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Autophagy as a Therapeutic Target in Gastrointestinal Cancer

Michiko Shintani

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Abstract

Autophagy is a bulk protein and organelle degradation system and is an important homeostatic cellular recycling mechanism. The following kinds are the three types of autophagy: macroautophagy, microautophagy, and chaperone-mediated autophagy. In general, the term "autophagy" indicates macroautophagy. Autophagy is mediated by double-membrane-bound structures called autophagosomes. During the autophagic process, cytoplasmic components are sequestered and engulfed by autophagosomes. Autophagosomes then fuse with lysosomes to form autolysosomes where the sequestered components are digested by lysosomal hydrolases. Microtubule-associated protein 1 light chain 3 (LC3) is an autophagosomal ortholog of the yeast protein ATG8. Autophagy stimulates the upregulation of LC3 expression, and a cytosolic form of LC3 (LC3-I) is conjugated to phosphatidylethanolamine to form LC3-II which is recruited to autophagosomal membranes. Subsequently, LC3-II is degraded by lysosomal hydrolases after the fusion of autophagosomes with lysosomes. Therefore, LC3 is a specific marker of autophagosome formation. Additionally, beclin 1, the mammalian ortholog of the yeast protein ATG6, has been known to play a crucial role in autophagy. Beclin 1 acts in conjunction with the phosphoinositide-3 kinase pathway to enhance the formation of the autophagic vacuole.

Recently, autophagy has been reported to play roles in both cell death and survival. Autophagy is a multifaceted process, and alterations in autophagic signaling pathways are frequently observed in cancer. Cancer is a disease caused by mutation, selection, and genome instability in tumor tissues, and the role of autophagy in cancer is unclear.

One anticancer treatment strategy is to trigger tumor-selective cell death. Apoptosis is regarded as the central mediator of programmed cell death in response to radiation and chemotherapy. Our previous report suggested that different cell-death pathways are activated in gastric and colorectal carcinomas and the extrinsic and

intrinsic apoptotic pathways could be mutually regulated in gastric adenocarcino-
mas. In contrast, in colorectal carcinomas, autophagy may function as a cellular
guardian to prevent caspase-9-dependent apoptosis (intrinsic apoptotic pathway).
LC3 positivity was less frequent in gastric adenocarcinomas than in colorectal ade-
nocarcinomas. Therefore, we suggested that LC3 expression in colorectal carcino-
mas is likely to aid cancer therapy, owing to its involvement in apoptosis and/or
autophagy.

In this chapter, we discuss the following: (1) the detection of autophagy using im-
munohistochemistry, (2) autophagy and tumor suppression and/or progression,
and (3) autophagy as a therapeutic target in gastrointestinal carcinomas.

1. Introduction

Autophagy is a bulk protein and organelle degradation system and is an important homeo-
static cellular recycling mechanism. The following are the three types of autophagy: macro-
autophagy, microautophagy, and chaperone-mediated autophagy. In general,
macroautophagy is believed to be the major type of autophagy. Autophagy is mediated by
double-membrane-bound structures called autophagosomes [1–3]. During the autophagic
process, cytoplasmic components are sequestered and engulfed by autophagosomes. Auto-
phagosomes then fuse with lysosomes to form autolysosomes where the sequestered compo-
nents are digested by lysosomal hydrolases. Microtubule-associated protein 1 light chain 3
(LC3) is an autophagosomal ortholog of the yeast protein ATG8. LC3 exists in two forms, LC3-
I and LC3-II. LC3-I is localized in the cytoplasm. Autophagy stimulates the upregulation of
LC3 expression, and LC3-I conjugates with phosphatidylethanolamine to form LC3-II. LC3-II
binds to autophagosomes and it is degraded by lysosomal hydrolases after the fusion of
autophagosomes with lysosomes [4–6]. Therefore, LC3 is a specific marker of autophagosome
formation. The autophagic pathway includes several phases: initiation, vesicle elongation,
maturation, fusion, and degradation (Figure 1). Recent studies have suggested that additional
membranes are derived from the Golgi complex, mitochondria, and plasma membrane;
however, this phenomenon has not been confirmed [1–2, 7–10].

