Minireview

Why Target Apoptosis in Cancer Treatment?

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Abstract
Defective apoptosis (programmed cell death) represents a major causative factor in the development and progression of cancer. The ability of tumor cells to evade engagement of apoptosis can play a significant role in their resistance to conventional therapeutic regimens. Our understanding of the complexities of apoptosis and the mechanisms evolved by tumor cells to resist engagement of cell death has focused research effort into the development of strategies designed to selectively induce apoptosis in cancer cells. This article will review the underlying mechanisms of apoptosis and the ways in which tumor cells modulate these processes to promote their survival and evaluate the efficacy of current clinical approaches aimed at exploiting these defects to selectively induce apoptosis in tumor cells.

Introduction
Until relatively recently, the underlying cause of cancer was attributed to accelerated or dysregulated proliferation leading to cellular expansion and accumulation of tissue mass. Without question, key regulators of the cell cycle are frequently altered in many tumor types, with a consequent impact on elements of proliferative control such as cell cycle checkpoints and the response to DNA damage. For that reason, many current chemotherapeutic strategies are designed to exploit such abnormalities to induce cytotoxicity and tumor regression or cytostasis to halt tumor progression. Abnormal proliferative capacity, however, is only part of the picture. Recent progress has broadened our understanding of cancer and its underlying etiology to encompass aberrant cellular survival, as a consequence of failing to appropriately induce apoptosis or cell death, as a major contributor to the transformed state.

The recognition that aberrant apoptosis may constitute a major clinical hurdle to overcome in the treatment of cancer has spawned a variety of strategies aimed at exploiting this pathway in the hope of being able to trigger tumor-selective cell death. The goal of any therapeutic strategy is to impact on the target tumor cells with limited detrimental effect to normal cell function. It is often the case that perturbation of tumor cell function so severely compromises normal cellular homeostasis that therapeutic intervention is of limited clinical value. The challenge lies in the fact that all normal cells also have the capacity to engage the apoptotic program and, furthermore, often do so more readily than their tumorigenic counterparts. The problem of attaining drug selectivity is no more clearly demonstrated than by many of the conventional chemotherapeutics that are designed to exploit the accelerated proliferative response seen in many tumor types (1), with devastating consequences for healthy proliferating cells. So can we really hope to exploit apoptosis as a therapeutic strategy and achieve tumor-selective killing without compromising normal cell function? In principle, that may be possible if we can exploit the expression and/or function of apoptotic-related molecules that are exclusively inherent to maintaining tumor cell function or molecules that are regulated in a different manner in tumor cells and in normal cells.

Apoptosis is just one form of cell death. Cells may be eliminated by a number of alternative mechanisms including necrosis. Necrosis is typically described as a “nonspecific” form of cell death, characterized by rupture of the plasma membrane with a consequent localized inflammatory response and damage to surrounding cells and tissues (2). In contrast, apoptosis is associated with the rapid engulfment and removal of cell corpses by phagocytic cells that recognize “eat-me” signals displayed on the outer surface of the apoptotic cell (3). Furthermore, apoptotic cell death is the consequence of a series of precisely regulated events that are frequently altered in tumor cells. This provides the opportunity for selective clinical intervention to bring about the death of the tumor cell without damage to normal cells. These basic differences between these two processes of cell death underscore the reason why apoptosis, and not necrosis, represents the most desirable target mechanism for the induction of cell death in tumor cells.

Cancer and Apoptosis: First Principles
Cancer is a highly heterogenous disease, arising from multiple tissue types and displaying great genetic diversity. However, recent insight has encouraged some to suggest that the underlying etiology and progression of the disease can be reduced to two lesions, mutations that give rise to excessive proliferation and a compensatory disruption of survival signaling pathways that ensures the persistence of these hyperproliferative cells (4). This theory forges the fundamental link between neoplasia and apoptosis, as exemplified by the ability of oncogenes including Myc, and tumor suppressors such as p53 to actively engage apoptosis. The ability of Myc to drive apoptosis in addition to providing a potent proliferative signal is interpreted as a failsafe mechanism to offset its oncogenic capacity (5). Although this

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mechanism seems to function most of the time, as it should, it creates a selective pressure for a "would-be" tumor cell expressing high levels of Myc to acquire compensatory mechanisms to suppress myc-driven cell apoptosis. Likewise, p53, which can engage apoptosis in response to stresses such as DNA damage to avert the potential acquisition of lethal mutations, is frequently mutated or deleted in tumor cells. Either scenario, the functional compensation for or the deletion of proapoptotic signals, generates a tumor cell with a distinct survival advantage over normal cells. Can we exploit this to our advantage for the successful treatment of cancer rather than allowing it to hinder our progress with conventional chemotherapeutics? Hope may lie in strategies designed to disengage the survival-promoting mechanism such that any tumor cell with a lesion driving accelerated proliferation will instead drive itself into apoptosis or to reintroduce the capacity of proapoptotic molecules to effectively engage the cell death machinery.

Central Biochemical Machinery and Pathways to Death

The unifying features of apoptosis, irrespective of cell type and inducing stimulus, are largely morphological and include chromatin condensation, DNA fragmentation, blebbing of the plasma membrane, and cell shrinkage. The underlying mechanistic basis for these changes focuses on the activity of aspartate-specific cysteine proteases, the caspases (cysteinyl, aspartate-specific proteases), which cleave to inactivate or activate target substrates within a cell.

Caspases, totaling 14 family members to date, are synthesized as inactive zymogens, which must be proteolytically cleaved at two (or three in some cases) aspartate residues to generate the active mature enzyme. These cleavage events remove an NH_2-terminal peptide and separate the small and large subunits of the proenzyme to generate a mature heterotetrameric caspase comprising two large and two small subunits. The generation of active caspases forms a cascade in which "initiator" caspases interact with specific adapter molecules to facilitate their own autoprocessing. These now active initiator caspases in turn cleave and activate the downstream "executioner" caspases. These then cleave their target substrates to orchestrate the proteolytic dismantling of the cell (6–9).

This sequence of events culminating in the activation of caspases has been broadly categorized into two pathways, the "extrinsic" pathway characterized by the engagement of cell surface "death receptors" and the "intrinsic" pathway involving key mitochondrial events. In both cases, an initiator caspase, via its interaction with a specialized adapter molecule, mediates its self-activation and, ultimately, activation of the downstream effector or executioner caspases. It is the activity of these caspases that ensure destruction of the cell.

