

# Autophagy as a Target for Anticancer Therapy and Its Modulation by Phytochemicals

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## ABSTRACT

Autophagy is a catabolic process and has an important homeostatic role, mediating the removal of dysfunctional or superfluous proteins and organelles. Dysfunction of autophagy is involved in various physiologic and pathologic conditions, such as developmental cell death, clearance of pathogens, aging process, neurodegenerative diseases, and cancers. Cancer cells which are inherently vulnerable to metabolic stress and also defective in apoptosis are more dependent on autophagy for cell survival than normal cells. Autophagy might have a dual but conflicting role in carcinogenesis, tumor-suppressing and tumor-promoting role. The roles of autophagy in specific type cancers *in vivo*, however, have not been determined clearly. In this review, we discussed the role of autophagy especially in ovarian cancer cells in relation to cancer cell metabolism and its modulation by natural compounds.

Key words: autophagy, cancer, metabolism, phytochemicals

## INTRODUCTION

Cancer is the leading cause of death in developed countries, and the global burden of cancer is increasing substantially<sup>(1)</sup>. Ovarian cancer, for instance, is the seventh leading cause of cancer deaths in women worldwide, and is the most lethal gynecologic malignancy<sup>(1)</sup>. The poor clinical outcome mainly comes from the high percentage of cases being diagnosed at an advanced stage and frequent emergence of chemoresistance. There have been numerous studies on the mechanisms of chemoresistance, including alterations in drug transport, mutations in drug-binding sites, defects in apoptotic pathways, and, recently, presence of cancer stem cells. *TP53* mutation is one of the most frequent genetic abnormalities found in epithelial ovarian cancer and is present in up to 96% of cases with high-grade serous ovarian carcinoma<sup>(2)</sup>. Although *TP53* mutation has been suggested to be involved in above mentioned mechanisms of chemoresistance, studies on the relationship between *TP53* mutation and chemoresistance have shown conflicting results<sup>(3,4)</sup>. Recent evidences have suggested autophagy as another mechanism of cancer cell survival, which is related to sustaining cell viability under

metabolic stress, such as nutrient starvation or hypoxia. Autophagy is a catabolic process and has an important homeostatic role, mediating the removal of dysfunctional or superfluous proteins and organelles, which are engulfed, digested and recycled to sustain cellular metabolism<sup>(5)</sup>. Characteristics of cancer cells, including enhanced cell proliferation, altered apoptotic pathways, and reprogrammed cellular metabolism, altogether have shown to influence the autophagic pathway.

Here, we review the role of autophagy especially in ovarian cancer cells in relation to cancer cell metabolism and *TP53* mutation, and its modulation by natural compounds.

## CANCER CELL METABOLISM AND AUTOPHAGY

Cancer cells are characterized by 'aerobic glycolysis', first described by Otto Warburg in 1920s (Warburg effect). Aerobic glycolysis refers to the high level of glycolytic activity even in the presence of oxygen, which is an inefficient way of ATP production compared to oxidative phosphorylation but is beneficial

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in production of macromolecular precursors necessary for protein, lipid and nucleic acid biosynthesis. The reprogrammed cancer cell metabolism is the basis of a diagnostic imaging modality such as  $^{18}\text{F}$ -fluorodeoxyglucose positron emission tomography (FDG-PET), which is shown to be sensitive in detection of recurrent ovarian cancer<sup>(6)</sup>.

The switch from oxidative phosphorylation to glycolysis is induced by the activation of several oncogenes, such as Ras, Src, and myc, as well as activation of phosphatidylinositol 3-kinase (PI3K) pathway<sup>(7)</sup>. Loss of p53 function by *TP53* mutation also promotes glycolysis through decreased levels of TP53-induced glycolysis and apoptosis regulator (TIGAR)<sup>(8)</sup>. TIGAR is known to inhibit glycolysis and decrease intracellular reactive oxygen species (ROS) levels through re-directing glucose metabolism toward the pentose phosphate pathway. The enhanced glycolysis by inhibition of TIGAR, subsequently, leads to induction of autophagy through expression of glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH), which promotes the transcription of autophagy-related protein Atg12.

