

Review

The role of apoptotic or nonapoptotic cell death in determining cellular response to anticancer treatment

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Abstract

Aims: Apoptosis, an early response cell death, is a useful marker for predicting tumour response after anticancer treatment; however, late-response cell death or nonapoptotic cell death, autophagy, can also be observed. This article reviews a rational model for predicting tumour response by assessing the influence of nonapoptotic cell death, and thereby developing a more efficient strategy for enhancing the therapeutic effect of anticancer treatment.

Method: Literature search of clinical and experimental studies on ‘cell death and cancer’ using established databases, including PUBMED.

Findings: Although induction of apoptosis may not contribute to overall tumour response, nonapoptotic cell death such as autophagy, which may be triggered by apoptosis, still occurs. Anticancer treatment-induced apoptosis is regulated by the balance of proapoptotic and antiapoptotic proteins through mitochondria, and resistance to apoptosis is mediated by Bcl-2-dependent and Akt-dependent pathways. Bcl-2 partially inhibits nonapoptotic cell death as well as apoptosis, whereas Akt inhibits both apoptotic and nonapoptotic cell death through several target proteins.

Conclusions: Drug sensitivity is likely correlated with the accumulation of apoptotic and nonapoptotic cell deaths, which may influence overall tumour response in anticancer treatment. The ability to predict overall tumour response from the modulation of several important cell death-related proteins may result in a more efficient strategy for improving the therapeutic effect.

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Keywords: Apoptosis; Nonapoptotic cell death; Tumour response; Cancer therapy

Introduction


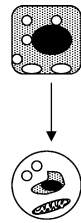
Over the past decade, the molecular mechanisms by which anticancer treatment such as anticancer drugs and irradiation induces apoptosis have been clarified.^{1–3} Induction of apoptotic cell death after treatment with anticancer drugs and irradiation is correlated with tumour response. In addition, failure in anticancer treatment is due to drug resistance in cancer cells, and the phenomenon of drug resistance is considered to be almost equal to resistance to apoptosis.^{4–6} Despite the strong correlation

between induction of apoptosis and increased drug and irradiation sensitivity, the early response cell death, apoptosis, is not necessarily correlated with overall tumour sensitivity.^{5,7,8} Anticancer drug-induced cell death is closely associated with an increase in apoptosis by the caspase-dependent pathway,^{1,9} however, pancaspase inhibitor cannot completely block drug-induced cell death,^{10,11} suggesting that anticancer drug-induced cell death also involves a caspase-independent pathway. Furthermore, in p53 knockout cells⁸ and Bax/Bak-double knockout cells,¹² the long-term drug sensitivity assay—clonogenic survival rates of apoptosis-deficient cells after treatment with etoposide (VP-16)—was similar to that of wild type cells. These findings strongly suggest that even

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Table 1
Differences in features between apoptotic and autophagic cell death

Feature	Apoptosis (type I)	Autophagic cell death (type II)
Cytoplasm	Condensation/blebbing/fragmentation Cytoskeletal protein breakdown (fodrin, myosin light chain, actin)	Autophagic vacuoles (AVs) Cytoskeletal protein preservation
Nucleus	Pyknotic	Pyknotic
Nuclear protein breakdown (SAF-A, p40, lamin B)	+	–
DNA fragmentation		
High molecular weight	+	+
Low molecular weight	+	–
Morphological change		
ATP requirement	Yes	Yes
Detection	Non-inflammatory DNA ladder formation assay TUNEL and annexin V FACS analysis Electron microscopy	Non-inflammatory Electron microscopy Monodansylcadaverine (MDC) Autophagosome membrane (LC3)

SAF-A, scaffold attachment factor A; p40, laminin-binding receptor-precursor p40.

though apoptotic cell death was blocked, nonapoptotic cell death could be activated, leading to cell death.

Previous studies indicated that anticancer drugs and irradiation induced nonapoptotic cell death, such as autophagy, which has different characteristics from apoptosis.^{13–15} Autophagy is also induced by specific conditions such as hypoxia and glucose starvation, which may be found in the microenvironment of solid tumours. Thus, autophagy may play a crucial role in influencing overall tumour response after treatment with anticancer drugs and irradiation. This article presents a rational model for predicting tumour response by assessing the influence of nonapoptotic cell death, such as autophagy, and thereby developing a more efficient strategy for enhancing the therapeutic effect of anticancer treatments.

