Cell, Vol. 108, 153–164, January 25, 2002, Copyright ©2002 by Cell Press

Review

Apoptosis: A Link between Cancer Genetics and Chemotherapy

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Defects in apoptosis underpin both tumorigenesis and drug resistance, and because of these defects chemotherapy often fails. Understanding the molecular events that contribute to drug-induced apoptosis, and how tumors evade apoptotic death, provides a paradigm to explain the relationship between cancer genetics and treatment sensitivity and should enable a more rational approach to anticancer drug design and therapy.

Introduction

Chemotherapy for the treatment of cancer was introduced into the clinic more than fifty years ago. Although this form of therapy has been successful for the treatment of some tumors such as testicular cancer and certain leukemias, its success for the treatment of common epithelial tumors of the breast, colon, and lung has been less than spectacular. Ideally, chemotherapeutic drugs should specifically target only neoplastic cells and should decrease tumor burden by inducing cytotoxic and/or cytostatic effects with minimal "collateral damage" to normal cells. In reality, the effectiveness of chemotherapy has suffered from a range of confounding factors including systemic toxicity due to a lack of specificity, rapid drug metabolism, and both intrinsic and acquired drug resistance. The problem of multidrug resistance has been the least understood, and most unpredictable factor affecting chemotherapy. Given the adaptability of tumor cells, it seems likely that drug resistance will continue to be an important clinical problem, even in the age of targeted therapeutics and tailored treatment regimes.

Why are tumors often inherently resistant to chemotherapeutic drugs or become resistant after an initial round of treatment? Our understanding of drug resistance has evolved over time due to a clearer understanding of how conventional drugs actually kill tumor cells. Initially, the development of chemotherapeutic agents was based on the observation that tumor cells proliferate faster than normal cells. Thus, drugs that interfered with DNA replication or cellular metabolism were chosen. Predictably, these agents also affected rapidly dividing normal cells of the bone marrow and gut, thereby reducing the therapeutic window in which these agents could be used. At that time, drug resistance was thought to arise from molecular changes inhibiting the drug-target interaction (Figure 1). Indeed, the discovery of drug pumps such as P-glycoprotein and intracellular detoxifiers such as antioxidants (e.g., glutathione) overexpressed in drug-resistant tumor cells was consistent with this view (Johnstone et al., 2000; Volm, 1998).

Although proteins that interfere with either drug accumulation or stability can contribute to clinical drug resistance, other factors that act downstream of the initial drug-induced insult also play an important role. Chemotherapeutic agents can induce a series of cellular responses that impact on tumor cell proliferation and survival (Lowe and Lin, 2000) (Figure 1). Perhaps the best studied of these cellular responses is apoptosis, a physiological cell death program that controls normal cell numbers during development and disease. We now understand many of the molecular events necessary for activation, amplification and execution of the apoptotic process, and it is evident that diverse drugs can kill tumor cells by activating common apoptotic pathways. Thus, single mutations that disable apoptosis can produce multidrug resistance.

The realization that apoptosis contributes to the antitumor activity of many chemotherapeutic drugs has allowed us to rethink how intrinsic drug resistance may arise. For a tumor cell to propagate, it must survive drastic structural and/or metabolic alterations, as well as an extremely stressful microenvironment (i.e., hypoxic conditions and nutrient deprivation). It also must evade the host antitumor immune response. As discussed herein, altered expression or mutation of genes encoding key apoptotic proteins can provide cancer cells with both an intrinsic survival advantage and inherent resistance to chemotherapeutic drugs. This "double whammy" results in the growth and expansion of neoplastic cells in the first instance and may thwart subsequent therapy.

The overall contribution of apoptotic defects to clinical multidrug resistance remains under debate (Brown and Wouters, 1999). However, accepting that there is a causal relationship between apoptosis and drug-induced cytotoxicity has several implications that we will address in this review. First, the similarity between physiological and drug-induced apoptotic programs establishes a clear link between tumor development and intrinsic resistance to anticancer treatment, and thus provides a biological basis for how tumor genotype can determine treatment outcome. Second, the fact that defects in apoptosis can promote drug resistance downstream of the drug-target interaction raises the possibility that genotoxic agents may induce further genetic mutations owing to "damage without death." Third, the efficiency with which apoptosis can eliminate tumor cells when engaged indirectly by conventional agents provides a strong rationale for strategies to target the process more directly. Finally, the sensitivity of normal cells to drug-induced apoptosis may explain many of the toxic side effects of conventional chemotherapy and suggests strategies to minimize them. It follows that a detailed

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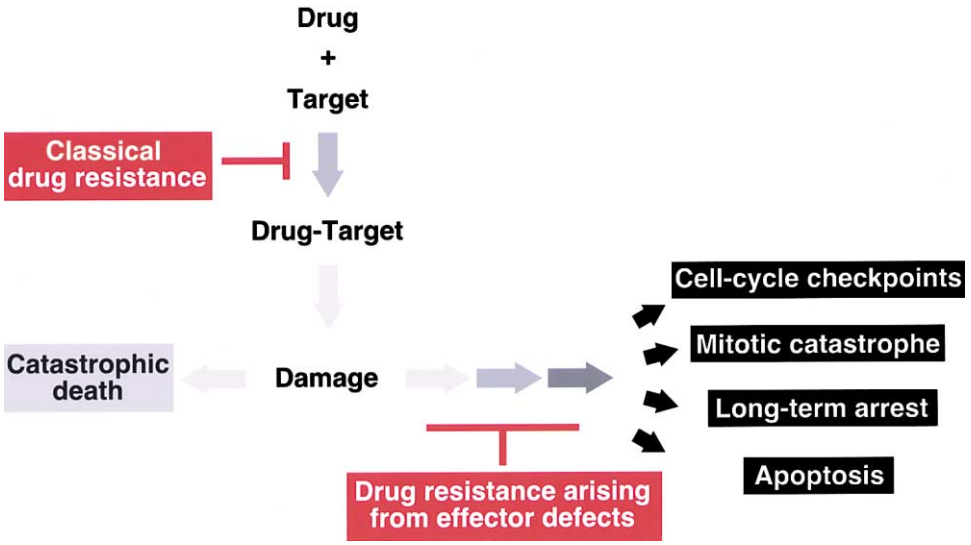

Figure 1. Mechanisms of Drug Action

Addition of chemotherapeutic drugs to tumor cells results in interaction between the drug and intracellular targets, and induction of the primary effect. Classical drug resistance proteins such as drug efflux pumps can inhibit the primary effect by affecting drug-target interactions. Depending on the severity of the initial insult, drug-induced damage may result in catastrophic death or initiate a series of secondary effects mediated by various stress signaling pathways leading to cell death or cell cycle arrest. Consequently, mutations in these downstream events can produce multidrug resistance.

understanding of how anticancer agents induce cell death, and how defects in death pathways promote resistance, will revolutionize the way chemotherapeutic drugs are designed and used, moving from "trial and error" to a more rational strategy that may be tailored to each cancer patient.

Disabling Apoptosis: An Important Process in Tumor Formation

To appreciate how alterations in apoptotic pathways impinge on drug action, it is necessary for us to first outline their role in tumorigenesis. Tumorigenesis is a multistep process in which mutations in key cellular genes produce a series of acquired capabilities that allow the developing cancer cell to grow unchecked in the absence of growth-stimulating signals, while overcoming growth-inhibitory signals and host immune responses. They also allow the tumor to replicate indefinitely, maintain an oxygen and nutrient supply, and invade adjacent and distant tissues. Importantly, the ability of cells to evade apoptosis is also an essential "hallmark of cancer" (Hanahan and Weinberg, 2000).

