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The Role of Cell Autophagy in Cancer and Its Application in Drug Discovery

Ming Hong, Ning Wang and Yibin Feng

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Abstract

Autophagy is a vital basic phenomenon that widely exists in eukaryotic cells. As one type of programmed cell death, autophagy has gained much more attention in the past few years. Recent studies suggest that the alterations in autophagy are associated with the genesis and development of cancers. It can affect cell apoptosis, angiogenesis, and treatment of tumor. Others' and our studies have found that some herbal medicines can induce autophagic cell death in cancer cell models. As herbal medicines are very important recourses for drug discovery and lead compounds of anticancer drugs, we have summarized the role of autophagy in inhibitive effect of natural products in cancer cell growth and metastasis. Finally, we present summary and critical comments on problems in current autophagy study and its future prospect.

Keywords: Autophagy, cancer, autophagic cell death, drug discovery, natural products

1. Introduction

Autophagy is a highly conserved cellular process that widely exists in eukaryotic cells. As one type of programmed cell death autophagy has gained great attention in the past few years. Recent studies suggest that autophagy is associated with the tumorigenesis and progression of cancers. It can affect tumour cell apoptosis, angiogenesis as well as the treatment outcome of chemotherapeutics. In this chapter, we have summarized the up-to-date research about cell autophagy, including but not limited to the molecular mechanisms underlying autophagy initiation and the potential targets that autophagy regulates. As many studies have demonstrated that autophagy plays an important role in cancer initiation and progression, we also critically reviewed the double-edged sword effect of autophagy in cancer, which is reflected as the cytoprotective autophagy and cytotoxic autophagy. The role of

autophagy in treatment and resistance of several present conventional anticancer agents is also discussed in our chapter. As Chinese medicines have a long history in treating cancer in Asian countries, we also introduced some anticancer natural products from Chinese medicines that target on autophagy in this chapter.

1.1. Cell autophagy overview through the up-to-date research information

Autophagy means “self-eating” in cells. It is a highly conserved cellular process among eukaryotes by which long-lived proteins and damaged organelles are packaged in the double-membrane autophagosomes and transported to lysosomes for degradation. Autophagy is one type of programmed cell death (PCD) for maintaining cell homeostasis by removing damaged or abnormal cells. It is a vital basic phenomenon that widely exists in eukaryotic cells, which has gained much more attention in the recent decades. In the process of autophagy, the intracellular damaged proteins or organelles are wrapped by the double-membrane structure autophagy vesicles and sent into the lysosome (for animal) or vacuoles (for yeast and plant) for further degradation and recycle. As autophagosome belongs to subcellular structures, normal optical microscope cannot observe it, so transmission electron microscope is needed for observing the process of autophagy. Generally, the features of phagophore are described as cup-shaped or crescent-shaped double-membrane or multilayer-membrane structure with the tendency of wrapping the cytoplasmic components. The characteristics of autophagosomes are described as double-layer or multilayer vacuole-like structure with cytoplasmic components such as mitochondria, endoplasmic reticulum, and ribosome. Autophagy lysosome is a monolayer-membrane structure in which the cytoplasmic components undergo degradation. In the study of the relationship between autophagy and cell death, the presence of a large number of autophagosomes or autophagic lysosomes were found in the cytoplasm before cell death, but these cells lack typical apoptosis features such as nuclear pyknosis and karyorrhexis or cell shrinkage and the formation of apoptotic bodies. So this is a new type of programmed cell death which is different from apoptosis. In order to distinguish between these two types of cell death, autophagy is also called Type II cell death. In contrast, apoptosis is called Type I cell death. However, whether autophagy is the direct cause of cell death is still controversial. Some researchers believe that cell death is caused by the process of autophagy, while some think that autophagy is not the direct reason for cell death.