Definitions of cell death

Anticancer treatment-induced cell death can be classified into two types of cell death: programmed cell death and nonprogrammed cell death. Programmed cell death has been classified based on morphological criteria into several categories.¹⁶ The most extensively studied is apoptosis, or type I cell death, which is characterized by cell rounding, membrane blebbing, cytoplasmic condensation and fragmentation, nuclear pyknosis, and chromatin condensation/fragmentation. Apoptotic bodies are rapidly phagocytized and digested by macrophages or neighboring cells.¹⁷ The second type of programmed cell death is autophagy, or type II cell death, which is characterized by the appearance of

abundant autophagic vacuoles in the cytoplasm, and enlargement of the endoplasmic reticulum and the Golgi apparatus.^{18,19} In some cases, apoptotic and autophagic cell death coincide *in vivo* in certain tissues, and both morphologies may coincide within the same cells (Table 1). It should be noted that initially the terms autophagy and autophagic cell death were used interchangeably, but recent studies indicate that autophagy can play dual roles in cell survival and cell death, dependent on the nutritional status of the cells.²⁰ During nutrient starvation, autophagy contributes to cell survival by producing amino acids. In contrast, in the absence of nutrient starvation, autophagy contributes to cell death, termed autophagic cell death, which is induced by anticancer drugs particularly in connection with inhibition of apoptosis.²¹ The type III cell death is necrosis, which is apparently uncontrolled cell death, in accordance with the loss of ATP or membrane pumps. This is most commonly seen as osmotic swelling of cell membranes and organelles.

Methods of causing cell death

Spontaneous apoptotic and autophagic cell death in tissue remodeling

Apoptosis is a physiological process for cell removal that functions to balance mitosis in the development and maintenance of tissue homeostasis.²² An opposed to necrosis, apoptosis is a nontoxic model of cell death, which affects single cells in the midst of living tissues

without eliciting an inflammatory response. Macrophage engulfment of apoptotic cells is known to be important in the remodeling of tissues, and it contributes to the resolution of inflammation through the removal of effete cells prior to the release of noxious cellular constituents. Moreover, apoptotic cells are a potential source of self-antigens, and clearance of cell corpses is thought to preclude the induction of autoimmune responses. Tissue homeostasis is dependent not only on the balance between mitosis and apoptosis, but also on the rate of apoptosis vs that of cell clearance.

Intracellular protein degradation systems can be roughly classified into two groups: the ubiquitin-protease system in the cytoplasm and a process called autophagy in the lysosome. Autophagy is a physiological process that starts with the formation of an autophagosome, enclosed within a double membrane that engulfs part of the cytoplasm. Autophagosomes undergo a maturation process by fusing with endocytic compartments and lysosomes.²³ Autophagy occurs constitutively at low levels even under growth conditions. However, since the hepatocytes of live-specific conditional autophagy-specific gene 7 (Atg7) knockout mice developed ubiquitin-positive aggregates, autophagy plays a critical role in intracellular clearance.²⁴ Although the mechanism underlying the accumulation of ubiquitin-positive aggregates is unknown, aggregates may be degraded naturally by autophagy. Recent studies indicate that nutrient starvation induces autophagy, which relieves the nutrient starvation and secures cell survival. When the nutrient starvation continues or is beyond the baseline of the control, the cells are committed to autophagic cell death.²¹

Anticancer drug-induced apoptotic and autophagic cell death

Many anticancer drugs induce apoptosis, and the magnitude of cell death is well correlated with tumour response. The molecular mechanisms by which anticancer drugs induce apoptosis are mediated by mitochondrial dysfunction, which is regulated by the balance of proapoptotic and antiapoptotic proteins in the Bcl-2 family.²⁵ DNA damage to cancer cells induces the activation of proapoptotic proteins such as Bax and Bak, which translocate from the cytosol to mitochondria. The proteins are then inserted into mitochondrial membranes, where they interact with a voltage-dependent anion channel (VDAC)/adenine nucleotide translocator (ANT) complex to release cytochrome *c*, resulting in the activation of caspase cascades.²⁶ Antiapoptotic proteins, such as Bcl-2 and Bcl-xL inhibit the release of cytochrome *c*, thereby blocking the activation of caspase cascades. Although truncated Bid (tBid), Bim, Noxa, and Puma, can release cytochrome *c*,²⁷ these proapoptotic proteins also interact with Bax or Bak to release cytochrome *c* from mitochondria. A functional defect of a proapoptotic protein or overexpression of an antiapoptotic protein causes resistance to apoptosis; both equally represent drug resistance in anticancer treatment.

