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# Autophagy as a molecular target for cancer treatment

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*Abstract*

***Autophagy is a progressive conserved catabolic mechanism, by which eukaryotic cells recycle or degrades internal constituents through the membrane-trafficking pathway. Thus, autophagy provides the cells with a sustainable source of biomolecules and energy for the maintenance of homeostasis under stressful conditions such as tumor microenvironment.It is generally believed that modulating autophagic activity, through targeting specific regulatory actors in the core autophagy machinery, may impact disease processes. Both autophagy upregulation and down regulation have been found in cancers, suggesting its dual oncogenic and tumor suppressor properties during malignant transformation. Identification of the key autophagy targets is essential for the development of new therapeutic agents. In this review the two-faced role of autophagy in cancer as a tumor suppressor or as a pro-oncogenic mechanism discussed and the shared regulatory pathways that play a role in autophagy and malignant transformationillustrated. Finally, anti-cancer therapeutic agents used as either inhibitors or inducers of autophagy have been discussed.***

## Introduction

Autophagy is a catabolic process in which cytoplasmic materials are directed to the lysosomes for degradation. This process is evolutionarily conserved from yeast to man and its activity is required for maintaining cellular homeostasis through the elimination of dysfunctional organelles, protein aggregates or even long-lived proteins. So far, three main classes of autophagy have been identified: Macroautophagy, microautophagy and chaperon-mediated autophagy (CMA). Macroautophagy (autophagy herein) is the main pathway that is divided into bulk and selective autophagy according to the specificity of targeted cytoplasmic constituents. In bulk autophagy, degradation targets are mainly wrapped within a double-membrane vesicle (autophagosome) as portions of cytoplasm in a non-selective manner. On the other hand, in selective autophagy, particular substrates

such as mitochondria(1), peroxisomes(2), lysosomes(3), ER(4), ribosomes(5), lipid droplets(6), pathogenic intracellular invaders(7) and even certain free proteins and RNAs (8)are targeted into the autophagosome. The ability to recycle macromolecules through autophagy gives cells an advantage for survival under stressful conditions such as nutrient starvation, oxidative stress, hypoxia, ER stress, metabolic stress, etc(9). Moreover, selective autophagy allows cells to control the number of the organelles based on the requirement, eliminating dysfunctional compartments and disposing of pathogens by combining the ubiquitin-proteasome system (UPS) and autophagic machinery(10). However, under certain conditions excess or deregulated activity of autophagy may also lead to cell death. Whether autophagy is an executioner or a savior is still a matter of debate and it is often determined in a context- and cell type-dependent manner(11).

To survive under stressful conditions within tumors such as hypoxia and/or nutrient deprivation or oxidative stress, cancer cells frequently exploit autophagy(12).Additionally, tumor cells could benefit from autophagy for adaptation to metastasis for withstanding the environmental stress they face during the several steps of metastasis including migration into the systemic circulation, adherence to the vessel walls, extravasation, and colonization(13). Thus, the recycling of cytoplasmic materials by autophagy provides a continuous supply of energy as well as essential ingredients for cancer cells to survive(13) and promotes metastatic recurrence of tumors(14).

**Molecular mechanisms of autophagy**

The autophagic process is initiated by the formation of double-membrane vesicles known as autophagosomes. Various cargos are engulfed into autophagosome and autophagosome eventually fuses with lysosomes that form autolysosomes. (15). Engulfed materials were degraded by the action of lysosomal hydrolases and newly generated building blocks (e.g., amino acids from protein degradation) are transferred back to the cytosol for reuse (Fig. 1). A series of stimuli, including amino acid deprivation, serum starvation and growth factor deprivation, hypoxia, exposure to various chemicals and stress conditions are capable of activating autophagy. Genetic studies in the yeast provided initial discoveries of autophagy-related (ATG) genes and enlightened the details of the molecular signaling pathway of the autophagic process (16). The autophagic pathway can be divided into several different phases: Initiation, nucleation, maturation, fusion, and degradation (Fig. 1).

