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REVIEW ARTICLE

Autophagy and its potent modulators from phytochemicals in cancer treatment

Chao-Yue Sun[1] · Qian-Yu Zhang[2] · Guang-Juan Zheng[1] · Bing Feng[1]

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Abstract

Autophagy is a ubiquitous catabolic process by which damaged or harmful intracellular components are delivered to the lysosomes for self-digestion and recycling. It is critical in cancer treatment. Therapy-induced autophagy predominantly acts as a pro-survival mechanism, but progressive autophagy can lead to non-apoptotic cell death, also known as autophagic cell death. Plants or herbs contain various natural compounds that are widely used in the treatment of many types of malignancies. Emerging evidence indicates that phytochemicals targeting the autophagic pathway are promising agents for cancer treatment. However, these compounds play different roles in autophagy. In this review, we discussed the role of autophagy in cancer development and therapy, and focussed on elucidating the anti-cancer activities of autophagic modulators, especially phytochemicals. Notably, we described a novel premise that the dynamic role of phytochemicals should be evaluated in regulation of autophagy in cancer.

Keywords Autophagy · Modulators · Phytochemicals · Cancer · Degree

Introduction

Before Yoshinori Ohsumi received the 2016 Nobel Prize in Physiology or Medicine for discovering the mechanisms governing autophagy, the role of autophagy in cancer was already a research hotspot. Autophagy is a dynamic process that involves the formation of double-membrane autophagosomes in which cytoplasmic components, known as autophagic cargo, are sequestered. These autophagosomes deliver their cargo to lysosomes for degradation (Fig. 1).

Autophagy has been studied in the progression of many human diseases, such as microbial infection [1], inflammatory disease [2], immune disease [3], pulmonary disease [4], heart and cardiovascular disorders [5], kidney disease [6], metabolic disease [7], and neurodegenerative disorders (Alzheimer's disease [8], amyotrophic lateral sclerosis [9] and Parkinson's disease [10]). As compared to these diseases, tumor or cancer is more autophagy dependent and occasionally involves "autophagy addiction" [11]. The impact of autophagy in cancer is highly context dependent, and as such can be neutral, tumor-suppressive, or tumor-promoting. Over the past several decades, the overarching questions of whether autophagy is a friend or foe and whether it can be modulated to kill cancer cells have remained unanswered. The general consensus is that autophagy serves as a survival mechanism in response to diverse environmental stresses including cancer therapy, providing a baseline for clinical trials designed to potentiate the therapeutic efficacy via autophagy inhibition [12]. However, there is now substantial evidence showing that autophagy can also act as a distinct mode of cell death, but only in a specific context when cancer cells are unable to undergo apoptosis [13]. Thus, a better understanding of the interplay between cancer therapy and autophagy and specific attempts to manipulate the autophagic process to kill cancer cells, are indispensable.

Plants or herbs have been widely used in the treatment of various diseases including malignant tumors for centuries. Advanced technologies of pharmaceutical analysis

✉ Guang-Juan Zheng
zhengguangjuan@gzucm.edu.cn

✉ Bing Feng
bing19831@163.com

[1] Guangdong Provincial Hospital of Chinese Medicine, The Second Clinical College, Guangzhou University of Chinese Medicine, Guangzhou 510120, China

[2] School of Pharmaceutical Sciences, Guangzhou Higher Education Mega Center, Guangzhou University of Chinese Medicine, no 232, Waihuandong Road, Guangzhou 510006, China