Nonapoptotic cell death is mainly attributed to autophagy. Many chemotherapeutic agents reportedly induce autophagic cell death. Death-inducing agents, such as tumour necrosis factor (TNF) and staurosporin, kill MCF-7 cells without producing apoptotic changes.²⁸ Tamoxifen and its downstream mediator ceramide induce autophagic cell death in MCF-7 cells.²⁹ Ceramide, as well as the proapoptotic Bcl-2 family protein BINP3, induces autophagic cell death in malignant glioma cells.³⁰ Similarly, the alkylating agent temozolomide kills malignant glioma cells through a pathway of autophagic cell death.³¹ Other recent reports showed that resveratrol induced autophagic cell death in ovarian cancer cells, coupled, in part, with apoptotic cell death.³² The resveratrol-induced autophagic cell death was not abrogated by the transfection of *bcl-2* and *bcl-xL* genes, suggesting that autophagic cell death plays a bigger role than apoptosis in exerting an antitumour effect. Likewise, several previous reports indicated that treatment with anticancer drugs, such as paclitaxel and vinblastine, induced autophagic cell death as well as apoptotic cell death.^{13,16,33}

In addition, one of the few anticancer drugs that specifically target an autophagy-regulated protein is rapamycin. Rapamycin and its analogues, such as CCI-779, RAD001, and AP23573, inhibit the mammalian target of rapamycin (mTOR), which is a kinase that normally suppresses autophagy and is active when nutrients are abundant. A preclinical study indicates that mTOR inhibitors have a broad spectrum of antitumour activity, particularly in tumours that have inactivated the PTEN tumour suppressor with the survival signaling pathway of Akt.³⁴ In clinical trials, mTOR inhibitors have been shown to induce tumour progression in patients with metastatic renal cancer.³⁵

Radiation-induced apoptotic and autophagic cell death

Radiation-induced DNA damage as well as anticancer drugs induce apoptosis in association with the therapeutic effect, and the significance of apoptosis as a process of cell loss from normal tissue and tumours has been critically evaluated. Various factors that may modulate the apoptotic response to DNA damage include the p53 status of the cell, levels and activity of the Bax and Bcl-2 families of proteins, activation of NF- κ B, PI3K/Akt kinases, ceramide generation and the Fas-signaling pathway.³ Alteration of the factors in the apoptotic pathway causes less susceptibility to apoptosis in response to DNA damage. Given that DNA damage signals must be transmitted via afferent biochemical pathways to proteins, such as p53, that determine which cellular responses are activated, the responses include cell cycle arrest, apoptosis and DNA repair, all of which relate closely to loss of clonogenic capacity and the outcome of treatment in cancer patients. In fact, the general lack of a correlation between radiation or drug-induced apoptosis and cell survival responses (using the clonogenic assay) in

tumour cells has been demonstrated because there are still many different forms of cell death (terminal differentiation, micronucleation, mitotic catastrophe or multinucleation) that, like apoptosis, are regulated by the cell.³⁶

Low-dose ionizing radiation (IR) induced massive autophagic cell death in M059J malignant glioma cells that lack the DNA-dependent protein kinase (DNA-PK), which plays a major role in the repair of DNA double-strand breaks induced by IR.³⁷ In contrast to M059J cells, most M059K cells in which DNA-PK is expressed at normal levels, survived and proliferated, although a small portion of the cells underwent apoptosis. Low-dose IR inhibited the phosphorylation of p70S6-kinase, a downstream molecule that is the mammalian target of rapamycin and is associated with autophagy in M059J cells but not in M059K cells. In another study, UMSSC-6 head and neck and A549 lung cancer cells could be radiosensitized in combination with the nucleoside analogue gemcitabine through nonapoptotic mechanisms.³⁸ The effect of autophagy on radiosensitization may be dependent on the magnitude of DNA damage and radiosensitivity of the treated cells. In turn, if the autophagy exceeds the baseline for cell survival, the cells are committed to autophagic cell death.

