MINI-REVIEW

Autophagy-associated Targeting Pathways of Natural Products during Cancer Treatment

Shu-Fang Zhang[1,2], Xiao-Lu Wang[2], Xiao-Qi Yang[1], Ning Chen[3]*

Abstract

It is well known that conventional chemotherapy and radiation therapy can result in toxicity to both normal cells and tumor cells, which causes limitations in the application of these therapeutic strategies for cancer control. Novel and effective therapeutic strategies for cancers with no or low toxicity for normal cells are a high priority. Therefore, natural products with anticancer activity have gained more and more attention due to their favorable safety and efficacy profiles. Pre-clinical and clinical studies have demonstrated that several representative natural compounds such as resveratrol, epigallocatechin-3-gallate, curcumin, allicin and ginsenosides have obvious anticancer potential. In this article, we summarize autophagy-associated targeting pathways of such natural products for inducing the death of cancer cells, and discuss the core autophagic pathways involved in cancer treatments. Recent advances in the discovery, evaluation and exploitation of natural compounds as therapeutic agents for cancers will provide references and support in pre-clinical and clinical application of novel natural drugs for the treatment of primary and metastatic tumors in the future.

Keywords: Autophagy - resveratrol - epigallocatechin-3-gallate - curcumin - allicin - ginsenoside

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Introduction

According to current large number of epidemiological studies, many cancers have high correlation with dietary habits and life styles (Baena and Salinas, 2014). Meanwhile, due to the alteration of life styles and high work pressure, the morbidity of various cancers reveals a gradually increasing trend (Rocque and Cleary, 2013). Nowadays, although a lot of chemotherapeutic agents have been developed for cancers, the treatment efficacy of many anticancer drugs is still limited or unsatisfactory (Weber, 2009). Therefore, developing effective and low-toxic anticancer drugs or strategies is highly urgent and desired. In the past 5 decades, a series of natural products with the capability to regulate physiological functions have been isolated and exploited from plants, animals and microorganisms, and most of which have revealed obvious anticancer activity (Kim and Park, 2002; Balunas and Kinghorn, 2005; Kinghorn et al., 2009; Vidanarachchi et al., 2012). Natural products enriched flavonoids from fruits have confirmed their anti-carcinogenic, anti-proliferative, co-chemotherapeutic and estrogenic effects through various mechanisms such as modulating cell cycles, inducing apoptosis, inhibiting ERK phosphorylation, and so on (Meiyanto et al., 2012). Thereby, natural compounds are expected to become potential effective drugs for the prevention and treatment of cancers in the future. *In vitro* and *in vivo* studies have shown that many dietary agents from fruits, tea and some herbs with both medicinal and food functions are able to fight against and prevent cancers via regulating cellular fate through apoptosis and autophagy (Kallifatidis et al., 2013; Kma, 2013; Lachumy et al., 2013; Zhong et al., 2013; Lao et al., 2014; Larocque et al., 2014).

Autophagy, as an evolutionary conserved catabolic process, can capture the damaged, denatured and aged cytoplasmic proteins, protein aggregates and organelles by the formation of autophagsome vesicles that are delivered to the lysosomal compartment for degradation (Levine and Kroemer, 2008). Up till now, many natural products have been reported to able to cause cancer cell death by regulating the functional status or signal pathways of autophagy (Ajabnoor et al., 2012; Lao et al., 2014; Larocque et al., 2014). In this article, the application of representative natural products in cancer treatments and their action modes associated with autophagy signal pathway have been reviewed and discussed in an attempt to inspire the promising treatment strategies for cancers in the future.

