Recent Advances in Understanding the Cell Death Pathways Activated by Anticancer Therapy

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Over the past two decades, the role of apoptosis in the cytotoxicity of anticancer drugs has become clear. Apoptosis may occur via a death receptor-dependent (extrinsic) or independent (intrinsic or mitochondrial) pathway. Mitochondria play a central role in cell death in response to DNA damage, and mediate the interaction(s) of various cytoplasmic organelles, including the endoplasmic reticulum, Golgi apparatus, and lysosomes. The mitochondrial pathway of cell death is mediated by Bcl-2 family proteins, a group of antiapoptotic and proapoptotic proteins that regulate the passage of small molecules, such as cytochrome c, Smac/Diablo, and apoptosis-inducing factor, which activates caspase cascades, through the mitochondrial transition pore. In addition, apoptosis can induce autophagic cell death via crosstalk between the two pathways upon treatment with anticancer drugs. The current review focused on recent advances surrounding the mechanism(s) of cell death induced by anticancer agents and discussed potential molecular targets for enhancing the chemotherapeutic effect(s) of anticancer agents. ***Cancer* 2005;103:1551–60.** *© 2005 American Cancer Society.*

KEYWORDS: anticancer drug, apoptosis, autophagy, cell death.

Programmed cell death occurs during organ development, and plays an important role in cellular homeostasis.[1] Three types of cell death are known: apoptosis (type I), autophagy (type II), and necrosis (type III).[2] Apoptosis is induced by a number of stimuli, including anticancer drugs, growth factor deprivation, and irradiation. The molecular mechanism by which apoptosis occurs has been extensively examined. Apoptosis in response to chemotherapy is known to correlate with a reduction in tumor volume, and resistance to apoptosis causes drug resistance.[3–5] Introduction of a proapoptotic gene such as Bax,[6,7] and inhibition of an antiapoptotic gene such as Bcl-2,[8,9] enhances the effects of chemotherapy. Thus, apoptosis clearly influences the antitumor effect(s) of chemotherapeutic agents.

In solid tumors, hypoxia and glucose deprivation enable tumor progression,[10] whereas depletion of adenosine 5′-triphosphate (ATP) results in necrosis. The production of lactic acid under anaerobic conditions does not necessarily induce necrosis, rather, autophagic cell death may depend on susceptibility to apoptosis, which can be inhibited by activation of Akt by environmental stimuli.[10,11] Drug resistance might involve resistance to autophagic cell death, as well as apoptosis. In fact, treatment with tamoxifen causes autophagy of MCF-7 breast carcinoma cells, as well as apoptotic cell death via a caspase 3-independent pathway.[12] Autophagy is also induced by aurintricarboxylic acid via activation of the ERK1/2 signaling pathway in Ras-mutated HT-29 colon carcinoma cells.[13] In addition, a human tumor suppressor gene, known as Beclin 1, induces autophagy.[14,15]

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Received October 26, 2004; revision received December 3, 2004; accepted December 10, 2004.

DOI 10.1002/cncr.20947
Published online 1 March 2005 in Wiley InterScience (www.interscience.wiley.com).

Reduced expression of this gene occurs in breast and ovarian carcinoma due to a 40–75% deletion of the gene.[16] Although resistance to autophagy and apoptosis is likely involved in tumor progression and acquisition of drug resistance by solid tumors, the exact molecular mechanism(s) by which this occurs, and the relation between autophagy and apoptosis, remain unknown. Here, we review evidence regarding the molecular mechanism(s) of anticancer drug-induced cell death and discuss potential therapeutic targets by which signal transduction pathways leading to cell death might be modulated by cancer therapy.

APOPTOTIC PATHWAYS OF CELL DEATH

Caspase-Dependent Pathway

Given that a number of anticancer agents induce apoptosis, a lot of research has focused on the various pathways that mediate apoptosis. Two are known to mediate anticancer drug-induced apoptosis, a death receptor-dependent (extrinsic) and a mitochondria-dependent (intrinsic) pathway.[17] The death receptor-dependent pathway involves activation of death receptors, such as Fas and TRAIL receptors (DR4, DR5). Death receptor activation is mediated by a death-inducing signaling complex, which is formed by recruitment of a Fas-associated death domain and procaspase 8 to the death receptor, thereby activating caspase 8.[3,18] Caspase 8 directly activates caspase 3, leading to apoptosis (type I cell death).[19] The mitochondria-dependent pathway involves cleavage of a proapoptotic protein, Bid, resulting in the production of truncated Bid (tBid), by caspase 8, within the mitochondria. Heterodimerization of tBid and Bak results in release of cytochrome c from mitochondria and activation of caspase 3 and caspase 9 (type II cell death).[19] The predominance of one pathway over another depends on cell type.