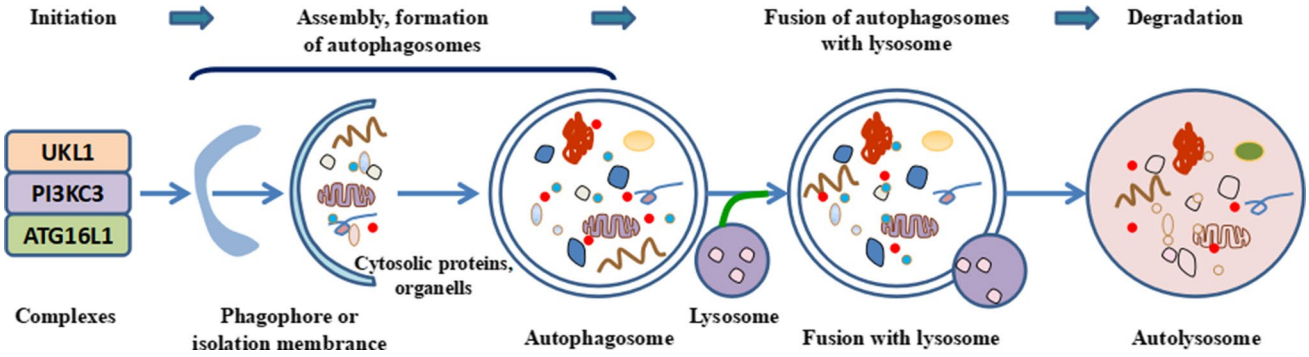

Fig. 1 Overview of the autophagy process. Under stress, three key protein complexes: *ULK1* complex (comprising *ULK1, FIP200, ATG13* and *ATG101*), *PI3KC3* complex and *ATG16L1* complex are involved in autophagy initiation. Autophagy begins with the sequestration of cytosolic proteins and organelles into a double membrane called the isolation membrane. After enveloping the engulfed contents, the isolation membrane forms an autophagosome that then fuses with the lysosome to generate autolysosomes for degradation and recycling

and pharmacology have facilitated extraction of numerous natural compounds from herbs, such as alkaloids, terpenes, flavonoids, and glucosinolates. Numerous phytochemicals have varied biological activities, but with less toxic effects than synthetic chemicals. Notably, several phytochemicals can act as autophagic modulators, with therapeutic potential in cancer. Three aspects of these chemicals in regulating of autophagy have been examined. First, certain phytochemicals can inhibit autophagy to sensitize cancer cells to radiation or chemotherapy, which act like the autophagy inhibitor, chloroquine. Second, phytochemicals induce cytoprotective or cytotoxic autophagy. For instance, a new cardenolide called 3′-epi-12β-hydroxyfroside isolated from the roots of *Calotropis gigantea*, induces cytoprotective autophagy in lung cancer cells, and combination treatment with chloroquine enhances its anti-tumor activity [14]. In contrast, enforced over-activation of autophagy facilitates cell death, termed as autophagic cell death (ACD). Our laboratory has previously reported that scutellarin extracted from *Erigeron breviscapus Hand-Mazz*, increases cisplatin-induced autophagy, which enhances the cytotoxicity of cisplatin, and inhibition of autophagy attenuates these combined effects [15]. In addition, we also found that norcantharidin, as an autophagy inducer, causes ACD in hepatocellular carcinoma (HCC) [16]. These results suggested that phytochemical-regulated autophagy can mediate cell death or survival. In this review, we explored the dynamic role of autophagy induced by phytochemicals. We elucidated the functions of autophagy induced by phytochemicals and focused on the context-dependent role of autophagy, to evaluate the role of phytochemicals in regulation of autophagy in cancer.

The definite role of autophagy in cancer

Autophagy is defined as a double-edged sword in many scientific articles. In general, autophagy displays a dichotomous role in cancer, where it can either prevent tumor initiation or facilitate tumorigenesis [17]. In specific contexts, cancer initiation can be robustly suppressed by autophagy, and autophagy defects are drivers of tumorigenesis, such as chronic inflammation, genomic damage response, and chromosomal instability [18–20]. For example, in a mouse model of deletion of autophagy gene autophagy related 5 (*Atg5*), autophagy loss resulted in *p62* accumulation, which contributed to tumor progression [21]. Other studies showed that autophagy's role in cancer development depends on the status of *p53*, and inhibition of autophagy activates *p53*, which is unrelated to *p53*-independent mechanisms that suppress tumor growth [22]. In addition, basal autophagy is blocked by human epidermal growth factor receptor 2 (*HER2*) amplification, and increased autophagy protects against *HER2*-mediated tumorigenesis [23]. Accordingly, autophagy is a tumor suppressor, and inhibition of autophagy predisposes healthy cells to malignant transformation.

