Chapter 53
Natural Product Regulates Autophagy in Cancer

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Abstract Anti-cancer effect of natural products has been widely known. As a sort of multi-target anti-cancer agents, natural compound's regulation on autophagy in cancer cells has been studied as a promising research to reveal the mechanism in oncogenesis, as well as a potential short way to anti-cancer drug discovery. In this chapter, we reviewed the cancer-autophagic-related studies on several natural product compounds. It was concluded that natural product compounds directly or indirectly regulated most of the target proteins on the autophagic signal pathways. Considering we have not seen the whole clear atlas of autophagy in oncogenesis yet, it is hard to raise up any conclusion that autophagy is always playing a positive role in oncogenesis and cancer progression.

Keywords Natural product · Autophagy · Cancer · Oncogenesis · Multi-target

53.1 Inroduction

The role of autophagy in tumorigenesis and development has been elaborated in the previous chapters of this book, and the duality of autophagy on tumor regulation remains a controversial subject for further study. Tumorigenesis may be prevented through autophagic regulation, but once tumorigenesis happened, an increase in autophagy flux tends to promote tumor cell survival and growth (Levy et al. 2017).

There is evidence that autophagy blocked the conversion of normal cells to cancer cells, autophagy cleared the accumulation of damaged organelles or proteins, and further activated programmed cell death when cells were severely damaged, which also provides checkpoint to avoid tumorigenesis. In addition, autophagy inhibits tumorigenesis by maintaining chromosome stability, inhibiting inflammation and angiogenesis, and promoting oncoprotein degradation (Zhan et al. 2018). However, in the absence of nutrients, hypoxia, metabolic stress, and treatment-induced cellular stress or drug resistance, autophagy may promote the survival of established

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tumor cells and produce resistance to radiation, chemotherapy, and targeted drug therapy (Rebecca and Amaravadi 2016). If autophagy is over-activated, it may lead to caspase-independent non-apoptotic cell death. Autophagy under certain special conditions may cause apoptosis, such as dramatic changes in Bcl-2 family protein levels. In recent years, immunotherapy has attracted widespread attention in the treatment of tumors. However, autophagy also plays a double-edged role in immunotherapy. The exact role and mechanism of autophagy in cancer immunotherapy is still unclear.

It should be mentioned that cisplatin, alkaloids, antimetabolic anti-cancer drugs, tyrosine kinase inhibitors, and other targeted therapeutic drugs, as well as radiotherapy, induce autophagy in cancer cells. However, the relationship between the effects of these treatments and the occurrence of autophagy still requires extensive research. A clinical trial with more than 30 samples investigated the anti-cancer effects of autophagy modulators in combination with cytotoxic chemotherapeutic drugs or targeted drugs in a variety of cancer cases. For example, chloroquine and hydroxylated chloroquine, which are commonly used in the treatment of malaria and rheumatoid arthritis, inhibit autophagosome acidification, therefore inhibiting autophagy. In anti-cancer therapy, chloroquine was combined with cisplatin or PI3K inhibitor LY294002 or mTOR inhibitor rapamycin, and the results showed that there is no significantly increased sensitivity to the anti-cancer treatment or autophagy inhibitors used above. Knockdown of Atg12, Beclin 1, or the use of bottromycin failed to simulate the above results. Clinical trial results provide important evidence for our in-depth understanding of autophagy and its role in tumor physiology and provide fresh ideas for the development of adjuvant anti-cancer therapy targeting autophagy.

It has been shown that natural products affect autophagy by regulating ROS levels in tumor cells, as well as directly regulate autophagy (induced autophagy or autophagy inhibition) (Table 53.1).

53.2 Polyphenol

A large number of studies have reported that polyphenols showed inhibitory effects on tumors alone or in combination with other anti-cancer therapy, and the mechanisms involve the regulation of autophagy. Such as catechins, resveratrol, quercetin, and curcumin, these polyphenols prevent the tumorigenesis by inducing autophagy, and may also contribute to its anti-aging effect. In addition to the induction of autophagy, it has been reported that some polyphenols have an inhibitory effect on autophagy, which has certain benefits for the treatment of cancer, especially for radiotherapy, chemotherapy, and targeted drugs. The latest research found that polyphenols can be used as an adjunct to the development of cardiotoxicity induced by doxorubicin, a chemotherapeutic drug (Shabalala et al. 2017). In the animal experiments and clinical researches, the study of polyphenolic natural products as adjuvant therapy in chemo or targeted therapy proves that regulating autophagy is a key mechanism for

Table 53.1 Natural product regulated cancer cell lines (or tumor tissue), autophagy-related mechanisms, and possible targets

