancer cells in tumor microenvironment. Cell survival and cell death mediated by autophagy can be regulated by UPR pathways [48]. Besides, although ER stress and autophagy are two independent response mechanisms, the crucial relationship between them is that inhibition of autophagy plays a positive role in chemotherapeutic effects by up-regulating apoptosis mediated by ER stress [49].

According to a previous study, in human cervical cancer cells, cisplatin can trigger ER stress by advancing the formation of misfolded ubiquitinated proteins and ER stress can increase autophagy to degrade ubiquitinated proteins, which alleviates ER stress. 3-methyladenine (3-MA) and chloroquine (CQ), as two types of autophagy inhibitors, can upregulate the level of ubiquitinated proteins, thereby increasing ER stress and leading to a higher apoptotic rate of these cells. Besides, it is demonstrated that autophagy can clean up misfolded proteins and exert a positive influence on cell survival against ER stress under some conditions [79]. In addition, it has reported that bortezomib can activate apoptosis signal, and activation of ER stress and inhibition of autophagy play a role in the cytotoxicity of bortezomib in cervical cancer [80].

Many of the UPR signaling outputs related to autophagy also play a crucial role in the regulation of apoptosis. For example, when NF\(\kappa\)B activity is suppressed, caspase-8 and apoptosis are activated except autophagy in antiestrogen resistant breast cancer cells. Antiestrogens can induce apoptosis and autophagy in sensitive cells, while in resistant cells which have sensitivity to antiestrogens again by suppressing BCL2 and/or BCL-W, these cells die through an autophagy-associated necrosis instead of apoptosis [105]. It is found that UPR, which is induced as an ER stress response, may induce autophagy. Activation of GRP78, which is an ER chaperone participating in protein folding and assembly and ER-mediated stress signaling, has been related to enhanced melanoma development, oncogenic signaling, drug resistance,
and inhibition of cell death [22]. Additionally, it is reported that in normal and transformed cells, various stimuli can induce autophagy including starvation, ER stress, reactive oxygen species stress, or pharmacologic inhibition of mTOR [106]. All these studies show that ER stress and autophagy have close relationship and they play important roles in tumors and tumor drug resistance, which may have many related pathways.

11. ER Stress-Based and Autophagy-Based Therapeutics in the Clinical Use: Now and Future

The roles of ER stress and autophagy in tumors provide a new method to discover drug resistance mechanism and diagnosis, thereby finding effective treatments for malignant tumors. First, the progress made in terms of ER stress and autophagy makes contributions to the study of tumor drug resistance mechanism. As mentioned in this review, the roles of ER stress and autophagy in tumors and tumor drug resistance have been illustrated and the related drug resistance mechanisms were also expounded. It is known to us that both ER stress and autophagy play dual roles in tumors. As for ER stress, UPR plays a significant part in regulating various kinds of tumor behavior including cell proliferation, cell death, angiogenesis and invasion. For example, the specific PERK inhibitor GSK2656157 downregulates cancer growth in vivo, which is most likely through impaired angiogenesis and amino acid metabolism [107]. In addition, it has shown that bortezomib can kill hypoxic tumor cells through activation of UPR because hypoxia is a reason for protein misfolding and inhibition of the proteasome through bortezomib has been regarded as a new therapeutic method for malignant cancers like pancreatic cancer [107,108]. As for autophagy, on one hand, autophagy can inhibit tumor growth. For instance, Beclin1 (an essential autophagy gene) heterozygous mutant mice are tend to the progression of liver and lung tumors and lymphomas with long latency. On the other hand, autophagy contributes to tumor growth. For instance, basic autophagy increases in hypoxic tumor regions, in which it is important for tumor cell survival and autophagy also rises in RAS-transformed tumor cells and contributes to their growth, survival, tumorigenesis, invasion, and metastasis [109]. Besides, in many cases it has also shown that autophagy inhibition can decrease cancer cell death and promote tumor development after drug treatment [24]. Autophagy serves as a prosurvival pathway in the metabolically stressed tumor microenvironment. For example, it has been found that epidermal growth factor receptor (EGFR) inhibition by drugs like erlotinib (a standard treatment in EGFR-mutant lung cancer) needs autophagy to be induced to maximize suppression of lung cancer growth [110]. At present, the combination of autophagy inhibitory drugs like chloroquine and hydroxychloroquine with EGFR inhibitors are regarded as clinical trials to treat NSCLC (non-small cell lung cancer) [110]. Additionally, a previous study has found that the derivative of tamoxifen 4-dehydroxy-tamoxifen (OHT) can trigger K-Ras degradation by inducing autophagy, which is important for survival of malignant peripheral nerve sheath tumor (MPNST) cells as well as breast, colon, glioma and pancreatic cancer cells, which shows that the role of autophagic death induced by tamoxifen and OHT in tumor cells may be clinically significant in tumor treatment [111].