However, autophagy is typically considered as a survival mechanism that can be deliberately employed by cancer cells in response to various conditions of cellular stress, which is more broadly applicable. In retrovirus-associated DNA sequence (*RAS*)-driven cancer cells, up-regulated basal autophagy facilitates tumorigenesis, and blocking essential autophagy proteins *Atg5* or *Atg7*

impairs cell growth [24]. However, with a good prototype of pancreatic cancer, elevated autophagy is a reactive survival mechanism, whereas inhibition of autophagy results in tumor regression [25]. A recent study showed that autophagy is critical for tumor maintenance via tumor cell-intrinsic and host mechanisms, and autophagy inhibition causes tumor suppression in pancreatic ductal adenocarcinoma [26]. In these cases, autophagy inhibition by genetic or pharmacological methods may improve anti-cancer therapeutics. Thus, the tumor suppression by autophagy prevents healthy cells from developing into a neoplastic precursor. However, in malignant cells, moderate autophagy only helps cells to thrive under various cytotoxic insults.

Autophagy as a promising target for cancer treatment

In malignant cells, therapy-induced autophagy can be a protective response to resist therapeutic challenges, including chemotherapy, radiation, and targeted therapy. Numerous studies have reported that increased autophagy contributes to chemoresistance, and therapeutic inhibition of autophagy augments the chemosensitivity of cancers [27–29]. Docetaxel, for instance, triggers autophagy that serves as a general mechanism of drug resistance, and inhibitors of autophagy can improve its therapeutic index [30–33]. Induction of protective autophagy is often observed in radiation therapy, which serves as an important mechanism of radioresistance. Numerous reports have hypothesized that combination of radiation exposure and inhibitors of autophagy could be a potential therapeutic strategy to increase radiosensitivity [34, 35]. In line with this hypothesis, inhibition of autophagy was found to improve the cytotoxicity of photodynamic and photothermal therapies [36, 37]. In addition, activated autophagy, which is essential for cell survival, frequently appears in cancer cells undergoing targeted drug therapy. Pharmacological autophagy inhibition increases cell death of B-type Raf kinase (*BRAF*) inhibitor vemurafenib-resistant brain tumors in vitro and in clinical trials [38]. Accordingly, regulation of autophagy, especially inhibition of autophagy, is a promising method to sensitize cancer cells to diverse therapies.

Contradictory to its cytoprotective role, however, there is increasing evidence for cell death by autophagy, also known as ACD. However, therapy-induced ACD is highly contextual and occurs in very few cases with defective apoptotic machinery. In *Bax*- or *PUMA*-deficient human colon cancer cells undergoing defective apoptosis, 5-FU elicits ACD, and inhibition of autophagy with 3-MA impairs cell death [39]. Similarly, triptolide induces ACD in pancreatic adenocarcinoma cell lines with disability of apoptosis, S2-013 and S2-VP10 cells, and loss of autophagy-specific genes rescues triptolide-mediated cell death [40]. These data suggested that ACD can be considered as an alternative cell death when cells fail to undergo apoptosis. Notably, ACD manifests with extensive cytoplasmic vacuolization that is correlated with increased autophagic flux, which culminates with phagocytic uptake and consequent lysosomal degradation [41, 42].

In summary, for cancer treatment, autophagy is a friend to cancer cells when it is cytoprotective and an enemy when it is induced as an alternative death pathway. Chemotherapeutic drugs or phytochemicals, which elicit moderate autophagy, facilitate the resistance of tumor cells to anticancer agents, and therapeutic inhibition of autophagy is expected to enhance the chemosensitivity of cancers. However, excessive autophagy induced by drugs can lead to ACD, if it proceeds to completion. Regulation of autophagy is therefore a promising strategy to kill cancer cells. Notably, the role of autophagy in cancer therapy can improve and more sensitively reflect its cytoprotective function in cancer cells.

Phytochemicals induce cytotoxic autophagy in cancer cells

Phytochemicals are considered as non-toxic and effective for treating cancer patients, prolonging their life span. Several recent studies have shown that numerous herbal compounds can trigger ACD, and hence could be powerful cancer therapy candidates. Listed below are several phytochemicals that can induce cytotoxic autophagy in various cancers. Plumbagin, a quinonoid component isolated from the root of *Plumbago zeylanica* L., inhibits cell proliferation by inducing cells to undergo ACD, and autophagy inhibitor bafilomycin can suppress its anti-cancer activity [43]. Saikosaponin-d (Ssd), a triterpenoid saponin extracted from a medicinal plant, induces cytotoxic autophagy in cervical and breast cancer cells, and the addition of autophagy inhibitor 3-MA suppresses Ssd-induced ACD [44]. Berberine is a natural product derived from *Coptidis rhizoma* that triggers autophagy in HCC cells, and co-treatment with 3-MA diminishes berberine-mediated ACD, as shown by the decreased cell survival rate [45]. A natural polyphenol from culinary herb, carnosol, induces apoptosis and ACD in breast cancer cells, and the cell viability increases after adding an autophagy inhibitor [46]. Bufalin, a soluble digoxin-like component extracted from toad's skin, plays a critical role in stimulating ACD in human HCC, and inhibition of autophagy by 3-MA leads to the loss of apoptotic ratio, suggesting that bufalin-induced autophagy can promote apoptosis [47]. Licarin A is a novel compound from *Myristica fragrans*, which can induce autophagy and promote death