Compound name	Cell line and tissue	Mechanism and target protein
Curcumin	Brain Bladder Prostate Colon Brain Mesothelioma Colon Endothelial Colon	Akt/mTOR/p70S6 K ERK1/2 CML Bcl-2 Akt Bcl-xL ROS PI3K/Akt/mTOR Beclin 1 and p62/SQSTM PI3K/Akt/mTOR and FOXO1 TFEB/Lysosome
EGCG (Epigallocatechin-3-gallate)	Hep3B Macrophage-like cell line Raw264.7 Mesothelioma cells BAEC HepG2 4T1 CAR	Atg5 Beclin 1 NOS LC3 ROS ROS PKC-β LC3 II LC3-I, LC3 II Beclin 1, ATG5, LC3B Atg5, Atg7, Atg12, Beclin 1, and LC3B-II AKT/STAT3
Resveratrol	Ovarian Salivary gland Ovarian Lung Colorectal Breast Cervical Gastric Brain Fibroblast, Cervical CML Lung Hepatoma Liver Colon Brain Cervical Osteosarcoma Melanoma Cervical, breast Lung	PELP1/HRS Akt/mTOR/p70S6K PELP1/HRS PI3K/Beclin 1/Lamp2b Akt/PKB/mTOR/p70S6K Cathepsin L Dihydroceramide desaturase Beclin 1 p70S6K JNK/p62, AMPK/mTOR SIRT1/PARP-1 SIRT1, AMPK, HIF-1α SIRT1 ATAD3A WIPI-1 Inhibiting autophagy

(continued)

Table 53.1 (continued)

Compound name	Cell line and tissue	Mechanism and target protein
Quercetin	Gastric carcinoma Colon Fibroblast-breast Gastric Rat mesothelial Breast, Cervical Ovarian Breast	Akt-mTOR and HIF-1α Ras ROS Akt/mTOR and HIF-1 HSP72/jnk and Beclin 1 mTOR/eIF4E-BP1/p70S6K Akt-mTOR and glycolysis
Genistein	Rat hepatocytes Ovarian Lung A549	Cytokeratin Akt PDE4A4vand p62/SQSTM1 N-CoR/Hsc70 Autophagic flux
Rottlerin	Prostatic carcinoma Fibrosarcoma Breast cancer SGC7901 and MGC803	PKCδ/TG2 pathway NF-κB PKCδ/TG2 independent pathway mTORC1 Rapamycin kinase and Skp-2
Berberine	HepG2 and MHCC97-L NCI-H2452	Atg5 Akt P38/MAPK Beclin 1 mTOR LC3 - II, p62, inhibiting autophagy
Matrine	HepG2 and SGC-7901	Pancreatic cancer Beclin 1 STAT3

natural products to overcome anti-cancer drug resistance and enhance the therapeutic effect of chemotherapy drugs. But more and more extensive and in-depth research is needed.

53.2.1 Curcumin

As a type of polyphenol compound extracted from *Curcuma longa,* Curcumin's regulation on autophagy is involved in the PI3K/Akt/mTOR signaling pathway and NF-κB-regulated proteins. A number of studies have confirmed that curcumin induces G2/M arrest and autophagy by inhibiting the activation of Akt/mTOR/p70S6K and ERK1/2 signaling pathways in cancer cells. Shinojima et al. observed that curcumin inhibited solid tumor proliferation mainly through autophagy rather than NF-κB (Shinojima et al. 2007).

Studies have shown that curcumin reduced the expression of Sp protein, and the overexpression of this protein in gastric cancer and pancreatic cancer is closely related to tumor invasion and poor prognosis, downregulation of EGFR (Sp protein regulatory gene, autophagy inhibition) expression, inhibition of Akt phosphorylation, induction of increased LC3 expression, and death of bladder cancer cells. Mosieniak et al. (2012) demonstrated that the senescence of colon cancer cell lines is accompanied by the development of autophagy with upregulated expression of Beclin 1 and p62/SQSTM1 proteins. Inhibition of autophagy by Atg5 siRNA interference reduces curcumin-induced cellular senescence but does not increase cell death. This study reveals that curcumin-induced cellular senescence is associated with autophagy, and its specific mechanisms require more deep research.