Components of Apoptotic Pathways

Apoptosis is defined by distinct morphological and biochemical changes mediated by a family of cysteine aspases (caspases), which are expressed as inactive zymogens and are proteolytically processed to an active state following an apoptotic stimulus. Two separable pathways leading to caspase activation have been characterized (Green, 2000; Wang, 2001) (Figure 2). The extrinsic pathway is initiated by ligation of transmembrane death receptors (CD95, TNF receptor, and TRAIL receptor) to activate membrane-proximal (activator) caspases (caspase-8 and -10), which in turn cleave and activate effector caspases such as caspase-3 and -7. This pathway can be regulated by c-FLIP, which inhibits upstream activator caspases, and inhibitor of apoptosis proteins (IAPs), which affect both activator and effector caspases. The intrinsic pathway requires disruption of the mitochondrial membrane and the release of mitochondrial proteins including Smac/DIABLO, HtRA2, and cytochrome c. Cytochrome c functions with Apaf-1 to induce activation of caspase-9, thereby initiating the apoptotic caspase cascade, while Smac/DIABLO and HtrA2 bind to and antagonize IAPs (Wang, 2001; Suzuki et al., 2001).

Mitochondrial membrane permeabilization is regulated by the opposing actions of pro- and antiapoptotic Bcl-2 family members. Multidomain proapoptotic Bcl-2 proteins (e.g., Bak and Bax) can be activated directly following interaction with the BH3-only Bcl-2 protein Bid. Alternatively, binding of other BH3-only proteins (e.g., Noxa, Puma, Bad, and Bim) to antiapoptotic Bcl-2 proteins (e.g., Bcl-2 and Bcl-X_L) results in activation of Bax and Bak (Adams and Cory, 1998; Huang and Strasser, 2000). Whether Bcl-2 proteins control mitochondrial membrane permeability by directly forming pores in the outer membrane, and/or by regulating the opening and closing of the permeability transition pore remains the topic of much debate (Martinou and Green, 2001). The net effect however, is the regulated release of proapoptotic factors from the mitochondria, induction of downstream caspases, and potential loss of mitochondrial function (Figure 2).

There is considerable cross-talk between the extrinsic and intrinsic pathways. For example, caspase-8 can proteolytically activate Bid, which can then facilitate cytochrome c release (Green, 2000). This apparently amplifies the apoptotic signal following death receptor activation, and different cell types may be more reliant on this amplification pathway than others (Fulda et al., 2001).

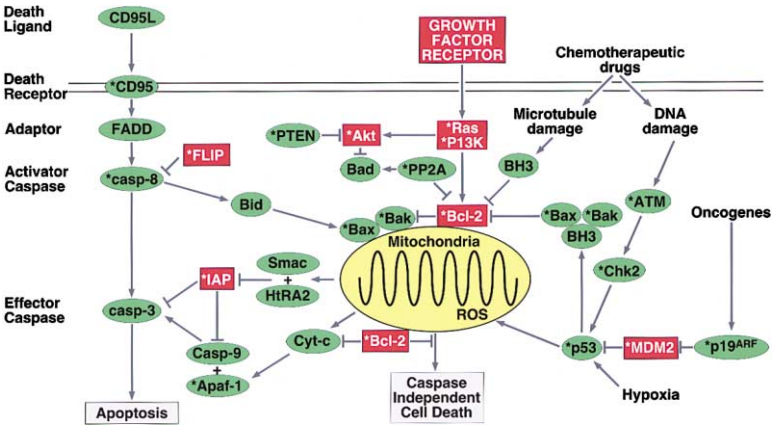

Figure 2. The Integrated Apoptotic Pathways

A schematic diagram showing some of the known components of the intrinsic and death receptor apoptotic programs that may modulate tumor development and therapy. An asterisk denotes components that are frequently mutated or aberrantly expressed in human cancers. Components in red inhibit apoptosis while those in green promote apoptosis. Abbreviations used: casp, caspase; cyt, cytochrome.

Conversely, activators of the intrinsic pathway can sensitize the cell to extrinsic death ligands (see below).

Regulation of the Intrinsic Apoptotic Pathway

"Intrinsic stresses" such as oncoproteins, direct DNA damage, hypoxia, and survival factor deprivation, can activate the intrinsic apoptotic pathway. As a sensor of cellular stress, p53 is a critical initiator of this pathway (Lowe and Lin, 2000) (Figure 2). For example, proteins that sense DNA damage, such as ATM and Chk2, phosphorylate and stabilize p53 directly, and inhibit MDM2-mediated ubiquitination of p53 (Khanna and Jackson, 2001). Mitogenic oncogenes can also activate p53 through a mechanism that is distinct from DNA damage, and can involve $p19^{ARF}$, the alternative reading frame product of the *INK4a/ARF* tumor suppressor locus. $p19^{ARF}$, in turn, binds and inactivates Mdm2, leading to p53 activation (Lowe and Lin, 2000; Sherr and Weber, 2000).

p53 can initiate apoptosis by transcriptionally activating proapoptotic Bcl-2 family members (e.g., Bax, Bak, PUMA, and Noxa) and repressing antiapoptotic Bcl-2 proteins (Bcl-2, Bcl-X_L) and IAPs (survivin) (Bartke et al., 2001; Hoffman et al., 2001; Ryan et al., 2001; Wu et al., 2001). However, p53 can also transactivate other genes that may contribute to apoptosis including *PTEN*, *Apaf-1*, *PERP*, *p53AIP1*, and genes that lead to increases in reactive oxygen species (ROS) (Hwang et al., 2001; Moroni et al., 2001; Ryan et al., 2001; Stambolic et al., 2001). In addition, p53 can transcriptionally activate both CD95 and TRAIL receptor 2 (TRAIL-R2/DR5), thereby sensitizing cells to death-receptor-mediated apoptosis (Herr and Debatin, 2001; Ryan et al., 2001). p53 may also have transcription-independent activities that potentiate cell death once the transcription-dependent functions initiate the process (Ryan et al., 2001). Therefore, it appears that p53 can function as a "master regulator" of the apoptotic program, capable of coordinating the process at multiple levels via several mechanisms. Still, p53 is not the only activator of the intrinsic apoptotic pathway. Some studies suggest that a related family member, p73, might substitute for p53 in certain circumstances (Yang and McKeon, 2000). Undoubtedly, additional p53-independent activities are also important.

In addition to transcriptional regulation by p53, the activities of Bcl-2 proteins can be modulated by post-translational modifications such as phosphorylation (Figure 2). For example, phosphorylation of Bad by several prosurvival kinases including Akt, p90S6K, p70S6K, and PKA, inhibits its interaction with antiapoptotic Bcl-2 proteins, and induces sequestration of Bad away from the mitochondria following binding of 14-3-3 adaptor proteins (Bonni et al., 1999; Harada et al., 2001; Wang, 2001). Moreover, Bcl-2 can be directly phosphorylated by MAP kinases and dephosphorylated by the PP2A tumor suppressor, leading to changes in its activity (Blagosklonny, 2001).

Tumors Dysregulate the Intrinsic Pathway

Disruption of the intrinsic apoptotic pathway is extremely common in cancer cells (Table 1). Indeed, the p53 tumor suppressor gene is the most frequently mutated gene in human tumors, and loss of p53 function can both disable apoptosis and accelerate tumor development in transgenic mice (Attardi and Jacks, 1999; Ryan et al., 2001). Moreover, functional mutations or altered expression of p53 downstream effectors (*PTEN*, *Bax, Bak,* and *Apaf-1*), or upstream regulators (*ATM*, *Chk2*, *Mdm 2*, and $p19^{ARF}$), occur in human tumors (Table 1). As a result, the presence of wild-type p53 does not necessarily indicate that the pathway is intact, thus complicating efforts to correlate p53 gene integrity with a functional p53 pathway (Schmitt et al., 1999).

Given the importance of Bcl-2 family members in regulating the intrinsic apoptotic pathway, it is not surprising that these genes are altered in tumor samples (Table 1). In fact, *Bcl-2* was first identified based on its translocation in follicular lymphoma, and is overexpressed in a variety of cancers (Reed, 1999). Moreover, Bcl-2 overexpression can accelerate tumorigenesis in transgenic mice (Adams et al. 1999). Conversely, proapoptotic Bcl-2 proteins are inactivated in certain cancers and disruption of these genes also promotes tumorigenesis in mice (Table 1). In addition, mutations or altered expression of upstream regulators of Bcl-2 proteins are associated with cancer. For example, the Bad-kinase Akt, is positively regulated by various oncoproteins, and negatively regulated by the PTEN tumor suppressor (Datta et al., 1999). Amplified *Akt* and mutated *PTEN* have been found with high frequency in a variety of solid cancers, indicating the importance of this pathway in regulating tumorigenesis (Table 1).