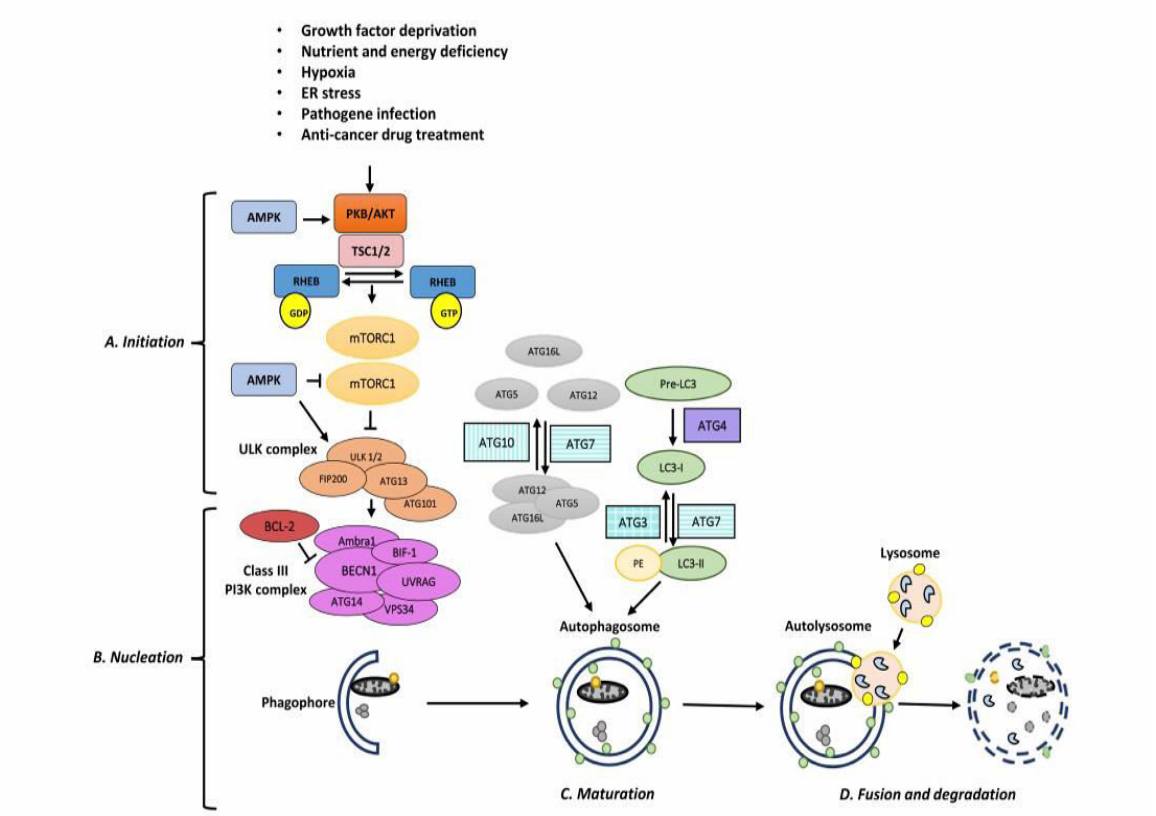


**Fig. 1.** Molecular mechanism of autophagy regulation in mammals.
Autophagic process consists of several phases such as initiation (A), nucleation (B), maturation (C), fusion and degradation (D). Same colours express the involvement of proteins or molecules in respective complexes or pathways.

## Autophagy in cancer

Cancer was one of the first diseases to be associated with autophagy(17-21).At early stages, autophagy usually acts as a tumor suppressor allowing cells to discard damaged cellular contents, decreasing ROS and DNA damage,while in more advanced stages of tumor development, it may help cancer cells to survive under low- oxygen and low-nutrient conditions, acting as a tumor promoter(22)(23). The dependence of tumor cells on autophagy is highly variable. While some tumor models (like pancreatic cancer) display increased autophagy levels in basal situations (including in plenty of nutrient conditions), with autophagy having a role in the maintenance of tumor growth (24), results from other studies. The outcomes of therapy-induced autophagy in cancer cells may represent also a "double-edged sword" and depend on the particular type of cancer, on the stage of disease progression or even on the type and duration of autophagy(25)(26). Indeed, several studies showed that increased autophagy leads to resistance to both chemo- and radiotherapy, while several others show that many anticancer drugs induce autophagy-related cell death in cancer cells(27)(28).