Methods of measuring cell death

Apoptosis represents energy-requiring spontaneous single cell death, with specific morphologic and biochemical features. Apoptosis is characterized by a stereotypic pattern of morphologic features, which can be illustrated mostly by electron microscopy. DNA and biochemical assays, based on the specific pattern of nucleosomal fragmentation can detect apoptosis. In vitro methods for identification of apoptosis include regular cleavage of DNA into internucleosomal 180–200 base pair fragments (DNA laddering) as opposed to DNA from necrotic cells, which appears as a smear of randomly degraded DNA; DNA fragmentation (terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling, or TUNEL) that is used in the in situ labeling techniques for demonstrating apoptosis in paraffin sections; various morphological changes (hematoxylin and eosin staining); and externalization of phosphatidylserine (PS) from the cytoplasmic to the extracellular membrane side (annexin V staining).³⁹ In vivo detection, identification, and characterization of apoptosis are even more difficult. There has only been limited success so far in monitoring apoptosis by standard, noninvasive in vivo imaging modalities such as magnetic resonance imaging and spectroscopy (MRI/MRS), computed tomography (CT), positron emission tomography (PET), and radionuclide imaging methods.³⁹ Nevertheless, since in vivo monitoring of the apoptotic process is vital in clinical situations and in guiding effective treatment for cancer patients, a somewhat more invasive but more sensitive approach using an extracellular marker such as PS may be useful.

For detection of autophagy, there is only a limited number of good diagnostic methods available. Since, the autophagic phenomenon was initially discovered by electron microscopy, this has been the only reliable method for monitoring autophagy. Although electron microscopy is a useful method for detection of autophagy, it requires skill and much time because it is sometimes difficult to distinguish autophagic vacuole from other structures, such as the endoplasmic reticulum, by morphology. Some biochemical methods have been utilized to measure autophagic activity, and recently, the molecular basis of autophagosome formation has been extensively studied using yeast cells, which have provided useful marker proteins for autophagosomes in mammals.⁴⁰ The LC3 protein, which is located in the membrane of an autophagosome, can be used as a general marker for the autophagic membrane. These locations are easily examined by generating chimeric proteins fused with green fluorescent protein (GFP) or anti-LC3 antibody for immunohistochemical staining in situ in tissues. Examination of GFP–LC3 localization is a simple method, and real-time observation is feasible by only a fluorescence microscope, but one of the limitations is the requirement for GFP–LC3 gene transfer. In contrast, immunohistochemical staining with anti-LC3 antibody may be easier and useful for detection of autophagy in tissues.⁴¹

Molecular and genetic models implicated in the regulation of cell death

Early and late response cell death

Previous studies on anticancer treatment-induced apoptosis have revealed that the early response cell death is well correlated with the cytotoxic effect, assessed by a short-term drug sensitivity assay such as the MTT assay. In some cases, the early response cell death was also evaluated by a long-term drug sensitivity assay such as the clonogenic assay. The detection of early response cell death is useful for the prediction of overall tumour response to irradiation in esophageal and colon cancer cells, and the responsiveness is associated with the clonogenic survival.^{42,43} These findings suggest that the early detection of apoptotic cell death may be more useful for predicting overall tumour response than the treatment is completed, which depends on the hypothesis that induction of apoptosis is coincident with overall tumour sensitivity to anticancer treatment (Fig. 1(a)).^{42,43} In this case, the accumulation of apoptotic and autophagic cell deaths in time sequence equivalently affects the overall tumour response, and both cell deaths are attenuated in low-sensitivity cells. In contrast, if there is dissociation between the effects of early response cell death (apoptosis) and late response cell death (autophagy), the overall tumour response is not necessarily correlated with the result of apoptotic cell death (Fig. 1(b)).^{7,8} The