Unlike the death receptor-dependent pathway, the mitochondria-dependent pathway is mediated by Bax/Bak and involves the release of cytochrome c.[3,18] In general, Bax is associated with 14-3-3 protein, which anchors Bax in the cytoplasm. However, in the presence of DNA damage, c-Jun NH(2)-terminal kinase phosphorylates 14-3-3, resulting in dissociation of Bax from this protein.[20] Homodimerization of Bax, or heterodimerization of Bax ank Bak, results in translocation of Bax from the cytoplasm to mitochondria.[21] Bax homodimers and heterodimers interact with a voltage-dependent anion channel (VDAC) in the mitochondrial outer membrane to release cytochrome c by increasing mitochondrial membrane permeability (MMP) via opening of the mitochondrial permeability transition pore.[22] The mitochondrial permeability transition pore complex (PTPC) mediates permeability of the mitochondrial membrane during apoptosis, necrosis, and autophagy. PTPC is composed of an adenine nucleotide translocator and VDAC, both of which are targeted by a variety of proapoptotic inducers. The release of cytochrome c results in the formation of an apoptosome, consisting of apoptosis activating factor 1 and procaspase 9, after which dATP activates caspase 9, leading to apoptosis.[23] The activated initiator caspase, caspase 9, leads to activation of one of the effector caspases, caspase 3 or caspase 7, which cleaves an inhibitor of caspase-activated DNAase and produces poly (ADP-ribose) polymerase (PARP), resulting in DNA fragmentation. The release of cytochrome c through the VDAC-mediated permeability transition pore is inhibited by Bcl-2 and Bcl-xL. In addition to cytochrome c, Smac/Diablo is released from mitochondria when there is a loss of membrane potential. Smac/Diablo binds to X chromosome-encoded IAP, cellular IAP-1 (cIAP-1), and cellular IAP-2, as well as survivin, to inhibit apoptosis,[24] and it directly activates caspase 3. Inhibitors of apoptosis proteins (IAPs) block apoptosis by binding to and inhibiting caspases, as well as by activating caspase-independent mechanisms.[25] IAPs can inhibit activation of executioner caspases activated by extrinsic or intrinsic pathways. The Smac/Diablo complex induces apoptosis via the apoptosome-dependent pathway,[26] and release of Smac/Diablo is inhibited by Bcl-2 and Bcl-xL.[27] There is also a report that Smac induces cytochrome c release and apoptosis in the absence of Bax/Bcl-xL via activation of caspase 3 in human HCT116 and DU145 colon carcinoma cells.[28] Smac is capable of circumventing defects in mitochondrial apoptosis signaling, such as loss of Bax or overexpression of Bcl-xL, which frequently occurs in tumor cells resistant to anticancer therapy. A recent study suggests that an IAP family protein, Apollon, binds to, ubiquitinates, and facilitates the proteasomal degradation of Smac and caspase 9, thereby preventing Smac-induced apoptosis.[29] The role of Apollon in drug resistance remains to be clarified. Translocation of endogenous Smac into the cytosol, and release of Smac/Diablo during anticancer drug-induced apoptosis, does not appear to play a major role in cell death after treatment of human lung carcinoma with etoposide,[30] because cytochrome c and mitochondrial protease Omi/HtrA2 are still detectable in the cytosol in the absence of Smac. Thus, the significance of Smac/Diablo in anticancer drug-induced mitochondrial pathway-mediated apoptosis remains unclear.

Caspase-Independent Pathway

Although caspase cysteine proteases execute apoptosis, a mitochondrial serine protease released into the