of lung cancer cells, and co-treatment with CQ reduces its anti-cancer effect [48]. Isoliquiritigenin, an active flavonoid of *Glycyrrhiza uralensis*, activates autophagy and inhibits growth of ovarian cancer cells, and its cytotoxicity can be inhibited in the presence of 3-MA [49].

Phytochemicals induce cytoprotective autophagy in cancer cells

Some phytochemicals are capable of inducing ACD, while others can trigger protective autophagy in tumor cells. Triptolide, a diterpene triepoxide extracted from *Tripterygium wilfordii Hook F*., induces protective autophagy in prostate cancer cells, and combined with autophagy inhibitors 3-MA and CQ promotes triptolide-induced prostate tumor growth inhibition [50]. This seems contradictory to the above-mentioned cytotoxic autophagy induced by triptolide in pancreatic adenocarcinoma cells. However, pancreatic cancer cell lines, including S2-013 and S2-VP10 cells, undergo non-apoptotic cell death in response to triptolide, indicating that autophagy is a preferred mechanism of cell death when apoptosis is defective, which merits further investigation [40]. Eriocalyxin B (EriB), a type of the ent-kaurane diterpenoid isolated from *Isodon eriocalyx var. laxiflora*, can induce accumulation of autophagosomes in breast cancer cells, and co-treatment with autophagy inhibitors or knockdown of autophagy gene Atg5 increases cell death, indicating EriB-induced autophagy is an adaptive mechanism for cell survival [51]. Isobavachalcone, a natural chalcone in the seeds of *Psoralea corylifolia* L., induces autophagy and apoptosis in multiple myeloma cells, and its cytotoxicity can be potentiated by autophagy inhibitors [52]. β-Elemene, a novel lipid-soluble compound extracted from *Curcuma zedoaria*, promotes autophagy in breast cancer, and inhibition of autophagy with CQ decreases cell viability, suggesting that β-Elemene-induced autophagy protects against cancer cell death [53–55]. Ginsenoside F2 is an active compound in ginseng that induces cytoprotective autophagy, and co-treatment with CQ results in enhancement of ginsenoside F2-induced cell death in breast cancer [56]. Toxicarioside O, a natural product derived from *Antiaris toxicaria*, increases autophagy in colorectal cancer cells, whereas combined with CQ enhances toxicarioside-induced apoptotic cell death [57]. Oridonin, a compound from *Rabdosia rubescens*, stimulates autophagy in prostate cancer cells, and combination with 3-MA reduces cell viability [58]. A novel sesquiterpene lactone called isodeoxyelephantopin (ESI) obtained from *Elephantopus scaber* L. induces protective autophagy, and pretreatment with 3-MA enhances its anti-cancer effect on lung cancer cells [59]. Oleanolic acid, a natural compound, triggers autophagy in breast cancer cells, and cell viability is reduced when oleanolic acid is used in combination with 3-MA, indicating that oleanolic acid-induced autophagy acts as a protective mechanism against its anti-tumor activity [60]. Cucurbitacin E, a natural triterpenoid widely distributed in the plant kingdom, induces protective autophagy in lung cancer cells, and autophagy inhibition facilitates cell death [61].