As a main intracellular degradation and recycling process, autophagy is important for maintaining energy homeostasis and cellular remodeling during physiological process. Recent studies have suggested that alterations in autophagy can affect the genesis and development in several diseases such as inflammatory disease, heart disease, neurodegenerative disease, and cancer. A common characteristic in these diseases is a dysfunction of autophagy, which influenced the susceptibility of programmed cell death. Our knowledge of the functions and regulation of this programmed cell death pathway has increased substantially in recent years from studies conducted in *Drosophila* and yeast, as well as in mammalian cells and tissues.

Generally, there are three types of autophagy: chaperone-mediated autophagy, microautophagy, and macroautophagy. For chaperone-mediated autophagy, single intracellular proteins are recognized by a chaperone complex that improves target-motif-governed transport,

lysosomal membrane binding, migration, and subsequent internalization. For microautophagy, it utilizes lysosomal limiting membrane invagination, septation, or protrusion to transport cytoplasmic materials. Macroautophagy is a highly conserved cellular process by which cells sequester a part of their cytoplasm or organelles into the autophagosomes that will fuse with lysosomes for degradation and recycling of the enclosed materials. Of the above three types of autophagy, most of our current studies are focused on macroautophagy (hereafter referred to as autophagy), which is also the focus of this chapter. Although all these three types of autophagy differ in the way by which cargo material is transported to the lysosome, they are similar in the last step of lysosomal protein degradation by hydrolase exposure.

Autophagy is very important for cellular homeostasis. It can promote the catabolism of denatured proteins or damaged organelles and the elimination of long-lived proteins. In addition, autophagy is an important adaptive mechanism. It is augmented obviously when the cells need to cope with certain cellular stresses such as starvation. Recent studies have demonstrated that autophagy also plays pathological roles sometimes. But the molecular mechanism of these pathological effects remains unknown.

2. The molecular mechanisms of autophagy

2.1. The initial stage of autophagy

In the initial stage, serine–threonine protein kinase ULK1 promotes the autophagy process. Autophagy inducers such as the defective nutrition or exposure to some chemotherapy agents cause dephosphorylation of ULK1; the ULK1 complex consists of ULK1, ATG13, FIP200 and receives stress signals from mTOR complex 1. When mTORC1 kinase activity is inhibited, autophagosome formation occurs. ULK1 can activate the phosphorylation of ATG13 and FIP200 to start the initial stage of autophagy.

2.2. Double-membrane vesicle formation stage of autophagy

In this stage, the Beclin-1–Vps34 complex, which consists of Vps34, Beclin-1, ATG14L, and Vps15, takes part in the process of vesicle nucleation. Under certain circumstances that induce autophagy, the dephosphorylated ULK1 complex musters the Beclin-1–Vps34 complex to form the autophagosome by phosphorylation of Ambra1. In this stage, TRAF6 interacts with Ambra1, which acts as an E3 ubiquitin ligase for ULK1, which results in stabilization and self-association of ULK1 by K63 ubiquitination. The dephosphorylated ULK1 complex also facilitates the activity of the ATG14L-containing Vps34 complex by phosphorylation of Beclin-1. Furthermore, EGFR and AKT regulate autophagy by phosphorylation of Beclin-1 without the impact of mTORC1. Next, the phosphatidylinositol-3-phosphate created by Vps34 musters an effector protein to facilitate the formation of double-membrane vesicle. The WIPI protein, which is the transcription product of a member of the ectopic P-granule subset of the metazoan-specific autophagy gene family, also participates in this stage.

2.3. Double-membrane vesicle elongation stage of autophagy

ATG proteins participate in the accomplishing of the autophagosome and fit out into two ubiquitin-like conjugation systems, ATG8 (LC3)–phosphatidylethanolamine and ATG12–ATG5–ATG16L. For the ATG12–ATG5–ATG16L system, ATG12 is connected to ATG5 by the synergistic action of ATG107 and ATG. The ATG12–ATG5–ATG16L complex consists of ATG12–ATG5 and ATG16L by conjugate binding, which takes part in the LC3–phosphatidylethanolamine conjugation. LC3, created by the ATG4 protease from Pro-LC3, is connected to phosphatidylethanolamine by ATG3, ATG7, and the ATG12–ATG5–ATG16L complex. After the above procedure, the lipid-conjugated LC3, which is positioned to the double-membrane of the autophagosomes, takes part in the formation and elongation of autophagosomes.