Death Receptor-mediated Apoptosis: The Extrinsic Pathway

The extrinsic pathway to caspase activation can be mediated by one of several death receptors when bound by the appropriate ligand (10). Currently, the most clearly understood of these pathways is interaction between the Fas receptor and its ligand, FasL,[2] and activation of the TNF-R1 by TNF. In both cases, the ligand binds to and induces trimerization of its receptor [although these receptors may also exist as preformed trimers in some cases (11, 12)]. This initial binding triggers a series of events, the physiological consequence of which is governed by the differential recruitment of specialized adapter molecules that selectively engage downstream signaling events.

Two major classes of adapter molecules have been described: TRAFs and DD-containing molecules. The selective recruitment of these molecules is determined according to whether the receptor itself contains a DD or TRAF binding motif. Both the TNF-R1 and the Fas receptors contain DDs and recruit the DD-containing adapter molecules TNF-R1-associated DD (TRADD) and FADD, respectively. In the case of Fas, the homotypic interaction between the DD of Fas and that of FADD induces the recruitment of procaspase-8 via interaction between death effector domains in FADD and the prodomain of the caspase. The FADD-bound dimer of procaspase-8 then autoactivates to release the active enzyme, which in turn, cleaves and activates the downstream effector caspases, caspases-3 and –7, which cleave their target substrates to bring about the death of the cell.

TNF is also able to trigger apoptosis, but it can do so by more than one route, and whereas our understanding of these events is extensive, it is by no means complete. The most commonly described pathway of TNF-induced apoptosis is regulated by TRADD-mediated recruitment of FADD to the TNF-R complex. In turn, FADD associates with procaspase-8 to initiate its cleavage, much like the scenario for Fas/FasL interaction as described above. However, FADD-deficient cells can still die in response to TNF, suggesting that an alternative apoptotic mechanism also exists.

The regulation of these pathways is complex, not least because activation of the TNF-R1 can trigger not only cell death but also NF-κB and JNK-mediated signaling events (13). This culminates in a myriad of physiological responses including enhanced cellular survival, which may be mediated in part by the transcriptional up-regulation of antiapoptotic molecules such as IAPs and TRAF-1 and TRAF-2 that inhibit caspase-8 activity (Ref. 14; see below). Other molecules that can directly regulate death receptor-mediated apoptosis include FLIP, an inactive procaspase-8-like molecule that can bind to the Fas receptor complex to inhibit the activation of caspase-8, and silencer of death domains (SODD), which binds to the TNFR-1 to prevent spontaneous receptor trimerization (15).

Mitochondria-mediated Apoptosis: The Intrinsic Pathway

The intrinsic mitochondrial pathway is characterized by the rapid release of cytochrome *c* from the mitochondrial inter-

[2] The abbreviations used are: FasL, Fas ligand; TNF, tumor necrosis factor; TNF-R, TNF receptor; DD, death domain; TRAF, TNF-R-associated factor; FADD, fas-associated DD; NF-κB, nuclear factor κB; JNK, c-Jun NH_2-terminal kinase; IAP, inhibitors of apoptosis protein; BH, Bcl homology; Hsp, heat shock protein; VEGF, vascular endothelial growth factor.

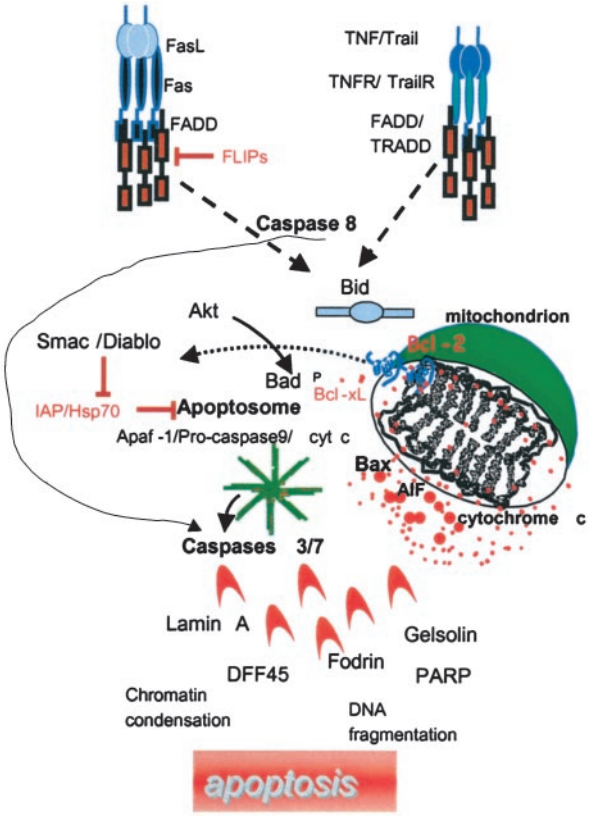

Fig. 1. Schematic of apoptosis pathways.

membrane space into the cytosol. Few would dispute that this event is absolutely required for caspase-dependent cell death (at least in mammalian cells), with the exception of granzyme B- and death receptor-induced caspase activation that can proceed independently of mitochondrial involvement (16). Although the precise mechanism of cytochrome *c* release remains unclear, it requires permeabilization of the outer mitochondrial membrane, proceeds in the absence of caspase activity, is associated with a loss of outer mitochondrial membrane potential [$\Delta\psi M$ (17)], and is tightly regulated by the pro- and antiapoptotic members of the Bcl-2 family (see below; Ref. 18–20).

Once released into the cytosol, cytochrome *c* binds to an adapter protein, Apaf-1, which, in the presence of dATP/ATP, self-oligomerizes to form an Apaf-1 multimer (21, 22). The Apaf-1 complex then recruits procaspase-9 via interaction between specialized CARDs (caspase recruitment domains) at the NH_2 termini of Apaf-1 and procaspase-9 (23). Formation of the "apoptosome" complex functions to bring several molecules of procaspase-9 into close proximity and, in doing so, promotes its self-processing (24). Active caspase-9 then recruits and cleaves procaspase-3, which is then released into the cytosol to effect the proteolytic degradation of its target substrates (Fig. 1).