Inhibition of autophagy by phytochemicals

Numerous studies have shown that the inhibition of autophagy by phytochemicals can improve anti-tumor therapeutic approaches, implying that autophagy inhibition could be a potential novel therapeutic strategy for the treatment of cancers. Deguelin, a retinoid extracted from *Mundulea sericea*, inhibits autophagy in human pancreatic cancer cells, sensitizing the cells to doxorubicin-induced cytotoxicity [62]. 20(S)-Ginsenoside Rg3,a compound isolated from *Panax ginseng C.A. Meyer*, exhibits autophagy inhibitory effect in HCC cells by increasing cell sensitivity to doxorubicin [63]. Oblongifolin C, a novel autophagic flux inhibitor, is a caged xanthone extracted from *Garcinia yunnanensis hu* that increases anti-tumor efficacy of nutrient deprivation associated with autophagy inhibition in various cell lines [64]. Astragaloside II, a key compound of *Radix Astragali*, blocks autophagy by disrupting lysosomal function to accelerate cisplatin-induced apoptosis, thereby increasing cell death [65]. Epigallocatechin-3-*O*-gallate, the most abundant catechin in *green tea*, is capable of repressing doxorubicin-induced autophagic flux to enhance its anti-cancer effects [66] (Tables 1, 2, 3).

Degree in philosophy applied to evaluate the role of phytochemicals in regulation of autophagy in cancer

The potency of any compound is a summation of its quality and quantity. Things in nature try to maintain their own existence; but when they exceed a specific range, it causes opposite changes. In line with this notion, therapy-induced autophagy may follow the principle of moderation. There is evidence showing that anti-cancer drugs, including phytochemicals, can modulate autophagy to play different roles. The degree of induced autophagy and whether its exact role depends on the defined extent of autophagy remain unclear. Here, we present a diagram to elaborate on the extent of autophagy. As shown in Fig. 2, under normal circumstances, basal autophagy is required to maintain host health via digestion of unwanted cytoplasmic materials. Therapy-induced autophagy exerts two opposite functions, cytoprotective and cytotoxic. Protective autophagy is a powerful tool that host cells employ to defend against stress, and the

Table 1 Herbal compounds induce cytotoxic autophagy

Compounds	Source	Cancer type	Pathways	Model	References
Anacardic acid	*Anacardiaceae adstringens*	Prostate cancer	DAPK3↑, Akt↓, mTOR↓	In vitro	[67]
Berberine	*Coptidis rhizoma*	Hepatocellular carcinoma	Akt↓, mTOR↓, p38↑	In vitro	[45]
Carnosol	*Rosmarinus officinalis* L.	Breast cancer	ROS↑, ERK1/2↑	In vitro, in vivo	[46]
Celastrol	*Thunder of god vine*	Osteosarcoma	ROS↑, JNK↑	In vitro, in vivo	[68]
Cucurbitacin B	Cucurbitaceous plants	Breast cancer	γH_2AX↑, ATM/ATR↑, ROS↑	In vitro	[69]
Falcarindiol	Dietary plants	Breast cancer	GRP78↑	In vitro	[70]
Guttiferone K	*Garcinia hanburyi Hook. F.*	Cervical cancer	Akt↓, mTOR↓, JNK↑	In vitro	[71]
Isoliquiritigenin	Licorice plant	Ovarian cancer	Beclin1↑	In vitro	[49]
Juglanin	*Juglans mandshurica*	Breast cancer	ROS↑, JNK↑	In vitro, in vivo	[72]
Licarin A	*Myristica fragrans*	Lung cancer	ROS↑, Beclin1↑	In vitro	[48]
Licochalcone K	Licorice	Breast cancer	PI3K↓, Akt↓, mTOR↓	In vitro	[73]
Maslinic acid	*Crataegus pinnatifida Bunge*	Pancreatic cancer	HSPA8↓, mTOR↓	In vitro	[74]
N-desmethyldauricine	*Menispermum dauricum*	Cervical cancer	Ulk-1↑, PERK↑ AMPK↑, mTOR↓	In vitro	[75]
Norcantharidin	*Mylabris phalerata Pallas*	Hepatocellular carcinoma	c-Met↓, mTOR↓	In vitro, in vivo	[16]
Ophiopogonin B	*Radix Ophiopogon Japonicus*	Lung cancer	PI3K↓, Akt↓, mTOR↓	In vitro	[76]
Platycodin-D	*Platycodon grandiflorum*	Lung cancer	PI3K↓, Akt↓, mTOR JNK↑, p38↑	In vitro	[77]
Plumbagin	*Plumbago indica L*	Breast cancer	PI3K↓, Akt↓, mTOR↓	In vitro, in vivo	[43]
Resveratrol	Grapes, nuts, and red wine	Ovarian cancer	None	In vitro	[78]
Saikosaponin-d	*Radix Bupleuri*	Breast cancer Cervical cancer	AMPK↑ mTOR↓	In vitro	[44]
Shikonin	*Lithospermum erythrorhizon*	Hepatocellular carcinoma	ERK↑ RIP↓	In vitro, in vivo	[79]
Tetraarsenic hexoxide	Arsenic ore	Colon cancer	PI3K↓, Akt↓ p38 MAPK↑	In vitro	[80]
Tetrandrine	*Stephania tetrandra*	Hepatocellular carcinoma	Wnt/β-catenin↓, MTA1↓	In vitro, in vivo	[81]
Triptolide	*Tripterygium wilfordii*	Pancreatic cancer	Akt↓, mTOR↓, p70S6K↓ ERK↑	In vitro	[40]
Tripchlorolide	*Tripterygium*	Lung cancer	PI3K↓, Akt↓, mTOR↓	In vitro	[82]
Ursolic acid	*Alectoria, Cladonia, Usnea*	Colorectal cancer	JNK↑	In vitro, in vivo	[83]
Usnic acid	*Lichen*	Gastric cancer	None	In vitro, in vivo	[84]