It has been reported that curcumin induced autophagy and apoptosis by downregulating Bcl-2 protein in chronic myeloid leukemia cells. The reverse of these effects by treating cells with autophagy/lysosomal inhibitor bafilomycin or caspase inhibitor zVAD-FMK, respectively, confirmed curcumin's autophagy-mediated inhibitory effect on chronic myeloid leukemia cells (Jia et al. 2009). In prostate cancer cells, curcumin induced autophagy and promoted cell death by downregulating Bcl-2 protein family member Bcl-xL. Curcumin does not induce cleavage of procaspase-8, −9, −3, and −7 or PARP, but results in the formation of LC3B-II isoforms and an increase in autophagosomes.

Curcumin treatment reversed the LC3-I/LC3-II ratio and promoted the breakdown of SQSTM1 and therefore induced the formation of autophagosomes in human colon cancer cells. Curcumin-induced autophagy can be blocked by the antioxidant NAC, suggesting that curcumin may act by promoting ROS production, autophagosome formation, and autolysosomal cleavage. Batroxomycin-induced SQSTM1 protein degradation further confirms that activation of autophagy may lead to cell death. Kim et al. reported that the anti-tumor effect of curcumin on oral squamous cell tumor involved the ROS production and presented anti-tumor activity through apoptosis and autophagy.

In addition, for glioblastoma, curcumin also induced autophagy in vitro and in vivo. Curcumin is less toxic to normal cells, especially in glial cells (GICs), the mechanism of action is through regulation of ERK1/2 signaling pathway (Zhuang et al. 2012).

Several studies have shown that curcumin regulated autophagosome and autolysosome formation, which enhanced the autophagic flux of human colon cancer HCT116 cells and mouse embryonic fibroblasts (MEFs), and then promoted lysosomal function. Curcumin-mediated lysosomal activation is mediated by mTOR inhibition and increased lysosomal acidification and enzymatic activity. Curcumin treatment activated several essential nuclear transcription factors that regulate autophagy and lysosomal and transcript factor EB (TFEB). Curcumin directly binds to TFEB, promotes nuclear translocation of TFEB, or increases the transcriptional activity of TFEB (Zhang et al. 2016). It has also been reported that a curcumin derivative (IHCH) inhibits the growth of A549 cells and induces the formation of autolysosomes in a dose- and time-dependent manner.

Curcumin has been shown to induce not only tumor cell apoptosis, but also synergistic effects with various FDA-approved drugs. However, the main reasons that prevent curcumin from becoming an anti-tumor drug are its low bioavailability, its low absorption rate, and poor in vivo distribution and biological metabolism. In order to solve the above problems, it is necessary to improve the absorption and bioavailability of curcumin by means of formulation modification, by using the nanomaterials, micelles, and phospholipid complex packaging. It should be emphasized that curcumin produces a variety of metabolites in the body, including glycosylation products, sulfation products, tetrahydro-, hexahydro-, and decahydrocurcumin, and all metabolites exhibit anti-tumor activity.

53.2.2 Epigallocatechin-3-Gallate (EGCG)

EGCG is a type of polyphenol extracted from green tea. Studies have shown that low concentrations of EGCG (10 μM) induced macrophage and tumor cell autophagy to degrade endotoxin-induced high mobility group B-1 (HMGB1) aggregation, resulting in anti-inflammatory effects. In contrast, high concentrations of EGCG (100 μM) inhibit autophagy, leading to apoptosis and anti-tumor effects. In addition, the combination of EGCG with certain anti-tumor drugs can also produce synergistic effects and inhibit autophagy.

As a biomarker of hepatocellular carcinoma (HCC), high level of α-fetal-associated protein (AFP) suggests malignant tumor differentiation and poor prognosis. Studies have found that EGCG effectively reduced AFP secretion in human hepatoma HepG2 cells and promoted autophagy-induced degradation of AFP aggregates in HepG2 cells. In addition, large-scale all-atom molecular dynamics simulation revealed a new molecular mechanism of EGCG. In addition, it was found that EGCG directly interacts with LC3-I protein and exposes the key site Gly-120 of LC3-I to other important binding partners, such as 1,2-divinyl-sn-glycerol-3-phosphoethanolamine, promoting the synthesis of autophagosome-labeled LC3-II, which provided a potential molecular basis for the prevention and treatment of hepatocellular carcinoma (Zhao et al. 2017).

