Cancer: Cell Structures, Carcinogens and Genomic Instability
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Abnormalities of cell structures in tumors: apoptosis in tumors

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Abstract. A conceptual shift has occurred in recent years from considering cancer as simply a disease of deregulated cell proliferation to a view that incorporates the aberrant control of apoptosis into the equation. Apoptosis is an organized, genetically programmed cell death process by which multicellular organisms specifically destroy, dismantle and dispose of cells. In cancer cells, this tightly controlled process is suppressed by genetic lesions, allowing cancer cells to survive beyond their normal life span even in hostile environments that are prone to hypoxia and lack many trophic factor supports. In the last two decades, cancer researchers have made great strides in our understanding of the underlying molecular mechanism of apoptosis in chemoresistance generation and tumorigenesis. This tremendous increase in our knowledge of apoptosis in tumors has greatly impacted our perspective on carcinogenesis. Key regulators of apoptosis such as members of the Inhibitors of Apoptosis family and Bcl-2 family have been shown to play a pivotal role in allowing most cancer cells to escape apoptosis. The identification of specific targets involved in the suppression of apoptosis in cancer cells has facilitated the design and development of therapeutic strategies based on rational molecular approaches that aim to modulate apoptotic pathways. Many promising apoptosis-dependent strategies have been translated into clinical trials in the continued assessment of regimens that can effectively eradicate cancers.

Key words: Apoptosis, bcl-2, death receptors, IAP, mitochondria, p53.

Background

In the 1960s, Lockshin and Williams introduced the term "programmed cell death" to refer to a gene-directed form of cell death [1]. The term "apoptosis" was coined in 1972 by Kerr, Wyllie and Currie to describe a form of ischemia-induced hepatic cell death. The term comes from the Greek (apo + ptosis) for "falling off" and depicts a distinct morphology of dying cells characterized by cell shrinkage, membrane blebbing, chromatin condensation and nuclear fragmentation [2]. The realization that apoptosis is a genetically invoked form of cell death has impacted our understanding of proliferative and degenerative diseases because of the implication that tissue homeostasis can be controlled by factors that regulate cell survival and death, as well as those that affect proliferation and differentiation. The fact that apoptosis is controlled by genetic programs renders it susceptible to disruption by mutations, and the acquired ability of cancer cells to evade apoptosis is one of the hallmarks of cancer [3, 4]. In this chapter, we first delineate the unique morphology of apoptotic cells.

However, since the process of apoptosis is very much dictated by genetic programs, the main thrust of this chapter is on the discussion of apoptosis in terms of the underlying molecular mechanisms. We also address the various approaches currently in use and under consideration to reactivate apoptosis for use in anticancer therapy. Our objective is that readers will gain a greater appreciation of the importance of apoptotic mechanisms underlying cancer pathogenesis, and thereby appreciate the subsequent impact this may have on newer modes of the medical management of tumors.

Introduction: apoptosis

Apoptosis is a gene-directed mechanism in which unnecessary or dangerous cells are triggered to undergo self-destruction without injuring neighboring cells or eliciting any associated inflammatory response [5]. The core apoptotic pathway was first described through genetic analysis in the nematode *Caenorhabditis elegans* and subsequently found in species as diverse as *Drosophila melanogaster* and humans [6]. In these multicellular organisms, the apoptotic process is crucial for normal development, differentiation, tissue physiology and defense against pathogens. The dysregulation of apoptosis is intricately involved in the etiology and pathogenesis of many diseases, including AIDS, autoimmune disorders, neurodegenerative diseases and cancer.

In general, apoptosis can be divided into the initiation phase, the effector phase, and the degradation phase [7]. In the initiation phase, a stimulus, either extrinsic or intrinsic to the cell, triggers the apoptosis process. This stimulus may arise from a variety of sources and some general inducers include radiation, UV, growth factor withdrawal and cytotoxic agents such as chemotherapeutic drugs. The potency of each of these stimuli to induce apoptosis, however, is cell-type dependent. Despite the differences in the initiation of apoptosis, the effector phase in which the apoptotic machinery is activated shares common biochemical features (see the section 'The apoptotic machinery'). Once cells have committed to apoptosis, the degradation phase begins and the process becomes irreversible. At this late stage, double-stranded breakdown of DNA into nucleosomal segments is manifested as DNA laddering in gel electrophoresis [5]. This DNA laddering is a defining feature of apoptotic cell death that contributes to the unique morphology of apoptotic cells.

Morphology of apoptotic cells

Apoptosis is characterized by a series of well-documented morphological changes that can be detected by light and electron microscopy [2, 8–12]. The most characteristic morphological change is seen within the nucleus, as compaction of nuclear chromatin leads to sharply delineated, uniformly granular masses marginated against the nuclear envelope followed by nuclear fragmen-

tation. As the nuclear outline convolutes, the cytoplasm also condenses and blunt blebs or protrusions appear on the plasma membrane. While the cytoplasm continues to condense, the cell disintegrates into the characteristic membrane-bound apoptotic bodies enclosing fragments of the nucleus. The integrity of the membrane encasing the apoptotic fragments is retained during the course of apoptosis until they are engulfed by phagocytes in a "contained" manner without eliciting an inflammatory response that might be harmful to the surrounding tissues [2, 13].