Inhibition of Postmitochondrial Death Processes

While mutations in cancer cells often target regulators of the intrinsic apoptotic pathway such as p53 and the

Table 1. Summary of the Roles of Apoptotic Initiators, Regulators or Executioners in Tumorigenesis, Apoptosis, and Drug Resistance

Protein	Role in tumorigenesis, apoptosis and drug resistance	References
Tumor Suppressor		
p53	Mutated or altered expression in many cancers. Initiates the intrinsic apoptotic pathway. $p53^{-/-}$ cells are resistant to drug induced apoptosis.	(Vogelstein et al., 2000)
$p19^{ARF}$	Mutated or altered expression in many cancers. Blocks MDM2 inhibition of p53. Enhances drug-induced apoptosis by p53.	(Sherr and Weber, 2000)
ATM	Mutated in ataxia-talangiectasia syndrome. Senses DNA double strand breaks and stabilizes p53. Deficiencies increase risk of developing haematological malignancies and breast cancer.	(Khanna and Jackson, 2001)
Chk2	Mutated in Li-Fraumeni syndrome. Senses DNA double strand breaks and phosphorylates and stabilizes p53.	(Khanna and Jackson, 2001)
Rb	Mutated in some cancers, and functionally disrupted in many cancers. Inhibits E2F-medidated transcription. Loss of Rb function induces p53-dependent and independent apoptosis.	(Harbour and Dean, 2000)
Bax	Mutated or decreased expression in some tumors. Mediates mitochondrial membrane damage. Sufficient but not necessary for drug-induced apoptosis.	(Rampino et al., 1997)
Bak	Mutated or decreased expression in some tumors. Mediates mitochondrial membrane damage. Sufficient but not necessary for drug-induced apoptosis.	(Kondo et al., 2000)
PTEN	Mutated or altered expression in cancers. Regulates Akt activation and subsequent phosphorylation of Bad. Loss of PTEN results in resistance to many apoptotic stimuli.	(Di Cristofano and Pandolfi, 2000)
Apaf-1	Mutated and transcriptionally silenced in melanoma and leukemia cell lines. Necessary for activation of caspase-9 following cytochrome c release. Apaf-$1^{-/-}$ cells are chemoresistant.	(Soengas et al., 2001)
CD-95/Fas	Mutated and down-regulated in lymphoid and solid tumors. Initiates the extrinsic apoptotic pathway. Loss of function is associated with resistance to drug-induced cell death.	(Muschen et al., 2000)
TRAIL-R1/R2	Mutated in metastatic breast cancers. Initiate the extrinsic apoptotic pathway. Mutations lead to suppression of death receptor-mediated apoptosis.	(Shin et al., 2001)
Caspase-8	Gene silenced in neuroblastomas. Activates both extrinsic and intrinsic apoptotic pathways. Silencing results in resistance to drug-induced apoptosis.	(Teitz et al., 2000)
Oncogene		
Bcl-2	Frequently overexpressed in many tumors. Antagonises Bax and/or Bak and inhibits mitochondrial membrane disruption. Inhibits drug-induced apoptosis.	(Reed, 1999)
MDM2	Overexpressed in some tumors. Negative regulator of p53. Inhibits drug-induced p53 activation.	(Sherr and Weber, 2000)
IAPs	Frequently overexpressed in cancer. Down regulation of XIAP induces apoptosis in chemoresistant tumors.	(Deveraux and Reed, 1999)
NF-κB	Deregulated activity in many cancers. Transcriptionally activates expression of anti-apoptotic members of the Bcl-2 and IAP families. Can inhibit both the extrinsic and intrinsic death pathways and induce drug-resistance.	(Baldwin, 2001)
Myc	Deregulated expression in many cancers. Induces proliferation in the presence of survival factors, such as Bcl-2, and apoptosis in the absence of survival factors. Can sensitise cells to drug-induced apoptosis.	(Evan and Vousden, 2001)
Akt	Frequently amplified in solid tumors. Phosphorylates Bad. Hyperactivation induces resistance to a range of apoptotic stimuli including drugs.	(Datta et al., 1999)
PI3K	Overexpressed or deregulated in some cancers. Responsible for activation of Akt and downstream phosphorylation of Bad. Inhibition of PI3K enhances chemotherapeutic drug-induced apoptosis.	(Roymans and Slegers, 2001)
Ras	Mutated or deregulated in many cancers. Activates PI3K and downstream pathways. Induces proliferation and inhibits c-myc and drug-induced apoptosis.	(el-Deiry, 1997)
FLIP	Overexpressed in some cancers. Prevents activation of caspase-8 and apoptosis induced by some chemotherapeutic drugs.	(Tepper and Seldin, 1999)

Bcl-2-related proteins, alterations that disrupt apoptosis downstream of the mitochondria have been reported (Table 1). For example, silencing of *Apaf-1* occurs in metastatic melanoma, and overexpression of IAPs and heat shock proteins (Hsp), which can inhibit caspase-9 activation, is commonly observed in human tumors (Deveraux and Reed, 1999; Soengas et al., 2001; Beere and Green, 2001). This implies that downstream defects in apoptosis contribute to tumorigenesis. Nevertheless, postmitochondrial mutations appear less frequently than those targeting upstream components of the apoptotic program. This could represent greater redundancy in the downstream pathway, or the difficulty in maintaining cell viability following damage to the mitochondria. Consistent with the latter possibility, cell death can sometimes proceed in the presence of caspase inhibitors (Herr and Debatin, 2001).

Tumor Cells Evade Death-Receptor-Induced Apoptosis

Tumorigenic disruptions in the death-receptor pathway occur less frequently than the intrinsic pathway. Nevertheless, tumor cells are often resistant to death-receptor-mediated apoptosis, and mutations in CD95, TRAIL receptors, and downstream signaling pathways do oc-

cur in cancer (Table 1). Autoimmune lymphoproliferative syndrome (ALPS) is caused by germline mutations in *CD95* resulting in inappropriate survival of activated T lymphocytes. ALPS patients have an increased incidence of lymphoma, possibly due to the expanded population of apoptosis-resistant T cells which can sustain further transforming mutations (Straus et al., 2001). In solid tumors and non-T cell leukemias, tumor surveillance and immune escape may also be important. While cytotoxic lymphocytes predominantly kill tumor cells via the granule exocytosis pathway (Trapani et al., 2000), CD95L and TRAIL are also utilized (Rosen et al., 2000; Takeda et al., 2001). Thus, inactivation of the death receptor pathway could allow escape from immune responses and provide a survival advantage to developing tumor cells. In fact, loss of CD95L or TRAIL function can promote tumor growth and metastasis (Rosen et al., 2000; Takeda et al., 2001)

Thus, apoptosis is regulated at many levels, including the initiation, transduction, amplification, and execution stages, and mutations that disrupt each of these stages have been detected in tumor cells. Because mutations in cancers necessarily produce a selective advantage to emerging tumor cells, the identification of mutated components and their frequency of mutation highlight critical regulatory points in survival and proliferative processes. The fact that apoptosis is disabled at distinct stages in different tumor types suggests that its critical control points are probably context dependent. As discussed below, this variability may contribute to the heterogeneity of treatment responses in human tumors. Moreover, the identification of these control points single out distinct "sites of attack" for targeting by novel chemotherapeutic drugs.

Cancer Therapy and Apoptosis

Since most cancer drugs were identified using empirical screens, the molecular events responsible for their antitumor effect were poorly understood. Over the last decade, our understanding of cellular damage responses and physiological cell death mechanisms has improved, leading in turn to new insights into drug-induced cell death. Drugs of differing structure and specificity induce the characteristic morphological changes associated with apoptosis, and it is now believed that apoptotic pathways contribute to the cytotoxic action of most chemotherapeutic drugs (Lowe and Lin, 2000). Collectively, these observations indicate that cells can interpret a drug-induced insult in the same way that a physiological insult, such as hypoxia or growth factor deprivation, is interpreted. Since the efficiency of apoptosis depends on the integrity of an elaborate molecular network, the killing of tumor cells by anticancer agents may be remarkably indirect.