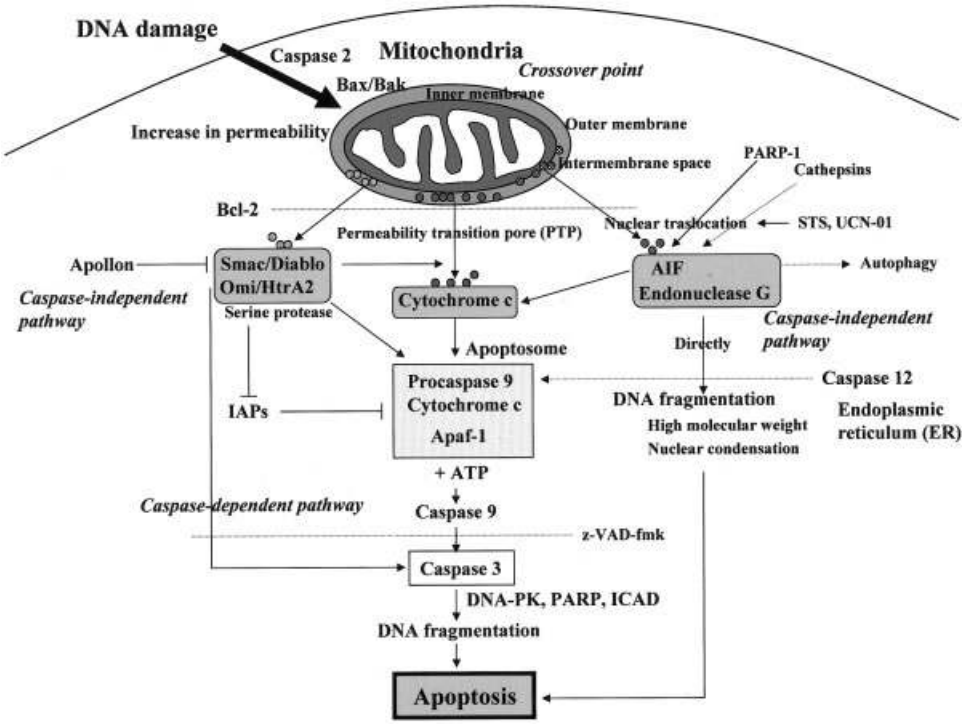

FIGURE 1. The role of mitochondria in cell death. Mitochondria play a central role in regulating the intrinsic and extrinsic apoptotic pathway responses to DNA damage. Apoptotic cell death can be induced by a number of triggers, resulting in the release of cytochrome c, Smac/Diablo, and apoptosis-inducing factor (AIF), from the mitochondrial outer membrane, inducing caspase-dependent and independent pathways of cell death. The release of these small molecules from mitochondria can be blocked by overexpression of Bcl-2. PARP-1: poly (ADP-ribose) polymerase-1; STS: staurosporine; ICAD: inhibitor of caspase-activated DNAase; PK: protein kinase.

cytosol during apoptosis, Omi/HtrA2, is required to antagonize inhibitors of apoptosis (IAPs), thereby enabling apoptosis to occur via an indirect mechanism.[31] Cleavage of c-IAP-1 by Smac/Diablo and Omi/HtrA2 is irreversible, thus, significant inactivation of IAPs occurs, enabling increased caspase activity after tumor necrosis factor apoptosis related ligand (TRAIL)-induced apoptosis.[32] IAP cleavage by Omi is independent of caspase activity. In addition, Omi/HtrA2 might activate more than one pathway of caspase activation because extramitochondrial expression of Omi-HtrA2 results in increased mitochondrial permeability and cytochrome c-induced caspase activation in HeLa cells.[33]

Apoptosis-inducing factor (AIF) is a phylogenetically conserved flavoprotein within the mitochondrial membrane, which has the ability to induce apoptosis via a caspase-independent pathway.[34] AIF induces nuclear chromatin condensation and large-scale DNA fragmentation (producing approximately 50-kilobase [kb] fragments), and is essential for programmed cell death. On lethal signaling, AIF translocates through the cytosol to the nucleus where it binds to DNA and provokes caspase-independent chromatin condensation. Responses to injection of anti-AIF antibodies, or knockout of the AIF gene, suggest that AIF may be required for cell death in response to certain stimuli.[35] Staurosporine (STS) induces mitochondrial dysfunction and translocation of AIF into the nucleus after activation of nuclear apoptosis in nonsmall cell lung carcinoma cells resistant to radiotherapy or chemotherapy.[36] This suggests that, although caspase-dependent and independent pathways coexist, the caspase-dependent pathway might be less efficient. Furthermore, resistant cells might be made more susceptible to treatment by activation of the AIF-mediated caspase-independent pathway. Moreover, recent reports indicate that AIF is necessary for PARP-1–dependent cell death.[37,38] PARP-1 generates a number of long, branched PARPS after DNA damage. Activation of PARP-1 initiates a nuclear signal that triggers the release of AIF from mitochondria. AIF then shuttles from mitochondria to the nucleus and induces peripheral chromatin condensation and large-scale DNA fragmentation.[38] Endonuclease G (Endo G) is a nuclear-encoded mitochondrial protein believed to be important for nuclear DNA fragmentation during apoptosis, as well as mitochondrial DNA replication. Cells from Endo G heterozygous mutant mice are resistant to tumor necrosis factor alpha and STS-induced cell death.[39]

Thus, increased MMP in response to DNA damage results in the release of a number of small molecules, such as cytochrome c, Smac/Diablo, and AIF from mitochondria. This results in activation of caspase-dependent and independent apoptotic pathways, depending on which triggers and cell types are involved.

Our current understanding regarding the role of mitochondria in cell death is summarized in Figure 1.

