



Autophagy Therapeutic Potential of Garlic in Human Cancer Therapy

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ABSTRACT

Cancer is one of the deadliest diseases against humans. To tackle this menace, humans have developed several high-technology therapies, such as chemotherapy, tomotherapy, targeted therapy, and antibody therapy. However, all these therapies have their own adverse side effects. Therefore, recent years have seen increased attention being given to the natural food for complementary therapy, which have less side effects. Garlic (大蒜 *Dà Suàn*; *Allium sativum*), is one of most powerful food used in many of the civilizations for both culinary and medicinal purpose. In general, these foods induce cancer cell death by apoptosis, autophagy, or necrosis. Studies have discussed how natural food factors regulate cell survival or death by autophagy in cancer cells. From many literature reviews, garlic could not only induce apoptosis but also autophagy in cancer cells. Autophagy, which is called type-II programmed cell death, provides new strategy in cancer therapy. In conclusion, we wish that garlic could be the pioneer food of complementary therapy in clinical cancer treatment and increase the life quality of cancer patients.

Key words: Autophagy, Cancer, Complementary therapy, Garlic

INTRODUCTION

Nature has precious treasures for potential cancer therapy. Humans with their technical skill have developed several therapies against cancer, but we still find some disorders and side effects in these artificial therapies. Hence to find more advanced therapy, several researchers try to identify novel materials bestowed in nature. Many natural components have incredible potential to cure diseases without any adverse side effects, such as the collagenase isolated from the King crab (*Paralithodes camtschatica*) as a the strongest antibiotic,^[1] the alkaloids from the skin of poison dart frogs (Dendrobatidae) toward the development of chemical defense.^[2]

Because cancer is the most deadliest disease, recent years have seen increased attention being given to cancer cell study.^[3] Cancer cells are characterized by apoptosis evasion, insensitivity to antigrowth signals, tissue invasion or metastasis, and limitless explicative potential.^[4] Induction of apoptosis had been the dominant research focus in anticancer field for the past decade. Therefore, it is important to study tumor microenvironment in cancer cells in the future.^[5] In this minireview, we focus on the regulating mechanisms of apoptosis, autophagy, and necrosis in anticancer research. This review is an attempt to update the recent research progress related to garlic research against liver cancers carried out in the recent years with a special emphasis on the cell death process.

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There are three famous types of cell death, which are apoptosis, autophagy, and necrosis, each having their own phenomenon. Cell cycle arrest, DNA fragmentation, caspase activation, and apoptosome formation are characteristics of apoptosis.^[6] However, autophagy, called type-II programmed cell death, is characterized by autophagosome induction, organelles degradation, and metabolic stress.^[7] As for necrosis, it may accompany with inflammation and lesions in cell surface.^[8] In this review, we concentrate on how active components of garlic regulate autophagy in cancer cells.

Garlic and cancer

Garlic (大蒜 *Dà Suàn*; *Allium sativum*), a member of Liliaceae family is a globally consumed food and is bestowed with immense medicinal benefits. Numerous research findings have attributed these health benefits mainly resulting from the organosulfur components, such as alliin, γ -glutamylcysteine, and their derivatives. Besides these organosulfur compounds, garlic is rich in trace elements (zinc, magnesium, copper, selenium, and iodine), protein content, dietary fiber, vitamins, ascorbic acid, and polyphenols. Historically almost all the civilizations in the world had knowledge of the medicinal properties of garlic and garlic has been used in treating a variety of ailments, including leprosy, diarrhea, constipation, and infections.^[9] However, garlic as a potent anticarcinogen came to light in the late 1950s after Weisberger and Pensky demonstrated that thiosulfates extracted from garlic possessed antitumor properties.^[10] With the therapeutic potential of garlic and the advent of modern analytical techniques, there has been a surge in garlic research by many research groups around the world.

Classification of autophagy

Four pathways are employed to induce autophagy, including macroautophagy, microautophagy, chaperone-mediated autophagy, and crinophagy.^[11-14]

Macroautophagy

Macroautophagy is the most common intracellular degradation system in autophagy. In yeast, it starts with the formation of preautophagosomal structure (PAS), followed by autophagosome formation, whose molecular basis is well-conserved from yeast to higher eukaryotes. The fusion of the autophagosome with lysosomal compartments causes the formation of the digestive vacuole of autophagy, known as autophagolysosome.