Autophagy Signaling Pathways

Autophagy is a catabolism process through utilizing lysosomes to degrade damaged, denatured and aged proteins and organelles in cells. Under normal physiological circumstances, autophagy is at the basal

*[1]Graduate School, [2]College of Sports Science and Technology, Wuhan Sports University, [3]College of Health Science, Hubei Provincial Collaborative Innovation Center for Exercise, Intervention and Health Promotion, Wuhan Sports University, Wuhan, China *For correspondence: nchen510@gmail.com*

level, while it can be up-regulated during nutritional deficiency, DNA damage, hypoxia and other stresses. As a cellular protection mechanism, autophagy can execute the elimination of redundant or damaged organelles, but overactive autophagy will cause cell death (Chen and Karantza, 2011). Usually, autophagy is regulated by autophagy related gene (Atg), and currently over 30 types of Atg are identified from yeasts to mammals (Mizushima et al., 2011). Atg1, also known as Unc51-like kinase 1 (Ulk1), is a serine-threonine kinase and plays an important role in the initial activation of autophagy (Mizushima, 2010). Autophagy activation also needs the activated phosphatidylinositol 3-kinase (PI3K). Meanwhile, the activation of PI3K usually is coupled with the formation of multiple complex containing Atg6 and Vps34, among which Vps34 acts as the catalyst (Juhasz et al., 2008).

During the induction of autophagy, a double-layer vesicle is first formed and gradually expanded to generate an autophagosome. Autophagosome formation is usually involved in two ubiquitin-like binding pathways including Atg5-Atg12 complex and microtubule-associated protein 1 light chain 3 (LC3) (Romanov et al., 2012). Ubiquitin-like binding of phosphatidylethanolamine (PE) and LC3 can promote LC3 translocation from cytoplasm to the membrane of autophagosome. In addition, the location of LC3-PE in autophagosome membrane becomes a reliable signal marker for the initiation and induction of autophagy. In the final step of autophagy, the autophagosomes integrate with lysosomes for functioning the degradation of autophagosome contents to generate small molecule amino acids, which will provide the fuels for protein synthesis and cell survival as well as intracellular homeostasis through the recycling of damaged, denatured and aged proteins and organelles (Satoo et al., 2009). On the other hand, lysosome acidifier such as chloroquine and protease inhibitors such as bafilomycin A and pepsin inhibitor A can lead to the inhibition of autophagy (Yang et al., 2011).

The Double-Edged Sword Role of Autophagy in Cancer

As us well known, autophagy, a process of cytoplasm and cellular organelle degradation in lysosomes, has been implicated in homeostasis, thus functioning as an important biological mechanism in targeting human cancers for executing a double-edged sword role in cancer, as both a tumor suppressor and a cancer cell survival protector (Chen and Karantza-Wadsworth, 2009; White and DiPaola, 2009; Pandey and Chandravati, 2012). The accumulation of autophagosome in dying cells is highly correlated with the autophagic cell death (ACD), as also defined as a non-apoptotic form of programmed cell death (PCD) or type II PCD with a potential function of tumor suppression similar to apoptosis (Clarke, 1990). Due to the discovery and characterization of Atg, the suppressive function of autophagy in cancer has been validated (Yang and Klionsky, 2010). Among a series of Atg genes, Beclin 1 (Atg6) is a key tumor suppressor through modulating the initiation and regulation of autophagy, and the high frequent allelic BECN1 deletion is often determined in human breast, ovarian and prostate cancers and aging $Becn1^{+/-}$ mice are prone to tumors including lymphomas, lung and liver cancers (Eccles et al., 1990; Russell et al., 1990; Futreal et al., 1992; Saito et al., 1993; Gao et al., 1995; Liang et al., 1999; Qu et al., 2003; Yue et al., 2003). On the other hand, as a tumor suppressive role, the cell survival function of autophagy during cancer progression under stress environments has been also validated. For example, immortalized, apoptosis-defective, IL-3-dependent bone marrow cells in response to growth factor deprivation could result in the extension of cell survival due to the autophagy induction; correspondingly, the accelerative cell death in the presence of autophagy inhibition has also been confirmed (Boya et al., 2005; Lum et al., 2005).

Another excellent example is that defective autophagy resulting from allelic Becn1 deletion or constitutive AKT activation could enhance the susceptibility of apoptosis-incompetent immortalized baby mouse kidney (iBMK) cells to metabolic stress (Degenhardt et al., 2006; Mathew et al., 2009). Moreover, increasing evidences have clearly documented that autophagy in cancer cells is up-regulated in response to metabolic and genotoxic stress induced by hormonal deprivation, chemotherapy and radiation as a cell survival mechanism, thereby contributing to treatment resistance (Chen and Karantza, 2011). Furthermore, the alteration of autophagic flux may be highly correlated with the cell or tissue type, correspondingly determining the cell fate such as cell survival and cell death under stress conditions.