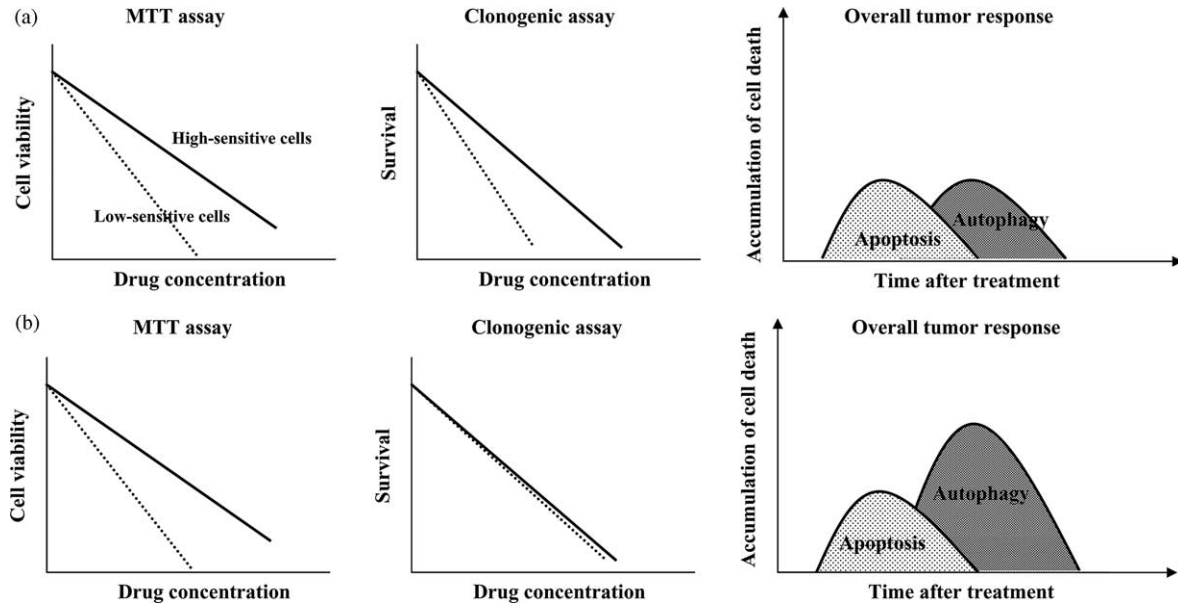


Figure 1. Comparison of tumour sensitivity to DNA damage in different cancer cell lines assessed by MTT and clonogenic assays. (a) Tumour sensitivity, evaluated by the MTT assay, is equivalent with that evaluated by the clonogenic assay in these two cell lines. (b) The differential tumour sensitivity detected by the MTT assay does not correspond to the assessment by the clonogenic assay.

attenuation of apoptotic cell death may be compensated by activation of autophagic cell death in low-sensitivity cells. Thus, early response cell death does not necessarily predict overall tumour sensitivity to anticancer treatment.

The late response cell death is observed within several days after DNA damage. Despite the fact that treatment of p53-deficient cells with VP-16 or irradiation attenuated drug sensitivity compared to the wild type cells (as assessed by the MTT assay), the clonogenic assay of the p53-deficient and wild type cells showed similar decreases in the survival after the treatment.⁸ In addition, although apoptotic cell death after the treatment with VP-16 or irradiation was increased in the wild type cells more than in the p21-deficient cells in HCT-116 human colon cancer cells, no significant difference was found in the clonogenic assay comparison between these two types of cells.^{8,44} These findings clearly suggest that the p53 genotype is not a critical determinant for tumour sensitivity to DNA damage, and that attenuation of apoptotic cell death did

not necessarily influence overall tumour response. Further, a recent study indicates that treatment with VP-16 of Bax/Bak-double-deficient cells still were committed to cell death through autophagic cell death.¹² Thus, another type of cell death, nonapoptotic cell death such as autophagy, may be involved in DNA damage-induced late response cell death.

Given that anticancer treatment-induced cell death involves not only apoptotic cell death but also autophagic cell death, these two pathways may be overlapping or run in parallel, leading to cell death depending on the type of cells and triggers. A potential pathway for apoptotic and autophagic cell death in the sequential process for determining overall tumour response after DNA damage can be considered. Cancer cells with DNA damage were primarily stopped at the cell-cycle checkpoint of the G1 and G2 phases. If the DNA damage was lethal, the cells were committed to apoptosis; if it was not lethal, the cells became senescent or possibly died through autophagic cell death.