## Functional forms of autophagy and their implications for cancer therapy

Although traditionally, autophagy has been seen as a pro-survival (cytoprotective) mechanism. Currently, at least four distinct functional forms of autophagy have been described(29)(30):( i) Cytoprotective, when cells die or arrest if autophagy is inhibited; ii) Cytotoxic, when autophagy induction results in cell death and its blockage results in cell survival; iii) Cytostatic when autophagy induction results in cell growth arrest and iv) Non-protective if autophagy does not affect cell growth once blocked. These forms are distinguished only based on their functional characteristics, having similar morphologic, biochemical or molecular profiles (29).

**Autophagy modulation as a therapeutic strategy to improve anticancer strategies**

The knowledge of whether autophagy is cytoprotective or is cytotoxic/cytostatic will help to define strategies for its modulation (through its decrease or increase, respectively) to interfere with the cellular sensitivity to therapy.

Targeting cytoprotective autophagy has been at the basis of multiple clinical trials. Indeed, if increased autophagy confers tumor resistance to death-inducing agents, its inhibition will allow an enhanced response to treatment (31). There are several autophagy inhibitors already identified and that have been classified as early-stage inhibitors, if blocking autophagosome formation [such as 3-Methyladenine (3-MA), wortmannin, and LY294002] orlate-stage inhibitors, acting at the level of the autophagosome-lysosome fusion and degradation steps [such as chloroquine (CQ), hydroxychloroquine (HCQ), bafilomycin A1, and monensin]. Studies using, not only these pharmacological autophagy inhibitors but also genetic silencing or knockdown of autophagy-associated genes, resulted in increased tumor cell sensitivity to the autophagy-inducing stimulus, usually via the promotion of apoptosis(29)(31).

Several clinical trials have been evaluating the use of autophagy inhibitors (particularly HCQ) in combination with chemo- and radiotherapy to improve its efficacy(32)(33).

A study performed in melanoma patients using HCQ in combination with the mTOR inhibitor (temsirolimus) showed an improvement of the median progression-free survival to 3.5 months and increased the rate of stable disease in patients(32)(34). Also, its combination with a proteasome inhibitor (bortezomib) in relapsed/refractory myeloma patients resulted in a higher rate of partial response and stable disease. Recently, the use of HCQ in combination with gemcitabine in pancreatic ductal adenocarcinoma patients caused significant decreases in the disease biomarker, CA 19–9, with the mean overall survival being extended to nearly 3 years(33)(35). Although clinical trials with these compounds indicate that autophagy inhibition in patients is possible, there is still room for improvement, since CQ/HCQ has also shown significant variability of autophagy inhibition levels among patients. Moreover, these types of compounds, although being already FDA approved, have to be administered in higher concentrations to inhibit autophagy and are retained for long periods in patients (some studies showing patients retaining HCQ) in their system up to 5 years(33)(36).

Besides autophagy induction may help improve the effectiveness of anticancer therapies when autophagy is cytotoxic, by inducing cell death by itself or by the activation of other cell death mechanism, namely apoptosis (37)(38). Several drugs/natural extracts, some of which already used in the clinic, have been described to induce autophagy-mediated cell death in different cancer cells. For example, the combination of Vitamin D with radiation promoted cytotoxic autophagy in breast tumor cells. Resveratrol and curcumin caused cell death in several human tumor cell lines through apoptosis and autophagy. Naphthazarin, a naphthoquinone compound acting as microtubule depolymerizing agent was shown to induce cell death in lung cancer cells (28)through apoptosis and autophagy. Besides, the small molecule STF-62247 induced autophagic cell death in Von Hippel Lindau (VHL)-deficient renal cell carcinoma cells (39)and TXA1, a thioxanthone small molecule, decreased the viability of melanoma and breast cancer cells through the induction of autophagy antitumor immune response(40).

**Autophagy as a tumor suppressor mechanism**

Autophagy has been implicated as a favorable mechanism for suppression of cancer formation at multiple stages through its established roles in the preservation of genomic stability; elimination of endogenous sources of reactive oxygen species (ROS); the maintenance of bioenergetic functions; degradation of oncogenic proteins and induction of immune response mechanisms against malignant transformations(41).