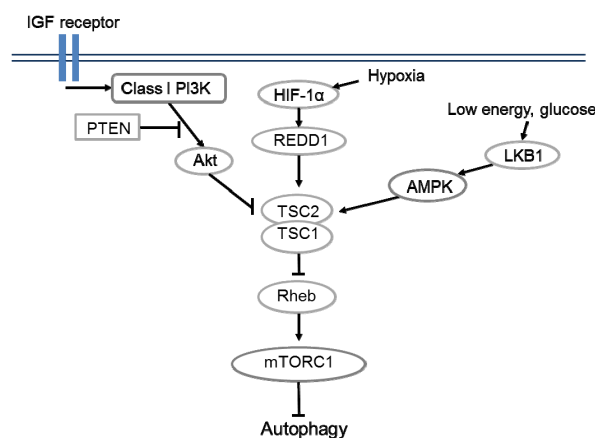
In addition to intrinsic metabolic stress derived from inefficient energy production due to aerobic glycolysis, cancer cells suffer from extrinsic metabolic stress from lack of nutrition and hypoxia, frequently encountered during carcinogenesis. As tumor grows larger, it outgrows its local blood supply leading to inadequate supply of oxygen, nutrients, and growth factors to cancer cells. These intrinsic and extrinsic metabolic stress trigger autophagic pathway to counterbalance the high metabolic demand of proliferating cells and to provide a survival mechanism to cancer cells<sup>(5)</sup>. Moreover, cancer cells which frequently have defects in apoptosis by *TP53* mutation, for example, are more dependent on autophagy for cell survival in metabolic stress. When autophagy is inhibited in apoptosis-defective cells under conditions of nutrient limitation, the cancer cells fail to tolerate metabolic stress and undergo necrotic cell death.

## ROLES OF AUTOPHAGY IN CANCER

Autophagic process is initiated with formation of the double-membrane autophagosome, which then fuses with the lysosome into the autophagolysosome where the contents are degraded and recycled. This process is controlled by several autophagy-related (Atg) proteins, including Beclin1 (Atg6) and LC3 (Atg8). Signals triggering autophagy include nutrient deprivation, hypoxia, endoplasmic reticulum stress, DNA damage, and specific pathogens.

The mammalian target of rapamycin complex 1 (mTORC1) is a key negative regulator of autophagy signaling, which integrates signaling through PI3K/Akt pathway and cellular nutrient status or energy levels (Figure 1). In response to nutrient and growth factor availability, mTORC1 downregulates the autophagic

cascade by phosphorylating a complex of autophagy proteins. Under the metabolic stress, however, mTORC1 activity is inhibited, de-repressing the autophagic pathway. At low energy level, AMP-activated kinase (AMPK) is activated by the LKB1, and then AMPK activates tuberous sclerosis protein (TSC) 2 to inhibit mTOR activity. Hypoxia also reduces mTOR activity in a similar way by inducing expression of REDD1, which stimulates TSC activity and, in turn, suppresses mTOR activity. In addition, amino-acid starvation, which inhibits mTORC1, induces autophagy by affecting the lysosomal positioning and influencing autophagosome formation<sup>(9)</sup>.



**Figure 1.** Regulation of autophagy by mTOR signaling. AMPK, AMP-dependent kinase; HIF, hypoxia-inducible factor; IFG, insulin-like growth factor; mTORC1, mammalian target of rapamycin complex 1; PI3K, phosphoinositide 3-kinase; PTEN, phosphatase and tensin homolog; TSC1/2, tuberous sclerosis 1/2 proteins.

Autophagy is involved in various physiologic and pathologic conditions, such as developmental cell death, clearance of pathogens, aging process, and neurodegenerative diseases. When autophagy is compromised, proteins such as  $\alpha$ -synuclein and huntingtin accumulate in neurons, causing Parkinson's disease and Huntington's disease, respectively<sup>(10)</sup>. The role of autophagy in cancer, however, is relatively less defined.

Autophagy may have a dual but conflicting role in carcinogenesis, tumor-suppressing and tumor-promoting role. As an evidence of its tumor-suppressive role, *Beclin-1* which is involved in autophagosome formation was found to be localized to a region of chromosome 17q21 which is commonly deleted in sporadic breast, ovarian and prostate cancers<sup>(11,12)</sup>. In addition, mice with monoallelic deletion of *Beclin1* (*Beclin1*<sup>+/-</sup>) demonstrated the decreased activity of autophagy and the highly increased frequency of spontaneous malignancies<sup>(13)</sup>. Functional autophagy may suppress cancer development and progression through prevention of DNA instability by removing oncoproteins and inhibiting necrotic cell death and inflammation in apoptosis-deficient cells. On the other hand, accumulating evidences suggest that

autophagy plays a more prominent role in promoting cancer progression<sup>(7)</sup>. Autophagy can provide a survival advantage to cancer cells which suffer inherent metabolic stress during carcinogenesis, especially in established tumors. Studies on apoptosis-defective cells with or without functional autophagy (for example, *Beclin1*<sup>+/+</sup> or *Beclin1*<sup>-/-</sup>) have shown that cell survival of metabolic stress is dependent on autophagy<sup>(14)</sup>. Cells impaired in both apoptosis and autophagy undergo necrotic cell death *in vitro* and *in vivo*. In addition, autophagy enables cells affected by metabolic stress to recover properly when nutrient supply is restored<sup>(15)</sup>. These capacities for long-term survival of metabolic stress and recovery through autophagy might be important in cancer cells which remain viable after treatment, recurrent tumor cells, and possibly cancer stem cells.