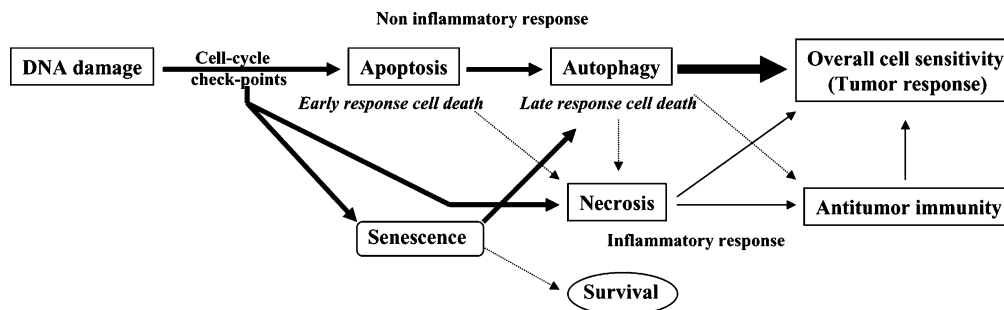


Figure 2. Overall tumour response is determined by accumulation of both types of apoptotic and nonapoptotic cell death. The role of necrosis in contributing to overall tumour response to DNA damage is obscured.

Autophagic cell death may be activated in a sequential fashion by triggering of apoptosis (Fig. 2).

Regulation of autophagic cell death

The regulation of autophagy is complex, and signaling pathways such as the target of the rapamycin (mTOR) kinase pathway play important roles in tumorigenesis.⁴⁵ Beclin 1, an autophagic protein, has been found to be a tumour suppressor.⁴⁶ Conversely, cancer cells may exploit autophagy as a means to adapt to hypoxic and nutrient-limiting environments. The relative importance of autophagic cell death and apoptosis in carcinogenesis remains to be established, and the Bcl-2 family of proteins may be instrumental in coordinating the two pathways of programmed cell death.

In hepatocytes, the phosphorylation of the ribosomal protein S6 correlates with the inhibition of autophagy.⁴⁷ The activity of p70S6-kinase is regulated by mTOR kinase, and inhibition of S6 phosphorylation by the inactivation of mTOR with rapamycin induces autophagy even under nutrient-rich conditions. In addition, protein phosphatase inhibitors, such as okadaic acid, block autophagy in hepatocytes through the activation of cAMP-activated protein kinase,⁴⁸ indicating that a protein phosphatase, such as PTEN, is involved in autophagic cell death. The tumour suppressor PTEN, which is an inhibitor of the PI3K/Akt pathway, has been shown to promote autophagy in HT-29 colon cancer cells.⁴⁹ The phosphoinositide phosphatase activity of PTEN blocked the Akt pathway and induced autophagy; conversely, constitutive activation of Akt inhibited autophagic activity. Activation of the Akt pathway switches on the survival pathway, which leads to the inhibition of apoptosis and activates the mTOR protein, which antagonizes autophagy. Thus, activation of the Akt pathway and mTOR signaling, due to a loss of the PTEN tumour suppressor, contributes to tumorigenesis by the simultaneous inhibition of apoptosis and autophagic cell death.

The other autophagy-regulating protein is Beclin 1, a product of a tumour suppressor gene, identified as a Bcl-2-interacting protein, which is involved in the elimination of cancer cells by triggering nonapoptotic cell death.^{46,50} Beclin 1 promotes autophagy when overexpressed in MCF-7 cells, and its single allele deletion is found in 40–75% of sporadic breast and ovarian cancers.⁵⁰ Treatment of MCF-7 cells with tamoxifen has been shown to induce autophagic cell death, which is mediated by the production of ceramide and the upregulation of Beclin 1.²⁹ In addition, death-associated protein kinase (DAPK) and DAPK-related protein kinase (DRP)-1, which are members of a family of Ca^{2+} /calmodulin-regulated serine/threonine death kinases, triggered caspase-independent cell death.⁵¹ Membrane blebbing and extensive autophagy occurred, and either the dominant mutant of DRP-1 or antisense DAPK reduced autophagy. DAPK is thought to function as a tumour

suppressor, and DAPK gene methylation was observed in a major fraction of lymphomas as well as lung, breast, colon, head, and neck cancers.⁵²