Anticancer Drugs Activate the Intrinsic Apoptotic Pathway

Mutations in *p53* or in the p53 pathway can produce multidrug resistance in vitro and in vivo, and reintroduction of wild-type *p53* into *p53* null tumor cells can re-establish chemosensitivity (Wallace-Brodeur and Lowe, 1999). However, *p53* status is not a universal predictor of treatment response, in part because not all drugs absolutely require p53 for their apoptotic function (Herr and Debatin, 2001) and, in some settings, p53 loss can enhance drug-induced cell death (Bunz et al., 1999). Still, loss of p53 function correlates with multidrug resistance in many tumor types (Wallace-Brodeur and Lowe, 1999).

Mutations or altered expression of Bcl-2-related proteins can drastically alter drug sensitivity in experimental models (Reed, 1999; Schmitt et al., 2000; Wei et al., 2001; Zhang et al., 2000), and are associated with multidrug resistance in human cancers (Reed, 1999). Although Bcl-2 does not promote long-term proliferation following drug treatment in all settings (Brown and Wouters, 1999), evidence now suggests that this reflects limitations of certain in vitro assays. For example, in primary lymphomas derived from c-*myc* transgenic mice, overexpression of Bcl-2 significantly inhibits drug-induced death in short term assays, but does not enhance clonogenic potential (Schmitt et al., 2000). Nevertheless, Bcl-2-overexpressing lymphomas are highly drug resistant in vivo. Similarly, Bcl-X_L-overexpressing breast carcinoma lines, which lose clonogenic potential following exposure to chemotherapeutic drugs in vitro, are resistant to drug-induced cell death in vivo (Liu et al., 1999).

How can the discrepancy between clonogenic assays in culture and tumor responses in vivo be reconciled? As clonogenic assays not only represent survival of a cell but also cell proliferation, Bcl-2 may not block drug-induced cytostasis, which would be indistinguishable from cell death in this assay. Indeed, IL-7 is a growth stimulatory cytokine that can increase the clonogenic potential of Bcl-2-overexpressing lymphomas (Schmitt et al., 2000), and a combination of microenvironmental survival signals cooperates with Bcl-X_L to promote clonogenic survival following drug treatment in B lymphoma cells (Walker et al., 1997). Therefore, the microenvironment can have a substantial impact on the ability of Bcl-2 to promote long-term survival. Moreover, even when anticancer agents induce cytostasis in the presence of an apoptotic block, the increase in tumor burden may provide a window of opportunity for survival of a more malignant variant with a defect in drug-induced cytostasis.

Postmitochondrial Events and Drug Action

Although the contribution of postmitochondrial events to drug action are less well defined, defects at this level can also promote drug resistance. For example, epigenetic inactivation of *Apaf-1* in malignant melanoma, and increases in IAP and Hsp expression in various tumors, correlates with drug resistance (Creagh et al., 2000; Deveraux and Reed, 1999; Soengas et al., 2001). Interestingly, melanoma cells with reduced *Apaf-1* expression efficiently activate p53 in response to a chemotherapeutic drug, but fail to activate caspase-9 and initiate an apoptotic response. Importantly, reintroduction of *Apaf-1* can reactivate caspase-9 and restore drug-induced apoptosis. In addition, loss of Apaf-1 expression or activity in human leukemia and ovarian carcinoma cell lines correlates with a decrease in drug-induced apoptosis, and these cells can be resensitized to drug following transfection of Apaf-1 (Jia et al., 2001; Wolf et al., 2001).

These findings suggest that release of cytochrome c from the mitochondria may not always be the "point of no return" with respect to cell survival following drug treatment. Although mitochondrial membrane damage triggers the caspase cascade, it also releases other pro-

apoptotic factors and disrupts essential metabolic processes such as ATP production by oxidative phosphorylation (Wang, 2001). Nevertheless, in some settings, cells with apoptotic defects apparently downstream of the mitochondria survive despite perturbation of the mitochondrial membrane. For example, mice lacking *apaf-1*, *caspase-9*, or *caspase-3* display massive cell accumulation in the developing nervous system (Los et al., 1999), and loss of *apaf-1* rescues some, but not all, of the aberrant apoptosis observed in Rb-deficient embryos (Guo et al., 2001). Similarly, transformed fibroblasts from *apaf-1* or *caspase-9* knockout mice are resistant to diverse apoptotic stimuli, including chemotherapeutic drugs (Los et al., 1999; Soengas et al., 1999). However, this is not universally true, since *apaf-1*$^{-/-}$ and *caspase-9*$^{-/-}$ lymphoid and myeloid cells still undergo developmentally programmed cell death in vivo, and are sensitive to cytokine withdrawal and drug-induced apoptosis in culture (V. Marsden, J.M. Adams and A. Strasser, personal communication). Moreover, fibroblasts from these mice are not as resistant to Bid-induced apoptosis as those lacking Bax and Bak, or overexpressing Bcl-2 (Cheng et al., 2001).

The ability of some tumor cells with postmitochondrial defects to survive chemotherapy presents a conundrum. Even in the absence of downstream caspase activation, damage to the mitochondrial membrane and release of cytochrome c still disrupts the electron transport chain and enhances ROS production. How might the cell tolerate this damage? Following initiation of the intrinsic apoptotic pathway and in the absence of caspase activation, mitochondria can restore transmembrane potential and maintain ATP production (Waterhouse et al., 2001). This can occur following the relocalization of cytoplasmic cytochrome c back into the mitochondria, a truly remarkable feat that infers that mitochondrial structure is preserved, or can rapidly recover, after cytochrome c release. Thus, in a situation where *Apaf-1* or downstream caspases are inactivated, the mitochondrial membrane damage that accompanies apoptosis may not always result in a "lethal hit" to the cell. Interestingly, the capacity of some tumor cells to tolerate mitochondrial dysfunction may be because they frequently express elevated levels of antioxidants (Volm, 1998), and are often growing in glycolytic conditions and therefore rely less on oxidative phosphorylation than normal cells (Dang and Semenza, 1999). Thus, the long-term survival of cells containing postmitochondrial defects in apoptosis probably depends on several factors including the intensity of the stimulus, the cellular context, and the physiological microenvironment.

Other Resistance Mechanisms

The contribution of the death receptor pathway in chemotherapeutic drug-induced cell death is controversial (Herr and Debatin, 2001). Treatment of tumor cells with drugs can induce CD95 and TRAIL receptors, and downregulate c-FLIP and the IAPs (Asselin et al., 2001; Chatterjee et al., 2001; Herr and Debatin, 2001). However, experiments using cells derived from mice with functional mutations in the death receptor pathway (i.e., FADD and caspase-8 knockout) indicate that this pathway is dispensable for the cytotoxic action of chemotherapeutic agents (Los et al., 1999). It has been argued that certain cell types require both the death receptor and mitochondrial pathways for drug-induced death, while others require only the mitochondrial pathway (Fulda et al., 2001). Given the ability of p53 to transactivate *CD95* and *DR5*, it is also possible that this contributes to drug-induced cell death in microenvironments where there is substantial ligand present (Herr and Debatin, 2001; Ryan et al., 2001).

In some cases, the very stimulus that induces apoptosis also initiates an antagonistic antiapoptotic program. For example, TNF engagement with its receptor can simultaneously signal apoptosis and upregulate the pro-survival transcription factor NF-κB, and its ability to induce cell death may depend on the balance between these two processes (Baldwin, 2001). This presumably allows life and death decisions to be more tightly regulated. Strikingly, certain chemotherapeutic drugs also activate the NF-κB pathway and inhibition of NF-κB sensitizes cells to drug-mediated death (Baldwin, 2001). This "inducible drug resistance" observed experimentally implies that many of the agents used to treat cancer initiate a protective response that thwarts their intended action. While the generality of this effect is not certain (Ryan et al., 2000), these data provide novel insight into the molecular mechanisms of drug resistance and have therapeutic implications.