The apoptotic machinery

Apoptosis is first and foremost defined by its morphological features. Apoptotic cells confer a distinctive constellation of biochemical changes that underlie the structural changes. Given that diverse cell types across species exhibit morphological similarity when subjected to various death-inducing stimuli, an intuitive suggestion would be that there exists a common apoptotic mechanism operating in most cells of an organism [8]. The core of the apoptotic machinery is, in fact, composed of a set of conserved molecules operating within metazoan cells [14], and is induced by a cascade of molecular events that may be initiated in a distinct manner, culminating in the activation of caspases. For the purpose of this chapter, we focus on the molecular pathways that have been defined in mammals, and in particular, humans (see Fig. 1 for a schematic overview).

Caspases and apoptotic pathways

The central component of the apoptotic machinery is a proteolytic system involving a family of aspartate-specific cysteine proteases, termed caspases, which cleave many vital cellular proteins and proteolytically activate enzymes that contribute to the disassembly of a cell, such as the DNase DFF40/CAD [15]. Caspases exist as zymogens in cells, but can become activated in response to apoptotic stimuli. They are organized in a cascade and can be divided functionally into two groups: initiator and effector caspases, with upstream initiator caspases being responsible for the activation of downstream effector caspases [16]. Although caspases share distinct similarities in amino acid sequence and structure, they are highly specific in their substrate preferences [17, 18]. The specificity of caspases allows them to function in an orchestrated fashion that guides the apoptotic cell to sever contacts with surrounding cells, reorganize the cytoskeleton, shut down DNA replication and repair, destroy DNA, disrupt the nuclear structure and eventually induce the cell to display signals that mark it for phagocytosis [15].

Initiation of apoptosis occurs by signals from two distinct but convergent pathways: the extrinsic and intrinsic pathway. These two pathways make use

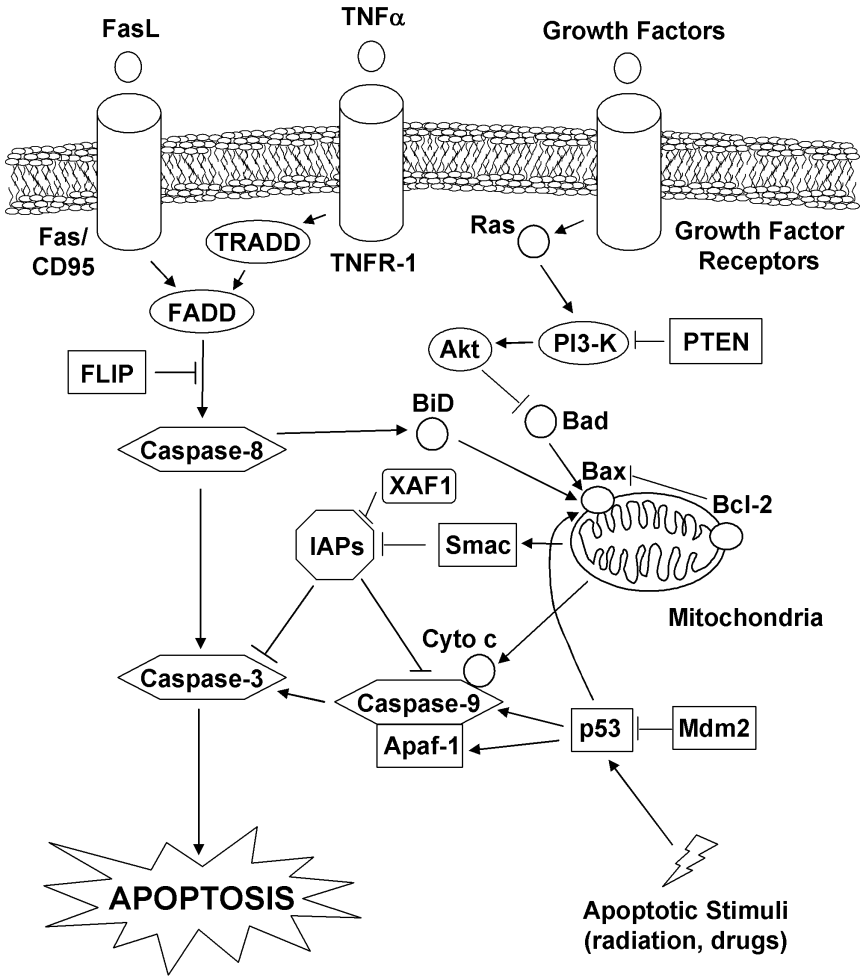

Figure 1. Apoptotic pathways. Key regulators in both the extrinsic and the intrinsic apoptotic signaling pathways are highlighted. See text for details.

of largely distinct molecular interactions and utilize different caspases, but are also interconnected at numerous steps and ultimately converge at the level of effector caspase activation [19]. However, for the sake of simplicity, we shall initially treat the two as being mutually exclusive.