It was also found that EGCG can sensitize the efficacy of several chemotherapeutic drug doxorubicin (DOX) to enhance its therapeutic effect on hepatocellular carcinoma. Electron microscopy and fluorescence microscopy confirmed that DOX significantly increased autophagic vesicles in hepatoma Hep3B cells. Results of immunoblotting and trypan blue assays showed that DOX increased the autophagic flow of about 45% of dead cells. In contrast, quantitative RT-PCR and immunoblotting showed that EGCG dose dependently inhibited autophagy signals, and 40 μg/ml EGCG treatment decreased the expression of Atg5 and beclin 1. In addition, EGCG treatment significantly enhanced the role of DOX inhibition of tumor cell growth. Combination therapy increases cell death by approximately 40–60% and synergistically enhances apoptosis, antagonizing DOX-induced autophagy. As a kind of autophagy inhibitor, rapamycin significantly inhibited the anti-cancer effect of DOX

or a combination of EGCG. On the other hand, the use of small interfering RNA targeting chloroquine-related autophagy genes Atg5 and beclin 1 inhibited autophagy, resulting in a significant increase in liver cancer cell death. In the subcutaneous transplantation of the Hep3B cell tumor model, the combination treatment of DOX and EGCG inhibited tumor growth by about 25% and apoptotic cells by 50% compared with DOX treatment. In addition, immunohistochemistry analysis indicated that the suppressed tendency of autophagic hallmark microtubule-associated protein LC3 expressions was consistent with thus combined usage in vitro (Chen et al. 2014).

Malignant mesothelioma is an asbestos-related fatal disease and there is currently no effective treatment. It was found that EGCG induced apoptosis in five sorts of human mesothelioma cell lines in a dose-dependent manner, which was related to EGCG-induced increase in reactive oxygen species (ROS) and damage to mitochondrial membrane potential. It was also found that EGCG induced autophagy, but when autophagy was inhibited by chloroquine, EGCG-induced cell death was enhanced (Satoh et al. 2013). These results indicate that the inhibitory effect of EGCG on mesothelioma is related to its induction of apoptosis and autophagy.

Recent studies have shown that EGCG induced autophagy in breast cancer 4T1 cells by regulating the expression levels of autophagy-related proteins beclin 1, ATG5, and LC3B in a concentration-dependent manner. The research on the molecular mechanism of EGCG on drug-resistant oral squamous cell carcinoma illustrated that EGCG inhibited cisplatin-resistant oral cancer CAR cell line and significantly increased Bax, cleaved caspase-9, cleaved caspase-3, Atg5, Atg7, Atg12. Expression of proteins such as beclin 1 and LC3B-II significantly decreased the expression of Bcl-2, phosphorylated AKT (Ser473), and STAT3 (Tyr705) in CAR cells. Importantly, EGCG showed a dose-dependent inhibition of protein and gene expression of multidrug resistance 1 (MDR1). It is clear that downregulation of MDR1 levels and changes in AKT/STAT3 signaling pathway promoted EGCG-induced apoptosis and autophagy in CAR cells. It suggests that EGCG has the potential to treat oral cancer and may play a role in the prevention of long-term oral cancer (Yuan et al. 2017).

53.2.3 Rottlerin

Rottlerin (5, 7-dihydroxy-2, 2-dimethyl-6-(2, 4, 6-trihydroxy-3-methyl-5-acetylbenzyl)-8-cinnamoyl-1, 2-chromine), also known as crude purotoxin, is extracted from Philippine bitter tea. Rottlerin induces autophagy through multiple pathways such as PKCδ/TG2 pathway, PKCδ-independent pathway, and mTORC1 pathway in prostate cancer, fibrosarcoma, breast cancer, gastric cancer, bladder cancer, and other types of cancers.

In the breast cancer and colon cancer cell models, rottlerin has antioxidant activity and inhibits NF-κB (Maioli et al. 2009). Upregulation of PKCδ and TG2 levels led to NF-κB activation, and inhibition of this pathway results in autophagy and cell death. Rottlerin has been recognized as the inhibitor of PKCδ. Rottlerin induced excessive autophagy by PKCδ and TG2 leading to prostate cancer cell death. Recent

studies have demonstrated that rottlerin induced apoptosis in fibrosarcoma cells via a PKCδ-independent pathway.

In breast cancer cell lines under normal nutrient deficiencies, rottlerin induced autophagosomes aggregation by blocking the mTORC1 pathway (Balgi et al. 2009). Rottlerin induced AMPK activation, reduced intracellular ATP levels, induced autophagy in tumor cells, or activated cyclin-dependent kinase (CDK) inhibitory protein p27 through AMPK and SIRT1/FOXO pathways, as a sequence, promoted autophagy. Rottlerin-induced autophagy may involve a number of signaling pathways and many mechanisms that induce autophagy, thereby causing cell death. However, the decisive role is still the external environment, the critical state of cells that trigger or inhibit apoptosis, and activation and inhibition of related signaling pathways. Pharmacokinetic results in a mouse-transplanted solid tumor model showed that tumor tissue has a good absorption effect on rottlerin, so rottlerin or its derivatives have the potential to be developed to induce autophagy and lead to the promising drugs.