Current studies are examining the molecular regulation and function of autophagy. Addi-
tionally, autophagy is believed to play a role in various diseases such as cancer, infectious
diseases, cardiovascular diseases, metabolic diseases, pulmonary diseases, and neurodege-
nerative disorders [11–20]. Recently, in clinical trials, several autophagic inhibitors, including
hydroxychloroquine and chloroquine, have been examined as targets in diseases. In cancer,
these autophagic components are being studied to enhance chemotherapeutic efficacy. Thus,
autophagy is now an important and widely studied topic in human health and disease [11–15].
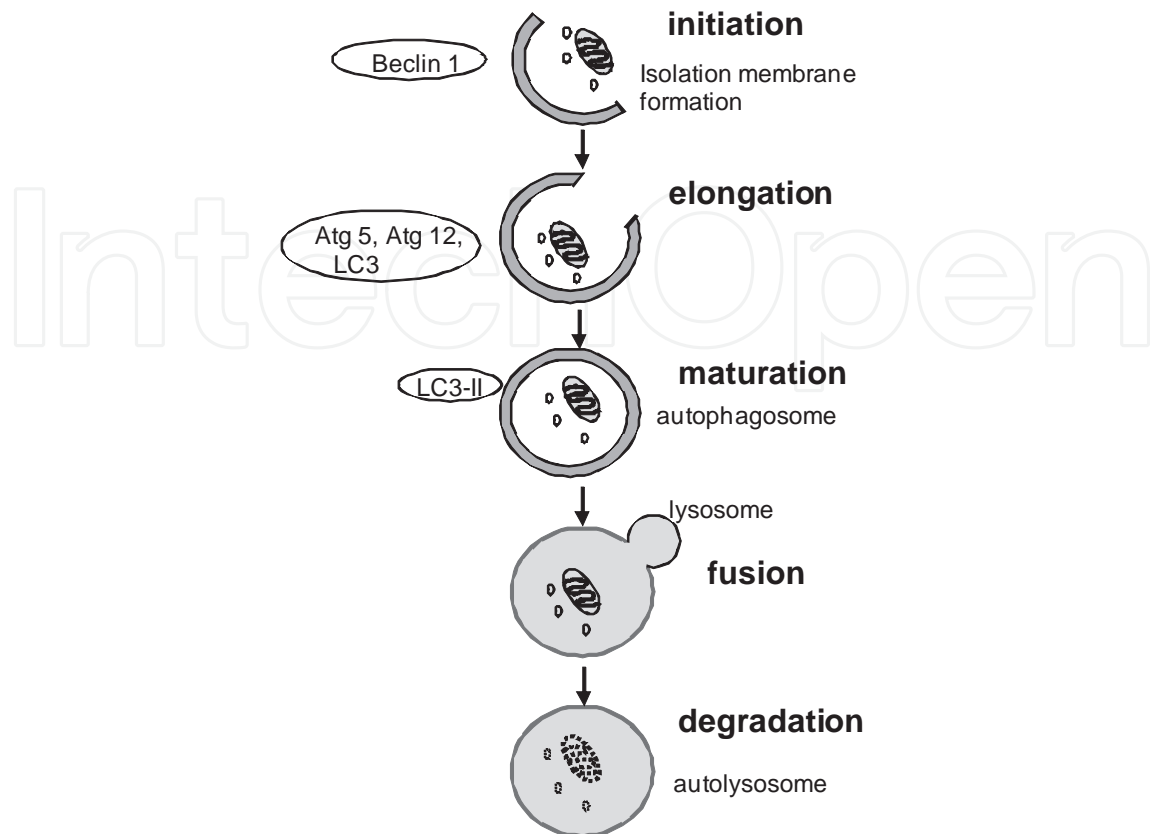
Figure 1. Cellular mechanism of autophagy. The autophagic pathway includes several phases: initiation, vesicle elongation, maturation, fusion, and degradation.

2. Detection of autophagy using immunohistochemistry

The role of autophagy in cancer development and progression has been studied by investigating autophagy-related proteins, LC3, beclin 1, and p62 using immunohistochemistry [21–23].

2.1. LC3

LC3 is an autophagosomal ortholog of the yeast protein ATG8 and is a specific marker of autophagosome formation. LC3-I is localized in the cytoplasm whereas LC3-II binds to autophagosomes. LC3 is presently used as an autophagy marker [21–26].

2.2. Beclin 1

Beclin 1 is the mammalian homolog of the yeast protein ATG6 and it has a central role in autophagy. The expression of beclin 1 has been reported in tumors such as breast, ovarian, prostate, lung, brain, stomach, and colorectal tumors. Beclin 1 may play a role in the tumorigenesis and/or progression of human cancers. However, beclin 1 has several physiological functions other than autophagy [21–22, 27–31].