The inflammation occurrence and vascular insufficiency in tumors can lead to the depletion of glucose and/or oxygen, thus perturbing the osmotic milieu causing extracellular acidosis in tumor microenvironment and eventually inducing the autophagy. Therefore, the functional status of autophagy will in govern tumor metastasis and subsequent carcinogenesis or be a determinant for the therapeutic strategies of cancers (Chen and Karantza, 2011; Pandey and Chandravati, 2012). Targeting or manipulating autophagic signaling pathways may be an innovative strategy during the exploration caner-associated biomarkers, and the prevention and treatments or even combinatorial treatments of cancers in the near future. Moreover, the elucidation of the specific role of autophagy at different stages in cancer progression and the application of functional status of autophagy in different cell types and genetic contexts in cells will be benefit to the development of novel and more effective therapeutic and preventative strategies for cancers.

Nowadays, besides chemotherapy, radiation, cell or genetic therapeutic strategies as well as combinatorial strategies, the intervention via natural products for the prevention and treatment of cancers has gained tremendous attention due to their favorable safety and efficacy profiles (Kim and Park, 2002; Balunas and Kinghorn, 2005; Kinghorn et al., 2009; Vidanarachchi et al., 2012). The resource utilization of novel, effective, and low-toxic natural products for the development of anticancer drugs will result in gigantic medical values and social benefits for cancer patients based on the rational regulation of autophagy functional status.

Autophagy-Associated Anticancer Natural Products

In order to fully achieve the benefits from natural resources, so many scientists have conducted or are being involved in the exploitation and utilization of natural products for the prevention and clinical treatment of cancers. Accumulating documents and evidences have supported that a large amount of natural products play a positive role in cancer prevention and treatment through adjusting oxidative stress response, inhibiting cancer cell proliferation and modulating autophagy functional status. Herein, the representative natural products from plants with the clear and obvious anticancer efficacy through regulating autophagy signal pathways or autophagy functional status are discussed.

Resveratrol

Resveratrol (RSV), a polyphenol, is usually extracted from red grape skin, peas, nuts, blueberries, mulberries, cranberries, spinaches and lilies (Kiraly-Veghely et al., 1998; Klinge et al., 2003; Bertelli and Das, 2009). RSV, with chemical name of 3, 5, 4'-thrihydroxystilbene and molecular formula of $C_{14}H_{12}O_3$, adopts cis-isomer and trans-isomer as its natural existence forms (Sabolovic et al., 2007). In plants, trans-isomer of RSV is the dominant form and reveals stronger physiological activity when compared with cis-isomer. RSV has been confirmed to have a potent growth-inhibitory effect against various human cancer cells as well as in *in vivo* preclinical cancer models (Aras et al., 2014). One report in 1997 has demonstrated that local application of RSV can reduce the morbidity of mice bearing cutaneous tumor, which has gained tremendous attention due to its anti-tumor activity (Jang et al., 1997). Further studies have confirmed that RSV can inhibit and even reverse cancer stages such as initiation, proliferation and progression stages (Aziz et al., 2003). In a variety of tumor cells, the major anti-tumor mechanism of RSV is the induction of apoptosis. RSV can induce the apoptosis of human breast cancer T47D cells without any influence on normal peripheral lymphocytes (Alkhalaf, 2007). Similarly, the application of RSV can result in the lecithoid asymmetry and DNA damage of human leukemia HL-60 cells in a concentration-dependent manner. In addition, RSV-induced HL-60 cell death is the apoptosis associated with CD95 signal transduction (Clement et al., 1998). Recent studies have documented that RSV can also inhibit tumor cell growth by inducing autophagic death, and RSV can result in the autophagy of ovarian cancer A2780 cells through modulating the expression of Bcl-2 and Bcl-XL (Opipari et al., 2004; Cheng et al., 2008; Lao et al., 2009). However, the application of RSV does not cause the change in the expression of Beclin 1 protein in MCF-7 cells, suggesting that RSV, different from other natural products, still can induce the autophagy in MCF-7 cells regardless of Beclin 1 expression level (Scarlatti et al., 2008). In addition, another report has also demonstrated that RSV can inhibit the phosphorylation of p70 ribosomal protein S6 kinase (p70S6K), a substrate of mammalian target of rapamycin (mTOR) through inhibiting the phosphorylation of protein kinase B (PKB)/Akt signaling pathway, thus consequently restraining mTOR signal pathway (Scarlatti et al., 2004). Since mTOR can up-regulate the expression of PKB and adenosine monophosphate kinase (AMPK), and AMPK plays a pivotal role in RSV-induced autophagy, RSV can induce autophagy by regulating the activity of PKB and AMPK. Furthermore, RSV can retard the growth of mouse hepatoma 22 (H22) at S stage, synergistically enhance anti-tumor effects of fluorouracil (5-FU), and simultaneously reduce the toxicity of 5-FU (Wu et al., 2004).