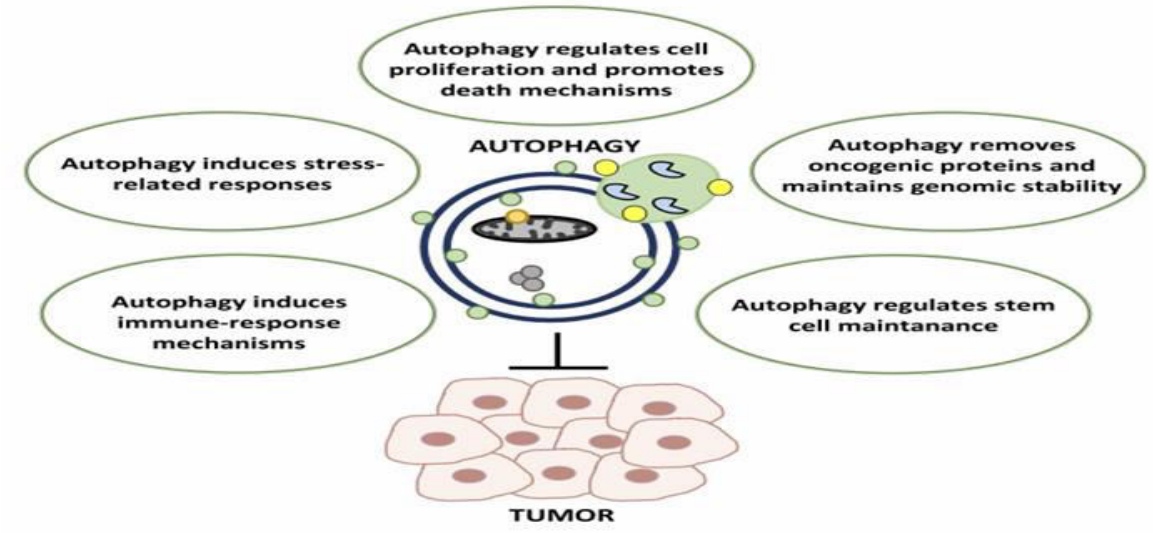


**Fig. 2.** Tumor suppressor role of autophagy.
Autophagy is involved in a variety of cellular mechanisms, each of which inhibits tumor progression by activating multiple molecular pathways.

**Autophagy as a pro-oncogenic mechanism**

In addition to its tumor-suppressive role, autophagy also contributes to malignant transformation and/or metastatic cascade by supporting cancer cells under stress conditions (e.g. exposure to metabolic, hypoxic, genotoxic, and oxidative stress) or tumor microenvironment (e.g. survival in the circulatory system, oxygen and glucose deprivation in solid tumors). Studies also suggested that autophagy provides resistance to cancer cells against chemo-/radio-therapies and cell death. The pro-oncogenic function of

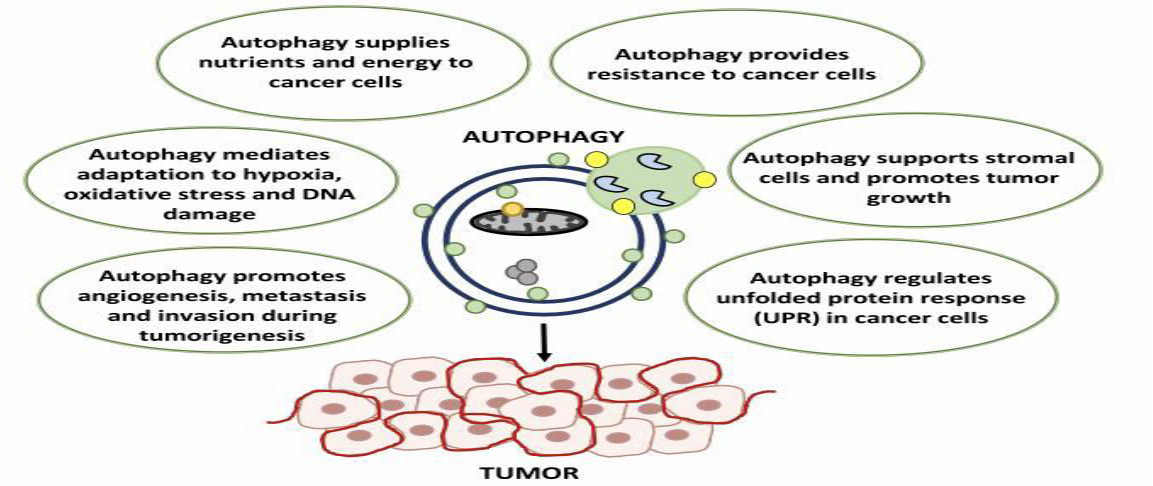


**Fig. 3.** Tumor promoting role of autophagy.
Autophagy contributes to tumorigenesis in a variety of stages ranging from proliferation to metastasis and invasion as well as sustain its improvement by providing resistance to death mechanisms.

autophagy is summarized in Fig. 3(42).