Intrinsic pathway

The intrinsic pathway is activated in response to intracellular stress, such as DNA damage, hypoxia, growth factor deprivation and some chemotherapeutic

drugs [20]. This pathway is sometimes referred to as mitochondrion-mediated cell death, and results in increased mitochondrial permeability, defined by mitochondrial outer membrane permeabilization (MOMP) that is executed by proteins from the Bcl-2 family [21] (see section 'Bcl-2 family' below). The increase in permeability leads to the release of proteins normally found in the space between the inner and outer mitochondrial membranes [22]. A pivotal protein released into the cytosol is cytochrome *c*, well known for its role in mitochondrial respiration and recognized as an essential component of a high molecular weight caspase-activating complex known as the apoptosome [23]. Apoptosome formation is caused by cytochrome *c* binding to Apaf-1, which in the presence of dATP facilitates the association and the activation of initiator caspase-9 [24]. Subsequently, effector caspase-3 is recruited to the apoptosome, where it is activated by caspase-9, leading to the degradation phase of apoptosis. It should be noted, however, that recent research has also pointed to the endoplasmic reticulum (ER) as an important modulator of both mitochondrion-mediated apoptosis [25], as well as an ER-specific, unique pathway for caspase activation and apoptosis [26–30].

Extrinsic pathway

The extrinsic pathway, also known as the death receptor-induced pathway, is initiated by the ligation of death receptors belonging to the tumor necrosis factor receptor (TNF-R) superfamily, such as Fas/APO-1/CD95 and TNF-R1 found on a variety of cells [19]. Members of the TNF-R family are characterized by a cytoplasmic death domain (DD) involved in protein-protein interactions that is essential for delivering apoptotic signals [31, 32]. Binding of ligands promotes oligomerization of the death receptors, and their cytoplasmic domains then recruit DD-containing adaptor proteins FADD and TRADD via DD-DD interactions, leading to the formation of a death-inducing signaling complex (DISC) [33–35]. FADD then causes the sequestration of the proenzyme forms of caspase-8 and -10 through the homotypic interaction of DDs known as death effector domains (DEDs) to DISC [36, 37]. The proximity-induced activation of multiple caspase-8 molecules by DISC [38] in turn activates effector pro-caspase-3 [39], at which point the intrinsic and the extrinsic pathways converge [40].

Evidently, caspases occupy a central role in the regulation of apoptosis in both the intrinsic and the extrinsic pathways. The apoptotic process is, thus, also tightly controlled by regulators of caspases. An important family of endogenous caspase inhibitors, termed the inhibitors of apoptosis (IAPs), was identified as a central regulatory factor that blocks the execution of apoptosis.

Inhibitors of apoptosis

Although other proteins have been identified that inhibit initiator caspases, only the IAPs (see Fig. 2) have been demonstrated to be endogenous direct repressors of the terminal caspase cascade [41, 42]. In humans, members of this family of proteins include neuronal apoptosis inhibitory protein (NAIP), X-linked inhibitor of apoptosis (XIAP/hILP), cellular IAP1 (c-IAP1/HIAP2), cellular IAP2 (c-IAP2/HIAP1), Survivin, Livin, testis-specific IAP (Ts-IAP) and Apollon/BRUCE. The anti-caspase activity of IAPs may be attributed to their characteristic 70–80-amino acid baculoviral IAP repeat (BIR) domains. XIAP, arguably the most potent IAP identified, possesses three BIR domains, of which BIR3 is an inhibitor to the initiator caspase-9 and BIR2 an inhibitor to effector caspase-3 and -7 [43, 44]. Moreover, some IAPs also contain a RING domain that functions as E3 ubiquitin ligase, capable of recruiting target proteins to a complex containing an E2 enzyme for ubiquitin conjugation and proteasomal degradation [45]. In particular, c-IAP2 and XIAP can trigger the ubiquitination of caspase-3 and -7 [46, 47], suggesting that targeting of caspases to the proteasome may be another anti-apoptotic mechanisms of the IAPs. During the course of apoptosis, the caspase-inhibitory function of IAPs is negated by antagonists Smac/DIABLO and Omi/Htra2, which normally reside in mitochondria but are proteolytically processed and released into cytoplasm once a cell receives an apoptotic stress [48]. In addition, XIAP-associ-