2.4. Fusion and degradation stage of autophagy

The captured proteins or organelles waiting for degradation in an engulfing or developing autophagosome are promoted by autophagy adaptor proteins, or receptors act as a connection. The complete autophagosomes will fuse with lysosomes to become autolysosomes. In these structures, the captured organelles and materials will be digested by lysosomal enzymes.

2.5. The final stage of autophagy

In the final stage, an essential component of the mTORC1 complex (mTOR) is reactivated by nutrients created by the autolysosomes. This process is very important for depressing the redundant activation of autophagy when the cells are in the periods of starvation. The mTOR generated from this stage can produce raw materials to form new lysosomes so that the lysosomes do not reduce during the autophagy procedure.

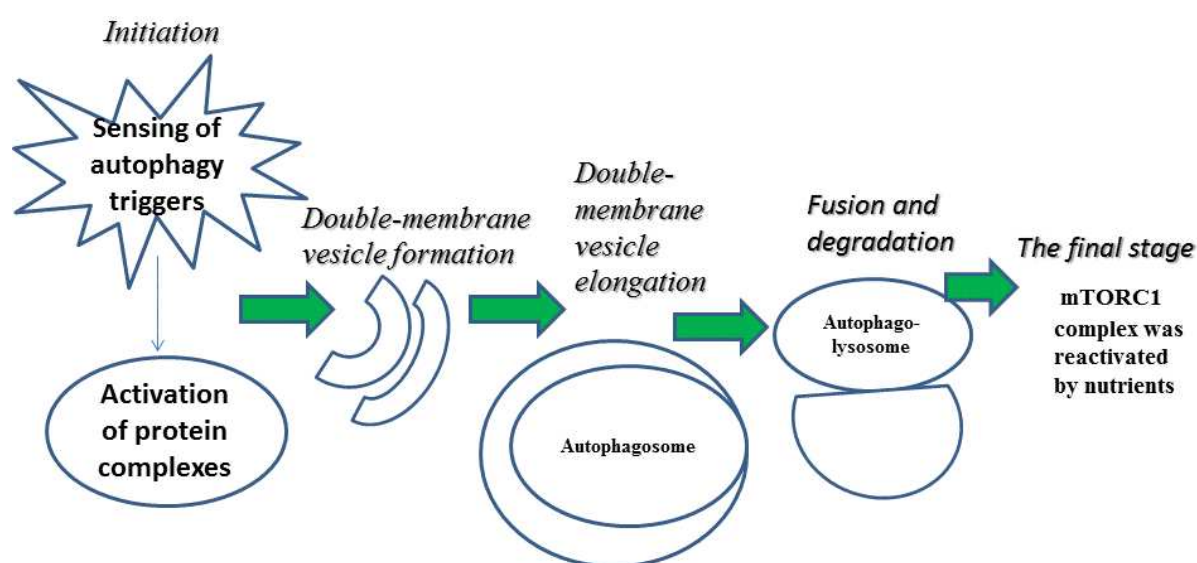


Figure 1. The process of autophagy

3. Major autophagy regulation target

cAMP-dependent protein kinases A (PKA), mammalian target of rapamycin complex 1 (MTORC1), and AMP-activated protein kinase (AMPK) are three major kinases for regulating cell autophagy. These kinases, along with some other molecules such as CAMKK2/CaMKK β and TSC1/2, participate in a wide range of intracellular or extracellular signal pathways to regulate autophagy.