In addition to cytochrome *c*, several other molecules are released from the mitochondria in response to apoptotic stimuli that have a variety of roles in the apoptotic cascade.

Table 1 Molecules with roles in the apoptotic cascade

Protein	Function	Ref. no.
AIF (apoptosis inducing factor)	Proapoptotic factor required for cell death during cavitation in embryonic development	25
Endo G	Induces DNA fragmentation	26
HtrA2/Oma	Death-inducing serine protease	27
Smac/DIABLO	Reverses caspase inhibition by IAPs	28 and 29
Hsp60/10	Promotes caspase activation	30 and 31
Procaspase	Promotes apoptosis	32

These are summarized in Table 1. It remains unclear whether mechanisms exist to selectively release one or more of these molecules, or whether permeabilization of the mitochondrial membrane permits the nonselective release of multiple proteins to regulate the apoptotic cascade.

"Upstream" Regulators of the Mitochondrial Pathway

Both the release and activity of cytochrome *c* are under strict regulation by several different mechanisms. These are broadly categorized into upstream regulators that impact on the release of cytochrome *c* and "downstream" events that regulate the biological consequences of its release.

One of the primary regulators of the mitochondria-mediated pathway to apoptosis is the family of Bcl-2 proteins (19, 20). These proteins are broadly characterized into proapoptotic members that include Bax, Bak, Bik, Bad, and Bid and antiapoptotic proteins such as Bcl-2 and Bcl-x_L, based on their ability to either suppress or induce the release of cytochrome *c*. Despite their broadly opposing activities, all members of the Bcl-2 family contain at least one of the four so-called BH domains (BH1–BH4); a subset of the proapoptotic members, including Bad, Bid, and Bim, contain only the BH3 domain. The BH3 domain mediates interaction between various members of the Bcl-2 family to generate a number of heterodimers and homodimers. BH3-only proteins, *e.g.,* Bid, function to facilitate the activity of proapoptotic family members such as Bax and Bak (33, 34) and represent the target for the antiapoptotic function of Bcl-2 and Bcl-x_L (35, 36). Bax-mediated release of cytochrome *c* coincides with its translocation to the mitochondria (37) and the formation of large multimers that have been suggested to represent the formation of pores in the outer mitochondrial membrane through which cytochrome *c* and other apoptosis-inducing factors may exit into the cytosol (38). The precise nature of the Bax-mediated opening in the outer mitochondrial membrane remains unclear. Interaction between Bax and the adenine nucleotide translocator as part of the permeability transition pore complex (39) and interaction between Bax and the voltage-dependent anion channel (40) are just two proposed mechanisms by which Bax can regulate the release of cytochrome *c* from the inter mitochondrial membrane space. However, one recent report indicates that Bax can function in the absence of any additional protein component but instead is dependent upon the lipid cardiolipin (41). It is the relative amounts of pro- and antiapoptotic proteins that determine cellular sensitivity to damage and, ultimately, whether a

cell will live or die. Recent studies indicate that this may be regulated, at least in part, by ubiquitin-dependent proteasomal degradation (42).

Apaf-1-independent Cell Death

A number of recent studies have defined a form of cell death that proceeds independently of Apaf-1 and caspase activation but is subject to regulation by factors typically associated with the apoptotic cascade. Following a variety of apoptotic stimuli, apoptosis inducing factor (AIF) is released from mitochondria via a mechanism regulated by Bcl-2 (25) and engages a form of cell death characterized by a number of biochemical and morphological alterations, none of which are altered by the caspase inhibitor zVAD-fmk (43). These observations appear to define a regulated form of cell death that retains certain features of apoptosis but cannot be classified as necrosis. Recent descriptions of an alternative Apaf-1-independent but caspase-dependent form of cell death also imply the existence of a novel mechanism for the activation of caspases (44–46). These studies describe one or more forms of Apaf-1-independent death engaged by specific stimuli, including endoplasmic reticulum stress (46), serum withdrawal (44) that can be suppressed by Bcl-2 (44), caspase inhibitors, and significantly attenuated by the overexpression of catalytically inactive forms of caspases-9 and -12 (46). This series of observations indicates a complexity of caspase-dependent cell death that is not currently understood.

"Downstream" Regulators of the Mitochondria Pathway

One viewpoint held is that once a cell has released its cytochrome *c*, it is doomed to die. That said, why, if mitochondrial events irrevocably commit a cell to die, are there regulatory mechanisms downstream of cytochrome *c* release that halt the apoptotic process? Examples include the family of IAPs, some of which can bind and inactivate caspases (47). This may be attributed to the ability of certain IAPs to promote the ubiquitination and proteasomal degradation of the associated caspase (48). The potential of IAPs as targets for anticancer therapy was highlighted by the identification of Smac/DIABLO, a protein that binds and inhibits IAPs and, by doing so, releases active caspases that are then able to mediate apoptosis. Small peptides from the NH_2 terminus of Smac/DIABLO are sufficient to displace IAPs from their target caspases, suggesting that small molecule design to disrupt such interactions in drug-resistant tumor cells may be possible (49). The Hsps are reported to mediate inhibition of the apoptotic cascade at points downstream of the release of cytochrome *c*. These include sequestration of cytochrome *c* by Hsp27, inhibition of Apaf-1 oligomerization by Hsp70 and Hsp90, and prevention of procaspase-9 recruitment to Apaf-1 by Hsp70 (50). Interestingly, many tumor types express elevated levels of Hsps that often correlate with a drug-resistant phenotype. It remains to be seen whether one or more of the Hsps represent feasible targets for the successful treatment of cancer.

Intracellular Signaling Pathways, Cancer, and the Regulation of Apoptosis

Although a variety of intracellular signaling events are activated in response to apoptotic stimuli, their precise role in regulating the downstream core apoptotic machinery is unclear. Attempts to integrate the complexity of intracellular signaling events with the regulation of apoptosis have generated a confusing number of possibilities, some or all of which may be physiologically relevant.

Activation of the JNK signaling pathway is frequently observed in association with the engagement of apoptosis. Whether JNK activation mediates a proapoptotic role or favors enhanced cellular survival is unclear (51, 52). However, a number of apoptotic molecules are targets for JNK-mediated phosphorylation, including p53 (53, 4), c-Myc (55), and the antiapoptotic Bcl-2 proteins, Bcl-2 and Bcl-x_L (56). Each of these proteins is capable of regulating the release of cytochrome *c*, a key event in the activation of caspases, and one reported to depend upon the activity of JNK (57).