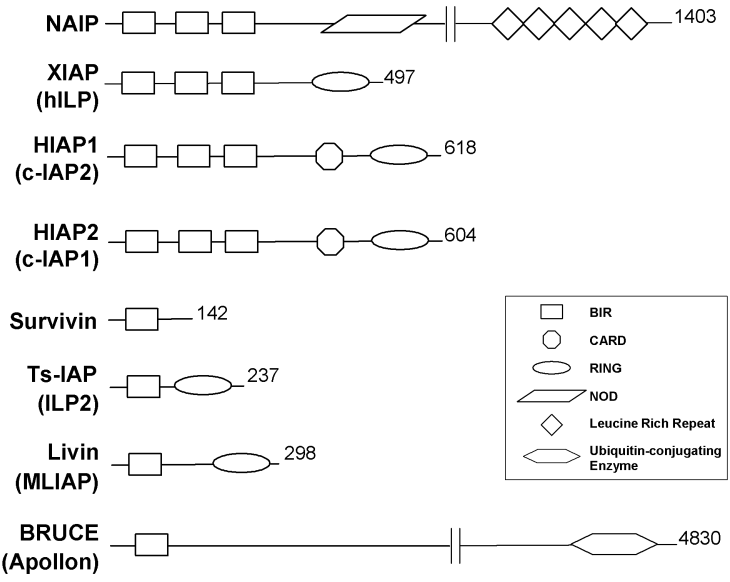

Figure 2. Domain structure of the IAP family. BIR, baculovial IAP repeat; CARD, caspase recruitment domain; RING, RING zinc-finger; NOD, nucleotide-biding oligomerization domain.

ated factor 1 (XAF1) has been identified as an antagonist of XIAP that promotes apoptosis by allowing unrestricted caspase activity [49].

Thus, the determination of whether a cell commits to the apoptotic process is tightly regulated, and is essentially a function of the severity and not merely the specificity of the apoptotic stimulus. As we shall see, it is this function that researchers are aiming to exploit in making cancer cells more susceptible to current modes of therapy (see section 'Therapeutic opportunities').

Bcl-2 family

The Bcl-2 family proteins may regulate apoptosis by altering the integrity of the mitochondria and by controlling calcium homeostasis [50–52]. Members of the Bcl-2 family can be divided into three classes: (1) anti-apoptotic (Bcl-2, Bcl-X_L, Bcl-w and Mcl-1); (2) pro-apoptotic Bax-like (Bax, Bak, Bok/Mtd and Bcl-X_S); and (3) pro-apoptotic BH3-only (Bad, Bid, Bik/Nbk, Bim_L/Bod, Hrk/DP5, PUMA/Bbc3, BNIP3, Noxa and Bmf) [51] (see Fig. 3). Through interactions between various pro- and anti-apoptotic Bcl-2 family members, calcium and mitochondrial protein release, including that of cytochrome *c*, is regulated.

The reader will note that we mentioned earlier that the two apoptotic pathways would be treated as mutually exclusive. However, at this point we must digress from that statement to provide a clearer picture of the complexity of cross-talk between the two pathways, and how certain members of the Bcl-2 family play a significant and vital role in bridging the two. For example, in response to Fas signals, these two death pathways might cross-talk via the function of cytosolic Bid. The full-length p22 Bid is inactive and is a substrate of caspase-8. Cleavage of p22 Bid gives rise to truncated p7/p15 Bid, exposing a glycine that is *N*-myristoylated, which enables the targeting of a complex of p7 and myristoylated p15 fragments of Bid to the mitochondria [53]. Upon activation, Bid induces intramembranous oligomerization of mitochondrion-resident Bak [54], as well as oligomerization and integration of cytosolic Bax in the outer mitochondrial membrane [55]. Multimers of Bak and Bax form a proposed pore on the mitochondria for cytochrome *c* efflux, thereby inducing caspase activation through the formation of apoptosomes [54, 56–58]. It is, thus, possible for an apoptotic stimulus acting through the extrinsic pathway to induce activation of the intrinsic pathway as well. By contrast, Bcl-2 inhibits apoptosis by preserving mitochondrial membrane integrity. Bcl-2 inserted into the outer mitochondrial membrane may, by a mechanism that has yet to be elucidated, prevent Bax/Bak oligomerization and subsequent release of apoptogenic molecules from the mitochondria [59].

In addition to controlling mitochondrial apoptotic process, Bcl-2 family proteins also regulate apoptosis by affecting calcium homeostasis. The ER is a major organelle involved in intracellular calcium homeostasis and calcium signaling [50]. Calcium released from the ER can induce a prolonged increase in