3.1. cAMP-dependent Protein Kinase A (PKA)

When the cell faces some stresses such as absence of growth factors, nutrient starvation, hypoxia, or endoplasmic reticulum stress, the survival of these cells can be dependent on autophagy. Recent research has shown that Ras/PKA (cAMP-dependent protein kinase) signaling pathway was associated with the early stage of autophagy in yeast. The studies showed that Ras/PKA signaling pathway can suppress the early stage of autophagy in *Saccharomyces cerevisiae* cells when faced with nutrient-rich conditions [20]. In mammals, this suppression is conducted at least partially by the phosphorylation of microtubule-associate protein 1 light chain 3 by PKA.

3.2. Mammalian Target of Rapamycin Complex 1 (MTORC1)

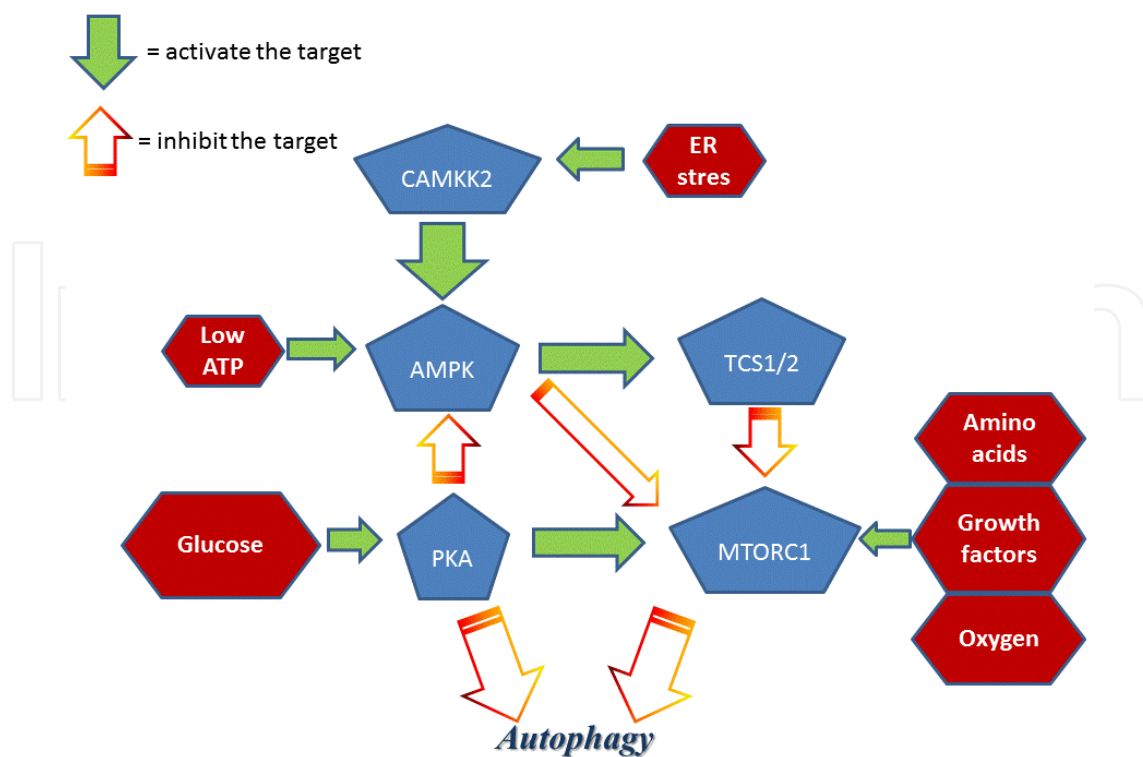
Studies have shown that MTORC1 is activated by the presence of amino acids. Amino acids can activate RAG (RAS-GTPases) proteins that regulate MTORC1. Recent research has demonstrated that in *Drosophila melanogaster* S2 cells, knockdown of Rag gene inhibited the activation effect of amino acids on TORC1. The constitutively active (GTP-bound) Rag gene expression can activate TORC1 without the presence of amino acids signals while expression of dominant-negative Rag can inhibit the activation effects of amino acids signals on TORC1. Researchers believe that there is some cross talk between these pathways. For example, PKA can directly activate MTORC1 and also indirectly activate MTORC1 by suppression of the AMPK.