AUTOPHAGIC PATHWAY OF CELL DEATH

Autophagic cell death, otherwise known as type II cell death, is characterized by the appearance of large autophagic vacuoles in the cytoplasm. It occurs during embryogenesis and differs from type I programmed cell death.[40] Autophagy is a bulk protein degradation system that is essential for normal cell activity and survival when nutrients are scarce.[41] The first step that occurs in autophagic cell death is the formation of a double-membrane vacuole, the autophagosome, which is derived from part of the endoplasmic reticulum[42] or from the cytoplasmic lipid pool.[43] The autophagosome fuses with a lysosome, after which its contents are degraded by lysosomal hydrolytic proteases. Despite recent advances regarding the molecular mechanism(s) underlying autophagy, many details remain poorly understood. Of note, class I phosphoinositide 3-kinase (PI 3-kinase) inhibits, whereas Class III PI 3-kinase enhances, autophagy in HT-29 human colon carcinoma cells.[44] Class I PI 3-kinase mediates various cellular functions, including insulin and glycogen synthesis, and suppresses apoptosis through the Akt-signaling pathway, whereas Class III PI 3-kinase influences membrane trafficking. Despite the finding that insulin-like growth factor I (IGF-I) normally inhibits apoptosis, IGF-I accelerates cell death when glucose and amino acids are limited, leading to accumulation of autophagic vacuoles within the cytoplasm and evidence of chromosome condensation.[40] Autophagic cell death is inhibited by 3-methyladenine (3-MA) and LY294002, which inhibit PI 3-kinase, but not by the pancaspase inhibitor, z-VAD-fmk. Of importance, LY294002 has no effect on cell death in PC12 and HepG2 carcinoma cells during glucose deprivation,[45] suggesting that interference with the PI 3-kinase/Akt signaling pathway causes autophagy of cancer cells but not normal cells.

A mammalian gene capable of inducing autophagy, Beclin 1, is a component of the PI 3-kinase complex responsible for autophagic vesicle nucleation.[16] This gene is deleted in 75% of ovarian, 50% of breast, and 40% of prostate carcinoma cases.[16] Frequent allelic deletions are observed in breast carcinoma cell lines in association with significant decreases in Beclin 1 protein levels. Beclin 1 functions as a tumor suppressor, because there is an attenuation of MCF-7–mediated tumorigenicity in nude mice overexpressing Beclin 1.[46] Although Beclin 1 is known to interact with Bcl-2 in yeast, the relation between this association and tumor suppression and autophagy is not yet clear. Downregulation of Bcl-2 by antisense Bcl-2 in HL-60 cells induces autophagy independent of caspase-dependent and mitochondria-dependent pathways,[47] suggesting a potential role of Bcl-2 in autophagic cell death, as well as apoptosis. Other autophagy-inducing genes, including bridging integrator 1 (Bin 1) and death-associated protein kinase (DAPK), exist. Bin 1 interacts with c-Myc and inhibits its transformation activity.[48] Bin 1 is regulated by alternate splicing, and the splice forms that interact with c-Myc have tumor suppressor properties. This gene is frequently missing or functionally inactivated in malignant melanoma, breast, and prostate carcinoma cells, and loss of Bin 1 expression may promote tumor progression, while limiting susceptibility to farnesyltransferase inhibitors (FTIs). Bin 1 adapter proteins act downstream or parallel to RhoB to exert their effect on cell signaling.[49] DAPK is a calcium-regulated serine/threonine kinase, and an important mediator of autophagy induced by nutrient deprivation, antiestrogens, and tumor necrosis factor.[50] Cancer and cancer cell lines frequently demonstrate decreased DAPK expression due to DNA methylation.[51] Transfection of Beclin 1, Bin 1, or an active form of DAPK, into cancer cells induces autophagic cell death. A human homolog of the *Drosophila* spin gene product (HSpin 1) exists, which binds to Bcl-2 and Bcl-xL. HSpin 1-induced cell death results in formation of autophagic vacuoles and release of a mature form of cathepsin D, suggesting a novel caspase-independent cell death, pathway resulting in autophagy.[52]