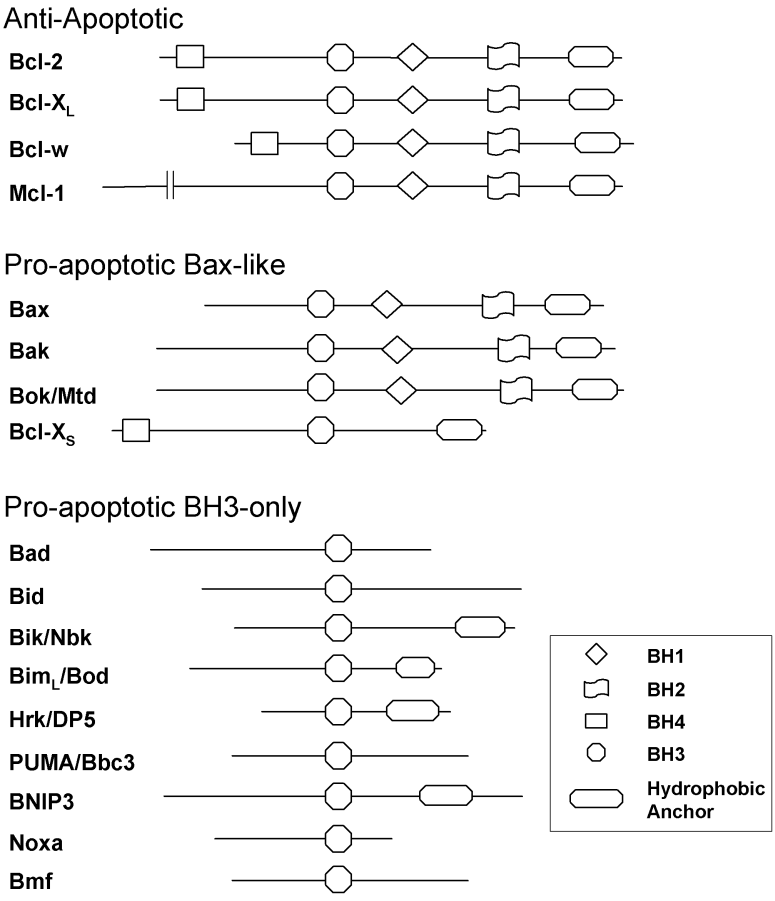

Figure 3. Classification of the Bcl-family. BH refers to Bcl-2 homology domain. The BH3 domain in the pro-apoptotic members is a ligand for the hydrophobic groove formed by the BH1-BH3 domains of the anti-apoptotic members. The hydrophobic C terminus consists of a 17–23-amino acid α-helix that anchors the protein in intracellular membranes.

mitochondrial calcium concentration followed by swelling of the mitochondria and rupture of the mitochondrial network [60]. This increase in mitochondrial free calcium may also be responsible for the release of cytochrome *c* into the cytosol [61]. Released cytochrome *c* can in turn translocate back to the ER where it selectively binds $InsP_3R$, resulting in sustained, oscillatory cytosolic calcium increases, creating a feed-forward loop, amplifying the apoptotic signal [62]. The effect of Bcl-2 on calcium concentration within the lumen of the ER is controversial. Conflicting studies indicating that Bcl-2 overexpression is associated with a decrease in ER luminal calcium contrast with those reporting that Bcl-2 either does not decrease luminal calcium or increases luminal

calcium [50]. However, mouse embryonic fibroblasts deficient of pro-apoptotic proteins Bax and Bak have a much reduced calcium concentration in the ER and are resistant to a variety of apoptotic stimuli [25], suggesting that the Bcl-2 family proteins may play a role in regulating the ER-mitochondria amplification loop of apoptotic signals.

Evading apoptosis: a hallmark of cancer

In malignant tumors, the balance between proliferation and cell death is lost, and defects in apoptosis mechanisms allow neoplastic cells to survive beyond normal levels of stress. Under normal circumstances, defects in DNA repair and chromosome segregation would lead to apoptosis as a defense mechanism for the removal of unstable cells. Clearly, defects in apoptosis would allow these unstable cell populations a survival advantage, providing opportunities for selection of progressively aggressive clones [63] with additional genetic alterations that further deregulate cell proliferation, interfere with differentiation, accelerate angiogenesis, and increase cell motility and invasiveness during tumor progression [64]. Anticancer treatments usually utilize cytotoxic agents and radiation to kill cancer cells causing irreparable cellular damage that, in turn, triggers apoptosis [65]. A major hurdle in cancer therapies is therefore quite apparent: inherent defects in apoptotic pathways render incipient cancer cells resistant to drugs and radiation, thereby requiring higher, more toxic doses for tumor killing, and ultimately contributing to the undesirable side effects of cancer therapy. In recent years, strategies aiming to overcome the aberrant control of apoptosis in cancer cells have become the focus of well-designed, rational anticancer regimens in an effort to increasing the sensitivity of these cells to conventional cytotoxic agents, thereby lowering the toxicity and burden on normal cells. Delineating the underlying mechanisms that cause cancer cells to escape from the apoptotic machinery has therefore been, not surprisingly, the subject of intense research.