Epigallocatechin-3-gallate (EGCG)

EGCG is one of the major bioactive components in green tea. EGCG can induce apoptosis of cancer cells or retard the growth cycles of cancer cells within certain physiological scope without influencing normal cells (Ahmad et al., 1997). The combinatorial therapy of EGCG and Cox-2 inhibitor can inhibit cell growth of prostate cancer, activate caspases, induce the apoptosis of cancer cells and restrain the activity of NF-κB (Shimizu et al., 2008). The complicated cascade reaction of signal modulation to cell death caused by EGCG pretreatment involves Fas-associated death domain (FADD) and Fas-associated death-like IL-beta-converting enzyme (FLICE) profiling (Siddiqui et al., 2008). EGCG, by depending on signal pathway of p53, can inhibit the growth cycle of cancer cells from isogenic lines, and induce the apoptosis of prostate cancer cells. The performance of EGCG is closely correlated with the functions of p21 and Bax, and the down-regulation of any of these two proteins will be benefit for cell growth (Thakur et al., 2012).

EGCG can inhibit self-renewal of prostate cancer cells and EGCG-induced apoptosis of tumor cells is due to the activation of caspase-3 and inactivation of Bcl-2 (Tang et al., 2010). EGCG can also sharply inhibit the expression of Fas-induced heregulin, HRG-β1 dependent on Fas mRNA in human breast cancer MCF-7 cells, and weaken the phosphorylation of Akt and Erk1/2. In human epidermoid carcinoma A431 cells, EGCG can significantly enhance caspase-3 activity in A431 cells, and strengthen the expression of caspases-3, caspase-8 and caspase-9; in contrast, caspase inhibitors can prevent EGG-induced cell apoptosis. In multiple myeloma cells, EGCG, through inducing expression of death-associated protein kinase 2, Fas ligand, Fas and caspase-4, inhibits the growth of tumor cells and induces the apoptosis of tumor cells (Shammas et al., 2006). In human pancreatic cancer cells, EGCG induces oligomerization of Bax genes and depolarization of mitochondrial membranes, to promote cytochrome C release to cytoplasm and strengthen caspase-dependent apoptosis (Qanungo et al., 2005).

Curcumin

Curcumin, a xanthein usually used as a food colorant, is the major bioactive component extracted from Curcuma longa L., Curcuma zedoaria (Christm). Rosc., and Curcuma petiolata. Curcumin has a wide range of biological functions, especially the anticancer activity. It can inhibit the proliferation of tumor cells, and induce the apoptosis of tumor cells including bladder cancer