Studies have shown that rottlerin induced autophagy and apoptosis of SGC-7901 and MGC-803 gastric cancer cell lines and inhibited cell migration and invasion. Moreover, rottlerin increased the expression of LC3β and enriched autophagosomes, while the expression levels of rapamycin kinase and S phase kinase-associated protein 2 (Skp-2) associated with autophagy were downregulated (Song et al. 2018). It is suggested that rottlerin may inhibit the invasion of gastric cancer cells and promote the apoptosis of gastric cancer cells, which may be mediated by autophagy activity.

In addition, it was found that rottlerin significantly increased apoptosis by inducing autophagy, inhibiting the viability of EJ human bladder cancer cells in a dose- and time-dependent manner. Rottlerin treatment induced autophagy, which was characterized by increased expression of LC3-II and increased autophagosomes. Elevated levels of LC3-II and autophagosomes suggest that autophagy may contribute to apoptosis in these cells (Qi et al. 2016).

53.2.4 Genistein

Genistein (4, 5, 7-trihydroxyisoflavone) is an isoflavone natural product extracted from legumes and has anti-tumor activity. Genistein induced cell death through apoptosis and autophagy pathways, and also reversed tumor chemoresistance by altering the role of apoptotic signals.

Genistein protected the cytokeratin network in stress, nutrient deficiencies, and growth factor deficiency. A number of studies have shown that genistein overcomes the okadaic (a potent inhibitor of autophagy)-induced damage of mouse liver cells through cytoskeleton and keratin recombination, suggesting that keratin filaments may be involved in autophagy. Genistein has a two-way cytotoxic effect on promoting apoptosis and autophagy in cervical cancer, inhibiting the glucose absorption and oxidative phosphorylation substrates and fatty acid synthesis substrate (methyl pyruvate), thereby effectively eliminating promoting autophagy of genistein.

Christian et al. (2010) reported that genistein inhibited autophagy in cervical cancer cells by inhibiting PDE4A4 aggregation. PDE4A4 interacts neither with autophagosomes nor with aggregates, but with p62 (SQSTM1) protein. Due to p62 and LC3, the interaction promoted the autophagosome formation, thus inhibiting the aggregation of PDE4A4 which may induce the development of autophagy.

Although genistein induces autophagy and apoptosis in a variety of tumor cells, pharmacokinetic or ADME studies have revealed that genistein has low oral bioavailability due to metabolic enzymes and transporters. Therefore, in the apoptosis-resistant cancer, the action efficiency of genistein still needs to be improved.

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is a transmembrane cytokine that selectively induces apoptosis in a variety of tumor cells and is a promising tumor suppressor gene (Nazim and Park 2015). Inhibition of autophagy flux has increasingly been recognized as a good and novel cancer therapy. Genistein induced TRAIL-mediated apoptosis in human adenocarcinoma A549 cells through TRAIL signal pathway. Notably, genistein treatment significantly increased LC3-II and p62 protein levels. The combination of genistein and TRAIL treatment increased the accumulation of LC3-II, p62, activated caspase-3, and activated caspase-8, inhibiting autophagy flux, indicating that genistein enhances drug-resistant A549 by inhibiting autophagy flux, as well as TRAIL-induced tumor cell death in adenocarcinoma cells.

53.2.5 Quercetin

Quercetin (3, 3′, 4′, 5, 7-pentahydroxyflavone) is a type of flavonoids natural product and is abundant in fruits, vegetables, plant stems, and leaves; it interacts with a variety of molecular, organelle, and tumor development related pathways, and therefore presents anti-tumor activity.

In order to confirm the effect of quercetin on autophagy, Psahoulia et al. (2007) used 3-MA to act on RAS gene-modified colon cells, inhibiting the formation of vacuoles, whereas the caspase inhibitor zVAD-FMK failed to inhibit quercetin-induced vacuolar formation, and the above results confirmed that quercetin induced autophagy, which was caspase-independent.

For gastric cancer cells, quercetin induced autophagy through activating several hub knots in autophagy, but after using the autophagy inhibitor chloroquine or knockout of Atg5 or beclin 1 gene, apoptosis of gastric cancer cells is significantly enhanced, suggesting quercetin-induced autophagy protected tumor cells against apoptosis. Further studies have shown that quercetin activates autophagy by modulating Akt-mTOR and HIF-1α signaling pathway (Wang et al. 2011). The above studies on tumor cells and xenograft tumor animal models have demonstrated that quercetin simultaneously induces autophagy and apoptosis.