**Targeting autophagy for cancer treatment**

The involvement of the shared regulatory pathways makes autophagy as a promising target in cancer treatment, even though the relationship between autophagy and cancer is still controversial. Regarding the dual roles of autophagy in tumor development mainly two different therapeutic strategies can be adopted. The first approach includes sensitizing the cancer cells for chemo−/radio-therapy through inhibition of the cytoprotective role of autophagy. The other strategy aims to target the induction of autophagic cell death in apoptosis-resistant cells. Targeted autophagic proteins and autophagy inhibitors for cancer treatment are listed in Tables 1 and 2, respectively(43).

**Table 1**

Role of autophagic proteins in cancer.

| Cancer type | Protein | Phase of autophagy | Status in cancer tissue | Reference |
|---|---|---|---|---|
| Tumor suppressor roles | | | | |
| Colorectal carcinomas | UVRAG | Initiation | Mutated | (Goi et al., 2003) |
| Colorectal carcinomas | UVRAG | Initiation | Mutated | (Ionov et al., 2004) |
| Gastric carcinomas | UVRAG | Initiation | Mutated | (Kim et al., 2008) |
| Colorectal carcinomas | AMBRA1 | Initiation | Mutated | (Cianfanelli et al., 2015) |
| Gastric and prostate carcinomas | Bif-1 | Initiation | Decreased | (Lee et al., 2006) |
| Breast carcinomas | FIP200 | Initiation | Mutated | (Chano et al., 2002) |
| Meningiomas | BECN1 | Initiation | Decreased | (Miracco et al., 2007) |
| Colorectal and gastric carcinomas | BECN1 | Initiation | Increased | (Ahn et al., 2007) |
| Breast carcinomas | BECN1 | Initiation | Decreased | (Liang et al., 1999) |
| Epithelial ovarian cancer | BECN1 | Initiation | Decreased | (Shen et al., 2008) |
| Melanoma | ATG5 | Elongation | Decreased | (Marino et al., 2007) |
| Benign liver tumor | ATG5 | Elongation | Decreased | (Takamura et al., 2011) |
| Colorectal and gastric carcinomas | ATG5 | Elongation | Mutated | (Kang et al., 2009) |
| Colorectal and gastric carcinomas | ATG12 | Elongation | Mutated | (Kang et al., 2009) |
| Leukemia | ATG3 | Elongation | Increased | (Ma et al., 2013) |
| Fibrosarcomas | ATG4C | Elongation | Decreased | (Marino et al., 2007) |
| Leukemia | RAB7A | Fusion | Mutated | (Kashuba et al., 1997) |
| Colorectal and gastric carcinomas | ATG2B | Fusion | Mutated | (Kang et al., 2009) |
| Colorectal and gastric carcinomas | ATG9B | Fusion | Mutated | (Kang et al., 2009) |
| Oncogenic role | | | | |
| Cervical carcinomas | PIK3CA | Upstream | Increased | (Ma et al., 2000) |
| Multiple myeloma | PDPK1 | Upstream | Increased | (Chinen et al., 2014) |
| Prostate carcinomas | RHEB | Upstream | Increased | (Nardella et al., 2008) |
| Chronic myeloid leukemia | ATG4B | Elongation | Increased | (Rothe et al., 2014) |
| Hepatocellular carcinomas | ULK1 | Initiation | Increased | (Xu et al., 2013) |
| Breast carcinomas | ULK1 | Initiation | Increased | (Pike et al., 2013) |
| Esophageal squamous cell carcinomas | ULK1 | Initiation | Increased | (Jiang et al., 2011) |
| Oral squamous cell carcinoma | ATG16L1 | Elongation | Increased | (Tang et al., 2015) |
| Thyroid carcinomas | ATG16L1 | Elongation | Mutated | (Huijbers et al., 2012) |
| Colorectal carcinomas | ATG16L1 | Elongation | Mutated | (Nicoli et al., 2014) |

Table 2
Regulating autophagy for cancer treatment.