However, although the studies of ER stress and autophagy have great potential for improving the treatment of tumors, there are still various questions to be answered. Since ER stress and autophagy both play dual roles in tumors, one of the problems is whether they should be inhibited or stimulated to improve clinical outcomes in patients [112]. Besides, it is known that ER stress and autophagy are closely related to tumor drug resistance, while the study of chemotherapy drug resistance mechanism is limited by small scale. Therefore, further study on them is very necessary.

In conclusion, in the future, after the relatively comprehensive research of ER stress and autophagy in tumor drug resistance, they can serve as valuable therapeutics in overcoming tumor drug resistance.

Funding: This research was supported by Doctoral Foundation of HuBei University of Science & Technology Science, grant number BK202028.

Conflicts of Interest: The author declares no conflict of interest.

Copyright Statement

©2020 the authors. This article is an open access article licensed under the terms and conditions of the CREATIVE COMMONS ATTRIBUTION (CC BY) LICENSE (http://creativecommons.org/licenses/by/4.0/).

References

1. Kibria G, Hatakeyama H, Harashima H. Cancer multidrug resistance: mechanisms involved and strategies for circumvention using a drug delivery system. *Archives of Pharmacal Research*, 2014, 37: 4–15.
2. Deng H, Zhang J, Shi J, Guo Z, He C, et al. Role of long non-coding RNA in tumor drug resistance. *Tumor Biology*, 2016, 37: 11623–11631.
3. Weeks JC, Catalano PJ, Cronin A, Finkelman MD, Mack JW, et al. Patients' expectations about effects of chemotherapy for advanced cancer. *The New England Journal of Medicine*, 2012, 367: 1616–1625.
4. Lukianova-Hleb EY, Ren X, Zasadzinski JA, Wu X, Lapotko DO. Plasmonic nanobubbles enhance efficacy and selectivity of chemotherapy against drug-resistant cancer cells. Advanced Materials, 2012, 24: 3831–3837.

5. Reyes-Habito CM, Roh EK. Cutaneous reactions to chemotherapeutic drugs and targeted therapy for cancer: Part II. Targeted therapy. Journal of the American Academy of Dermatology, 2014, 71: 217.

6. Watson JD, Crick FH. Molecular structure of nucleic acids; a structure for deoxyribose nucleic acid. Nature, 1953, 171: 737–738.

7. Bernard J, Boiron M, Jacquillat C, Weil M, Najeay Y. A new agent active in the treatment of acute leukemia: cytosine arabinoside. La Presse Médicale, 1966, 74: 799–802.

8. Bonadonna G, Monfardini S, De Lena M, Fossati-Bellani F. Clinical evaluation of adriamycin, a new antitumour antibiotic. BMJ British Medical Journal, 1969, 3: 503–506.

9. Dimarco A, Gaetani M, Orezzi P, et al. ‘Daunomycin’, a new antibiotic of the rhodomycin group. Nature, 1964, 201: 706–707.

10. Falkson G, de Villiers PC, Falkson HC, Fichardt T. Natulan (Procarbazine) combined with radiotherapy in management of inoperable malignant melanoma. BMJ British Medical Journal, 1965, 2: 1473–1474.

11. Johnson IS, Armstrong JG, Gorman M, Burnett Jr JP. The vinca alkaloids: a new class of oncolytic agents. Cancer Research, 1963, 23: 1390–1427.

12. Mathe G, Schweisguth O, Brule G, et al. Trial therapy of Hodgkin’s disease and other malignant reticulo-histiocytic diseases by vincleukoblastin. La Presse Médicale, 1962, 70: 1349–1352.