In addition, quercetin also promotes the removal of damaged mitochondria by autophagy/mitochondrial autophagy in oxidative stress. Therefore, it can be

considered that fibroblasts around cancer cells provide nutrition and energy for mitochondrial production of adjacent cancer cells by reversing the Warburg effect.

Quercetin induces the intracellular vesicle and autophagosome formation by upregulating autophagy-associated marker proteins in epithelial cancer cells, which form cell cycle arrest and induce apoptosis. Prior to the formation of autophagosomes, mTOR activity was detected to be inhibited, accompanied by a significant decrease in phosphorylated substrate levels, including the endoplasmic reticulum S6 subunit (phosphorylation by p70S6 kinase) and elF4 (via inhibiting phosphorylation of elF4 inhibitory protein 4E-BP1). Quercetin also induces excessive autophagy by inhibiting proteasome activity and mTOR activity, leading to cancer cell death. Therefore, quercetin has strong anti-tumor activity, not only through cell cycle arrest and apoptosis, but also through regulating key autophagy signaling pathways such as Akt-mTOR and HIF-1α.

Studies on the bioavailability and metabolic kinetics of quercetin in rats showed that after 53% of quercetin was administered by gavage, 93.8% of quercetin was present in the structures of sulfonylation and glycosylation in the blood circulation. The original structure of quercetin was not detected in the blood.

Tumor metastasis is one of the main causes of death in cancer patients. Inhibition of tumor metastasis by inhibiting glycolysis (the main pathway of tumor cell energy supply) is one of the popular research fields in cancer therapy. Studies have found that quercetin inhibits the breast cancer metastasis by inhibiting the Akt-mTOR pathway, inducing autophagy, and by inhibiting glycolysis. Quercetin inhibits glucose uptake and lactic acid production, and successfully blocks cell glycolysis and reduces glycolysis-associated proteins pyruvate kinase M2 (PKM2), glucose transporter 1 (GLUT1), and lactate dehydrogenase A (LDHA). It is suggested that quercetin may inhibit glycolysis by reducing the acidity of the tumor microenvironment. The application of autophagy inhibitor 3-MA and Akt-mTOR pathway inducer IGF-1 further demonstrated that quercetin inhibited cell migration and glycolysis by autophagy mediated by Akt-mTOR pathway. In vivo studies have shown that quercetin treatment inhibits tumor growth and metastasis by inhibiting p-AKT/AKT, which in turn induces autophagy, thus inhibiting glycolysis (Jia et al. 2018). This study found for the first time that quercetin inhibits cell migration and glycolysis through autophagy induced by Akt-mTOR pathway, thereby inhibiting the breast cancer oncogenesis and providing a potential therapeutic target for breast cancer treatment.

53.2.6 Resveratrol

Resveratrol (3,5,4-trihydroxystilbene) is a phytoalexin present in grapes, nuts, and red wine, which has chemopreventive and multi-target properties, and its targets depend on types of cell lines and environmental conditions. Opipari et al. (2004) confirmed that resveratrol induces cell death by promoting autophagy in five types of cervical cancer cell lines, suggesting that resveratrol may have a lethal effect on apoptosis-resistant tumors, and resveratrol initiates nutrient deficiency. The response

signaling pathway, for example, reduces the level of phosphorylated Akt in cervical cancer cells and the expression of mTOR. Studies have demonstrated that in chronic myeloid leukemia cells, resveratrol initiates autophagy and leads to cell death by activating JNK-mediated overexpression of p62/SQSTM1 and AMPK/mTOR signaling pathway activation machinery. Resveratrol also upregulates the expression of several tubulin subunits, which play an important role in the movement of autophagosomes.

It has been reported that resveratrol is not cytotoxic to human colorectal cancer LDL1 cells in acute short-term treatment (for 2 h), whereas repeated and prolonged (48 h) resveratrol exposure initiates autophagy-dependent cytotoxicity. Inactivation of PI3K/Beclin 1 and Lamp2b can significantly reduce the cytotoxicity of resveratrol (Trincheri et al. 2008). After gene silencing of Lamp2b, fusion of autophagosomes with lysosomes was abolished. In addition, studies using this model also found that the caspase inhibitor zVAD-FMK inhibited cell death but did not inhibit autophagy. This study shows us two new ways of producing cytotoxic effects of resveratrol, in which autophagy has a two-sided effect. Initial autophagy initiates a response to stress signaling and, in the later stages, responds to the mechanism of caspase-dependent apoptosis. In another study, resveratrol upregulated the ROS level by inducing caspase-8 and caspase-3 splicing and upregulating LC3-II expression in colon cancer cells. This effect was blocked by NAC (N-acetyl cysteine).