| Compound | Target | Tumor/Cancer cell type | Effect | Reference |
|---|---|---|---|---|
| Inhibition of autophagy | | | | |
| 3-MA(3-methyladenin) | PIK3C3 | Esophageal squamous cell cancer | Enhanced radiation sensitization | (Chen et al., 2011) |
| | | Colorectal cancer | Enhanced antitumor effect | (Li et al., 2009) |
| | | Lung cancer | Enhanced antitumor effect | (Liu et al., 2013) |
| Wortmannin | PIK3C3 | Mouse melanoma cell | Enhanced antitumor effect | (Lin et al., 2014) |
| SAR405 | PIK3C3 | Renal tumor cells | Reduced proliferation | (Pasquier, 2015) |
| Chloroquine | Lysosomal pH | Non-small cell lung cancer | Enhanced antitumor effect | (Selvakumaran et al., 2013) |
| | | Glioblastoma multiform | Enhanced antitumor effect | (Sotelo et al., 2006) |
| | | Colon cancer cells | Enhanced antitumor effect | (Sasaki et al., 2010) |
| | | Head and neck cancer cells | Enhanced radiation sensitization | (Cerniglia et al., 2012) |
| | | Glioblastoma | Enhanced radiation sensitization | (Cerniglia et al., 2012) |
| Hydroxychloroquine | Lysosomal pH | Melanoma | Enhanced antitumor effect | (Rangwala et al., 2014) |
| Bafilomycin A1 | Vacuolar-ATPase | Nasopharyngeal carcinoma cells | Enhanced antitumor effect | (Liu et al., 2015) |
| | | Gastric cancer cells | | (Li et al., 2016) |
| | | Osteosarcoma cells | | (Xie et al., 2014) |
| | | Colon cancer cells | | (Greene et al., 2013) |
| Spautin-1 | Inhibits ubiquitin-specific peptidases | Breast cancer cells | Induced cell death | (Liu et al., 2011) |
| | | Ovarian cancer cells | Induced cell death | (Liu et al., 2011) |
| | | Chronic myeloid leukemia cells | Enhanced antitumor effect | (Shao et al., 2014) |
| Pepstatin-A | Lysosomal protease inhibitor | Cervical cancer cells | Enhanced antitumor effect | (Hsu et al., 2009) |
| siRNAs | Autophagic proteins mRNA | Several cancer cells | Enhanced antitumor effect/ enhanced radiation sensitization | (Wu et al., 2012) |
| Activation of autophagy | | | | |
| Temsirolimus (CCI-779) | mTORC1 inhibitors | Mantle cell lymphoma | Enhanced antitumor effect | (Yazbeck et al., 2008) |
| Everolimus (RAD-001) | mTORC1 inhibitors | Acute lymphoblastic leukemia | Enhanced antitumor effect | (Crazzolara et al., 2009) |
| Rapamycin | mTORC1 inhibitors | Malignant glioma | Enhanced antitumor effect | (Carayol et al., 2010) |
| | | Chronic myeloid leukemia cells | | (Takeuchi et al., 2005) |
| Imatinib (Gleevec) | Tyrosine kinase inhibitors | Chronic myeloid leukemia cells | Enhanced antitumor effect | (Ertmer et al., 2007) |
| Dasatinib (Sprycel) | | Glioma | Enhanced antitumor effect | (Milano et al., 2009) |
| Erlotinib (Tarceva) | | Non-small cell lung cancer | Enhanced antitumor effect | (Gorzalczany et al., 2011) |
| Butyrate, suberoylanilide hydroxamic acid (SAHA) | HDAC inhibitors | Cervical cancer cells | Enhanced antitumor effect | (Shao et al., 2004) |
| | | Chronic myeloid leukemia cells | | (Carew et al., 2007) |
| Arsenic Trioxide | Toxin | Leukemia cells | Induced cell death | (Qian et al., 2007) |
| | | Malignant glioma | | (Kanzawa et al., 2005) |
| Resveratrol | Antioxidant | Ovarian cancer cells | Induced cell death | (Opipari et al., 2004) |
| Polygonatum cyrtonema lectin | Lectin | Murine fibrosarcoma | Induced cell death | (B. Liu et al., 2010, F. Liu et al., 2010) |
| | | Melanoma cells | | (Liu et al., 2009) |
| Epigallocatechin-3-gallate | Polyphenol | Oral squamous cell carcinoma | Induced cell death | (Irimie et al., 2015) |
| Curcumin | Polyphenol | Malignant glioma | Induced cell death | (Aoki et al., 2007) |
| | | Malignant glioma | | (Shinojima et al., 2007) |
| | | Breast cancer cells | | (Akkoç et al., 2015) |
| | | Lung cancer | | (Xiao et al., 2013) |
| Allicin | Thiosulfinate | Liver cancer cells | Induced cell death | (Chu et al., 2012) |
| Ginsenosides | Saponins | Breast cancer stem cells | Induced cell death | (Mai et al., 2012) |