13. Umezawa H, Ishizuka M, Maeda K, Takeuchi T. Studies on bleomycin. Cancer, 1967, 20: 891–895.

14. Felix W, Senn HJ. Clinical study of the new podophyllotoxin derivative, 4’-demethyllepipodophyllotoxin 9-(4,6-o-ethylidene-betad-glucopyranoside) (NSC-141540; VP-16-213), in solid tumors. CancerChemotherapy Reports, 1975, 59: 737–742.

15. Lippman AJ, Nelson C, Nelson L, Krakoff IH. Clinical trials of cisdiamminedichloroplatinum (NSC-119875). Cancer Chemotherapy Reports, 1973, 57: 191–200.

16. Holland JF. The chemical control of cancer. Public Health Reports, 1954, 69: 1151–1166.

17. Drinberg V, Bitcover R, Rajchenbach W, Peer D. Modulating cancer multidrug resistance by sertraline in combination with a nanomedicine. Cancer Letters, 2014, 354: 290–298.

18. Bach DH, Hong JY, Park HJ, Lee SK. The role of exosomes and miRNAs in drug-resistance of cancer cells. International Journal of Cancer, 2017, 141: 220–230.

19. Verfaillie T, Garg AD, Agostinis P. Targeting ER stress induced apoptosis and inflammation in cancer. Cancer Letters, 2013, 332: 249–264.

20. Wang M, Kaufman RJ. The impact of the endoplasmic reticulum protein-folding environment on cancer development. Nature Reviews Cancer, 2014, 14: 581–597.

21. Schonthal AH. Pharmacological targeting of endoplasmic reticulum stress signaling in cancer. Biochemical Pharmacology, 2013, 85: 653–666.

22. Riaz Ahmed KB, Kanduluru AK, Feng L, Fuchs PL, Huang P. Antitumor agent 25-epi Ritterostatin GN1N induces endoplasmic reticulum stress and autophagy mediated cell death in melanoma cells. International Journal of Oncology, 2017, 50: 1482–1490.

23. White E. Deconvoluting the context-dependent role for autophagy in cancer. Nature Reviews Cancer, 2012, 12: 401–410.

24. Maycotte P, Thorburn A. Autophagy and cancer therapy. Cancer Biology & Therapy, 2011, 11: 127–137.

25. Ozcan L, Tabas I. Role of endoplasmic reticulum stress in metabolic disease and other disorders. Annual Review of Medicine, 2012, 63: 317–328.

26. Liu Y, Jiang ZY, Zhou YL, Qiu H, Wang G, et al. β-elemene regulates endoplasmic reticulum stress to induce the apoptosis of NSCLC cells through PERK/IRE1α/ATF6 pathway. Biomedicine & Pharmacotherapy, 2017, 93: 490–497.

27. Soto-Pantoja DR, Wilson AS, Clear KY, Westwood B, Troizzi PL, et al. Unfolded protein response signaling impacts macrophage polarity to modulate breast cancer cell clearance and melanoma immune checkpoint therapy responsiveness. Oncotarget, 2017, 8: 80545–80559.

28. Gkouveris I, Nikitakis N, Asservatham J, Ogbaruere KUE. Interferon γ suppresses dentin sialophosphoprotein in oral squamous cell carcinoma cells resulting in antitumor effects, via modulation of the endoplasmic reticulum response. International Journal of Molecular Sciences, 2018, 53: 2423–2432.

29. Luciani DS, Giwiaza KS, Yang TL, Kalynyak TB, Bychkivska Y, et al. Roles of IP3R and Ryr Ca~(2+) Channels In Endoplasmic Reticulum Stress And β-cell Death. Diabetes, 2009, 58: 422–432.

30. Takeda K, Nagashima S, Shiiba I, Uda A, Tokuyama T, et al. MITOL prevents ER stress-induced apoptosis by IRE1α ubiquitylation at ER–mitochondria contact sites. The EMBO Journal, 2019, 38: e100999.

31. Xiao G, Chung TF, Pyun HY, Fine RE, Johnson RJ. KDEL proteins are found on the surface of NG108-15 cells. Molecular Brain Research, 1999, 72: 121–128.