Although drug resistance may occur upstream or downstream of the drug-target interaction, these two mechanisms are not mutually exclusive. In addition to actively effluxing chemotherapeutic drugs, the multidrug resistance protein, P-gp can also protect cells against apoptosis mediated by the death receptor pathway, UV-irradiation, and serum starvation (Johnstone et al., 2000). Similarly, the high levels of anti-oxidants often observed in drug-resistant tumors are thought to promote resistance upstream of the intracellular target (Volm, 1998), but may act downstream as well. ROS contribute to p53-mediated apoptosis following 5-fluorouracil (5-FU) treatment of colon carcinoma cells, and genetic manipulation to reduce ROS, or the addition of exogenous antioxidants, can dramatically reduce drug-induced apoptosis (Hwang et al., 2001). The potential for single drug resistance mechanisms to inhibit drug action at multiple levels illustrates the complexity of the problem.

In summary, there is growing evidence that disruption of apoptotic pathways contribute to drug resistance, with lesions upstream of mitochondrial damage producing the greatest effect. However, it is important to remember that other drug-induced effects such as cytostasis and mitotic catastrophe can augment apoptosis to help reduce the tumor burden. These additional effects may be inhibited by either the same mechanisms that impact on drug-induced apoptosis (e.g., overexpression of P-glycoprotein), or by other resistance mechanisms occurring downstream of the drug-target interaction (Figure 1). Although the relative contribution of each resistance mechanism to clinical multidrug resistance is not yet known, it is likely to depend on the specific activity of the chemotherapeutic drug, as well as the tissue origin and genetic background of the tumor.

Apoptosis Links Cancer Genetics and Cancer Therapy

Our discussion of apoptosis during tumor development and following drug treatment reveals that these pro-

cesses are remarkably similar. Thus, the very genetic alterations that induce tumorigenesis can also mediate intrinsic resistance to both physiological (growth factor withdrawal and hypoxia) and nonphysiological death stimuli (drugs). As a result, tumors that have never been challenged with drug can be inherently resistant to conventional chemotherapeutic agents. Put simply, these observations imply that tumor genotype is the most important parameter underpinning successful chemotherapy using current drugs and regimes. This concept has enormous implications for the future of cancer diagnosis and treatment, for it suggests that more rational approaches to chemotherapy will require both an intimate knowledge of the genetic lesions that give rise to tumor development, as well as a detailed understanding of the molecular basis of drug action.

The principal described above couples the disruption of apoptosis during tumor development with intrinsic drug resistance. The best demonstration of this is in *Eμ-myc* transgenic mice crossed with mice containing only one functional *INK4a/ARF* (*INK4a/ARF*$^{+/-}$) or *p53* (*p53*$^{+/-}$) allele (Schmitt et al., 1999). The onset of *Eμ-myc* tumors in *INK4a/ARF*$^{+/}$ or *p53*$^{+/-}$ mice is greatly accelerated relative to control *Eμ-myc* animals, and the resulting tumors typically lose the wild-type *INK4a/ARF* or *p53* allele. This indicates a strong selective pressure to disengage the p53 pathway during lymphoma development. The *INK4a/ARF* null and *p53* null tumors are highly invasive and less prone to spontaneous apoptosis. Furthermore, they are more chemoresistant in vitro and in vivo compared to *Eμ-myc* tumors alone. These experiments demonstrate that drug resistance does not only occur in response to drug challenge, but can be a byproduct of the process that produces accelerated and aggressive tumor growth.

For tumors to progress and metastasize, they must circumvent cellular responses to hyperproliferative signals, hypoxia, nutrient factor deprivation, and altered cell adhesion. In addition, they must survive in a foreign environment and overcome or evade continual immune attack. Each hurdle provides further selective pressure to disable apoptosis, such that by the time a tumor metastasizes, it is not surprising that it is highly resistant to physiologic and pharmacologic death-inducing signals. This may explain, in part, why metastatic tumors are notoriously chemoresistant (Fidler, 1999). Indeed, the observations that metastatic tumors are enriched for *p53* mutations or overexpressed Bcl-2 support this notion (Sierra et al., 2000).

What are the implications of these findings in terms of future treatment of cancer with cytotoxic drugs? If inhibition of apoptosis is a necessary event for tumorigenesis and most chemotherapeutic drugs utilize intact apoptotic pathways to induce cell death, it is little wonder that drug resistance is such a major clinical problem. While this may greatly impede our chances of successfully treating tumors with conventional drugs and treatment regimes, our increasing knowledge of the molecular links between tumorigenesis and apoptosis provides new opportunities for a more tailored approach to chemotherapy.

The Potential Problem of Damage without Cell Death

Most conventional anticancer agents produce some form of cellular damage and, indeed, directly or indirectly damage DNA. Whereas upstream drug resistance mechanisms such as P-gp overexpression prevent drug-induced damage, downstream resistance mechanisms do not (Figure 1). Hence, inhibition of apoptosis in drug resistant tumors not only affects the death-inducing activities of the drug, but also allows for the possibility of cells acquiring additional mutations following DNA damage. In principle, these mutagenized cells could become more malignant and even less sensitive to subsequent therapies, such that treatment of highly resistant tumors containing antiapoptotic lesions may be doing more harm than good.

At present there is no definitive proof that treatment of tumors harboring apoptotic lesions is deleterious. However, there is no doubt that the anticancer agents can be mutagenic and this property can have clinical ramifications. For example, many cancer patients encounter the problem of therapy-related leukemia, in which new primary tumors arise following the treatment of hematological and solid tumors with alkylating agents and topoisomerase inhibitors (Leone et al., 1999). These leukemias have relatively short latency times, indicating that they probably arise from premalignant cells, and are associated with an increase in mutation frequency. It seems likely that these agents would be at least as mutagenic to the primary tumors, perhaps producing a more advanced malignancy. Consistent with this possibility, median survival times for leukemias and certain solid tumors following relapse are often shorter when compared to newly diagnosed patients, and relapsed cancers infrequently achieve long-term remission with subsequent chemotherapy (Ihde et al., 1997).

Studies that have examined the relationship between p53 and mutation frequency following DNA damage are consistent with the possibility that resistance to apoptosis can produce genetically altered cells. One study shows that loss of p53 increases gene mutation frequency following UV irradiation (Corbet et al., 1999), while another fails to demonstrate any significant effect (Griffiths et al., 1997). Both studies, however, show that p53 loss increases the survival of cells following DNA damage, and that these survivors harbor additional mutations. Therefore, whether or not p53 affects the mutation rate directly, it clearly eliminates potentially mutated cells. The possibility that cytotoxic drugs might actually mutagenize tumors that cannot be killed highlights the necessity for a more rational approach for the future treatment of cancer where the "cure matches the cause."

A More Rational Approach to Cancer Therapy

Our understanding of the molecular links between tumorigenesis, apoptosis, and drug resistance provides the foundation for a new age of targeted cancer therapy. For example, information concerning key apoptotic proteins, their regulation, and the manner in which they are altered in tumor cells can be used for target selection in designing new anticancer agents. Alternatively, a "blueprint" of the proteins and pathways necessary for the cytotoxic action of a given drug, coupled with an understanding of the molecular basis of drug resistance, may provide the necessary information to tailor existing therapies for individual tumors. Although substantially more information is needed, both strategies are already being pursued with promising results.

New Therapeutics

Although conventional agents were not designed to induce apoptosis, the fact that they do so indirectly demonstrates that apoptosis *can be* an effective mechanism for eliminating tumor cells. In principle, agents that induce apoptosis *directly* would overcome many of the problems observed with existing drugs and, as a result, have great therapeutic potential. Such an approach would have the following benefits. First, cell death is preferable to cytostasis, since tumor cells are eliminated and hence unable to contribute to tumor relapse. Second, apoptosis is preferable to necrosis, since it is a particularly efficient mode of cell death that does not produce inflammation and damage to the surrounding normal tissue. Finally, agents that induce apoptosis directly should be less mutagenic than existing drugs and, because they engage the program further downstream, less prone to resistance. However, such agents will be just as toxic as conventional therapies unless they are designed to selectively kill tumor cells. Exploiting the genetic and/or physiological differences between tumor and normal cells may provide such an opportunity, and establishes the basis for a truly targeted approach to cancer therapy.