determination of cytoprotection is empirical in that pharmacological inhibitors of autophagy or genetic silencing can increase tumor cell sensitivity to autophagy inducer, whereas its cytotoxic role is determined when autophagy inhibition does not benefit cancer therapy [96]. In addition, therapy-induced autophagy also appears to serve as a novel mode of programmed cell death termed as increased autophagic flux, which represents a crucial event in the drug's anti-tumor activity. It is possible that the dynamic role of autophagy can be discriminated by its degree.

However, due to lack of effective measurement techniques to assess the extent of autophagy induction, this concept has not yet been verified. The exact degree should be quantified as a detailed number. Moderately increased autophagy is an optimal survival response for malignant cells. However, when therapy-induced autophagy exceeds a certain degree, it can benefit cancer treatment. Thus, the degree of autophagy can decide the fate of the host cells. The key point is to effectively measure the degree of autophagy. However, the quantification of autophagic flux remains challenging due to poorly defined parameters [97]. Many tools exist to monitor ongoing autophagy, such as Western blot for the microtubule-associated protein 1 light chain 3 (*LC3*) or *p62*, fluorescent-tagged probes, and transmission electron microscopy (TEM) [98]. Among them, the fluorescent GFP-LC3 processing assay permits a quantitative measurement to depict autophagy induction or autophagic flux [99]. For this purpose, an objective number of *LC3* puncta per cell can be obtained by computerized assessment to assess the efficiency of autophagy modulator [100]. Thus, we propose for the first time that the exact number of *LC3* puncta per cell after treatment with a certain concentration of herbal compound represents the degree of autophagy. In this context, the key dose is when autophagy inhibition cannot alter tumor cell

Table 2 Herbal compounds induce cytoprotective autophagy

Compounds	Source	Cancer type	Pathways	Model	References
Actein	Black cohosh	Bladder cancer	ROS↑, JNK↑ Akt↓	In vitro, in vivo	[85]
Alisol B	*Alisma orientale*	*Breast cancer*	AMPK↑ mTOR↓	In vitro	[86]
Berberine	*Coptidis rhizoma*	Pleural mesothelioma	None	In vitro	[87]
Cucurbitacin E	*Cucumis melo* L.	Lung cancer	Akt↓, mTOR↓	In vitro	[61]
Curcumin	Turmeric	Pancreatic cancer	Akt↓, mTOR↓	In vitro	[88]
Eriocalyxin B	*Isodon eriocalyx laxiflora*	Breast cancer	Akt↓, mTOR↓, p70S6K↓	In vitro, in vivo	[51]
Ginsenoside F2	Ginseng	Breast cancer	Atg7↑	In vitro	[56]
Honokiol Magnolol	*Magnolia officinalis*	Glioblastoma	PI3K↓, Akt↓, mTOR↓	In vitro, in vivo	[89]
Honokiol	*Magnolia officinalis*	Prostate cancer	mTOR↓, Akt↓, 4E-BP-1↓	In vitro	[90]
Isobavachalcone	*Psoralea corylifolia*	Multiple myeloma	PKCδ↑	In vitro	[52]
Isodeoxyelephantopin	*Elephantopus scaber* L.	Lung cancer	Nrf2↑, p62↑, HO-1↑	In vitro	[59]
Oleanolic acid	*Olea europaea* L.	Pancreatic cancer Breast cancer	JNK↓, mTOR↓	In vitro	[60]
Onjisaponin B	*Radix Polygalae*	Adrenal pheochromocytoma	AMPK↑, mTOR↓	In vitro	[91]
Oridonin	*PC-SPES*	Prostate cancer	P21↑	In vitro	[58]
Pentagalloylglucose	Oriental medicinal herbs	prostate cancer	S6K↓, 4EBP1↓	In vitro	[92]
Quercetin	A variety of plants	Uterine cancer	S6K1↓	In vitro	[93]
Toxicarioside O	*Antiaris toxicaria*	Colorectal cancer	SIRT1↑, Akt↓, mTOR↓	In vitro	[57]