Clinical implications and new drugs

Several previous studies have reported the interaction of these two cell death pathways. A study on TNF- α -induced cell death in acute T-lymphoblastic leukemic cells showed apoptotic cell death that was preceded by autophagy.⁵³ An inhibitor of autophagosomes, 3-MA inhibits the cytolysis and DNA fragmentation induced by TNF- α . However, the inhibition of the fusion of lysosomes with autophagosomes by asparagine did not block TNF- α -induced apoptosis. These findings suggest that the same stimulus for cell death may lead to the activation of distinct and independent signaling pathways. For example, the early stage of autophagy in TNF- α -induced cell death does not necessarily result in apoptosis; rather, the cell may switch between apoptosis and autophagy as the dominant form of cell death. On the other hand, TNF-related apoptosis-inducing ligand (TRAIL) regulates autophagic activity during acinar morphogenesis, which together with caspase-mediated apoptotic events, results in lumen formation in MCF-10A human mammary epithelial cells.⁵⁴ Furthermore, another study on 3-MA showed that it increased the sensitivity of HT-29 colon cancer cells to apoptosis induced by a COX inhibitor, sulindac sulfide.⁵⁵ A mutant with a low rate of autophagy was more sensitive to sulindac sulfide-induced apoptosis than the parent cells. These findings suggest that autophagy may delay apoptosis by sequestering mitochondrial death-promoting factors, such as cytochrome *c*, in the presence of direct interaction of autophagy and apoptosis leading to cell death. The three possible patterns of the interaction between apoptosis and autophagy are shown in Fig. 3: (i) independent pathways for each type of cell death. Cancer cells may switch either way in accordance to the triggers and tumour microenvironment if either pathway was abrogated; (ii) apoptotic pathway inhibited by autophagy. The inhibition of autophagy enhances apoptotic

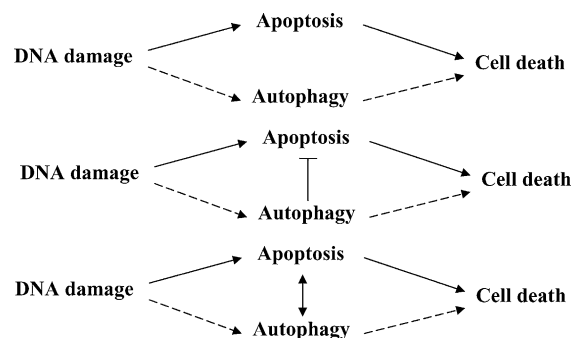


Figure 3. Typical patterns of interaction between apoptotic and autophagic cell death. (a) The independent pathways for each type of cell death. (b) The apoptotic pathway inhibited by autophagy. (c) The overlapping or compensatory interaction in two cell death pathways.