The tumor suppressor phosphatase and tensin homolog deleted on chromosome10 (PTEN), which is an inhibitor of the PI 3-kinase/Akt signaling pathway, promotes autophagy in HT-29 colon carcinoma cells.[53] PTEN PI phosphatase activity inhibits Akt activity, resulting in induction of autophagy. Loss of function of PTEN increases the expression of Class I PI 3-kinase and Akt in human cancer cells. Activation of Akt and constitutive expression of active Akt block autophagic cell death, leading to inhibition of apoptosis and activation of a mammalian target of rapamycin (mTor) protein. It is likely that activation of Akt and the mTor signaling pathway, due to loss of PTEN, contributes to malignant transformation by simultaneous inhibition of autophagic and apoptotic cell death. Another oncoprotein, Ha-Ras, induces autophagic cell death in glioma and gastric carcinoma cells[54] via a p53-independent pathway that is not blocked by Bcl-2. Another Ras family, including K-Ras and N-Ras proteins, also induces autophagic cell death. Increased Ras expression is observed in spontaneous regression of neuroblastoma, which is characterized by autophagic cell death and a lack of apoptosis.[55] In addition, Ha-Ras overexpression induces autophagic cell death in neuroblastoma cell lines. Ha-Ras–induced autophagy in HT-29 colon carcinoma cells has

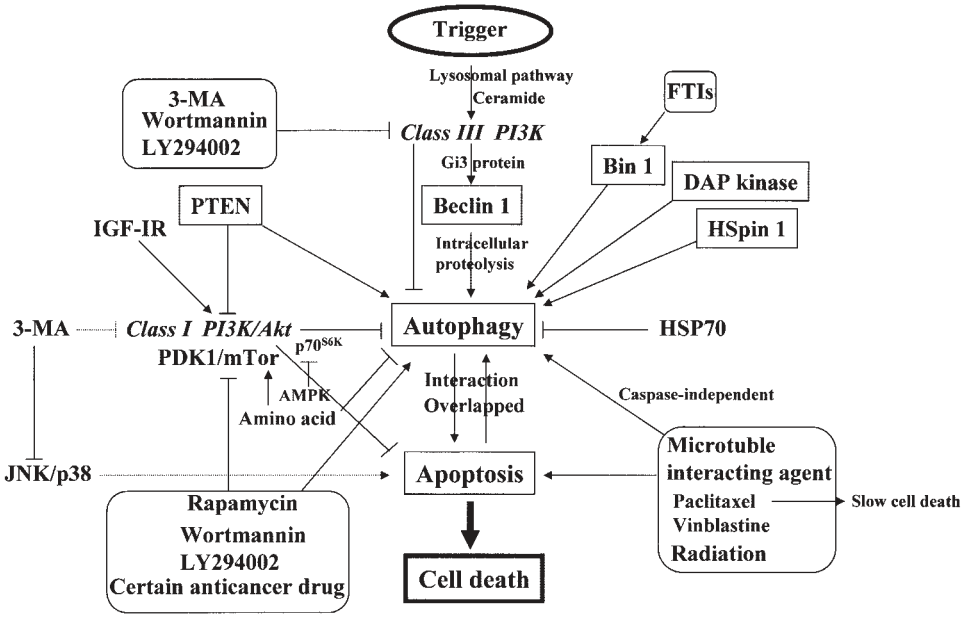

FIGURE 2. A proposed mechanism for regulation of autophagic cell death. A mammalian gene capable of inducing autophagy, Beclin 1, is activated via a Class III phosphoinositide 3-kinase (PI 3-kinase)–dependent pathway, which is stimulated by lysosomal activation, ceramide, and Gi3 protein. A number of other genes, including Bin 1, DAPK, and HSpin 1 genes, are also involved in autophagic cell death. Autophagy can be induced by antimicrotubule agents, such as vinblastine and paclitaxel, as well as by irradiation. A tumor suppressor gene, PTEN, induces autophagic cell death, and interference with PTEN expression results in activation of the Class I PI 3-kinase/Akt pathway, which inhibits apoptosis. Activation of the PI 3-kinase/Akt signaling pathway leads to activation of the mammalian target of rapamycin (mTor) through PDK1, which is inhibited by rapamycin, and induces autophagic cell death. Autophagic cell death is inhibited by 3-methyladenine (3-MA), and treatment with PI 3-kinase inhibitors, such as wortmannin and LY294002, also inhibits autophagy, even if apoptosis is activated. There is likely some overlap in the pathways mediating autophagy and apoptosis. However, further research is required to clarify the molecular mechanism(s) underlying these pathways. FTI: farnesyltransferase inhibitors; IGF-IR: insulin-like growth factor-I receptor.

some overlap with the Raf 1/ERK signaling pathway of autophagic cell death.[56] The ability of Ras to cause autophagy might involve activation of ErbB2 and ErbB3. It is interesting to note that one of the signaling pathways regulated by Ras, the Raf1/ERK pathway, triggers autophagy, whereas the PI 3-kinase/Akt pathway, which is downstream of Ras signaling, inhibits it. Ras-induced increases in the expression and trafficking of lysosomal cathepsins might contribute to Ras-induced autophagy. A proposed molecular model of autophagy is illustrated in Figure 2.