p53

p53 is a multi-faceted tumor suppressor gene that is capable of inducing temporary growth arrest and DNA repair, irreversible growth arrest, terminal differentiation, or apoptosis in response to potentially oncogenic cellular stress such as DNA damage [66]. Therefore, it is imperative that functional p53 be present *in vivo* for tumor growth suppression [67]. The function of the p53 gene is lost by mutation in over 50% of human cancer and a loss of heterozygosity often accompanies tumor progression [68, 69]. Unlike many other tumor suppressor genes, more than 85% of p53 mutations result in single amino acid substitutions rather than deletions or frame shifts [70]. Most of the missense mutations occur in the DNA binding core domain (amino acids

102–292) region of p53 that is evolutionarily conserved between p53 and its homologues from *Drosophila* and *C. elegans*. In human tumors, amino acid residues that are essential for contact with DNA target sequence (two repeats of PuPuPuC(A/T)(A/T)GpyPyPy; in which Pu is a purine and Py is a pyrimidine) are frequently found to be mutated [69]. In addition, mutations of residues that do not contact DNA directly but are required for structural maintenance also cause disruption of the p53-DNA interaction. Frequently, mutations in one allele are sufficient to interfere with p53-dependent apoptosis by a dominant negative mechanism since in most cases mutant p53 negates wild-type p53 function through heteromerization.

Under normal conditions, p53 has a short half-life and is maintained at very low levels by Mdm2-mediated degradation [71]. However, in response to stress by DNA damage, hypoxia, oxidative stress and oncogene activation, p53 is stabilized and activated by post-translational modification [69]. In tumor cells, transcriptionally inactive mutant p53 is unable to induce the expression of the Mdm2 protein which would normally provide a feedback mechanism that downregulates p53 protein levels [72]. Moreover, some p53 mutants exhibit lower affinity for association with Mdm2 [73]. Hence, mutant p53 proteins that are impervious to these negative regulations accumulate to high levels in cancer cells and negate the functions of the wild-type protein.

Pathways through which p53 induces apoptosis may involve both transcriptional transactivation and transrepression of multiple p53-target genes, as well as transcription-independent mechanisms that engage the mitochondrial-apoptotic pathways [70]. In general, apoptotic target genes of p53 may be divided into two major categories: (1) proteins acting at the level of receptor signaling for apoptosis, and (2) proteins acting downstream by activating apoptotic effector proteins [74]. The former includes the insulin-like growth factor-1-binding protein 3 (IGF-BP3), which induces apoptosis by blocking the IGF-1 survival signal [75] and Fas/APO-1/CD95, which functions in the T cell killing triggered by anticancer drugs [76]. Essential downstream p53-targeted apoptotic effector proteins are primarily associated with mitochondrial changes, including caspase-9 and its cofactor Apaf-1 in myc oncogene-induced apoptosis [77], and Bax, necessary for p53-mediated cell death in brain tumors [78]. In addition to acting as a regulatory gene coordinating the expression of many proteins involved in apoptosis, recent research also suggests that p53 is involved in mediating apoptosis at the mitochondrial level by directly and physically interacting with the Bcl-2 member Bak, resulting in the release of cytochrome *c* from the mitochondria [79, 80].

Bax, Bak and Bcl-2

The pro-apoptotic proteins Bax and Bak are mediators of mitochondrial membrane damage that are mutated or downregulated in gastric and colorectal cancers [81–83]. In particular, combined mutation of p53 and Bax results in an

extremely aggressive tumor progression and poor clinical prognosis [83]. Bax and Bak were found to be sufficient but not necessary for drug-induced apoptosis [84]. By contrast, increased copy numbers of the anti-apoptotic Bcl-X_L occurs in breast carcinoma, glioblastomas, and Hodgkin lymphoma and other specific tumor types [52]. Similarly, the anti-apoptotic Bcl-2 protein is frequently overexpressed in many tumors including acute lymphoblastic leukemia (ALL), precursor B-lymphoblastic leukemia/lymphoma and diffuse large B cell lymphoma [52]. Bcl-2 is an antagonist to Bax and Bak and inhibits mitochondrial membrane disruption, a mechanism that likely accounts for drug resistance in Bcl-2-overpressing lymphomas [85].

Akt, PI3K and PTEN

The pro-apoptotic Bad is a substrate for Akt/protein kinase B [86] and acts as a negative regulator for other anti-apoptotic family members. Upon phosphorylation by Akt, Bad dissociates from anti-apoptotic Bcl-X_L, allowing it to hinder the progression of apoptosis [87, 88]. Amplification of *akt* has been found in ovarian, pancreatic, breast and gastric malignancies [89, 90] and hyperactivation of Akt is known to induce resistance to a range of apoptotic stimuli including chemotherapeutic drugs [91, 92]. Akt activity can be induced indirectly by Ras in various growth factor receptor-initiated signaling cascades [93]. This Ras-mediated survival signal is connected to the effector phosphatidylinositol 3-kinase (PI3K), a lipid kinase responsible for the activation of Akt [94, 95]. Ras and PI3K are deregulated in many cancers, and the inhibition of PI3K enhances chemotherapeutic drug-induced apoptosis [94, 95]. The PI3K-Akt pathway is negatively regulated by PTEN (phosphatase and tensin homologue deleted on chromosome 10), a lipid phosphatase that inhibits PI3K-induced signaling by dephosphorylating PI3K-generated 3'-phosphorylated phosphatidylinositides [96, 97]. PTEN is frequently mutated in advanced stages of several human tumors, notably in glioblastoma, endometrial and prostate cancers [97], and some PTEN mutations are associated with a higher risk in the development of malignant breast tumors [98].