Apoptosis is negatively regulated by the activity of the phosphatidylinositol 3′-kinase to Akt kinase signaling cascade. This pathway is readily engaged by a number of survival factors including insulin-like growth factor I and platelet-derived growth factor and functions as a potent prosurvival signal in a variety of tumor types. The elevation of Akt activity in transformed cells may be attributed, at least in part, to the frequently observed accompanying mutations in PTEN, a phosphatase that opposes the activity of Akt. The precise mechanism of Akt-mediated survival is unclear but appears to involve the regulation of one or more apoptotic-related proteins (58). For example, Akt-mediated phosphorylation of Bad, a proapoptotic member of the Bcl-2 protein family, promotes the dissociation of Bad from Bcl-x_L and, instead, its binding to 14-3-3 (59).

Akt-mediated phosphorylation can also impact on the transcriptional regulation of apoptosis. For example, Akt-mediated phosphorylation and inactivation of forkhead (FKHRL1) serve to limit the transcription of its target genes including FasL, insulin-like growth factor-binding protein (IGF-BP1; Ref. 60), and Bim (61), all of which function to promote apoptosis. In contrast, Akt is able to potentiate the activity of NF-κB by accelerating the degradation of its endogenous inhibitor, IκB (62). This can lead to an elevation in the expression of its target genes including the antiapoptotic Bcl-2 protein A1 (63), TRAF1 and TRAF-2, and the caspase inhibitors c-IAP1 and c-IAP2 (14, 64). Such activities of NF-κB may explain the frequently observed elevation of constitutive NF-κB activity in many tumor cells (65).

Oncogenes, Tumor Supressors, and Apoptosis

Our current understanding of how transformation is able to proceed focuses on the opposing activities of oncogenes such as Myc, which drive relentless cellular proliferation, and tumor supressors (for example, p53), which can counter the consequences of these oncogenes by engaging apoptosis (see "Cancer and Apoptosis: First Principles"). In an effort by the cell to limit the devastating consequences of uncontrolled oncogene-driven proliferation, oncoproteins including

Myc, Ras, and E2F also have the capacity to induce apoptosis, often via a p53-dependent mechanism.

Myc can influence apoptosis via a number of different routes (66, 67). These include the induction of p19ARF, an inhibitor of the p53 functional suppressor Mdm-2, leading to the induction of p53 and consequent apoptosis (68). In addition, Myc can induce death ligand expression (67, 69) or sensitize cells for the induction of apoptosis by Fas, TNF, and TNF-related apoptosis-inducing ligand (TRAIL) death receptors and via the mitochondrial-mediated pathway to apoptosis by elevating cellular sensitivity to BH3-containing proteins and the consequences of their ability to induce cytochrome *c* release (70, 71).

p53 represents the most well characterized of tumor supressors with a clear role in the induction of apoptosis or cellular arrest in response to stresses such as DNA damage. As such, this gene is frequently mutated in cancers, thereby inactivating the protective proapoptotic role of p53 and contributing to the drug-resistant phenotype. Although it is clear that p53 is able to induce apoptosis, the precise mechanism remains unclear, with both transcriptional-dependent and -independent mechanisms being attributed to its ability to induce cell death (72–74).

In keeping with its role as a transcription factor, p53 is able to regulate the expression of a number of genes that have a direct role in regulating cellular sensitivity to apoptosis via both the death receptor signaling pathway and mitochondria-mediated events. These include Bax, Noxa, a BH3-containing proapoptotic protein that interacts with Bcl-2 (75), p53AIP1 (p53 apoptosis-inducing protein) (76), Fas, DR5, and Pidd, a novel death domain containing protein (77).

Apoptosis Modulators: Potential Drug Targets in the Death Pathway

As discussed, the development of tumors arises as a consequence of dysregulated proliferation and a suppression of apoptosis, and each of these primary defects provides an obvious opportunity for clinical intervention. Many of the current chemotherapeutics designed to perturb proliferation do so in such a crude manner that the resulting damage to normal cells limits their clinical efficacy. However, targeting and overcoming abnormalities in tumor cells that suppress apoptosis could generate a potent proapoptotic stimulus by virtue of the growth-deregulating mutations, for example Myc, which can drive apoptosis.

Reversal of the malignant phenotype by the introduction and/or restoration of wild-type p53 function constitutes the strategy of several pharmacological-based and gene therapy-focused approaches. Replacement of wild-type p53 using both viral and nonviral delivery systems, although promising in preclinical studies, has fallen short of expectation in the clinic as a consequence of limited target specificity, inefficient delivery, and adverse immune reaction (78). A number of small molecule-based approaches designed to modify the function of p53 include peptides and antibodies able to revert the conformation of mutant p53 to that of the wild type and, in doing so, restoring its transcriptional competence (79–81). The activity of p53 is negatively regulated by its association with Mdm-2, an E3 ubiquitin ligase that promotes the rapid proteasomal degradation of p53. Attempts to exploit this regulatory pathway to prolong the half-life of p53 include antisense treatment to reduce the levels of Mdm-2 and small peptides designed to disrupt the interaction between p53 and Mdm-2 (82).

The Bcl-2 proteins also represent a promising target for modulating tumor cell sensitivity to apoptosis (83). Overexpression of antiapoptotic Bcl-2 proteins is observed in many tumor types, which may contribute to the drug-resistant state and help mediate the expansion of a transformed population by disrupting normal cell turnover. In theory, overcoming a blockade induced by Bcl-2 or Bcl-x_L would restore normal cellular homeostasis, reverse the drug-resistant phenotype, and restore tumor cell sensitivity to conventional chemotherapeutics. Clinical success using antisense oligonucleotides (84) and antibodies against Bcl-2 has been reported (85, 86). Additional small molecule-based strategies for disrupting the activities of antiapoptotic Bcl-2 proteins have arisen from our extensive understanding of the structures of these proteins. These include the design of small molecules able to bind to and inactivate the hydrophobic pocket in Bcl-2 and Bcl-x_L, which is essential for its antiapoptotic function and which houses the docking site for the death-promoting members of the family (Bax, Bak, and Bad) via their BH3 domains (87). Alternative approaches include the use of BH3 peptides (88) or molecules that mimic BH3 domains to induce apoptosis (89).