3.3. AMP-activated protein Kinase (AMPK)

AMPK can respond to the change of ATP/AMP levels in cells and act as an intracellular ATP/AMP-sensing kinase. It is also a substrate of PKA and participates in regulating autophagy. AMP can activate the activity of AMPK, while ATP binding suppresses this kinase activity. AMPK can phosphorylate the TSC1/2 complex when activated by low ATP/AMP levels. Then, the phosphorylated TSC1/2 complex can indirectly or directly suppress the activity of MTORC1. Several studies have also demonstrated that AMPK can induce autophagy by activating ULK1.

3.4. Other regulating mechanisms

Some studies have proved that ER stress can cause autophagy by leading to Ca²⁺ concentrations' upregulation and inducing calcium/calmodulin-dependent protein kinase 2, beta



(Modified from: Antioxid Redox Signal. 2014 Jan 20; 20(3): 460–473)

Figure 2. The regulation mechanisms of autophagy

(CAMKK2/CaMKK β), to activate AMPK [29]. (UPR) signaling also plays an important role in ER stress-related autophagy. This signal pathway has been proved in both mammals and yeast models. However, the final results of autophagy in response to ER stress are still not clear. Some researchers insist that it will improve cell survival while others believe that it may lead to cell death.

4. The relationship of cell autophagy and cancer

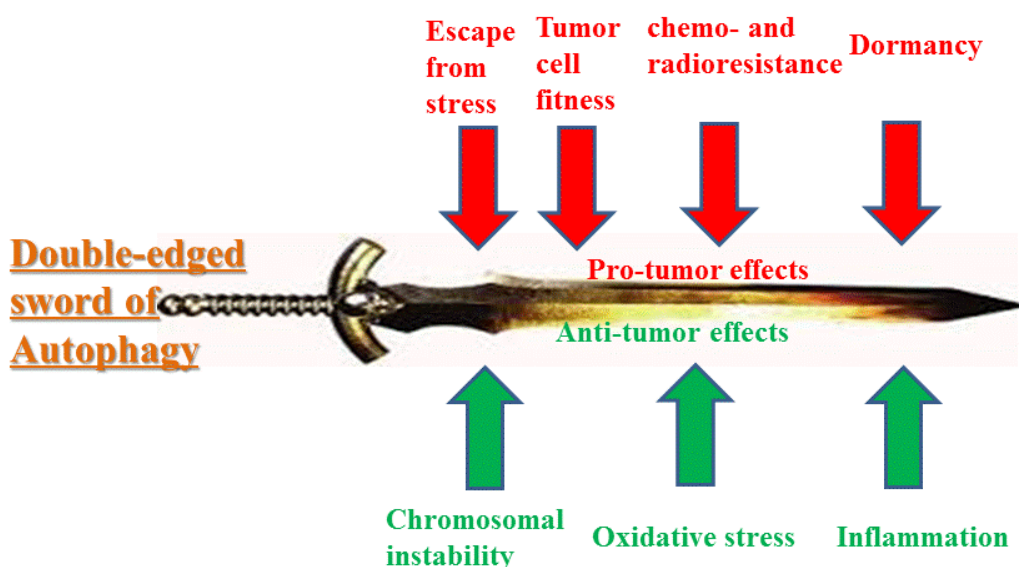
4.1. Autophagy: A double-edged sword in cancer

Recent studies have demonstrated that autophagy plays an important role in cancer initiation and progression stages. In some studies, autophagy has shown tumor inhibition effect by inducing autophagic cell death. But some studies have also proved that autophagy can facilitate cell survival during cancer treatment or other stresses. Many cancer researchers have recognized that autophagy is a double-edged sword in cancer. Usually, the role of autophagy in cancer depends on the tumor stage, the tumor genotype and microenvironment, as well as the type of therapy. For example, some recent studies have found that different kinds of autophagy-inducing drugs can play different roles in cancer treatment. In one study, the researchers used TRAIL and Fas ligand to treat cell lines with different autophagic levels

respectively. These two compounds can both activate the cell death receptors and induce programmed cell death.