Given that many of the apoptotic regulators altered in multidrug resistant tumors have been identified, one new approach to therapy is to restore apoptotic potential through genetic or pharmacological methods. The direct relationship between p53, apoptosis, and drug action implies that restoring p53 activity in p53 null tumors, or activating apoptotic pathways that are directly downstream of p53, would have clinical benefits. Reintroduction of wild-type *p53* into *p53* null tumors can directly induce apoptosis and restore sensitivity to chemotherapeutic drugs, while adenoviral gene transfer of *Bax* activates apoptosis downstream of p53 and can synergize with chemotherapy to promote tumor regression in vivo (Swisher et al., 1999; Tai et al., 1999). Small molecules that reactivate mutant p53 provide a pharmacological approach that is not prone to the limitations of gene therapy. For example, CP-31398 promotes conformational stability of mutant p53 and restores the DNA binding and transcriptional regulatory functions of mutant protein (Foster et al., 1999). In preclinical studies, CP-31398 can induce p53 transcriptional targets and inhibit the growth of p53 mutant cells in vitro and in vivo. However, a concern with this approach is that most tumors are usually hemizygous for the p53 mutant and are genomically unstable. Therefore, a substantial number of tumor cells may become p53 null by chance, thereby producing resistance.

In certain circumstances, apoptotic pathways may be inactivated in cancer by gene silencing rather than mutation. Silencing of *INK4a/ARF*, *caspase-8*, and *Apaf-1* by DNA methylation has been linked to drug resistance (Robertson and Jones, 1998; Soengas et al., 2001; Teitz et al., 2000). This provides the exciting possibility that targeting the silencing mechanism may reactivate these latent killers. In cultured cells, this can be achieved using demethylating agents and/or histone deacetylase (HDAC) inhibitors, and these agents are now in clinical trials (Marks et al., 2001). However, these agents lack specificity, since normal gene expression can be controlled by similar mechanisms. Thus, epigenetically silenced genes such as *hTERT* can be reactivated by HDAC inhibitors, which might contribute to cellular immortalization and tumor progression (Takakura et al., 2001). Furthermore, HDAC inhibitors might reactivate imprinted genes which, in other settings, are associated with several human diseases, including cancer (Paulsen and Ferguson-Smith, 2001). As the mechanisms of gene silencing at specific loci are elucidated, it is possible that more specific strategies to reactivate discrete genes will be developed.

Antisense approaches to decrease expression of a wide variety of antiapoptotic genes including *Bcl-2*, *Ras, X-IAP*, *and Mdm2* are in various stages of preclinical development (Cunningham et al., 2001; Jansen et al., 2000; Sasaki et al., 2000; Tamm et al., 2001). Experiments in vitro and in vivo provide proof-of-principle that such approaches may work. However, efficient delivery of the DNA to every tumor cell in vivo has not been perfected, ensuring that such therapies are still some way from being adapted for common clinical use. Specific small molecule inhibitors of Bcl-2, which block Bcl-2 homo- and heterodimerization leading to cytochrome c release, have now been developed (Wang et al., 2000). It is unclear whether these inhibitors will have specificity for tumor cells or will be useful as "stand alone" therapies, however, as with CP-31398, the use of such molecules in combination with other agents may have clinical benefit.

Other promising therapeutic targets include components of the prosurvival signal transduction pathways involving Ras, Akt, or NF-κB that contribute to intrinsic or inducible drug resistance. For example, inactivation of NF-κB by overexpression of I-κB can restore sensitivity of tumor cells to chemotherapy (Baldwin, 2001). Since I-κB is normally kept at low levels by ubiquitin-mediated proteolysis, one pharmacological approach to upregulate it is through proteosome inhibition, and there have been exciting preclinical and early phase clinical trial results using the proteosome inhibitor PS-341. This agent inhibits the degradation of I-κB, thereby suppressing transcriptional activation by NF-κB and sensitizing cells to drug-induced apoptosis (Adams, 2001). PS-341 also causes the induction of genes that inhibit cell cycle, and suppression of genes that promote angiogenesis, events that can all enhance the antitumor action of the drug (Adams, 2001). The drug is remarkably nontoxic, has few adverse side effects and might be of great benefit in combination therapies using reduced doses of existing chemotherapeutic drugs (Adams, 2001). In addition, small molecule inhibitors of PI-3 kinase/Akt (Ng et al., 2000), and farnysyltransferases, necessary for the activity of Ras (Omer et al., 2000), can induce apoptosis in vitro and in vivo, are relatively nontoxic to normal cells, and mediate tumor regression in mice. The usefulness of such agents in the clinic is currently under investigation.

Rather than attempting to reactivate the intrinsic apoptotic pathway, an alternative approach would be to engage a fundamentally different apoptotic program to kill tumor cells. As discussed above, mutations in the death receptor pathway are not as frequent as those in the intrinsic apoptotic pathway, such that this program might remain available to trigger an antitumor response. Recently, a recombinant form of TRAIL has been tested as an antineoplastic agent. TRAIL induces the death receptor pathway and is not affected by overexpression

of Bcl-2 or Bcl-X_L (Walczak et al., 2000). TRAIL specifically targets tumor cells and is relatively nontoxic to untransformed cells, although the molecular events underlying this specificity are controversial. Importantly, TRAIL can synergize with subtoxic doses of conventional chemotherapeutic drugs to kill drug resistant tumor cells (Ashkenazi et al., 1999). These data highlight the potential for combination therapies that simultaneously activate both the extrinsic and intrinsic apoptotic pathways.

Most conventional anticancer agents were selected based on their ability to selectively kill tumor cells at clinically achievable doses. This tumor specificity has been attributed to the increased proliferation rate of cancer cells, but this is almost certainly not a sufficient explanation. Instead, the answer may return to the notion that many cells possess mechanisms that couple inappropriate cell division to apoptosis. While some degree of apoptosis inhibition must accompany tumorigenesis, it is clear that tumor cell survival reflects a fine balance between hyperproliferative, proapoptotic, and antiapoptotic events. Thus, the addition of yet another apoptotic stimulus in the form of a chemotherapeutic drug may tip the balance in favor of apoptosis, at least in those cells containing a partially functional apoptotic program. In contrast, normal cells, not "living on the edge" may be less sensitive to these signaling alterations. This property of malignant cells may therefore be exploited to develop new, rationally designed therapeutic agents.

This concept has been put into practice by Kaelin and colleagues who designed novel drugs that target only those cells containing a specific tumorigenic lesion. Disruption of the pRb tumor suppressor protein by direct mutation or altered expression of upstream regulators such as cyclins, cyclin-dependent kinases (cdks), or cdk inhibitors is commonly seen in human tumors (Harbour and Dean, 2000). pRb converts the E2F-1 transcription factor from an activator to a repressor and, like c-Myc, active E2F-1 can promote both proliferation and apoptosis (Harbour and Dean, 2000). While pRb regulates E2F-1 activity during the G_0/G_1 phase of the cell cycle, cyclinA/cdk2 can bind and neutralize E2F-1 during S phase and mutation of the cyclinA/cdk2-binding motif in E2F-1 enhances its ability to induce apoptosis (Krek et al., 1995). Therefore, inhibition of cyclinA/cdk2 binding to E2F-1 in cells containing inactive pRb would result in activation of E2F-1 and induction of apoptosis. This hypothesis was tested using soluble peptides that block the interaction of cyclinA/cdk2 with E2F-1. Apoptosis was readily induced in tumor cells with inactivated pRb but not in normal cells, demonstrating the potential for rationally designed agents (Chen et al., 1999).