A death-promoting "BH3-only" family protein, Bcl-2/adenovirus E1B 19-kilodalton interacting protein 3 (BNIP3), differs from other proteins of this class. BNIP3 can directly integrate into the mitochondrial outer membrane via its BH3 domain in the absence of Bax/Bak, thereby disrupting the MMP, generating reactive oxygen species (ROS) and promoting autophagic degeneration.[57] Overexpression of BNIP3, a hypoxia-inducible protein with a BH3 domain, results in autophagic cell death in cancer cell lines, after cytochrome c or AIF nuclear translocation. BNIP3-induced autophagy can be attenuated by overexpression of Bcl-2, which interacts with BNIP3 protein. Autophagic cell death can be activated in cancer cells in response to various anticancer drugs. Treatment of MCF-7 breast carcinoma cells with tamoxifen induces autophagy before apoptotic cell death.[12] Treatment with estradiol and 3-MA inhibits tamoxifen-induced cell death. Similarly, other anticancer drugs, such as vinblastin, paclitaxel, and rapamycin, induce autophagic cell death.[58–60] Treatment of malignant glioma cells with arsenic trioxide induces autophagic cell death in association with G_2/M arrest.[61] In addition, radiotherapy induces autophagic cell death in several cancer cell lines, including breast, colon, and prostate carcinoma, as well as glioblastoma multiforme.[62] Despite several reports of induction of autophagy by treatment with anticancer drugs and/or irradiation, the clinical significance of autophagy as a result of chemotherapy remains unclear.

INTERACTION BETWEEN APOPTOTIC AND AUTOPHAGIC PATHWAYS OF CELL DEATH

Different types of cell death differ in their morphologic characteristics (Fig. 3 and Table 1). In fact, ceramide, which is believed to activate apoptosis, also induces

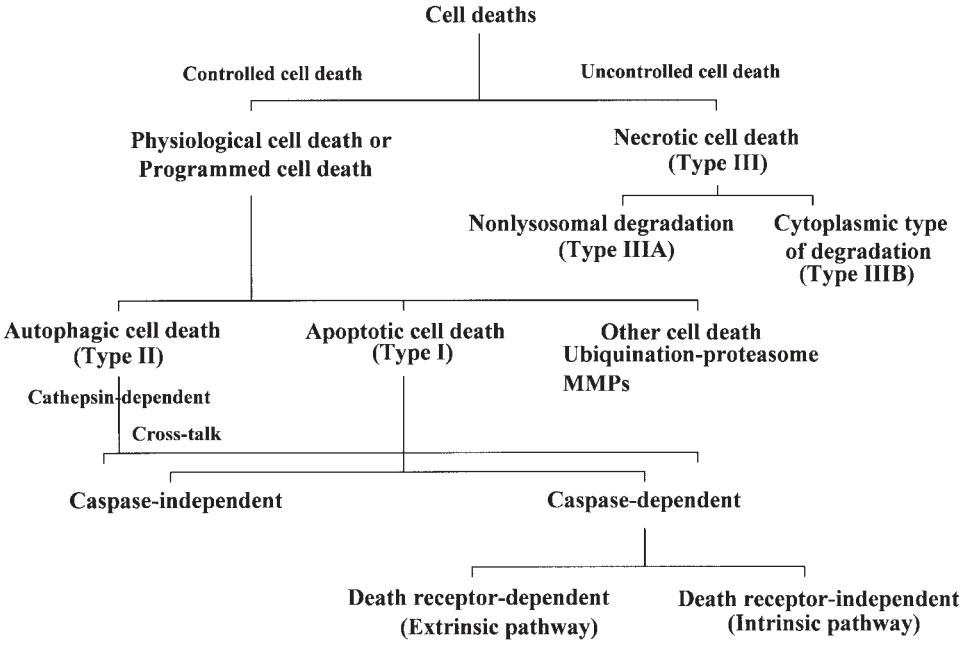

FIGURE 3. Schematic illustration of the different types of cell death. Cell death can be classified as controlled or uncontrolled, referring to programmed or necrotic cell death. Programmed cell death includes apoptosis, and autophagy, among other types of cell death. Apoptosis usually occurs through a caspase-dependent pathway. However, it sometimes occurs via an alternative pathway, which overlaps with that of autophagy. MMP: matrix metalloproteases.