4.2. The cytoprotective autophagy in cancer

Previous studies have confirmed that autophagy can lead to resistance against metabolic stress and protect tumor cells through recycling organelles or proteins. In a study of Kirsten rat model, the sarcoma-activating viral oncogene homolog (K-Ras) variations can upregulate autophagy and facilitate tumor cell survival by autophagy. Some studies also demonstrated that both radiation therapy and chemotherapeutic agents can lead to autophagy and facilitate tumor cell survival. Although these studies have proved that the conventional cancer therapy may induce autophagy and reduce tumor cell apoptosis, no direct evidence has proved that inhibition of cell autophagy can increase the sensitivity of chemotherapy and radiation for cancer treatment. So the cytoprotective role by autophagy in cancer still needs more supportive evidence. If we can prove this cytoprotective form of autophagy in cancer, we can reduce the therapy resistance by inhibiting tumor cell autophagy during conventional cancer treatment [37].



(Modified from: Semin Cancer Biol. 2013 Aug;23(4):252-61.)

Figure 3. Autophagy: a double-edged sword in cancer

4.3. The cytotoxic and cancer inhibitory autophagy

As we have discussed above, many studies have shown the cytotoxic form of autophagy in cancer. One study has combined vitamin D with radiation therapy for breast tumor model. The results showed that autophagy induced by the therapy can facilitate cell death in breast tumor. According to recent studies on autophagy, the cytotoxic form of autophagy was generated on directly killing cells by itself or further inducing apoptosis-related cell death. Some recent studies have also discovered that the autophagy-related genes such as Beclin 1

can suppress tumor devolvment in both human and mice cells. Further studies demonstrated that this tumor suppression effect by autophagy was related to the Akt pathway. Some antitumor agents also have been established with the activity of inducing autophagy-related cell death and tumor suppression [39]. Autophagy deficiency can induce the accumulation of the autophagy substrate P62 and promote the tumor growth through the NF- κ B pathway. The deficiency of autophagy-related genes such as Beclin-1, UVRAG, and Bif-1 has been confirmed in many different tumor types. These findings further strengthen the potential linking between autophagy and cancer inhibition. Autophagy-related cell death and tumor suppression are very important in recent anticancer research especially when tumor cells are deficient in essential apoptotic modulators such as caspases and Bcl-2 family. The cytotoxic and cancer inhibitory autophagy may be a potential target for developing novel cancer therapy, although there are still some unsolved problems in this field. In future studies, the molecular mechanisms of cytotoxic and cancer inhibitory autophagy still need further exploration.