Tailoring Cancer Therapy

A variation on the theme of rational drug design is the idea of individualized therapy whereby the "cure circumvents the cause." Theoretically, a more targeted approach to chemotherapy might involve genotyping individual tumors for their drug resistance profiles, and then employing agents known to work effectively despite the identified antiapoptotic lesions. The potential of this approach is illustrated by a study demonstrating that tumor cells with elevated levels of c-*myc* and wild-type p53 are selectively sensitive to 5-FU (Arango et al., 2001). 5-FU does not induce apoptosis in *p53* null colon carcinoma cells (Bunz et al., 1999) and tumors expressing low levels of c-Myc or mutant p53 are relatively resistant to 5-FU treatment (Arango et al., 2001). Consistent with these studies, retrospective analyses of data from a phase III clinical trial demonstrate that only those patients with colon carcinomas containing elevated c-Myc and wild-type p53 respond significantly to 5-FU treatment (Arango et al., 2001).

Although large-scale genotyping of tumors is still years from the clinic, the use of drug-resistance markers to tailor cancer therapy has already been applied in a more simplistic manner. For example, leukemias that acquire drug resistance after an initial round of therapy are often screened for the presence of P-gp. If P-gp is found to be expressed in the resistant tumors, new therapeutic regimes using drugs that are not P-gp substrates, or combination therapies involving P-gp inhibitors, are used. The recent findings that P-gp can both efflux chemotherapeutic drugs and inhibit caspase activation could lead to a further alteration to the criteria for selecting drugs capable of killing P-gp expressing tumors (Johnstone et al., 2000).

The use of markers to tailor therapy or predict treatment response has been on a gene-by-gene basis, and the power of this approach will undoubtedly improve as more information is obtained from each patient's tumor. The time frame for the application of such rational therapy will depend largely on the ongoing classification of the molecular mechanisms of drug action and on the ability to rapidly profile the genetic makeup of a given tumor. Recent experiments using DNA microarray to classify tumors based on gene expression patterns highlights the advances in technology that should enable rapid, high-throughput tumor profiling (Sorlie et al., 2001).

Apoptosis and the Side Effects of Cancer Therapy

The often debilitating side effects of chemotherapy are a major clinical problem. Although chemotherapeutic drugs would ideally specifically target only tumor cells, normal hemopoietic and intestinal epithelial cells, and hair matrix keratinocytes are often susceptible to the toxic effects of these agents (Komarova and Gudkov, 2000). It now appears that drug toxicity is due, in part, to apoptosis induced by p53. Hence, $p53^{-/-}$ mice treated with chemotherapeutic drugs or radiotherapy do not suffer the same degree of damage in susceptible tissues, and can survive doses that are lethal for wild-type animals (Komarova and Gudkov, 2000). This apparently results from disruption of the intrinsic apoptotic pathway since ectopic expression of Bcl-2 in bone marrow cells achieves a similar effect (Domen et al., 1998). Importantly, there is a direct correlation between *p53* activation and the sites of normal tissue toxicity (Komarova and Gudkov, 2001). These studies highlight the reasons underlying the general lack of success in developing drugs that can specifically kill target cells using an empirical approach. On one hand, p53 potentiates the action of most drugs but is functionally inactivated in most tumors. On the other hand, an intact p53 pathway in normal cells contributes to their own destruction.

In theory, it should be possible to reduce the "collateral damage" that gives rise to the side effects of chemo-

therapy by inhibiting p53 activity. This would enhance survival of normal cells while leaving p53 mutant tumor cells unaffected. A combination approach using a p53 blocker and an anticancer drug might eliminate toxicity while maintaining an antitumor effect. Gudkov and colleagues have moved toward testing this hypothesis by identifying Pifithrin-α (PFTα), a reversible small molecule inhibitor of p53-mediated transcriptional activation. PFTα inhibits p53-mediated, drug-induced cell death and protects mice from lethal doses of irradiation (Komarov et al., 1999). Furthermore, PFTα inhibits drug-induced immunosuppression, intestinal damage, and hair loss.

Treating patients with a genotoxic agent and an apoptosis inhibitor carries the risk of promoting tumorigenesis by allowing the survival of mutated cells. Although this is a concern in young cancer patients, the potential benefits may far outweigh the possibility of secondary malignancies that arise years later. Encouragingly, the initial animal studies did not observe increased tumor incidence following PFTα treatment. The key may be to use agents, such as PFTα, that only transiently inhibit p53 function. At the very least, this strategy warrants further consideration.

Summary

Historically, the idea behind combination chemotherapy was that by using agents with distinct targets, one could overcome the problem of resistance. This is simply due to the probability that individual cells will have, by chance, developed mutations that affect independent mechanisms of drug action. However, the predicted power of this approach has never been realized. The role of apoptosis and other cell responses in treatment sensitivity, and the striking realization that resistance and tumorigenesis can occur simultaneously, helps us understand this failure. It is more than likely that these agents did not actually kill through independent mechanisms but activated common death pathways that when mutated, could induce multidrug resistance. By developing more rational targets and understanding actual mechanisms of therapy-induced death and resistance (apoptotic or otherwise), we should be able to use chemotherapy cocktails that truly target independent pathways and provide us with the potency needed to control or cure cancer. It seems likely that such therapies will be more specific, less mutagenic, and less likely to encounter many of the same resistance mechanisms that have befallen conventional agents. Apoptosis is only one paradigm for understanding how drugs work and how tumors evade their action. As more information is uncovered about other cell death mechanisms, it can be applied in similar ways.

Acknowledgments

We apologize to those whose work was not cited or discussed due to space limitations. We thank Mark Smyth, Sarah Russell, Nigel Waterhouse, Clemens Schmitt, and members of the Johnstone and Lowe laboratories for discussions. R.W.J. is a Wellcome Trust Senior Research Fellow and is supported by the National Health and Medical Research Council of Australia. S.W.L. is a Rita Allen Foundation Scholar and is supported by the American Cancer Society and the National Cancer Institute.

References

Adams, J. (2001). Proteasome inhibition in cancer: development of PS-341. Semin. Oncol. *28*, 613–619.

Adams, J.M., and Cory, S. (1998). The Bcl-2 protein family: arbiters of cell survival. Science *281*, 1322–1326.

Adams, J.M., Harris, A.W., Strasser, A., Ogilvy, S., and Cory, S. (1999). Transgenic models of lymphoid neoplasia and development of a pan-hematopoietic vector. Oncogene *18*, 5268–5277.

Arango, D., Corner, G.A., Wadler, S., Catalano, P.J., and Augenlicht, L.H. (2001). c-Myc/p53 interaction determines sensitivity of human colon carcinoma cells to 5-fluorouracil in vitro and in vivo. Cancer Res. *61*, 4910–4915.

Ashkenazi, A., Pai, R.C., Fong, S., Leung, S., Lawrence, D.A., Marsters, S.A., Blackie, C., Chang, L., McMurtrey, A.E., Hebert, A., et al. (1999). Safety and antitumor activity of recombinant soluble Apo2 ligand. J. Clin. Invest. *104*, 155–162.

Asselin, E., Mills, G.B., and Tsang, B.K. (2001). XIAP regulates Akt activity and caspase-3-dependent cleavage during cisplatin-induced apoptosis in human ovarian epithelial cancer cells. Cancer Res. *61*, 1862–1868.

Attardi, L.D., and Jacks, T. (1999). The role of p53 in tumour suppression: lessons from mouse models. Cell. Mol. Life Sci. *55*, 48–63.

Baldwin, A.S. (2001). Control of oncogenesis and cancer therapy resistance by the transcription factor NF-κB. J. Clin. Invest. *107*, 241–246.

Bartke, T., Siegmund, D., Peters, N., Reichwein, M., Henkler, F., Scheurich, P., and Wajant, H. (2001). p53 upregulates cFLIP, inhibits transcription of NF-κB-regulated genes and induces caspase-8-independent cell death in DLD-1 cells. Oncogene *20*, 571–580.

Beere, H.M., and Green, D.R. (2001). Stress management—heat shock protein-70 and the regulation of apoptosis. Trends Cell Biol. *11*, 6–10.

Blagosklonny, M.V. (2001). Unwinding the loop of Bcl-2 phosphorylation. Leukemia *15*, 869–874.