Microautophagy

The process of microautophagy is nonselective degradation process by lysosome engulfing the cell membrane, and then degradation in the body. So far this situation is often found in yeast.^[12]

Chaperone-mediated autophagy

The process is more complex, involving the recognition of the hsc70 complex. By recognition and binding, some unfolding proteins may get transferred to lysosome, which then initiates degradation, and the target marker from lysosome in chaperone-mediated autophagy is lysosome-associated membrane protein (LAMP)-type 2A.^[13]

Crinophagy

Crinophagy is a cellular degradation process by which specifically secretory granules will be degraded by endocrine secretions or hormones.^[15] However, the process of crinophagy is still mediated by the fusion with lysosome.

Molecular mechanism of autophagy signaling transduction

Autophagy is a self-catabolic process that imparts a survival mechanism to cells undergoing nutrient deprivation or other stresses, and has been recently linked to the type-II programmed cell death process.^[16,17] For example, energy and amino acid exhaustion, unfolding protein response and virus infection stress could induce autophagic initiation. Notably, the most dominant phenomenon of autophagy is autophagosome formation. Except autophagosome formation, the discovery of autophagy-related gene (ATG) could help us to understand the cell signaling transduction of autophagy. Atg12-conjugation and LC3-modification are considered to be the necessary protein-binding systems at mammalian autophagosome formation.^[18]

ATG family protein and autophagy

ATG5 and ATG12 are located in PAS structure together. ATG12 covalently binds ATG5 at a lysine residue. This polymer is considered to help double membrane winding. On the other hand, LC3 (MAP-LC3, microtubule-associated protein 1 light chain 3), a small hydrophilic protein (16–18 kDa) located in autophagosome, is associated with the formation of autophagolysosome. The cytosolic form of LC3 (LC3-I) will be proteolytically cleaved to form the LC3-phosphatidylethanolamine conjugate (LC3-II or LC3B-II) by lysosomes during the conversion of autophagosome to autophagolysosome. More specifically, a C-terminal glycine 120 of LC3-I is lysed by Cys-protease ATG4. Then ATG7 and ATG3 will catalyze PE bind LC3-I as LC3-II (Atg8-PE in yeast). Therefore, most of the studies employ the increase in LC3-II as a biomarker in autophagy studies rendering it to be the best biomarker of autophagy.^[19]

However, autophagy is not only mediated by ATG family. Some studies demonstrated that the mammalian target of rapamycin (mTOR), Akt/phosphoinositide-3-kinase (PI3K) pathway,^[20] extracellular signal-regulated kinases (ERK1/2)/p38 mitogen-activated kinase (p38 MAPK) signaling pathway,^[21] Bcl-2/Beclin-1 signaling transduction,^[22] and p53 trigger AMP-activated kinase (AMPK) have also involved in autophagy.^[23]

mTOR signaling transduction

mTOR, a PI3K-related kinase, acts as a central regulator of cell growth in response to nutrients and growth factors. mTOR is usually deregulated in cancer cells by dephosphorylating Akt/PI3K or Akt/protein kinase B (PKB). When mTOR is inhibited, the mTOR-mediated phosphorylation of an autophagy protein ATG13 will also be inhibited. Therefore, the hypophosphorylated form of ATG13 can interact with ATG1 and ATG17 to form a complex, which is essential in the formation of two layers – double-membrane autophagosomes. At the same time, Beclin-1/Atg6 will combine with Vps34, a phosphatidylinositol 3-kinase, as a complex to lead to the formation of autophagosome precursors (vesicle nucleation).^[24]

Mitochondria and autophagy

Bcl-2/Bcl-xL, is the most well-known inhibitor of cell death related with mitochondria. For cytoprotective study, Bcl-2 has the ability to antagonize Bax/Bak, block MOMP, and then inhibit apoptosis. Recently, Bcl-2 was demonstrated to prevent autophagy.^[25] Because Beclin-1 can interact with Bcl-2/Bcl-xL by its Bcl-2-homology-3-only domain, some research groups consider that Bcl-2 will completely inhibit the binding of Beclin-1 and Vps34. In the end, the complex of ATG5-ATG12 and LC3-II will become autophagosome, and ATG5-ATG12 will release from autophagosome before lysosome binding. Then LC3-II and lysosomes will become autophagolysosome. In addition, the colocalization of LC3-II with MitoTracker-red (labeling mitochondria), resulting in drug-induced degradation of mitochondria, could be observed by confocal laser microscopy.^[26] Autophagosome engulfs the mitochondria and this situation is called “Mitophagy.”^[27] Recent studies indicate that oxidative stress could trigger aerobic glycolysis, autophagy, and mitophagy in cancer cells.