32. Yong J, Bischof H, Burgstaller S, Siirin M, Murphy A, et al. Mitochondria Supply ATP to the ER Through a Mechanism Antagonized by Cytosolic Ca~^2+. Elife, 2019, 9: e49682.
60. Lin P, Yang YZ, Li X, Chen F, Cui C, et al. Endoplasmic reticulum stress is involved in granulosa cell apoptosis during follicular atresia in goat ovaries. *Molecular Reproduction & Development*, 2012, 79: 423–432.

61. Chi KT, Zhou HJ, Wong CM, Lee JM, Chan CP, et al. The Liver-Enriched Transcription Factor CREB-H Is a Growth Suppressor Protein Underepressed in Hepatocellular Carcinoma. *Nucleic Acids Researc.* 2005, 33: 1859–1873.

62. Papaioannou A, Higa A, Jégou G, Jouan F, Pineau R, et al. Alterations of EDEM1 functions enhance ATF6 pro-survival signaling. *The FEBS Journal*, 2018, 285: 4146–4164.

63. Degracia DJ, Kumar R, Owen CR, Krause GS, White BC. Molecular Pathways of Protein Synthesis Inhibition During Brain Reperfusion: Implications for Neuronal Survival or Death. *Journal of Cerebral Blood Flow & Metabolism*, 2002, 22: 127–141.

64. Li GD, Wu DQ, Li BY. Research progresses on the role of cell autophagy in cancer. *Chinese Journal of Cancer*, 2009, 28: 445–448.

65. Wang L, Chen M, Yang J, Zhang Z. LC3 fluorescent puncta in autophagosomes or in protein aggregates can be distinguished by FRAP analysis in living cells. *Autophagy*, 2013, 9: 756–769.

66. Oku M, Sakai Y. Three Distinct Types of Microautophagy Based on Membrane Dynamics and Molecular Machineries. *BioEssays*, 2018, 40: e1800008.

67. Onorati AV, Dyczynski M, Ojha R, Amaravadi RK. Targeting autophagy in cancer. *Cancer*, 2018, 124: 3307–3318.

68. Yoshida GJ. Therapeutic strategies of drug repositioning targeting autophagy to induce cancer cell death: from pathophysiology to treatment. *Journal of Hematology & Oncology*, 2017, 10: 1–14.

69. An H, Statsyuk AV. An Inhibitor of Ubiquitin Conjugation and Aggresome Formation. *Chemical Science*, 2015, 6: 5235.

70. Nishida Y, Arakawa S, Fujitani K, Mizuta T, et al. Discovery of Atg5/Atg7-independent alternative macroautophagy. *Nature*, 2009, 461: 654–658.

71. Morselli E, Galluzzi L, Kepp O, Vicenzo JM, Criollo A, et al. Anti- and pro-tumor functions of autophagy. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, 2009, 1793: 1524–1532.

72. Johna D, Bahcreck EH. Life, death and autophagy. *Nature Cell Biology*, 2018, 20: 1110–1117.

73. White E, Mehnerd JM, Chan CS. Autophagy, Metabolism, and Cancer. *Clinical Cancer Research*, 2015, 21: 5037–5046.

74. Chen C, Lu Y, Yan S, Yi H, Yao H, et al. Autophagy and doxorubicin resistance in cancer. *Anti Cancer Drugs*, 2017, 29: 1–9.

75. Liu LD, Pang YX, Zhao XR, Li R, Jin CJ, et al. Curcumin induces apoptotic death cell and protective autophagy by inhibiting AKT/mTOR/p70S6K pathway in human ovarian cancer cells. *Archives of Gynecology & Obstetrics*, 2019, 299: 1627–1639.

76. Liao N, Zhang GC, An SL, Li XR. Inhibition of Autophagy Induced by PTEN Loss Promotes Intrinsic Breast Cancer Resistance to Trastuzumab Therapy. *Tumor Biology*, 2016, 37: 5445–5454.

77. Kim KY, Park KL, Kim SH, Yu SN, Park SG, et al. Inhibition of Autophagy Promotes Salinomycin-Induced Apoptosis via Reactive Oxygen Species-Mediated PI3K/AKT/mTOR and ERK/p38 MAPK-Dependent Signaling in Human Prostate Cancer Cells. *International Journal of Molecular Sciences*, 2017, 18: 1088.