The complexity of multiple kinase-mediated pathways that modulate cellular survival and the frequent dysregulation of these pathways in transformed cells have directed rational drug design toward a number of potential therapeutic tools designed to impact on these pathways. However, to date, the success of such strategies has been thwarted by disappointingly inefficient tumor regression, patient relapse, and associated tumor resistance. As a consequence, effort is often geared toward maximizing drug efficacy by the use of combination therapy. The outlook for the success of rational drug design, however, is not all bleak. Inhibitors of Bcr-Abl tyrosine kinase, the constitutive activation of which manifests in chronic myelogenous leukemia, showed extreme promise when first introduced (90). That said, more recent follow-up studies suggest that the long-term clinical use of this inhibitor is associated with the development of clinical resistance, such that its use may instead be confined to combination therapy (91). Receptor tyrosine kinases and their ligands also have important roles in tumor angiogenesis, the process by which new vasculature is developed, and one that is often dysregulated in tumors. VEGF is the primary endothelial growth factor whose activity is mediated by two high affinity receptors, VEGF receptor 1/FLT-1 and VEGF receptor 2/FLK-1/KDR. Inhibitors of the tyrosine kinases involved in tumor angiogenesis represent another optimistic anticancer therapy.

As highlighted earlier, one of the most conserved biochemical features of apoptotic cell death is the activation of caspases. Once activated, these destructive proteases proceed to systematically dismantle the cell to ensure its effective removal without damage to surrounding cells and tissue. Caspase activation represents the culmination of a sequence

of events and, by definition, the bypass of any inhibitory mechanisms that may have developed in tumor cells to arrest the progression of this cascade of events.

The rationale for discovery of novel therapeutics to overcome the above-mentioned growth advantage mechanisms and be able to activate caspases may therefore represent a possible approach for effective tumor treatment that has several advantages over both conventional therapies and the more current "designer" approaches. In theory, the activation of caspases can result from intervention at numerous points in the apoptotic cascade, and such an approach broadens the focus to encompass molecules that can target Bcl-2 protein function alongside those that can preferentially overcome the prosurvival effects of Akt, for example. The only scenario in which the activation of caspases, either directly or indirectly, may not induce the death of the cell is if there are protective mechanisms downstream of caspase activation. To date, there have been no convincing examples of long-term cell rescue once caspases have been activated.

The hope for a successful resolution to the design of agents that effectively induce tumor regression lies, at least in part, in the need to overcome the inherent resistance of many transformed cells to the engagement of apoptosis. We hope that rising insights into the field of apoptosis and cancer will uncover new and effective strategies to tackle the complexity of tumor chemoresistance.

References

1. Shapiro, G. I., and Harper, J. W. Anticancer drug targets: cell cycle and checkpoint control. J. Clin. Investig., *104:* 1645–1653, 1999.

2. Kroemer, G., Dallaporta, B., and Resche-Rigon, M. The mitochondrial death/life regulator in apoptosis and necrosis. Annu. Rev. Physiol., *60:* 619–642, 1998.

3. Henson, P. M., Bratton, D. L., and Fadok, V. A. Apoptotic cell removal. Curr. Biol., *11:* R795–R805, 2001.

4. Green, D. R., and Evan, G. I. A matter of life and death. Cancer Cell, *1:* 19–30, 2002.

5. Evan, G. I., and Vousden, K. H. Proliferation, cell cycle and apoptosis in cancer. Nature (Lond.), *411:* 342–348, 2001.

6. Nicholson, D. Caspase structure, proteolytic substrates, and function during apoptotic cell death. Cell Death Differ., *11:* 1028–1042, 1999.

7. Stennicke, H., and Salvesen, G. Caspases: controlling intracellular signals by protease zymogen activation. Biochim. Biophys. Acta, *1477:* 299–306, 2000.

8. Hu, S., Snipas, S. J., Vincenz, C., Salvesen, G., and Dixit, V. M. Caspase-14 is a novel developmentally regulated protease. J. Biol. Chem., *273:* 29648–29653, 1998.

9. Ahmad, M., Srinivasula, S. M., Hegde, R., Mukattash, R., Fernandes-Alnemri, T., and Alnemri, E. S. Identification and characterization of murine caspase-14, a new member of the caspase family. Cancer Res., *58:* 5201–5205, 1998.

10. Locksley, R. M., Killeen, N., and Lenardo, M. J. The TNF and TNF receptor superfamilies: integrating mammalian biology. Cell, *104:* 487–501, 2001.

11. Siegel, R. M., Frederiksen, J. K., Zacharias, D. A., Chan, F. K., Johnson, M., Lynch, D., Tsien, R. Y., and Lenardo, M. J. Fas preassociation required for apoptosis signaling and dominant inhibition by pathogenic mutations. Science (Wash. DC), *288:* 2354–2357, 2000.

12. Chan, F. K., Chun, H. J., Zheng, L., Siegel, R. M., Bui, K. L., and Lenardo, M. J. A domain in TNF receptors that mediates ligand-independent recpetor assembly and signaling. Science (Wash. DC), *288:* 2351–2354, 2000.

13. Baud, V., and Karin, M. Signal transduction by tumor necrosis factor and its relatives. Trends Cell Biol., *11:* 372–377, 2001.

14. Wang, C. Y., Mayo, M. W., Korneluk, R. G., Goeddel, D. V., and Baldwin, A. S. J. NF-κB anti-apoptosis induction of TRAF-1 and TRAF-2 and c-IAP1 and c-IAP2 to suppress capase-8 activation. Science (Wash. DC), *281:* 1680–1683, 1998.

15. Jiang, Y., Woronicz, J. D., Liu, W., and Goeddel, D. V. Prevention of constitutive TNF receptor 1 signaling by silencer of death domains. Science (Wash. DC), *283:* 543–546, 1999.

16. MacDonald, G., Shi, L., Vande Velde, C., Lieberman, J., and Greenberg, A. Mitochondria-dependent and -independent regulation of granzyme B-induced apoptosis. J. Exp. Med., *189:* 131–144, 1999.