(Gao et al., 2012), pancreatic cancer (Plengsuriyakarn et al., 2012), prostate cancer (Zhou et al., 2014) and uterine cervix carcinoma (Odot et al., 2004). Curcumin also exhibits favorable synergistic performance both in thermotherapy and γ-ray therapy for cancers (Bansal et al., 2011). The injection of curcumin in mice bearing breast cancer accomplishes an obvious inhibitory effect on the growth of breast cancer cells (Kang et al., 2009). Curcumin has also been found to greatly inhibit the metastasis of breast cancer cells. Previous reports have revealed that curcumin can inhibit cell proliferation of chronic granulocytic leukemia (CGL), glioblastoma, and esophageal cancer through inducing autophagy. It can also inhibit the growth of leukemia K562 cells accompanying with the up-regulation of LC3-II and Beclin 1 as well as the accumulation of autophagosomes. In contrast, in the presence of autophagy inhibitor Bafilomycin A1, the curcumin-induced death of K562 cells is obviously inhibited, suggesting that curcumin can induce autophagy and K562 cell death. In glioblastoma cells, curcumin can inhibit Akt/p70S6K signal pathway, activate extracellular signal-regulated kinases (ERK1/2), and finally induce autophagy (Shinojima et al., 2007). It can induce the generation of reactive oxygen species (ROS), up-regulate the expression of Beclin 1 and p53, activate autophagy, and eventually result in the death of human colon cancer cells (Lee et al., 2011). Serine/threonine protein phosphatases type-1 (PP1) and PP2A are key targets of phosphorylation of ERK, and curcumin can stimulate the phosphorylation of ERK by inhibiting PP1 (Aoki et al., 2007). Besides activating autophagy, curcumin also exhibits time- or concentration-dependent inhibition on the growth of K562 cells. Curcumin-induced cell death is highly correlated with the generation of apoptotic or autophagy complexes, mitochondrial membrane potential (MMP) and the activation of caspase-3. In addition, curcumin can cause the down-regulated expression of Bcl-2 protein in K562 cells (Jia et al., 2009). The combinatorial treatment of curcumin and adriamycin on human Hepatoma G2 (HepG2) facilitates to the apoptosis of HepG2 cells due to the reduced proportion of Bcl-2/Bax protein and caspase-3 activation. Moreover, curcumin can result in the mitochondrial fission of HepG2 cells, reduced potential of mitochondrial membrane and autophagy activation. These results have shown that curcumin is likely to strengthen adriamycin-induced HepG2 cell death rate through activating mitochondria-mediated autophagy (Qian et al., 2011).

Allicin

Garlic, belonging to allium of lily family, has great medicinal value and is widely used as a daily food all over the world. In garlic, the richest ingredient is allicin, and the most valuable ingredient in healthcare products is organic sulfides including alliin, γ-glutamyl cysteine and their derivatives. Besides these organic sulfur-containing compounds, garlic also has abundant trace elements such as zinc, magnesium, copper, selenium and iodine, protein, dietary fiber, vitamin, ascorbic acid and polyphenols (Amagase, 2006). Garlic has a long history in curing leprosy, diarrhea, constipation and infection. However, until late 1950s, the anti-neoplastic property of thiosulfinates extracted from garlic has been reported (Weisberger and Pensky, 1958). Due to therapeutic potential of garlic and the improvement in modern analytical techniques, the extensive exploration of garlic has been conducted and increasing achievements on garlic studies have made all over the world. Garlic can fight against various cancers including colon cancer (Altonsy and Andrews, 2011), glioma and hepatoma carcinoma. The anticancer properties of garlic are highly correlated with apoptosis. However, garlic-induced autophagy has also reported in many studies. For example, allicin can induce p53-mediated autophagy, and inhibit the survivability of human hepatoma cells, which reveals the declined p53 expression in the cytoplasm of HepG2 cells, inhibited PI3K/mTOR signal pathway, reduced Bcl-2 expression and enhanced signal transduction pathways of AMPK/TSC2 and Beclin 1 (Chu et al., 2012).