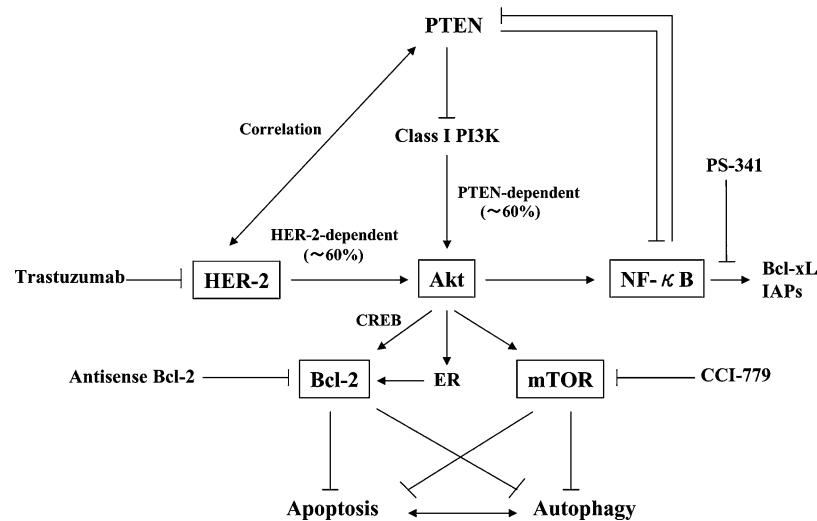


Figure 4. Possible key molecules for determining treatment response in HER-2-overexpressing breast cancer cells. Efficient inhibition of the signaling pathways associated with not only apoptosis but also autophagic cell death may produce greater enhancement of the therapeutic effect aimed at overcoming drug resistance in anticancer treatment. IAPs, inhibitor of apoptosis proteins; PTEN, phosphatase and tensin homologue deleted from chromosome 10; PI3K, phosphatidylinositol 3'-kinase; CREB, cyclic AMP-responsive element binding protein; mTOR, mammalian target of rapamycin; ER, estrogen receptor.

cell death; (iii) overlapping or compensatory interaction between two cell death pathways. The two pathways may increase cell death through overlapping or compensatory interactions, resulting in an increase in overall tumour response.

In our preliminary study, a multidrug-resistant MCF-7 cell line, which overexpressed P-glycoprotein, had a reversed sensitivity to doxorubicin in combination treatment with verapamil. The cell death was not apoptotic, but autophagic cell death with cytoplasmic vacuoles was observed in a way of slow progress for a week (unpublished results). This finding suggests that an abrogated apoptotic pathway can be replaced by the autophagic pathway in drug-resistant cells. Of importance, the failure of clinical chemosensitization in targeting apoptosis-related proteins may be overcome by activating autophagic cell death instead of apoptosis. In another approach to increase nonapoptotic cell death, the viral fusogenic membrane glycoproteins (FMGs) may be useful as a new treatment for the control of tumour growth.⁵⁶ FMGs kill cells by fusing them into large multinucleated syncytia, which die by sequestration of cell nuclei and subsequent nuclear fusion by a mechanism that is nonapoptotic. FMG-mediated cell killing combined with other anticancer treatment modalities may provide effective local tumour cell killing with the specific antitumour immunity.

Targeting apoptosis-related proteins and their modulation of the signaling pathway in apoptosis may increase tumour response, leading to prolongation of survival in patients with cancer. However, since resistance to apoptosis is due to multiple genetic alterations of the apoptotic pathways, activation of autophagy may be a more susceptible target for increasing the therapeutic effect or overcoming drug resistance. In breast cancer cells, for

example, several strategies for increasing drug response in HER-2-overexpressing tumours may be designed in terms of the relationship between apoptotic and autophagic signaling pathways. It is unlikely that modulation of drug response in combination with trastuzumab is sufficient. Although combination treatment with trastuzumab targeting the HER-2 protein increases the therapeutic effect of anthracyclines and taxanes, the resistance to trastuzumab can develop through the Akt-signaling pathway, which is associated with the activation of NF-κB, Bcl-2, and mTOR in tumour survival. The activated Akt, around 40% in a HER-independent fashion, may not be blocked by the treatment with trastuzumab. The inhibition of Akt and mTOR using mTOR inhibitors, such as CCI-779, may increase the therapeutic effect by producing autophagic cell death coupled with apoptotic cell death (Fig. 4). Future studies are needed to test the effect of other molecular targeting drugs in trastuzumab-resistant tumours by modulating the autophagic pathway.

Conclusions

Recent advances in understanding the molecular mechanisms of apoptotic and nonapoptotic cell death induced by anticancer treatment have provided critical information not only for understanding tumour response in terms of signal transduction pathways of cell death, but also for creating an opportunity to design targeting therapy for promoting cell death. Autophagy may be a critical contributor to the determination of tumour response as well as to caspase-dependent and -independent apoptotic cell death. In particular, in considering the microenvironment of a solid tumour, autophagy may be shifted to cell survival during nutrient starvation, which can be attributed to the drug

resistance coupled with resistance to apoptosis. A greater understanding of the clinical involvement and molecular mechanism of autophagic cell death coupled with apoptosis in cancer cells is required for predicting tumour response to both conventional anticancer drugs and molecular targeting agents, and for devising a more efficient strategy that will increase therapeutic effect in anticancer treatment.

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