2.3. p62

p62/SQSTM1 (p62) is an autophagy substrate protein which accumulates in autophagy-deficient cells after metabolic stress. p62 accumulation leads to mitochondrial damage, oxidative stress, and DNA damage. Additionally, p62 accumulation has been strongly suggested to result in cancer development [23, 32].

3. Autophagy and tumor suppression/progression

Recently, several studies have suggested that dysregulation of autophagy plays a critical role in tumorigenesis. Liang et al. (1999) reported that beclin 1 can inhibit tumorigenesis and that its levels decrease in human breast carcinoma [27]. Qu et al. (2003) presented the genetic evidence for the role of autophagy genes in tumor suppression. They suggested that beclin 1 is a haploinsufficient tumor-suppressor gene, and that mutation of beclin 1 or other autophagy genes may contribute to the pathogenesis of human cancers [28]. Autophagy may prevent normal cells from developing into tumor cells; however, it may also protect cancer cells by providing nutritional support. Yang et al. (2011) investigated the effects of autophagy in stressed and unstressed colon cancer cells. They found that, in unstressed cells, the inhibition of autophagy was associated with a significant growth advantage but, in biologically stressed cells, the inhibition of autophagy markedly reduced cell viability compared to that observed in controls. Therefore, they suggested that autophagy has a dual role in colon cancer cells; it is pro-survival under biological stress and pro-death under normal conditions [33].

Li et al. (2009) reported the dual role of autophagy in colon cancer. They found that the inhibition of autophagy enhances 5-fluorouracil-induced colon cancer cell apoptosis and improves the chemotherapeutic effect of 5-fluorouracil. This result indicates that autophagy plays a role in protecting some cells from chemotherapy-induced death [34].

Autophagy may play an important role in maintaining normal cellular homeostasis and may prevent normal cells from developing into cancer cells. However, autophagy is a cellular recycling mechanism and is active during metabolic stress [35–36]. Additionally, it may prevent cell death in tumor cells (apoptosis or autophagic cell death). Thus, autophagy has a role in both the suppression of cancer initiation and the promotion of cancer growth [27–28, 33–34].

4. Autophagy as a therapeutic target in gastrointestinal carcinomas

Autophagy has a role in both tumor promotion and tumor suppression. In cancer therapy, using the effect of autophagy, cell survival is inhibited or cell death is promoted by inhibition or induction [37–41].

Recently, autophagy has been used for cancer therapy in aggressive cancers. Particularly, a clinical trial of cancer therapy involving the combination of an autophagy regulator with

conventional anticancer agents or radiation therapy has been performed. In colorectal cancer, a combination of an autophagy inhibitor (hydroxychloroquine), oxaliplatin, leucovorin, 5-fluorouracil, and bevacizumab has been used. Several studies have reported the influence of apoptosis and autophagy on each other in cancer cells after chemotherapy [42–45].

Therapeutic agents in gastrointestinal cancer are summarized in Tables 1 and 2 [46–57].

5. Conclusion

Autophagy, an intracellular process involved in removing and recycling cellular components, may play a role in both protecting and promoting cancer cell death under different stress situations. The role of autophagy in tumorigenesis is controversial, because it can either protect or promote cell death. Recently, autophagy has been used in cancer therapy for aggressive cancers. Particularly, a clinical trial of cancer therapy involving the combination of an autophagy regulator with conventional anticancer agents or radiation therapy has been performed [5, 12, 37–41].

Our previous study suggested that different cell-death pathways are activated in gastric and colorectal carcinomas and the extrinsic and intrinsic apoptotic pathways could be mutually regulated in gastric adenocarcinomas. In contrast, in colorectal carcinomas, autophagy may function as a cellular guardian to prevent caspase-9-dependent apoptosis (intrinsic apoptotic pathway). LC3 positivity was less frequent in gastric adenocarcinomas than in colorectal adenocarcinomas [25]. Therefore, we suggested that LC3 expression in colorectal carcinomas is likely to aid cancer therapy. The detection of apoptosis and autophagy activity may help predict the treatment effect in colorectal cancer.

Presently, the anticancer agents that induce apoptosis are mainly being used. The development of a drug that induces autophagic cell death is expected in the near future. Therefore, the identification of a marker for determining the autophagic effect of drugs is important. Additionally, the development of a regulator specific for autophagy is needed.