Ginsenosides

Ginseng, a time-honored nourishment in China, has functions of tonifying five internal organs, stabilizing souls and spirits, eliminating pavor and pathological factors, improving eyesight, feeling delighted, reinforcing intelligence, and prolonging lifespan after long-term application according to Sheng Nong's Herbal Classic. Ginsenosides are major pharmacological active ingredients in Ginseng (Park et al., 2013). Many studies have shown that ginsenosides reveal excellent performance in inhibiting tumor cell growth, anti-fatigue, anti-aging, strengthening organism immunity, adjusting central nervous system and improving insufficient blood supply for heart and cerebral vessels (Zhang et al., 2002). Scholars all over the world have conducted extensive studies on ginsenoside's function in tumor prevention and inhibition, which exhibits that ginsenosides can promote apoptosis of tumor cells, accelerate differentiation of tumor cells, improve sensitivity of tumor cells to chemotherapy, inhibit formation of new tumor vessels, and restrain tumor growth and metastasis (Aggarwal et al., 2005). At present, approximately 40 kinds of ginsenosides have been identified. The widely used ginsenosides are Rb1, Rg1, Rg3, F2, Rd and Rh1. Ginsenosides Rg3 and Rh2 can greatly inhibit the growth of cancer cells. Rg3 is in favorable for strengthening curative effect of cancer chemotherapy, and the combinatorial treatment of Rg3 with docetaxel, paclitaxel, cisplatin or doxorubicin can strengthen the sensitivity of colon cancer cells to chemotherapeutic drugs. The combinatorial treatment of Rg3 and docetaxel is also applied for the treatment of prostate cancer cells via inducing apoptosis and retarding cell cycle of G1 phase more effectively (Kim et al., 2010). Combinatorial therapy of low-dose cyclophosphamide and Rg3 can inhibit the generation of tumor micro-vessels and result in the longest survival for patients (Zhang et al., 2006). The anticancer function of ginsenosides is associated with its function of regulating autophagy. Ginsenoside Rk1 can mitigate the proliferation of HepG2 cells at the G1 stage, and improve the survival rate from 53.3% to 91.9%. Meanwhile, Rk1 can induce autophagy and strengthen the expression of autophagy marker LC3,

especially LC3-II. Moreover, the combinatorial therapy of Rk1 and autophagy inhibitor can enhance antitumor efficacy of Rk1. Ginsenoside F2 can induce the apoptosis and inhibit the proliferative activity of breast cancer cells through activating intrinsic apoptosis pathway and mitochondrial dysfunction. Meanwhile, ginsenoside F2 can also induce the formation of acid vesicles and GFP-LC3-labeled autophagosomes and up-regulation of Atg7 expression, which indicates that ginsenoside F2 initiates autophagy of breast cancer cells (Mai et al., 2012). On the other hand, the application of ginsenoside F2 in the presence of autophagy inhibitor can strengthen F2-induced cell death.

Other natural products

Recently, other natural products have also been confirmed to induce cancer cell death via the regulation of autophagy or functional status of autophagy. Taxol, initially extracted from bark of Pacific taxus chinensis, is widely used in the therapy of lung cancer, ovarian cancer and breast cancer (Wessely et al., 2006). The anticancer property of taxol is realized through up-regulating autophagy and inhibiting the expression of apoptotic factors. Terpinen-4-ol, a monoterpene, as the major essential oil from many plants, can induce autophagic and apoptotic cell death in human leukemic HL-60 cells through inducing the accumulation of LC3-I/II, ATG5 and Beclin-1, and the release of cytochrome C from mitochondria, even decreasing Bcl-xl expression (Banjerdpongchai and Khaw-On, 2013). Oridonin, a diterpenoid extracted from Rabdosia rubescens, can kill melanoma cells and cervical cancer cells, thus inhibiting tumor growth and tumor cell proliferation through regulating apoptosis and autophagy transcription factors (Cui et al., 2006). Genistein, the major bioactive isoflavone in soybean, is reported to have the potential to cure various tumors and its anticancer function is achieved through inhibiting the activation of Akt (Gossner et al., 2007).

Conclusion

Since natural products reveal obvious damage on cancer cells without any adverse effects on the body, they will be regarded as perfect chemotherapeutic agents in the future. Some natural products from plants such as RSV, EGCG, curcumin, allicin and ginsenosides have been proven to play a significant role in autophagy-associated cell death signal pathway and network regulation in the past decades. In short, these studies have emphasized the fact that natural products associated with functional status of autophagy will become an innovative strategy in the prevention and clinical treatment of cancers in the future.

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