TABLE 1
Differences in Characteristics between Type I and Type II Cell Death

Factor	Apoptosis (type I)	Autophagy (type II)
Morphology		
Nucleus	Chromatin condensation	Partial chromatin condensation
	DNA laddering and fragmentation	No DNA laddering and fragmentation
	Pyknosis	Sometimes pyknosis
Cytoplasm	Cytoplasmic condensation	Many large autophagic vacuoles
	Fragmentation to apoptotic bodies	Many autophagosomes
	Increase in MMP	Lysosomal activation
	Activation of caspase cascade	Caspase independent
	Potential release of lysosomal enzyme	Potential involvement of MMP
	Organelles do not swell	
Membrane	Blebbing	Blebbing
Primary proteases	Caspases such as caspase 3	Cathepsins and proteasomal proteins
ATP requirement	Yes	Yes (AMPK, AMP/ATP ratio)
Inhibition	z-VAD-fmk	3-methyladenine (3-MA)
	XIAP	PI3K inhibitors
	Bcl-2/Bcl-xL	PI3K-I/Akt
	Sometimes with actinomycin D	Actinomycin D
	Sometimes with cyclophosphamide	Cyclophosphamide
Detection	DNA laddering test	Electron microscopy
	Caspase activation and the substrate	Lysosome activity test
	TUNEL and annexin V staining	Cytoplasmic sequestration test
	FACS analysis	LC3 associated with autophagosome membrane
	Electron microscopy	

MMP: mitochondrial membrane permeability; XIAP: X chromosome-encoded inhibitors of apoptosis proteins; PI3K: phosphoinositide 3-kinase; TUNEL: terminal deoxynucleotidyl transferase nick-end labeling; FACS: fluorescence-activated cell sorting; AMPK: AMP-activated protein kinase; AMP: adenosine 5′-monophosphate; ATP: adenosine 5′-triphosphate.

autophagy.[63] Similarly, proteins like DAPK are capable of inducing both apoptosis and autophagy depending on the cell type involved, suggesting that they may function as molecular switches or integrators of these two types of programmed cell death. Commitment to autophagy or apoptosis may depend on the cell type involved, as well as the microenvironment. Depending on the setting, initiation of autophagy may induce,

delay, or antagonize apoptosis. Three scenarios must be considered when contemplating the interaction between apoptosis and autophagic cell death.

First, autophagy may precede apoptosis. Treatment of sympathetic neurons with cytosine arabinoside, thereby depleting neural growth factor, results in a 30-fold increase in autophagy, followed by DNA degradation and apoptosis.[64] Delayed induction of apoptosis occurs with 3-MA, which inhibits cytochrome c release and caspase activation. These findings may model cell death after treatment with anticancer drugs or growth factor deprivation.

Second, autophagy may antagonize apoptosis. Conversely, a reduction in autophagy might increase the susceptibility of cells to apoptotic stimuli. Accelerated sulindac sulfide-induced apoptosis occurs in mutant clone HT-29 colon carcinoma cells, which lack autophagic capability due to overexpression of a mutant G (alpha i3) protein.[65] In contrast, treatment of parent HT-29 cells with 3-MA increases sensitivity to sulindac sulfide-induced apoptosis. Even though there are no differences in the expression patterns of COX-2, Bcl-2, Bcl-xL, Bax, and Akt/PKB among mutant and parent HT-29 cells, greater cytochrome c release occurs in mutant G (alpha i3) protein overexpressing cells, compared with parent cells. It is possible that autophagy retards apoptosis in colon carcinoma cells by sequestering mitochondrial death-promoting factors, such as cytochrome c.

Third, apoptosis and autophagy may be mutually exclusive. Inhibition of autophagy may lead to apoptotic cell death. A recent report reveals that treatment of malignant glioma cells with arsenic trioxide induces autophagic cell death. However, inhibition of autophagy by treatment with bafilomycin, a H^+-ATPase inhibitor, leads to apoptosis.[61] However, although treatment of neurons and HeLa cells with a pancaspase inhibitor prevents apoptosis, selective mitochondrial impairment results in autophagy.[66] In light of several reports indicating that treatment with caspase inhibitors causes a shift from apoptotic cell death to autophagy, it is possible that this also occurs under other circumstances. For example, inhibition of apoptosis in Bax/Bak double-deficient DKO cells induces autophagic cell death.[67] The ability of cells to revert between apoptosis and autophagy might be clinically significant because drug resistance to apoptosis might be overcome by activation of autophagic cell death. Along this line, multidrug-resistant MCF-7 cells can be made susceptible to doxorubicin by treatment with verapamil, after which autophagic cell death occurs, suggesting that the autophagic cell death pathway remains viable after inhibition of the apoptotic pathway (unpublished data). The molecular mechanism(s) by which reversion from apoptosis to autophagy occurs remains unclear. Although overexpression of Bcl-2 or Bcl-xL may inhibit autophagic cell death, the role of these antiapoptotic proteins in autophagic cell death requires further clarification.