Alisertib (ALS)	Yuan etal. [46]
ALS exerts potent inhibitory effects on cell proliferation, and inducing effects on cell-cycle arrest, mitochondria-dependent apoptosis, and autophagy with the involvement of PI3K/Akt/mTOR, p38 MAPK, and AURKA-mediated signaling pathways in AGS and NCI-N78 cells.	
Compound 1	Chun et al. [47]
Compound 1 effectively inhibits the growth of AGS cells by inducing apoptosis, as well as autophagy. Apoptosis after compound 1 treatment is associated with activation of caspases, release of cytochrome c, and an increased ratio of Bax/Bcl-2. Autophagy with compound 1 treatment is indicated by LC3-II protein expression.	
Klotho gene (klotho protein)	Xie et al. [48]

Klotho is a tumor suppressor in gastric cancer. Restoration of *klotho* gene expression significantly inhibits cell proliferation, induces cell apoptosis, and increases LC3-I/LC3-II expression in gastric cancer cells. Klotho regulates IGF-1R phosphorylation and the subsequent activation of IRS-1/PI3K/Akt/mTOR signaling, tumor cell proliferation, apoptosis, and autophagy.

Evodiamine	Rasul et al. [49]

Evodiamine significantly inhibits the proliferation of SGC-7901 cells and induces G2/M phase cell cycle arrest. Furthermore, both autophagy and apoptosis are activated during the evodiamine-induced death of SGC-7901 cells. Evodiamine is an effective natural compound for the treatment of gastric cancer, and it may be used in in vivo studies of monotherapies or combined antitumor therapies.

Table 1. Therapeutic agents in gastric cancer

Melphalan, bortezomib, and rapamycin	Song et al. [50]

Melphalan triggers apoptosis, bortezomib induces apoptosis and autophagy, and rapamycin induces autophagy. The combination treatment induces synergistic apoptosis, which is mediated through an increase in caspase activation in human colon cancer cell lines. Mitochondrial dysfunction induced by this combination treatment has been linked with altered cellular metabolism, which induces adenosine monophosphate-activated protein kinase (AMPK) activation. AMPK-induced apoptosis, through an interplay between autophagy and apoptosis, is triggered by this combination treatment.

DENSpm	Çoker-Gürkan *et al.* [51]

The effect of DENSpm, a polyamine analog, on cell death differs according to the p53 protein expression profile. In addition, DENSpm-induced autophagy may be critical in drug resistance in colon cancer cells.

Apigenin (4',5,7-trihydroxyflavone)	Lee et al. [52]

Apigenin is a natural flavonoid with apoptosis- and autophagy-inducing effects in HCT116 colon cancer cells. Autophagy plays a cytoprotective role in apigenin-induced apoptosis, and the combination of apigenin and an autophagy inhibitor may be a promising strategy for colon cancer.

Compound K (20-O-(β-D-glucopyranosyl)-20(S)-protopanaxadiol)	Kim et al. [53]

Compound K stimulates autophagy as well as apoptosis by disrupting the interaction between Atg6 and Bcl-2. The induction of autophagy and apoptosis by compound K is mediated through reactive oxygen species generation and c-Jun NH2-terminal kinase activation in human colon cancer cells.

LBH589	Gandesiri et al. [54]

The histone deacetylase inhibitor (HDACi) LBH589 has been verified as an effective anticancer agent. Death-associated protein kinase (DAPK) induces autophagy in response to HDACi treatment. In autophagy-deficient cells, DAPK plays an essential role in committing cells to HDACi-induced apoptosis.

MS-275	Zhan et al. [55]

MS-275 is a synthetic benzamide derivative of an HDACi. p38 MAP kinase plays a vital role in the switch from autophagy to apoptosis in MS-275-induced human colon cancer cells. High expression of p38 induces cell autophagy,

but low expression results in apoptosis. MS-275 may be a promising clinical chemotherapeutic agent with multiple effects.

11'-deoxyverticillin A (C42)	Zhang et al. [56]

C42 is an epipolythiodioxopiperazine. C42 enhances the cellular autophagic process, which requires both PARP and receptor-interacting protein 1 participation, and this precedes and possibly augments caspase-dependent apoptotic cell death.

Sulforaphane (SUL)	Nishikawa et al. [57]

SUL, a type of isothiocyanate and a pro-apoptotic agent, triggers the induction of autophagy by endothelial cells, similar to cancer cells, and the inhibition of autophagy potentiates the pro-apoptotic effect. This suggests the possible use of autophagy inhibitors in combination with anti-angiogenic agents.

Table 2. Therapeutic agents in colorectal cancer

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