78. Shen Y, Wang P, Li Y, Ye F, Wang F, et al. miR-375 is upregulated in acquired paclitaxel resistance in cervical cancer. *British Journal of Cancer*, 2013, 109: 92–99.

79. Xu Y, Yu H, Qin H, Kang J, Yu C, et al. Inhibition of autophagy enhances cisplatin cytotoxicity through endoplasmic reticulum stress in human cervical cancer cells. *Cancer Letters*, 2012, 314: 232–243.

80. Zhang Y, Bai C, Lu D, Wu X, Gao L, et al. Endoplasmic reticulum stress and autophagy participate in apoptosis induced by bortezomib in cervical cancer cells. *Biotechnology Letters*, 2016, 38: 357–365.

81. Hou LL, Gao C, Chen L, Hu GQ, Xie SQ. Essential role of autophagy in fucoxanthin-induced cytotoxicity to human epithelial cervical HeLa cells. *Acta Pharmacologica Sinica*, 2013, 34: 1403–1410.

82. Pan WR, Chen PW, Chen YL, Hsu HC, Lin CC, et al. Bovine lactoferricin B induces apoptosis of human gastric cancer cell line AGS by inhibition of autophagy at a late stage. *Journal of Dairy Science*, 2013, 96: 7511–7520.

83. Lim SC, Han SI. Ursodeoxycholic acid effectively kills drug-resistant gastric cancer cells through induction of autophagic death. *Oncology Reports*, 2015, 34: 1261–1268.

84. Ho JN, Byun SS, Lee S, Oh JJ, Hong SK, et al. Synergistic antitumor effect of triptolide and cisplatin in cisplatin resistant human bladder cancer cells. *Journal of Urology*, 2015, 193: 1016–1022.

85. Zhang Y, Wang Z, Yu J, Shi J, Wang C, et al. Cancer stem-like cells contribute to cisplatin resistance and progression in bladder cancer. *Cancer Letters*, 2012, 322: 70–77.

86. Buffen K, Oosting M, Quintin J, Ng A, Kleinijnenhuis J, et al. Autophagy controls BCG-induced trained immunity and the response to intravesical BCG therapy for bladder cancer. *PLOS Pathogens*, 2014, 10: e1004485.

87. Lin C, Tsai SC, Tseng MT, Peng SF, Kuo SC, et al. AKT serine/threonine protein kinase modulates baikalin-triggered autophagy in human bladder cancer T24 cells. *International Journal of Oncology*, 2013, 42: 993–1000.

88. Dey P, Kundu A, Sachan R, Park JH, Ahn MY, et al. PKM2 Knockdown Induces Autophagic Cell Death via AKT/mTOR Pathway in Human Prostate Cancer Cells. *Cellular Physiology & Biochemistry*, 2019, 52 : 1535–1552.
89. Xue JF, Shi ZM, Zou J, Li XL. Inhibition of PI3K/AKT/mTOR signaling pathway promotes autophagy of articular chondrocytes and attenuates inflammatory response in rats with osteoarthritis. *Biomedicine & Pharmacotherapy*, 2017, 89: 1252–1261.

90. Beiyun, Wang, Yuan, Cui L, Huang G. Autophagy of macrophages is regulated by PI3k/Akt/mTOR signalling in the development of diabetic encephalopathy. *Aging*, 2018, 10: 2772–2782.

91. Bilanges B, Posor Y, Vanhaesebroeck B. PI3K isoforms in cell signalling and vesicle trafficking. *Nature Reviews Molecular Cell Biology*, 2019, 20: 515–534.

92. Baron S, Manin M, Beaudoin C, Leotoing L, Communal Y, et al. Androgen Receptor Mediates Non-genomic Activation of Phosphatidylinositol 3-OH Kinase in Androgen-sensitive Epithelial Cells. *Journal of Biological Chemistry*, 2004, 279: 14579–14586.

93. Diblee CC, Cantley LC. Regulation of mTORC1 by PI3K signaling. *Trends in Cell Biology*, 2015, 25: 545–555.

94. Shohreh M, Power JHT, Chataway TK, Grantham HJM. A comparison of LKB1/AMPK/mTOR metabolic axis response to global ischaemia in brain, heart, liver and kidney in a rat model of cardiac arrest. *BMC Cell Biology*, 2018, 19: 7–18.