17. Waterhouse, N., Goldstein, J., von Ahsen, O., Schuler, M., Newmeyer, D., and Green, D. Cytochrome *c* maintains mitochondrial transmembrane potential and ATP generation after outer mitochondrial membrane permeabilization during the apoptotic process. J. Cell Biol., *153:* 319–328, 2001.

18. Martinou, J., and Green, D. Breaking the mitochondrial barrier. Nat. Rev. Mol. Cell. Biol., *2:* 63–67, 2001.

19. Green, D. R., and Reed, J. C. Mitochondria and apoptosis. Science (Wash. DC), *281:* 1309–1312, 1998.

20. Gross, A., McDonnell, J. M., and Korsmeyer, S. J. BCL-2 family members and the mitochondria in apoptosis. Genes Dev., *13:* 1899–1911, 1999.

21. Zou, H., Henzel, W., Liu, X., Lutschg, A., and Wang, X. Apaf-1, a human protein homologous to *C. elegans* CED-4, participates in cytochrome *c*-dependent activation of caspase-3. Cell, *90:* 405–413, 1997.

22. Zou, H., Li, Y., Liu, X., and Wang, X. An APAF-1·cytochrome *c* multimeric complex is a functional apoptosome that activates procaspase-9. J. Biol. Chem., *274:* 11549–11556, 1999.

23. Li, P., Nijhawan, D., Budihardjo, I., Srinivasula, S., Ahmad, M., Alnemri, E., and Wang, X. Cytochrome *c* and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. Cell, *91:* 479–489, 1997.

24. Srinivasula, S. M., Ahmad, M., Fernandes-Alnemri, T., and Alnemri, E. S. Autoactivation of procaspase-9 by Apaf-1-mediated oligomerization. Mol. Cell, *1:* 949–957, 1998.

25. Susin, S., Lorenzo, H., Zamzami, N., Marzo, I., Snow, B., Brothers, G., Mangion, J., Jacotot, E., Costantini, P., Loeffler, M., Larochette, N., Goodlett, D., Aebersold, R., Siderovski, D., Penninger, J., and Kroemer, G. Molecular characterization of mitochondrial apoptosis-inducing factor. Nature (Lond.), *397:* 441–446, 1999.

26. Li, L., Luo, X., and Wang, X. Endonuclease G is an apoptotic DNase when released from mitochondria. Nature (Lond.), *412:* 95–99, 2001.

27. Suzuki, Y., Imai, Y., Nakayama, H., Takahashi, K., Takio, K., and Takahashi, R. A serine protease, HtrA2, is released from the mitochondria and interacts with XIAP, inducing cell death. Mol. Cell, *8:* 613–621, 2001.

28. Du, C., Fang, M., Li, Y., Li, L., and Wang, X. Smac, a mitochondrial protein that promotes cytochrome *c*-dependent caspase activation by eliminating IAP inhibition. Cell, *102:* 33–42, 2000.

29. Verhagen, A., M., Ekert, P. G., Pakusch, M., Silke, J., Connolly, L. M., Reid, G. E., Moritz, R. L., Simpson, R. J., and Vaux, D. L. Identification of DIABLO, a mammalian protein that promotes apoptosis by binding to and antagonizing IAP proteins. Cell, *102:* 43–53, 2000.

30. Xanthoudakis, S., Roy, S., Rasper, D., Hennessey, T., Aubin, Y., Cassady, R., Tawa, P., Ruel, R., Rosen, A., and Nicholson, D. Hsp60 accelerates the maturation of pro-caspase-3 by upstream activator proteases during apoptosis. EMBO J., *18:* 2049–2056, 1999.

31. Samali, A., Cai, J., Zhivotovsky, B., Jones, D., and Orrenius, S. Presence of a pre-apoptotic complex of pro-caspase-3, Hsp60 and Hsp10 in the mitochondrial fraction of jurkat cells. EMBO J., *18:* 2040–2048, 1999.

32. Mancini, M., Nicholson, D. W., Roy, S., Thornberry, N. A., Peterson, E. P., Casciola-Rosen, L. A., and Rosen, A. The caspase-3 precursor has a cytosolic and mitochondrial distribution: implications for apoptotic signaling. J. Cell Biol., *140:* 1485–1495, 1998.

33. Eskes, R., Desagher, S., Antonsson, B., and Martinou, J. Bid induces the oligomerization and insertion of Bax into the outer mitochondrial membrane. Mol. Cell. Biol., *20:* 929–935, 2000.

34. Desagher, S., Osen-Sand, A., Nichols, A., Eskes, R., Montessuit, S., Lauper, S., Maundrell, K., Antonsson, B., and Martinou, J. Bid-induced conformational change of Bax is responsible for mitochondrial cytochrome *c* release during apoptosis. J. Cell Biol., *144:* 891–901, 1999.

35. Cheng, E., Wei, M., Weiler, S., Flavell, R., Mak, T., Lindsten, T., and Korsmeyer, S. BCL-2, BCL-X_L sequester BH3 domain-only molecules preventing BAX- and BAK-mediated mitochondrial apoptosis. Mol. Cell, *8:* 705–711, 2001.

36. Vieira, H., Boya, P., Cohen, I., El Hamel, C., Haouzi, D., Druillenec, S., Belzacq, A., Brenner, C., Roques, B., and Kroemer, G. Cell permeable BH3-peptides overcome the cytoprotective effect of Bcl-2 and Bcl-X_L. Oncogene, *21:* 1963–1977, 2002.

37. Wolter, K., Hsu, Y., Smith, C., Nechushtan, A., Xi, X., and Youle, R. Movement of Bax from the cytosol to mitochondria during apoptosis. J. Cell Biol., *39:* 1281–1292, 1997.

38. Antonsson, B., Conti, F., Ciavatta, A., Montessuit, S., Lewis, S., Martinou, I., Bernasconi, L., Bernard, A., Mermod, J., Mazzei, G., Maundrell, K., Gambale, F., Sadoul, R., and Martinou, J. Inhibition of Bax channel-forming activity by Bcl-2. Science (Wash. DC), *277:* 370–372, 1997.

39. Marzo, I., Brenner, C., Zamzami, N., Jurgensmeier, J., Susin, S., Vieira, H. L., Prevost, M., Xie, Z., Matsuyama, S., Reed, J., and Kroemer, G. Bax and adenine nucleotide translocator cooperate in the mitochondrial control of apoptosis. Science (Wash. DC), *281:* 2027–2031, 1998.