Table 3 Herbal compounds inhibit autophagy

Compounds	Source	Cancer type	Pathways	Model	References
Astragaloside II	*Radix Astragali*	Gastric cancer Liver cancer	PI3K↓, Akt↓	In vitro	[65]
Capsaicin	Pepper	Prostate cancer	PI3K↓, Akt↓, ROS↑	In vitro	[94]
Deguelin	*Mundulea sericea*	Pancreatic cancer	None	In vitro	[62]
(−)-Epigallocatechin-3-O-gallate	Green tea	Hepatocellular carcinoma	Atg5↓	In vitro, in vivo	[66]
20(S)-Ginsenoside	*Panax ginseng*	Hepatocellular carcinoma	None	In vitro, in vivo	[63]
Liensinine	*Nelumbo nucifera Gaevth*	Breast cancer	DNM1L↓	In vitro, in vivo	[95]
Oblongifolin C	*Garcinia yunnanensis hu*	Cervical cancer	None	In vitro, in vivo	[64]

sensitivity to certain dosage of drugs, which can be measured by the MTT assay. Notably, the degree of autophagy varies in different cancer types, cell lines, drugs and duration. It can guide the dose selection of autophagy inducer, and whether combination treatment with autophagy inhibitors is needed to kill cancer cells.

Conclusions and perspectives

As a catabolic process in mammalian cells, autophagy is a research hotspot in cell biology. It has been linked to many pathological conditions, such as viral infection, metabolic disease and neurodegeneration. In cancer, therapy-induced autophagy has either cytoprotective or cytotoxic properties. Phytochemicals can inhibit or activate autophagy, which holds immense potential for the treatment of cancers. It is possible that a certain degree can determine the dynamic role of autophagy. Although the degree depends on cancer types, cell lines, drugs and duration, it can guide the dose selection of autophagy inducer, and determine whether combination treatment with autophagy inhibitors is needed to kill cancer cells. It is critical to measure the degree of autophagy when using herbal compounds to treat malignant cells. In this review, we recommended the use of fluorescent GFP-LC3 processing assay to quantify the degree of autophagy, but it remains a challenge due to poorly defined parameters. Future efforts should

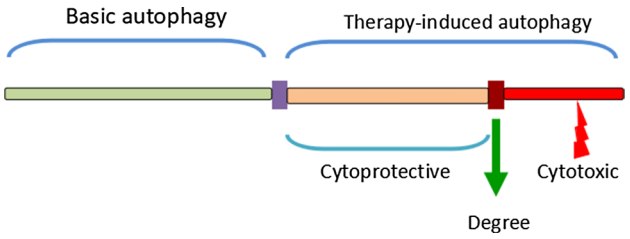

Fig. 2 The degree of autophagy in cancer treatment. Under normal circumstances, basal autophagy is required to maintain host health via digestion of unwanted cytoplasmic materials. Therapy-induced autophagy exerts two opposite functions, cytoprotective and cytotoxic. Protective autophagy is a powerful tool that host cells employ to defend against adverse stress. In addition, therapy-induced autophagy also appears to serve as a novel mode of programmed death termed as increased autophagic flux. There exists a degree that can discriminate the dynamic role of autophagy

be dedicated to identify novel tools to measure the degree of autophagy.

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Compliance with ethical standards

Conflict of interest All four authors declare that they have no conflicts of interest.

Ethical approval This review does not contain any experiments involving humans or animals, and hence ethical clearance was not required.

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