5. Anticancer drug discovery target on autophagy

5.1. Overview of conventional anticancer agent target on autophagy

One of the characteristics of tumor cells is their capacity to escape from apoptosis. Recent studies have suggested that in some conditions, nonapoptotic cell death can occur in tumor cells, which may related with autophagy. Although there are few antitumor drugs that target autophagy in present clinical cancer therapy, many existing antitumor drugs can suppress tumor growth with more or less connections with autophagy. Some drugs can induce autophagic cell death directly or indirectly by autophagic apoptosis or necrosis. Recent research has shown that alkali, vincalkebostinum, arsenic trioxide, rapamycin, and vitamin D derivatives can induce autophagic cell death in some cancer cells; while LAK (lymphokine activated killer cell) can induce autophagic cell death in T98G and U373MG glioblastoma cell lines 24–96 hours after the treatment. On the other hand, autophagy can also play a tumor cell protective role by promoting intracellular material cycle and isolating harmful substances. For example, conventional anticancer drugs, such as some hormonal agents, and chemotherapy drugs may induce treatment resistance by autophagy. In this case, combination use of autophagy inhibitor with these drugs is important for clinical therapy. Recent studies have found that in particular microenvironment or genetic backgrounds, autophagy can play cytotoxic or tumor inhibitory roles. Therefore, context-specific autophagy regulations can be a promising novel therapeutic attempt to facilitate the present available antitumor therapy. Representative autophagy inducers or inhibitors designed for the purposes of promoting or inhibiting cancer progression are described in Table 1. Among the present studied autophagy-based anticancer drugs, some autophagy-modulating agents such as and rapamycin have been approved for clinical use in cancer management ; thus, autophagy has been established as a promising therapeutic target in cancer treatment.

5.2. Anticancer natural products from Chinese medicines target on autophagy

Chinese medicines have a long history in treating cancer in some Asian countries and districts. It is one of the most popular alternative and complementary medicines used for cancer therapy. The effect of Chinese medicines on survival and improving the quality of life has been identified by many basic and clinical studies. Recent research has proved that some of these natural products from Chinese medicines can induce autophagy in tumor cells, while some of them can suppress autophagy in cancer. Y. Lao et al. found that oblongifolin C, which is isolated from the herb *Garcinia yunnanensis* Hu can improve the antitumor efficiency of nutrient deprivation by inhibiting autophagy. Normally, cancer cells can resist nutrient deprivation by inducing autophagy and escaping from cell death, but oblongifolin C can inhibit autophagy in this circumstance and thus exhibit antitumor effect. M. Chen et al. reported that Ophiopogonin B, which is isolated from the herb *Radix Ophiopogon Japonicus*, can suppress cell cycle, inducing autophagic cell death in lung cancer cells in vitro. M. Pedro et al. and their research teams found that seven natural prenylated flavones extracted from Chinese medicine herbs can induce autophagy in MCF-7 cells and suppress tumor cell growth. Honokiol, which is isolated from the herb *Mangnolia officinalis*, has been demonstrated with anticancer effect by inducing autophagic cell death on melanoma cells; further study of mechanisms has confirmed that the related signal pathways are Notch signaling and AKT/mammalian target of rapamycin. The herb *Syzygium samarangense*'s active compound dimethyl cardamonin can inhibit the growth of colorectal carcinoma HCT116 cells by inducing autophagy; the molecular mechanism study confirmed that the microtubule-associated protein (LC3)-I-LC3-II is related with the autophagic cell death by this agent. Law reported that the natural product from herb *Alisma orientale* can induce autophagy and cell cycle arrest in cancer cells through clearing cellular stress; this discovery has been further proved by another research team that also used a Chinese medicine active compound cucurbitacin B to induce cell autophagy. Research from our lab in The University of Hong Kong demonstrated that fangchinoline (isolated from Fangji, *Stephenia tetrandra* S Moore) can induce autophagic cell death in two human hepatocellular carcinoma cell lines HepG2 and PLC/PRF/5. Further descriptions of the mechanisms of some isolates from Chinese medicines targeting on autophagy are presented in Table 1. These natural products from Chinese medicines are very important sources for cancer treatment, but it still needs more molecular mechanism studies in the future.