40. Shimizu, S., Narita, M., and Tsujimoto, Y. Bcl-2 family proteins regulate the release of apoptogenic cytochrome *c* by the mitochondrial channel VDAC. Nature (Lond.), *399:* 483–487, 1999.

41. Kuwana, T., Mackey, M., Perkins, G., Ellisman, M., Latterich, M., Schneiter, R., Green, D., and Newmeyer, D. Bid, bax, and lipids cooperate to form supramolecular openings in the outer mitochondrial membrane. Cell, *111:* 331–342, 2002.

42. Li, B., and Dou, Q. P. Bax degradation by the ubiquitin/proteasome-dependent pathway: involvement in tumor survival and progression. Proc. Natl. Acad. Sci. USA, *97:* 3850–3855, 2000.

43. Cregan, S., Fortin, A., MacLaurin, J. G., Callaghan, S. M., Cecconi, F., Yu, S. W., Dawson, T. M., Dawson, V. L., Park, D. S., Kroemer, G., and Slack, R. S. Apoptosis-inducing factor is involved in the regulation of caspase-independent neuronal cell death. J. Cell Biol., *158:* 507–517, 2002.

44. Haraguchi, M., Torii, S., Matsuzawa, S., Xie, Z., Kitada, S., Krajewski, S., Yoshida, H., Mak, T. W., and Reed, J. C. Apoptotic protease activating factor 1 (Apaf-1)-independent cell death suppression by Bcl-2. J. Exp. Med., *191:* 1709–1720, 2000.

45. Sperandio, S. I., de Belle, I., and Bredesen, D. E. An alternative, nonapoptotic form of programmed cell death. Proc. Natl. Acad. Sci. USA, *97:* 14376–14381, 2000.

46. Rao, R., Castro-Obregon, S., Frankowski, H., Schuler, M., Stoka, V., del Rio, G., Bredesen, D. E., and Ellerby, H. M. Coupling endoplasmic reticulum stress to the cell death program. An Apaf-1-independent intrinsic pathway. J. Biol. Chem., *277:* 21836–21842, 2002.

47. Deveraux, Q., and Reed, J. IAP family proteins: suppressors of apoptosis. Genes Dev., *13:* 239–252, 1999.

48. Suzuki, Y., Nakabayashi, Y., and Takahashi, R. Ubiquitin-protein ligase activity of X-linked inhibitor of apoptosis protein promotes proteasomal degradation of caspase-3 and enhances its anti-apoptotic effect in Fas-induced cell death. Proc. Natl. Acad. Sci. USA, *98:* 8662–8667, 2001.

49. Chai, J. Structural and biochemical basis of apoptotic activation by Smac/DIABLO. Nature (Lond.), *406:* 855–862, 2000.

50. Beere, H. M., Wolf, B. B., Cain, K., Kuwana, T., Tailor, P., Morimoto, R. I., Cohen, G., and Green, D. R. Heat-shock protein 70 inhibits apoptosis by preventing recruitment of procaspase-9 to the Apaf-1 apoptosome. Nat. Cell Biol., *2:* 469–475, 2000.

51. Chen, Y. R., and Tan, T. H. The c-Jun N-terminal kinase pathway and apoptotic signaling. Int. J. Oncol., *16:* 651–662, 2000.

52. Davis, R. J. Signal transduction by the JNK group of MAP kinases. Cell, *103:* 239–252, 2000.

53. Fuchs, S. Y., Adler, V., Buschmann, T., Yin, Z., Wu, X., Jones, S. N., and Ronai, Z. JNK targets p53 ubiquitination and degradation in nonstressed cells. Genes Dev., *12:* 2658–2663, 1998.

54. Fuchs, S. Y., Adler, V., Pincus, M. R., and Ronai, Z. MEKK1/JNK signaling stabilizes and activates p53. Proc. Natl. Acad. Sci. USA, *95:* 10541–10546, 1998.

55. Noguchi, K., Kitanaka, C., Tamana, H., Kokubu, A., Mochizuki, T., and Kuchino, Y. Regulation of c-Myc through phosphorylation at Ser-62 and Ser-71 by c-Jun N-terminal kinase. J. Biol. Chem., *274:* 32580–32587, 1999.

56. Yamamoto, K., Ichijo, H., and Korsmeyer, S. J. BCL-2 is phosphorylated and inactivated by an ASK1/Jun N-terminal protein kinase pathway normally activated at G_2/M. Mol. Cell. Biol., *19:* 8469–8478, 1999.

57. Tournier, C., Hess, P., Yang, D. D., Xu, J., Turner, T. K., Nimnual, A., Bar-Sagi, D., Jones, S. N., Flavell, R. A., and Davis, R. J. Requirement of JNK for stress-induced activation of the cytochrome *c*-mediated death pathway. Science (Wash. DC), *288:* 870–874, 2000.

58. Datta, S. D., Brunet, A., and Greenberg, M. Cellular survival: a play in three Akts. Genes Dev., *13:* 2905–2927, 1999.

59. Zha, J., Harada, E., Yang, J., Jockel, J., and Korsmeyer, S. J. Serine phosphorylation of death agonist BAD in response to survival factor results in binding to 14-3-3 not BCL-XL. Cell, *87:* 619–628, 1996.

60. Brunet, A., Bonni, A., Zigmond, M. J., Lin, M. Z., Juo, P., Hu, L. S., Anderson, M. J., Arden, K. C., Blenis, J., and Greenberg, M. E. Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. Cell, *96:* 857–868, 1999.

61. Dikers, P. F., Medema, J-H., Lammes, J., Koenderman, L., and Coffer, P. J. Expression of the pro-apoptotic family member Bim is regulated by the Forkhead transcription factor, FKMR-L1. Curr. Biol., *10:* 1201–1204, 2000.

62. Kane, L. P., Shapiro, V. S., Stokoe, D., and Weiss, A. Induction of NF-κB by the Akt/PKB kinase. Curr. Biol., *9:* 601–604, 1999.

63. Zong, W-X., Edelstein, L. C., Chen, C., Bash, J., and Gelinas, C. The pro-survival Bcl-2 homolog Bfl-1/A1 is a direct transcriptional target of NF-κB that blocks TNF-α-induced apoptosis. Genes Dev., *13:* 382–387, 1999.