The role of Bcl-2 in apoptosis in response to mitochondrial damage is well established. However, signaling as a result of mitochondrial damage might also activate the autophagic pathway of cell death. It appears that mitochondria are involved in integrating the apoptotic and autophagic pathways of cell death. Lysosomal enzymes might activate apoptosis because lysosome rupture activates apoptosis. It is interesting to note that apoptosis after lysosome rupture is preceded by autophagy. Sequestration of mitochondria within autophagic vacuoles might protect cells from apoptosis by delaying the release of cytochrome c. Thus, autophagy might regulate apoptosis through lysosome formation and enzyme activity. With regard to the possibility of signaling between lysosomes and mitochondria, a recent study reveals that production of H_2O_2 from ROS in response to DNA damage activates lysosomal enzymes, such as cathepsin D and B.[68] Cathepsin B directly or indirectly results in translocation of proapoptotic proteins, such as Bid and Bax, from the cytoplasm to mitochondria. This alters MMP and results in caspase activation subsequent to the release of cytochrome c, Smac/Diablo, and AIF from the mitochondria. Conversely, under different circumstances, production of H_2O_2 from ROS in response to DNA damage may inhibit the release of small molecules, such as cytochrome c, Smac/Diablo, and AIF, from the mitochondrial membrane and activate lysosomal activity. Thus, crosstalk might occur between the pathways mediating autophagy and apoptosis by way of lysosomes and mitochondria. Further research is required to clarify the signaling that occurs between these pathways.

FUTURE PERSPECTIVES REGARDING CELL DEATH AND CANCER THERAPY

Increasing information is known regarding the molecular mechanism(s) by which cell death is mediated by anticancer drugs. Proapoptotic and antiapoptotic proteins are now being targeted to enhance the effect(s) of chemotherapy. In general, the antitumor effect of an anticancer agent is measured in terms of its ability to induce apoptotic cell death within tumors, which is well correlated with outcome in most preclinical and clinical studies. Nevertheless, despite significant reductions in tumor volume, high rates of apoptosis do not always occur within solid tumors after chemotherapy. This suggests that other types of cell death, such as autophagy and necrosis, might also occur as a result

of chemotherapy. Research into other types of cell death, particularly autophagic cell death, is important to better understand the effects of anticancer drugs. In addition, the relation between autophagic cell death and apoptosis needs to be examined, because several autophagy-related proteins, including Beclin 1, BNIP3, DAPK, and HSpin 1, may influence apoptosis. Because apoptosis and autophagy are mutually exclusive in some cell lines, molecular switches between the two processes must exist.

Resistance to autophagy, as well as apoptosis, might lend resistance to anticancer drugs. Likewise, induction of autophagic cell death in cancer cells may be of therapeutic benefit. Cells transformed with the tumor suppressor gene Beclin 1 are less likely to undergo autophagy and are more susceptible to oncogenesis. Alterations in autophagic genes reduce the incidence of autophagy in cancer cells. However, even in cells resistant to apoptosis or cells in which apoptosis is inhibited, nonapoptotic cell death pathways, such as autophagy, may be activated. Targeting alternative pathways of cell death might overcome drug resistance or explain why the effects of chemotherapy and irradiation complement each other in the clinical setting. Alterations or defects in apoptotic signal transduction pathways might result in drug resistance, however, irradiation induces autophagic cell death. Alterations of one or both pathways (autophagic or apoptotic) might determine susceptibility to cell death after chemotherapy because anticancer drugs might trigger both apoptotic caspase-dependent and autophagic caspase-independent pathways at the same time.

Autophagic cell death might also inhibit angiogenesis.[69] The angiogenesis inhibitor, endostatin, induces endothelial cell death via the caspase-independent autophagic pathway. In addition, the tumor suppressor gene, PTEN, which induces autophagy, inhibits tumor angiogenesis in nude mice with orthotopic brain tumors.[70] Reconstitution of wild-type PTEN has no effect on in vitro proliferation, but dramatically decreases tumor growth in vivo and prolongs survival in mice, suggesting that PTEN regulates tumor-induced angiogenesis via regulation of PI-dependent signals. The role of autophagy in tumor angiogenesis might be an important means of inhibiting tumor progression and increasing the effectiveness of anticancer therapy.

It is now clear that a number of different types of cell death, including apoptosis and autophagy, play a role in the antitumor effect(s) of treatment with anticancer drugs. Targeting therapy at genes related to apoptosis might increase the therapeutic efficacy of anticancer agents by modulating signal transduction pathways, and other cell death pathways might be influenced by changes in the microenvironment and by crosstalk between the various cell death pathways. Further research into the molecular mechanism(s) behind autophagic cell death as a result of chemotherapy might provide a new strategy for overcoming drug resistance by exploring this alternative pathway of cell death.

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