Death receptors

Fas/APO-1/CD95 and TRAIL-R1/R2 are sensors on the cell surface that, upon binding to their respective ligands, initiate the extrinsic apoptotic pathway (see above). Fas ligand and TRAIL are components of a tumor surveillance mechanism that partakes in the killing of cancer cells by cytotoxic lymphocytes [99, 100]. Tumorigenic disruptions, found in the intrinsic pathway, may also occur in the extrinsic pathway, albeit far less frequently. Fas is observed to be mutated and downregulated in lymphoid and solid tumors [101], whereas TRAIL-R1/R2 is mutated in metastatic breast cancers [102]. Suppression of the death

receptor pathway could allow immune escape and provide a survival advantage to tumor cells. This loss of function is also associated with resistance to drug-induced cell death.

Caspases and non-IAP regulators

Caspase-8 is the initiator caspase for the extrinsic apoptotic pathway, and it is also the mediator for cross-talk between the extrinsic and the intrinsic pathways (see the 'Bcl-2 family' section above). Caspase-8 is silenced through DNA methylation as well as through gene deletion in childhood neuroblastomas, rendering these cancers resistant to apoptosis triggered by death-receptor ligation and by doxorubicin, a chemotherapeutic drug [103]. The expression level of c-FLIP, an endogenous inhibitor to caspase-8, is upregulated in some cancers, thus preventing caspase-8-mediated apoptosis induced by some chemotherapeutic drugs [104]. In the intrinsic pathway, Apaf-1 is necessary for activation of caspase-9 following cytochrome *c* release for the early amplification of apoptotic signals. In malignant melanoma and leukemia cell lines, Apaf-1 is mutated and transcriptionally silenced. Notably, Apaf-1-negative melanomas are chemoresistant, failing to execute typical apoptosis in response to p53 activation [105, 106].

Inhibitors of apoptosis and antagonists

As the only known endogenous proteins that function as direct, physiological inhibitors of both initiator and effector caspases, the IAPs occupy a central position in the apoptotic cascade, representing an important survival factor in resistant cancer [42, 107]. The IAPs, especially XIAP, are frequently overexpressed in the NCI 60 cell line panel of cancer cells as well as in cancer tissues compared to normal tissues [108–112]. Interestingly, at least in breast, colon and pancreatic cancers, a strong positive correlation was found between the levels of XIAP and caspase-3 [113, 114], suggesting that opportunities exist in which the downregulation of XIAP might release caspase-3 inhibition and promote the execution of apoptosis in cancer cells. Indeed, the inhibition of XIAP by antisense oligonucleotides, peptide inhibitors or small-molecule antagonists has been shown to sensitize cancer cells to apoptosis in chemoresistant tumors [108, 115, 116].

The potential therapeutic utility of IAP suppression for cancer treatment had sparked an explosion of research into finding and identifying endogenous IAP antagonists. As discussed in the section 'Inhibitors of apoptosis' above, to date, Smac/DIABLO, Omi/Htra2 and XAF1 are the three negative regulators of IAPs activity. The expression of Smac is decreased in various types of cancer, including lung, prostate and hepatocellular carcinomas [117, 118]. Recombinant adenovirus carrying Smac is able to sensitize ovarian carcinoma

cells to chemotherapeutic drugs cisplatin and paclitaxel [119], whereas the combination of TRAIL and cell-permeable peptides that mimic Smac activity has been shown to eradicate established malignant glioma in mice [120]. Conversely, the elimination of endogenous Omi by RNA interference increases resistance to TRAIL-induced apoptosis [121]. While XAF1 is ubiquitously expressed in normal tissues and cells, it is found at less than 1% of control levels in the majority of the NCI 60 cell line panel of cancer cells [109]. Furthermore, overexpression of XAF1 by adenoviral vector transduction is capable of inducing apoptosis by unblocking caspase-3 and -9 inhibitions in certain pancreatic and colon cancer cell lines [114].

NF-κB

The nuclear factor of κB (NF-κB) family is composed of a number of heterodimeric transcription factors that regulate the expression of over 200 genes that are involved in the control of immune, inflammatory and stress responses, as well as growth and apoptosis [122, 123]. The activity of NF-κB is deregulated in many cancers, notably in B cell lymphomas [124]. Although NF-κB transcriptionally activates both anti- and pro-apoptotic genes, on a balance, NF-κB activation favors the suppression of apoptosis [123]. Key anti-apoptotic genes activated by NF-κB include the IAPs and the anti-apoptotic members of the Bcl-2 families [124, 125]. Since the IAPs and the anti-apoptotic members of the Bcl-2 families are crucial inhibitors to both the extrinsic and intrinsic death pathways, as expected, active NF-κB can inhibit these pathways and induce drug resistance in cancer cells [124].