64. You, M., Ku, P. T., Hirdlickova, R., and Bose, H. R. J. ch-IAP1, a member of the the inhibitor-of-apoptosis protein family, is a mediater of the antiapoptotic activity of the v-Rel oncoprotein. Mol. Cell. Biol., *17:* 7328–7341, 1997.

65. Rayet, B., and Gelinas, C. Aberrant rel/nfkb genes and activity in human cancer. Oncogene, *18:* 6938–6947, 1999.

66. Prendergast, G. Mechanisms of apoptosis by c-Myc. Oncogene, *18:* 2967–2987, 1999.

67. Kasibhatla, S., Beere, H., Brunner, T., Echeverri, F., and Green, D. A "non-canonical" DNA-binding element mediates the response of the Fas-ligand promoter to c-Myc. Curr. Biol., *10:* 1205–1208, 2000.

68. Sher, C. J., and Weber, J. D. The ARF/p53 pathway. Curr. Opin. Genet. Dev., *10:* 94–99, 2000.

69. Brunner, T., Kasibhatla, S., Pinkoski, M., Frutschi, C., Yoo, N., Echeverri, F., Mahboubi, A., and Green, D. Expression of Fas ligand in activated T cells is regulated by c-Myc. J. Biol. Chem., *275:* 9767–9772, 2000.

70. Juin, P., Hueber, A. O., Littlewood, T., and Evan, G. c-Myc-induced sensitization to apoptosis is mediated through cytochrome *c* release. Genes Dev., *13:* 1367–1381, 1999.

71. Juin, P., Hunt, A., Littlewood, T., Griffiths, B., Swigart, L., Korsmeyer, S., and Evan, G. c-Myc functionally cooperates with Bax to induce apoptosis, Mol. Cell. Biol., *22:* 6158–6169, 2002.

72. Urist, M., and Prives, C. p53 leans on its siblings. Cancer Cell, *1:* 311–313, 2002.

73. Vousden, K., and Lu, X. Live or let die: the cell's response to p53. Nat. Rev. Cancer, *2:* 594–604, 2002.

74. Ryan, K., Phillips, A., and Vousden, K. Regulation and function of the p53 tumor suppressor protein. Curr. Opin. Cell Biol., *13:* 332–337, 2001.

75. Oda, E., Ohki, R., Murasawa, H., Nemoto, J., Shibue, T., Yamashita, T., Tokino, T., Taniguchi, T., and Tanaka, N. Noxa, a BH3-only member of the Bcl-2 family and candidate mediator of p53-induced apoptosis. Science (Wash. DC), *288:* 1053–1058, 2000.

76. Oda, K., Arakawa, H., Tanaka, T., Matsuda, K., Tanikawa, C., Mori, T., Nishimori, H., Tamai, K., Tokino, T., Nakamura, Y., and Taya, Y. p53AIP1, a potential mediator of p53-dependent apoptosis, and its regulation by Ser-46-phosphorylated p53. Cell, *102:* 849–862, 2000.

77. Lin, Y., Ma, W., and Benchimol, S. Pidd, a new death-domain-containing protein, is induced by p53 and promotes apoptosis. Nat. Genet., *26:* 122–127, 2000.

78. Gallagher, W. M., and Brown, R. p53-orientated cancer therapies: current progress. Ann. Oncol., *10:* 139–150, 1999.

79. Selivanova, G., Iotsova, V., and Okan, I. Restoration of the growth suppression function of mutant p53 by a synthetic peptide derived from the p53 C-terminal domain. Nat. Med., *3:* 632–638, 1997.

80. Abarzua, P., Lsardo, J. E., and Gubler, M. L. Microinjection of monoclonal antibody PAb421 into human SW480 colorectal carcinoma cells restores the transcription activation function to mutant p53. Cancer Res., *55:* 3490–3494, 1995.

81. Foster, B. Pharmacological rescue of mutant p53 confomation and function. Science (Wash. DC), *286:* 2507–2510, 1999.

82. Bottger, A., Bottger, V., and Sparks, A. Design of a synthetic Mdm-2-binding mini protein that activates the p53 response *in vivo*. Curr. Biol., *7:* 860–869, 1997.

83. Baell, J., and Huang, D. Prospects for targeting the Bcl-2 family of proteins to develop novel cytotoxic drugs. Biochem. Pharmacol., *64:* 851–863, 2002.

84. Webb, A., Cunningham, D., Cotter, F., Clarke, P., DdiStefano, F., Ross, P., Corbo, M., and Dziewanowska, Z. Bcl-2 antisense therapy in patients with non-Hodgkin lymphoma. Lancet, *349:* 1137–1141, 1997.

85. Jansen, B., Schlagbauer-Wadl, H., Brown, B., Bryan, R., van Elsas, A., Muller, M., Wolff, K., Eichler, H., and Pehamberger, H. bcl-2 antisense therapy chemosensitizes human melanoma in SCID mice. Nat. Med., *4:* 232–234, 1998.

86. Piche, A., Grim, J., Rancourt, C., Gomez-Navarro, J., Reed, J. C., and Curiel, D. T. Modulation of Bcl-2 protein levels by an intracellular anti-Bcl-2 single-chain antibody increases drug-induced cytotoxicity in the breast cancer cell line MCF-7. Cancer Res., *58:* 2134–2140, 1998.

87. Degterev, A. Identification of small molecule inhibitors of interaction between the BH3 domain and BCL-XL. Nat. Cell Biol., *3:* 173–182, 2001.

88. Huang, Z. Bcl-2 family proteins as targets for anticancer drug design. Oncogene, *19:* 6627–6631, 1999.

89. Tzung, S. Antimycin A mimics a cell death-inducing Bcl-2 homology domain 3. Nat. Cell Biol., *3:* 183–192, 2001.

90. Druker, B., Tamura, S., Buchdunger, E., Ohno, S., Segal, G., Fanning, S., Zimmermann, J., and Lydon, N. Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. Nat. Med., *2:* 561–566, 1996.

91. Mahon, F., Deininger, M., Schultheis, B., Chabrol, J., Reiffers, J., Goldman, J., and Melo, J. Selection and characterization of BCR-ABL positive cell lines with differential sensitivity to the tyrosine kinase inhibitor STI571: diverse mechanisms of resistance. Blood, *96:* 1070–1079, 2000.

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