**Autophagy inhibitors as anti-cancer agents**

The role of autophagy as a mechanism that promotes resistance to chemo- or radio-therapies compromises the efficacy of anti-cancer treatment strategies.Therefore, inhibition of autophagy may serve as a tool for sensitizing the tumor cells for treatment. The most common autophagy-inhibiting molecules could be categorized into four groups according to their mode of action(44)(45)(46):

**i.** Repressors of autophagosome formation: Class III PI3K inhibitors 3- methyl adenine (3-MA), Wortmannin, LY294002, SAR405, and recently developed Viridiol were shown to block the formation of the autophagosome.

**ii.** Repressors of lysosomal acidification: Lysosomotropic agents including CQ, HCQ, Lys0569, and monensin prevent acidification of lysosomes and thus inhibit degradation of the cargo in the autophagosomes.

**iii.** Inhibitors of autophagosome-lysosome fusion: Vacuolar-ATPase inhibitors, including variants of Bafilomycin (Baf A1, Baf B1, and Baf C1) and Concanamycin variants (Con A, Con B, and Con C) interferes with the fusion of autophagosomes with lysosomes whereas, Spautin-1 targets Beclin-1 subunit of Vps34 complexes(47)(48).

**iv.** Silencing expression of autophagy-related proteins at transcription level: By utilizing siRNA- or miRNA-mediated silencing strategies, knockdown of autophagy-related genes subsequently inhibited autophagic activity.

**Autophagy activators as anti-cancer agents**

Since excessive autophagic activity acts as a pro-death mechanism, autophagy induction is a direct strategy that may promote tumor cell death. Inhibition of mTOR or disruption of Beclin-1/BCL-2 interaction is among the most common strategies implemented to induce autophagy directly complex(42).

**The combinatory approach in cancer treatment**

Tumors exhibit heterogeneous, irregular and branched blood vessel networks (49). These heterogeneities in vascularization resulted in permeability imbalances and inadequate blood supply to differential compartments of the tumor tissue further associated with metabolic stresses, including hypoxia and starvation, which in turn provided invasion and decreased immune response(50)(51). Hence , targeting cancer with combinatory therapy even at a single cell level provides an alternative strategy to combat tumor progression. The use of miRNAs and Nano sized carriers become an alternative therapeutic approach for targeted therapies. Besides from their increased usage and benefits, Nano-sized carriers tend to accumulate in spleen or liver by macro- phages-mediated endocytosis.

**Autophagy regulating miRNAs in cancer**

MicroRNAs (miRNAs) are involved in a class of short RNAs (~ 21nucleotides) that target partially complementary transcripts to control key biological processes post-

transcriptionally. miRNAs are transcribed from several different loci in the genome which encode for long RNAs (pri-miRNAs) with a hairpin structure. Then RNase III enzyme Drosha processes the pri-miRNAs to give the precursor miRNAs (pre-miRNAs)(52). Pre-miRNAs are subsequently transported into the nucleus and then processed further by the RNase III enzyme, DICER (also known as DICER1), to yield a mature miRNA(53). Mature miRNA is then loaded into an argonaute protein within the RNA-induced silencing complex (RISC) acting as a guide strand through the target-specific seed sequence(53). The miRNA-processing enzyme DICER and the main miRNA effector, AGO2 can be targeted for degradation by the selective autophagy receptor NDP52 (also known as calcium binding and coiled-coil domain 2 (CALCOCO2)(54).