Therapeutic opportunities

Given that apoptosis suppression is fundamental to cancer cell survival, it is not surprising that components of the apoptotic pathway have emerged as important therapeutic targets. A variety of antisense oligonucleotides, traditional small molecules, biologically active peptides, peptidomimetics, monoclonal antibodies and gene therapy pay loads have been incorporated into strategies that target apoptotic pathways in cancer cells [64, 126–130]. Although factors such as unexpected toxicities, poor pharmacokinetics, stability and oral bioavailability may limit the use of these compounds in anticancer treatment, these apoptosis-based antitumor agents might still serve as precursor molecules for the development of more effective therapies.

The importance of Bcl-2 in tumor cells resistant to most cytotoxic anticancer drugs has propelled this anti-apoptotic gene to the forefront as a candidate for antisense oligonucleotides (ASONs)-based therapies. ASONs are short pieces of DNA that hybridize to a specific target mRNA, thereby blocking its translation to a functional protein. *In vitro* experiments and xenograft

models have demonstrated that Bcl-2 ASONs chemosensitizes human cancer cells [131, 132]. In a phase I clinical trial, a combination of Bcl-2 ASON and mitoxantrone has been shown to be well tolerated in combination [133]. In fact, ASONs targeting the Bcl-2 mRNA have advanced to phase II clinical trials for a variety of solid tumors, and phase III for melanoma, myeloma, chronic lymphocytic leukemia and acute myeloid leukemia [64]. One concern for targeting Bcl-2 alone is the ability of some tumor cells to switch expression from Bcl-2 to Bcl-X_L, thereby potentially retaining their apoptosis resistance [134]. Therefore, the simultaneous inhibition of Bcl-2 and Bcl-X_L expression in tumors by a single bi-specific ASON [135] or by small-molecule antagonists [136] may represent an appealing approach in certain cancers. Alternatively, inducing the expression of pro-apoptotic Bax with p53 adenovirus is a potentially useful gene therapy, particularly in human brain tumors [137]. In addition, short peptides that represent the BH3 domains of Bid or Bim have been shown to be capable of inducing oligomerization and activation of Bak and Bax, promoting killings of leukemic cells [138].

By virtue of their anti-caspase activity, the IAPs serve as pivotal regulators of the core apoptotic machinery, thereby representing another promising target for enhancing the re-activation of the death program. Numerous proof-of-principle studies have demonstrated that the downregulation of XIAP leads to enhanced chemotherapy sensitivity in various types of cancer cells [42, 64, 139]. For example, in both *in vitro* and *in vivo* xenograft human lung cancer models, ASONs targeting XIAP induce apoptosis and enhance chemotherapeutic activity [140]. These validations for XIAP as an important gate keeper to the apoptosis cascade have led to the launching of phase I clinical trials of an XIAP-specific ASON designed to stimulate apoptosis in cancer cells [141]. An alternative approach to suppress IAP function utilizes short peptides or small molecules that mimic IAP antagonists. In an intracranial malignant glioma xenograft model *in vivo*, synthetic peptides that mimic IAP antagonists Smac and HtrA2 are able to induce complete regression of the tumors caused by TRAIL-mediated apoptosis without detectable toxicity to normal brain tissue [120]. Similarly, non-peptidyl small-molecule XIAP antagonists screened from combinatorial chemical libraries have been shown to sensitize cancer cells to chemotherapeutic drugs and to suppress growth of established tumors in xenograft models in mice, while displaying little toxicity to normal tissues [116, 142]. Clearly, the effective tumor suppression activities of these IAP antagonists warrant further studies into their applicability in anticancer regimens.

Conclusions

Cancer is the consequence of parallel pathways that lead to both inappropriate cell proliferation and aberrant control of apoptosis. The inherent suppression of apoptosis in cancer cells has emerged to be a fundamental mechanism of tumor formation, progression and resistance to therapy. Advances made in elu-

cidating the underlying mechanisms for the inhibition to apoptosis in tumor cells have identified important therapeutic targets and facilitated the development of novel strategies for resensitizing cancer cells to apoptosis. As evident in the leaps and bounds made in our understanding of apoptosis in cancer, clinical trials in progress are employing new approaches that are designed to directly modulate key apoptosis regulators. We anticipate that future advances will continually be made to these rational molecular approaches, such that apoptosis-based cancer therapies will match the diversity of the disease itself. Although much remains to be learned regarding apoptosis in cancers as well as other aspects of resistance and tumorigenesis, progress made to date indeed justifies our optimism that eradicating this disease will be a reality.

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