p53 signal transduction

p53 gene not only regulates apoptosis but also autophagy.^[23] When cell incurs some stress or damage, the nuclear p53 will be activated and the cytoplasmic p53 protein would be decreased. And then the low expression of cytoplasmic p53 protein will activate the phosphorylation of AMPK, which triggers the induction of tuberous sclerosis protein 2 (TSC2), and finally TSC2 would inhibit the protein expression of mTOR and trigger autophagy.^[28] However, p53 is a famous tumor suppressor gene and involves in many developments of diseases, including cancer. Although p53 molecular mechanism of autophagy is not clearly understood and very complicated, we try to narrow it down in cancer therapy field.

Garlic and autophagy

Many research groups consider that phytochemicals have a good potential of cancer chemoprevention.^[29] Garlic is one such potential phytochemical candidate that has been thoroughly studied against various cancer cells, such as colon cancer cells,^[30] glioblastoma cells,^[31] and hepatocarcinoma cells.^[32] Most of these studies show that apoptosis as the mechanism to be associated with the anticancer properties of garlic. However, some studies demonstrate that garlic could trigger other phenomena, such as autophagy.^[33] For example, the crude garlic, which has the protective effects on iron-mediated oxidative stress, proliferation, and autophagy in rats.^[34] In addition, allicin, which is the most abundant component in garlic, could induce autophagic cell death in Hep G2 cells.^[26] From many research outcomes, it could be perceived that garlic with its potent anticancer activities makes it a potential complementary medicine in cancer therapy.^[35]

CONCLUSION

Autophagy, type-II programmed cell death, is defined as alternative cancer therapy in addition to apoptosis.^[36] However, the fate of cell undergoing various types of stress is quite complex, and sometimes be contradictory. Even we could not draw the whole picture of cell death and survival, we can try our best to find

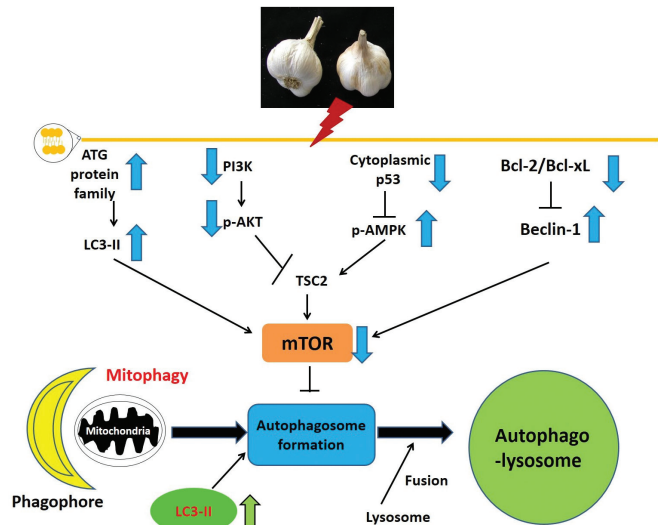


Figure 1. A possible mechanism of autophagy of garlic in cancer cells

complementary therapy, which is much safe and easy in our life.

The risks associated with the side effects of chemotherapy, radiation therapy, and surgery, makes us to look for the alternative treatments, such as complementary therapy from food or natural herbal medicines, such as resveratrol,^[37] curcumin,^[38] soybean fermentation products,^[39] and even active components from food such as anthocyanins from berries,^[40] triterpenes from ginsenoside,^[41] which have good tumor inhibition *via* autophagy and have the potential to be an alternative drug in apoptosis-resistant cancer cells.

Garlic, which is considered the most powerful anticarcinogenic agent by the Designer Food Program, NCI, 2005, not only induces apoptosis but also autophagy in cancer cells. Therefore, we try to figure out that garlic has good potential to kill cancer cells through apoptosis and induce autophagy in apoptosis-resisted cancer cells [Figure 1]. No matter what mechanism garlic triggers, we still consider that garlic as a very good alternative medicine for cancer therapy and to be the pioneer in complementary therapy of clinical cancer treatment in the future.

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