The complicated autophagy-mediated differential regulatory mechanism in carcinogenesis becomes even complex with the involvement of miRNAs(54)(55). For instance, autophagy inhibitor miR-101 was shown to be progressively lost during cancer progression(56). Moreover, miR-20a, miR-101, miR-106a/b and miR-885-3p targeted ULK1/2 while miR-155 regulated mTOR signaling (57). Other miRNAs function as inhibitors of autophagy include miR-30a, miR-34a, miR-204, miR-375 were linked to cancer with their reduced level of expression(54)(55)(57). Recently, miR-4487 and miR-595 were identified as novel biomarkers and ULK1-targeting miRNAs in the regulation of autophagy(58).

**Autophagy modulation through Nano-sized material systems in cancer**

Being a central participant in the regulation of metabolic and stress- response pathways, autophagy plays a dual role in drug resistance likewise in the case of carcinogenesis. Recent advances in designing Nano sized drug delivery systems opened a new perspective for targeted delivery of chemotherapeutics at specific sites and controlled drug release into tumor cells(59). Even some of the tested nanomaterials found to modulate autophagic activity in some cancer cells.

Giving the importance of nanoparticle usage in the clinical, it has been an emerging issue to combine CQ-derivatives with nanoparticles to target cancer cells, due to the decreased effect of CQ on the accumulation of Nano-sized carriers in liver or spleen(60). For instance, CQ was suggested as a promising candidate to decrease the accumulation of Nano-sized carriers in organs by inhibiting macrophage uptake, therefore promoted their distribution and localization on their targets for cancer therapy(61). As a multidrug complex example, CQ was included in the Nano capsule erlotinib and shRNA survivin co-delivery treatment system and CQ-mediated vessel normalization increased the targeting ratio of erlotinib and shRNA surviving(62).

Besides, C60 (Nd) nanoparticles were shown to promote autophagy-mediated chemosensitization of cancer cells(63)(64). The therapeutic use of iron core-gold shell nanoparticles was able to inhibit the growth of oral cancer through the induction of

reactive oxygen species and autophagy(65). Similarly, reports by others also showed that both the iron oxide(66) and alpha-alumina-nanoparticles(67) exhibited autophagy-induced anti-tumor effects. Furthermore, combining Nano-sized delivery systems with autophagy modulating agents may provide even a wider range of strategies to circumvent drug resistance mechanisms adopted by cancer cells. For example, in breast cancer cells, anti-cancer treatment was achieved by the utilization of chloroquine-loaded gold nanoparticle conjugates (GNP-Chl)(68). In a similar context, a single intravenous injection of the Nano-liposomal C6-ceramide together with vinblastine combination was shown to a tremendous decrease in tumor growth in both hepatocellular carcinoma and colorectal cancer(69). Additionally, chitosan nanoparticle-mediated delivery of miRNA-34a was reported to induce autophagy and decrease prostate tumor growth in the bone(70).

## Discussion

Autophagy plays an important role as a stress response mechanism to chemotherapeutic drugs and radiation in cancer cells. There are at least four functional forms of autophagy that may occur in response to chemotherapy or radiation: cytoprotective, nonprotective, cytotoxic and cytostatic. Currently, is not possible to predict which form will be induced by a particular therapy, since these forms of autophagy have no clear-cut morphologic, biochemical, or molecular distinctions. In some circumstances, autophagy protects tumor cells from cancer therapy while in others it is associated with cancer cell killing. Modulation of autophagy may represent an important therapeutic opportunity to enhance the efficacy of anticancer therapies. The future challenge for autophagy research in cancer therapy is to find ways to identify which functional form of autophagy is activated, in specific tumor models, and which tumors may be most effectively treated by autophagy modulation. A better understanding of the role of autophagy in different tumor models will provide new therapeutic tools for more effective cancer therapeutic strategies (71). However, there are still many obstacles to overcome to develop autophagic drugs; for example, there is a lack of specific biomarkers to distinguish autophagic cell death from other types of cell death. There is also a need to clarify which type of autophagy, cytoprotective, or cytotoxic should be targeted. Moreover, it is difficult but important to determine to what extent autophagy should be induced before the cells reach the point of no return and undergo autophagic cell death (72).

## Conclusion

The molecular mechanism and biological function of autophagy are now basically clear, and Janus's role of autophagy determines that it plays an important role in the antitumor process. I agree with autophagy since it may represent a new and promising pharmacologic target for future drug development and therapeutic applications in human diseases. Some compounds, plant extracts killing tumor cells through the regulation of

autophagy activity, especially induced autophagic cell death has also become an important strategy against the tumor.

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