The exact role in ovarian cancer *in vivo*, however, is still not determined clearly. The expression of autophagic proteins, LC3 and Beclin1, was demonstrated to be significantly decreased in ovarian cancer compared to benign and borderline tumors, suggesting that decrease in autophagic activity might be involved in development of ovarian cancer<sup>(16)</sup>. However, in malignant melanomas, LC3 expression was increased in advanced malignant tumors compared to early melanoma *in situ* lesions and normal melanocytes, implicating the role of autophagy in late stage of tumorigenesis<sup>(17)</sup>. To make things more complicated, tumor microenvironments have shown to influence the fate of tumor cells undergoing autophagy<sup>(18)</sup>. Autophagy induced by tumor suppressor gene aplasia Ras homolog member I (ARHI) resulted in autophagic cell death *in vitro* model, whereas it lead to cell survival and tumor dormancy in ovarian cancer xenografts, supposedly due to restored PI3K signaling from several factors derived from the tumor microenvironment.

#### **AUTOPHAGY IN ANTICANCER THERAPY AND THE ROLE OF PHYTOCHEMICALS**

Many anticancer drugs, including cytotoxic chemotherapeutic agents, mTOR inhibitors, or anti-angiogenic agents, induce autophagy. Inhibition of the autophagic pathway which is supposed to be a mechanism of chemoresistance may be a promising strategy to enhance treatment efficacy even in the apoptosis-defective tumor cells. Preclinical studies with autophagy inhibitors, such as chloroquine or hydroxychloroquine, demonstrated the enhanced efficacy in combination with other anticancer drugs<sup>(19)</sup>. On the other hand, over-stimulation of autophagy is another approach for anticancer therapy, inducing autophagic cell death in excessively stressed cells beyond a critical point<sup>(5,20)</sup>. In addition, autophagy inducers may be useful in the setting of cancer prevention, where autophagy may prevent tumor progression through preventing genetic instability. Several autophagy inducers or inhibitors have been evaluated for their anticancer activities in preclinical and clinical studies.

Phytochemicals, which are natural compounds derived from fruits and vegetables, have been widely investigated for their anticancer activities due to their safety, low toxicity and general availability. The major dietary sources of phytochemicals include garlic, soybeans, ginger, grapes, green tea, turmeric, and cruciferous vegetables. These natural compounds have shown potentials in chemoprevention, partly due to their ability to target multiple signaling pathways involved in carcinogenesis, such as cell proliferation, apoptosis, angiogenesis, and inflammatory signaling pathway<sup>(21)</sup>. In addition, recent studies have suggested the role of phytochemicals in modulating autophagic pathway. Resveratrol, a phytoalexin abundantly found in grape skins and red wine, induced cell death and growth inhibition in five ovarian cancer cell lines, through autophagocytosis<sup>(22)</sup>. Although resveratrol induced molecular features of apoptosis, including mitochondrial release of cytochrome *c* and caspase activation, resveratrol-treated cells exhibited the morphologic and ultrastructural changes indicative of autophagocytic death. Those cells demonstrated intact nuclei without fragmentation, absence of blebbing, and presence of cytoplasmic autophagosomes. The possible mechanism of resveratrol-induced autophagy was suggested to be the inhibition of glucose metabolism through reducing glucose uptake and downregulating Akt and mTOR pathways, which are the two rate-limiting steps in glycolysis<sup>(23)</sup>. A soy-derived isoflavone, genistein, was also shown to induce both apoptosis and autophagy<sup>(24)</sup>. Genistein induced a punctate pattern of LC3 distribution in ovarian cancer cells, indicating recruitment and localization of LC3-II to autophagosomes during genistein-induced autophagy.

#### **CONCLUSIONS**

Autophagy, a 'self-eating' catabolic degradation process, has been suggested to maintain cell survival during conditions of metabolic stress. Cancer cells which are inherently vulnerable to metabolic stress due to extrinsic and intrinsic metabolic stressors and also defective in apoptosis are more dependent on autophagy for cell survival than normal cells. Autophagy may have a dual but conflicting role in carcinogenesis, tumor-suppressive and tumor-promoting role. The exact roles of autophagy in specific type of cancers *in vivo*, however, have not been determined clearly, but it is clear that autophagy is deeply integrated into cell metabolism, stress response and cell-death pathways<sup>(20)</sup>. Moreover, responses to autophagy inducers or inhibitors might vary depending on the cell types and the specific mutations occurred in cancer cells. Therefore, further studies elucidating this complex process are essential for development of optimal strategies for anticancer therapy using targeted agents or phytochemicals.

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