95. Liang J, Shao SH, Xu ZX, Hennessy B, Ding Z, et al. The energy sensing LKB1–AMPK pathway regulates p27kip1 phosphorylation mediating the decision to enter autophagy or apoptosis. *Nature Cell Biology*, 2007, 9: 218–224.

96. Budanov AV, Karin M. p53 Target Genes Sestrin1 and Sestrin2 Connect Genotoxic Stress and mTOR Signaling. *Cell*, 2008, 134: 451–460.

97. Crighton D, Wilkinson S, O’Prey J, Syed N, Smith P, et al. DRAM, a p53-Induced Modulator of Autophagy, Is Critical for Apoptosis. *Cell*, 2006, 126: 121–134.

98. Amaravadi R, Kimmelman AC, White E. Recent insights into the function of autophagy in cancer. *Genes & Development*, 2016, 30: 1913–1930.

99. Pattingre S, Tassa A, Qu XD, Garuti R, Liang XH, et al. Bcl-2 Antiapoptotic Proteins Inhibit Beclin 1-dependent Autophagy. *Cell*, 2005, 122: 927–939.

100. Maejima Y, Isobe M, Sadoshima J. Regulation of autophagy by beclin 1 in the heart. *Journal of Molecular & Cellular Cardiology*, 2016, 95: 19–25.

101. Liang XH, Jackson S, Seaman M, Brown K, Kempkes B, et al. Induction of autophagy and inhibition of tumorigenesis by beclin 1. *Nature*, 1999, 402: 672–676.

102. Liu L, Li J. Communications Between the Endoplasmic Reticulum and Other Organelles During Abiotic Stress Response in Plants. *Frontiers in Plant ence*, 2019, 10: 749–762.

103. Piero DP, Stefanie R, Annika GS, Miriam LM, Hall P, et al. A Systems Study Reveals Concurrent Activation of AMPK and mTOR by Amino Acids. *Nature Communications*, 2016, 21:13254–13271.

104. Chaurasia M, Gupta S, Das A, Dwarakanath BS, Simonsen A, et al. Radiation induces EIF2AK3/PERK and ERN1/IRE1 mediated pro-survival autophagy. *Autophagy*, 2019, 15: 1391–1406.

105. Clarke R, Cook KL, Hu R, Facey CO, Tavassoly I, et al. Endoplasmic reticulum stress, the unfolded protein response, autophagy, and the integrated regulation of breast cancer cell fate. *Cancer Research*, 2012, 72: 1321–1331.

106. Mahoney E, Lucas DM, Gupta SV, Wagner AJ, Herman SE, et al. ER stress and autophagy: new discoveries in the mechanism of action and drug resistance of the cyclin-dependent kinase inhibitor flavopiridol. *Blood*, 2012, 120: 1262–1273.

107. Clarke HJ, Chambers JE, Liniker E, Marcinak SJ. Endoplasmic reticulum stress in malignancy. *Cancer Cell*, 2014, 25: 563–573.

108. Wissmioewski TT, Meister S, Hahn EG, Kalden JR, Voll R, et al. Mucin production determines sensitivity to bortezomib and gemcitabine in pancreatic cancer cells. *International Journal of Oncology*, 2012, 40: 1581–1589.

109. White E. The role for autophagy in cancer. *Journal of Clinical Investigation*, 2015, 125: 42–46.

110. Wei Y, Zou Z, Becker N, Anderson M, Sumpter R, et al. EGFR-mediated Beclin 1 phosphorylation in autophagy suppression, tumor progression, and tumor chemoresistance. *Cell*, 2013, 154: 1269–1284.

111. Kohli L, Kaza N, Coric T, Byer SJ, Brossier NM, et al. 4-Hydroxytamoxifen induces autophagic death through K-Ras degradation. *Cancer Research*, 2013, 73: 4395–4405.

112. Levy JM, Thorburn A. Targeting autophagy during cancer therapy to improve clinical outcomes. *Journal of Clinical Investigation*, 2011, 131: 130–141.