An inhibitory protein of endogenous cathepsin L, SCCA1, is widely expressed in uterus and cervical cells. Hsu et al. (2009) found that the lysosomal pathway of cathepsin L-SCCA1 and autophagy mediates the toxic effects of resveratrol on uterine cells. In this cell model, autophagy inhibitors wortmannin or asparagine was used to reduce resveratrol-induced cell death. In glioma cells, resveratrol-induced autophagy can inhibit resveratrol-induced apoptosis. Autophagy plays a different role in apoptosis, which leads to the death of glioma cells, whereas autophagy delays the apoptotic process and protects cell survival. It can be seen that autophagy inhibitors may have the potential to enhance the anti-tumor activity of resveratrol.

SIRT1 is one of the most popular targets for resveratrol, which activates SIRT1 and induces the development of autophagy and apoptosis (Wang et al. 2018a, b). However, Armour et al. found that resveratrol inhibits autophagy in response to nutrient deficiencies in this cell line by a SIRT1-independent pathway in some tumor cell lines. Resveratrol induces autophagy by regulating SIRT1 and PARP in lung cancer cell lines mediated by tobacco-mediated oxidative stress. Resveratrol decreased ATP concentration and upregulated SIRT1 expression in liver tissues of wild-type mice with endotoxin intervention, and also increased HIF-1α expression and promotes autophagy, whereas in SIRT1 knockout mouse model, the above effects cannot be observed.

However, it has recently been reported that resveratrol induced protective autophagy in non-small cell lung cancer by activating SIRT1, inhibiting Akt/mTOR, and activating p38-MAPK. They found that the combination of the autophagy inhibitor 3-MA or the SIRT1 inhibitor nicotinamide significantly inhibited proliferation and promoted apoptosis compared with the resveratrol 200 μM group alone. It indicated that resveratrol-induced autophagy may promote the survival of NSCLC

cells as a protective mechanism, while inhibition of autophagy may enhance the anti-tumor effect of resveratrol. Furthermore, resveratrol treatment inhibited Akt/mTOR, while p38-MAPK was activated in NSCLC cells in a dose-dependent manner. The combination of IGF-1 to activate the mTOR pathway or inhibit the p38-MAPK pathway with doramapimod significantly inhibited cell proliferation and increased apoptosis of non-small cell lung cancer cells compared to 200 μM resveratrol alone (Wang et al. 2018a, b). These studies indicate that resveratrol-induced autophagy may be a protective mechanism that promotes survival of NSCLC cells, thus inhibiting autophagy enhances the anti-tumor activity of resveratrol in non-small cell lung cancer.

Resveratrol is metabolized in the body to a glycosylated, sulfated form. Oral resveratrol bioavailability tends to zero due to its excessive metabolic rate (Wenzel and Somoza 2005). However, resveratrol induces autophagy in a variety of tumor cells, and its multi-target anti-tumor activity has led drug developers to attach great importance to structural modification and drug development.

53.3 Alkaloids

Alkaloids are rich treasure trove of natural products. Current research shows that alkaloids exert obvious anti-proliferative and metastatic effects on tumors. Among them, camptothecin and vinblastine have been successfully developed into anti-tumor drugs. It has also been reported that cyclovirobuxine D (CVB-D) has a dose- and time-dependent induction effect on autophagy in breast cancer cell lines. Berberine, evodia, corni, matrine, piperine, phloretin, and tetrandrine are other alkaloids under investigation.

53.3.1 Berberine

Berberine is an isoquinoline alkaloid, which has a wide range of biological activities such as anti-inflammatory, antibacterial, anti-diabetic, anti-ulcer, sedative, protecting against myocardial ischemia–reperfusion injury, dilates blood vessels, inhibiting platelet aggregation, and protecting liver and nerves. They have been used for the treatment of diarrhea, neurasthenia, arrhythmia, diabetes, and so on.

The in vitro and in vivo studies have shown that berberine has strong anti-tumor properties through a multifaceted mechanism that interferes with tumor progression. In addition, berberine was also found to induce apoptosis and autophagy in hepatoma cells HepG2 and MHCC97-L (Wang et al. 2010). In the presence of 3-MA or the interfering autophagy gene Atg5, berberine-induced hepatoma cell death is reduced. Further studies have found that berberine may induce apoptosis by increasing Bax expression and may also inhibit Akt activity and upregulation of P38/MAPK signaling, which in turn activates beclin 1, inhibits mTOR signaling, and induces autophagic

HepG2 and MHCC97-L cell death. Compared with clinically used anti-cancer drugs, its cytotoxicity is weak, but it inhibits invasion and metastasis as well as tumor angiogenesis. Combined with chemotherapy drugs or radiotherapy, berberine improved the treatment effect.