Agent	Target	Regulation mechanisms	Tumor type	Role in cancer
Rapamycin	mTORC1	mTORC1 inhibitors	Malignant glioma	pro-death
Berberine*			hepatocellular carcinoma	pro-death
aArsenic trioxide*			Melanoma	pro-death
			Ovarian cancer	
uUrsolic acid*	BCR-ABL	Tyrosine kinase inhibitors	Breast cancer	pro-death
INNO-406			Chronic myeloid leukemia	pro-death

Agent	Target	Regulation mechanisms	Tumor type	Role in cancer
			Gastrointestinal stromal tumor	pro-survival
Imatinib			Chronic myeloid leukemia	pro-survival
Dasatinib	SRC/ABL	Tyrosine kinase inhibitors	Ovarian cancer	pro-death
			Chronic lymphocytic leukemia	pro-survival
Baicalin*	Akt	Akt inhibitors	Bladder cancer cells	pro-survival
Honokiol *			Malignant melanoma cells	pro-death
Fangchinoline*	AMPK	AMPK activators	Hepatocellular carcinoma	pro-death
NPI-0052	Proteasome	Proteasome inhibitors	Ovarian cancer	pro-survival
			Prostate cancer	pro- death
Bortezomib			Prostate cancer	pro-survival
Vorinostat	Histone deacetylases	Histone deacetylases inhibitors	Gastric cancer	pro-survival
SAHA			Chondrosarcoma, endometrial stromal sarcoma, hepatocellular carcinoma	pro-death
Pseudolaric acid B*	BH3 domain	Bcl-2 inhibitors	Prostate cancer	pro-death
Oridonin*			Malignant glioma	pro-survival
Z18			Breast cancer	pro-death
Oridonin*	NF-κB	NF-κB inhibitors	Colon cancer	pro-survival
Silibinin*	NF-κB	NF-κB activators	Multiple cancer	pro-death
Etoposide*	Topoisomerase II		Hepatocellular carcinoma	pro-death
3-MA	PI3K	PI3K inhibitor	Colon cancer, esophageal squamous cell carcinoma	pro-survival
CQ	Lysosome	Lysosomotropic agents	Glioblastoma, colon cancer	pro-survival
HCQ			Breast cancer	pro-survival
Dimethyl cardamonin*	(LC3)-I-LC3-II	(LC3)-I-LC3-II activator	Colorectal carcinoma cells	pro-death

* represents natural products from Chinese medicines
(Modified from: *Acta Pharmacol Sin.* 2013 May; 34(5): 612–624)

Table 1. Representative antitumor agents target on autophagy

6. Summary: Problems in current autophagy study in cancer and future prospects

Autophagy is a homeostatic process that is highly conserved across different types of mammalian cells. Sustained and excessive autophagy may lead to cell death and tumor shrinkage. Autophagy is able to relieve tumor cell from oxidative and nutrient stress during the rapid expansion of cancer. The mechanisms and the role of autophagy in human cancer are complicated. Many recent discoveries in basic research have found that targeting autophagy may be a potential novel therapeutic solution for treating cancer. Technical variations in detecting autophagy affect data quality, and studies should focus on elaborating the role of autophagy in deciding cell fate. Although our knowledge on autophagy in cancer research has increased rapidly in the last few decades, there are still some problems in the current study of autophagy. How to monitor a dynamic autophagy process with improved technique and method? Why does autophagy have either tumor protective or inhibitor role at different cancer stages and in different microenvironments? How does autophagy regulate various of tumor generation or inhibition pathways? Would targeting the autophagy-related cancer pathways be a novel strategy for anticancer therapy? Also, as cancer is a systemic disease, many autophagy-related cross-talk pathways need to be further studied at a systems level in future research. According to previous studies, some chemical agents have been proved with the ability of inducing or suppressing autophagy in vitro, but few in vivo data are available in this field. More in vivo studies on detecting autophagy in tumor initiation and development are urgently required for drugs development in current cancer research and wide evidence-based clinical research on the relationship between autophagy and therapy efficacy are also necessary in future studies. It was shown in the literature that many anticancer natural compounds and extracts could initiate autophagy in tumor cells as summarized in this chapter. Natural-products-induced autophagy could protect tumor cells from apoptotic death in some cases. This is an emerging issue, and it is necessary to study the role of autophagy in tumor suppressive effect of natural products.

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