However, a study has shown that berberine induces mitochondria-mediated apoptosis in human malignant pleural mesothelioma NCI-H2452 cells but produces protective autophagy (Yao et al. 2018). This study found that berberine inhibited the proliferation of NCI-H2452 cells in a dose- and time-dependent manner and may induce apoptosis through a caspase-9-dependent mitochondrial pathway. In addition, berberine induced autophagy, showing accumulation of LC3-II and decreased expression of p62. Further use of apoptosis inhibitors and autophagy inhibitors, or autophagy inducers, found that apoptosis is the main pathway for berberine-induced cell death in NCI-H2452 cells. However, berberine-induced autophagy may be an adaptive response to anti-tumor drugs and has a protective effect on malignant pleural mesothelioma cells. Inhibition of autophagy enhances berberine-induced apoptosis. Therefore, inhibition of autophagy may be an effective strategy for the treatment of malignant pleural mesothelioma.

53.3.2 Evodiamine

Evodiamine is a quinolone alkaloid and is the main active compound of Chinese herbal medicine *Evodia*. It has anti-anxiety, analgesic, anti-inflammatory, anti-allergic, protective myocardial ischemia–reperfusion injury, vasodilation, and anti-tumor effects. The study found that Chinese herb medicine *Wujing* activated autophagy in tumor cells, which mainly showed protective effects on tumor cells. It is currently believed that redox of cells is extremely important for controlling apoptosis or autophagy. Evodia induces the production of ROS and NO in HeLa cells in a time-dependent manner, while Evodia also induces autophagy, but pretreatment with 3-MA, a specific inhibitor of autophagy, dose dependently reduces cell viability, indicating autophagy played a protective role in cancer cell survival. These findings may help to elucidate the regulation of autophagy and apoptosis in the redox state of cells and their effects in anti-tumor therapy (Yang et al. 2008).

Glioblastoma is one of the most aggressive types of brain tumors. The median survival rate for patients with glioblastoma (World Class IV) was < 15 months. WZY-321 is a novel evodiamine derivative. Studies have shown that WZY-321 inhibits the proliferation of SHG-44 cells in a dose- and time-dependent manner by promoting apoptosis and inducing cell cycle arrest. IL-3α and beclin 1 levels induce autophagy to produce an effect (Sun et al. 2019).

53.3.3 Matrine

Matrine is extracted from the plant *Sophora flavescens* and has a wide range of pharmacological activities. It has been used to treat bacterial dysentery, enteritis, arrhythmia, and anti-tumor. Although matrine inhibits the proliferation of cancer cells at a relatively high concentration (milli-mole), it has no significant effect on the survival rate of normal cells. Matrine can cause apoptosis and autophagy in cancer cells, such as liver cancer HepG2 cells and SGC-7901 gastric cancer cells. Studies have shown that matrine dose and time dependently inhibits the proliferation of HepG2 hepatoma cells and induces cell cycle arrest and HepG2 cell apoptosis in G1 phase. Electron microscopy studies showed that HepG2 cells formed abundant autophagic vacuoles after matrine treatment and showed more MDC-labeled particles. After the autophagy inhibitor 3-MA, the number of autophagic vacuoles was greatly reduced. The above results indicate that matrine induces autophagy and apoptosis in HepG2 cells. Real-time quantitative RT-PCR results showed that the expression levels of Bax and Beclin 1 increased, suggesting that Beclin 1 may be involved in matrine-induced autophagy, and the pro-apoptotic mechanism of matrine may be related to the upregulation of Bax gene expression (Zhang et al. 2010).

Recently, it has been reported that matrine significantly reduced the pancreatic cancer proliferation by reducing mitochondrial metabolism and energy levels in vitro and in vivo. Supplementation with pyruvate or α-ketoglutarate significantly reversed the growth of pancreatic cancer cells inhibited by matrine. The mechanism of action may be that matrine downregulates STAT3, inhibits autophagy, weakens the function of lysosomal proteases, and inhibits mitochondrial energy production. In addition, studies have shown that the inhibition of matrine on the growth of pancreatic cancer depends on the mutation of KRAS oncogene. Taken together, this study demonstrates that matrine can inhibit the growth of KRAS mutant pancreatic cancer by inhibiting autophagy-mediated energy metabolism (Cho et al. 2018).

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