Bonni, A., Brunet, A., West, A.E., Datta, S.R., Takasu, M.A., and Greenberg, M.E. (1999). Cell survival promoted by the Ras-MAPK signaling pathway by transcription-dependent and -independent mechanisms. Science *286*, 1358–1362.

Brown, J.M., and Wouters, B.G. (1999). Apoptosis, p53, and tumor cell sensitivity to anticancer agents. Cancer Res. *59*, 1391–1399.

Bunz, F., Hwang, P.M., Torrance, C., Waldman, T., Zhang, Y., Dillehay, L., Williams, J., Lengauer, C., Kinzler, K.W., and Vogelstein, B. (1999). Disruption of p53 in human cancer cells alters the responses to therapeutic agents. J. Clin. Invest. *104*, 263–269.

Chatterjee, D., Schmitz, I., Krueger, A., Yeung, K., Kirchhoff, S., Krammer, P.H., Peter, M.E., Wyche, J.H., and Pantazis, P. (2001). Induction of apoptosis in 9-nitrocamptothecin-treated DU145 human prostate carcinoma cells correlates with de novo synthesis of CD95 and CD95 ligand and down-regulation of c-FLIP(short). Cancer Res. *61*, 7148–7154.

Chen, Y.N., Sharma, S.K., Ramsey, T.M., Jiang, L., Martin, M.S., Baker, K., Adams, P.D., Bair, K.W., and Kaelin, W.G., Jr. (1999). Selective killing of transformed cells by cyclin/cyclin-dependent kinase 2 antagonists. Proc. Natl. Acad. Sci. USA *96*, 4325–4329.

Cheng, E.H., Wei, M.C., Weiler, S., Flavell, R.A., Mak, T.W., Lindsten, T., and Korsmeyer, S.J. (2001). Bcl-2, bcl-x(l) sequester bh3 domain-only molecules preventing bax- and bak-mediated mitochondrial apoptosis. Mol. Cell *8*, 705–711.

Corbet, S.W., Clarke, A.R., Gledhill, S., and Wyllie, A.H. (1999). p53-dependent and -independent links between DNA-damage, apoptosis and mutation frequency in ES cells. Oncogene *18*, 1537–1544.

Creagh, E.M., Sheehan, D., and Cotter, T.G. (2000). Heat shock proteins—modulators of apoptosis in tumour cells. Leukemia *14*, 1161–1173.

Cunningham, C.C., Holmlund, J.T., Geary, R.S., Kwoh, T.J., Dorr, A., Johnston, J.F., Monia, B., and Nemunaitis, J. (2001). A Phase I trial of H-ras antisense oligonucleotide ISIS 2503 administered as a con-

tinuous intravenous infusion in patients with advanced carcinoma. Cancer *92*, 1265–1271.

Dang, C.V., and Semenza, G.L. (1999). Oncogenic alterations of metabolism. Trends Biochem. Sci. *24*, 68–72.

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

Deveraux, Q.L., and Reed, J.C. (1999). IAP family proteins-suppressors of apoptosis. Genes Dev. *13*, 239–252.

Di Cristofano, A., and Pandolfi, P.P. (2000). The multiple roles of PTEN in tumor suppression. Cell *100*, 387–390.

Domen, J., Gandy, K.L., and Weissman, I.L. (1998). Systemic overexpression of BCL-2 in the hematopoietic system protects transgenic mice from the consequences of lethal irradiation. Blood *91*, 2272–2282.

el-Deiry, W.S. (1997). Role of oncogenes in resistance and killing by cancer therapeutic agents. Curr. Opin. Oncol. *9*, 79–87.

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

Fidler, I.J. (1999). Critical determinants of cancer metastasis: rationale for therapy. Cancer Chemother. Pharmacol. *43*, S3–10.

Foster, B.A., Coffey, H.A., Morin, M.J., and Rastinejad, F. (1999). Pharmacological rescue of mutant p53 conformation and function. Science *286*, 2507–2510.

Fulda, S., Meyer, E., Friesen, C., Susin, S.A., Kroemer, G., and Debatin, K.M. (2001). Cell type specific involvement of death receptor and mitochondrial pathways in drug-induced apoptosis. Oncogene *20*, 1063–1075.

Green, D.R. (2000). Apoptotic pathways: paper wraps stone blunts scissors. Cell *102*, 1–4.

Griffiths, S.D., Clarke, A.R., Healy, L.E., Ross, G., Ford, A.M., Hooper, M.L., Wyllie, A.H., and Greaves, M. (1997). Absence of p53 permits propagation of mutant cells following genotoxic damage. Oncogene *14*, 523–531.

Guo, Z., Yikang, S., Yoshida, H., Mak, T.W., and Zacksenhaus, E. (2001). Inactivation of the retinoblastoma tumor suppressor induces apoptosis protease-activating factor-1 dependent and independent apoptotic pathways during embryogenesis. Cancer Res. *61*, 8395–8400.

Hanahan, D., and Weinberg, R.A. (2000). The hallmarks of cancer. Cell *100*, 57–70.

Harada, H., Andersen, J.S., Mann, M., Terada, N., and Korsmeyer, S.J. (2001). p70S6 kinase signals cell survival as well as growth, inactivating the pro-apoptotic molecule BAD. Proc. Natl. Acad. Sci. USA *98*, 9666–9670.

Harbour, J.W., and Dean, D.C. (2000). Rb function in cell-cycle regulation and apoptosis. Nat. Cell Biol. *2*, E65–E67.

Herr, I., and Debatin, K.M. (2001). Cellular stress response and apoptosis in cancer therapy. Blood *98*, 2603–2614.

Hoffman, W.H., Biade, S., Zilfou, J.T., Chen, J., and Murphy, M. (2001). Transcriptional repression of the anti-apoptotic survivin gene by wild type p53. J. Biol. Chem. *19*, 19.

Huang, D.C., and Strasser, A. (2000). BH3-only proteins-essential initiators of apoptotic cell death. Cell *103*, 839–842.

Hwang, P.M., Bunz, F., Yu, J., Rago, C., Chan, T.A., Murphy, M.P., Kelso, G.F., Smith, R.A., Kinzler, K.W., and Vogelstein, B. (2001). Ferredoxin reductase affects p53-dependent, 5-fluorouracil-induced apoptosis in colorectal cancer cells. Nat. Med. *7*, 1111–1117.

Ihde, D.C., Pass, H.I., and Glatstein, E. (1997). Small cell lung cancer. In Cancer Principles & Practices of Oncology, 5th ed., V.T. De Vita, S. Helman, and S. Rosenberg, eds. (Philadelphia: Lippincott-Raven).

Jansen, B., Wacheck, V., Heere-Ress, E., Schlagbauer-Wadl, H., Hoeller, C., Lucas, T., Hoermann, M., Hollenstein, U., Wolff, K., and Pehamberger, H. (2000). Chemosensitisation of malignant melanoma by BCL2 antisense therapy. Lancet *356*, 1728–1733.

Jia, L., Srinivasula, S.M., Liu, F.T., Newland, A.C., Fernandes-Alnemri, T., Alnemri, E.S., and Kelsey, S.M. (2001). Apaf-1 protein deficiency confers resistance to cytochrome c-dependent apoptosis in human leukemic cell. Blood *98*, 414–421.

Johnstone, R.W., Ruefli, A.A., and Smyth, M.J. (2000). Multiple physiological functions for multidrug transporter P-glycoprotein? Trends Biochem. Sci. *25*, 1–6.

Khanna, K.K., and Jackson, S.P. (2001). DNA double-strand breaks: signaling, repair and the cancer connection. Nat. Genet. *27*, 247–254.

Komarov, P.G., Komarova, F.A., Kondratov, R.V., Christov-Tselkov, K., Coon, J.S., Chernov, M.V., and Gudkov, A.V. (1999). A chemical inhibitor of p53 that protects mice from the side effects of cancer therapy. Science *285*, 1733–1737.

Komarova, E.A., and Gudkov, A.V. (2000). Suppression of p53: a new approach to overcome side effects of antitumor therapy. Biochemistry *65*, 41–48.

Komarova, E.A., and Gudkov, A.V. (2001). Chemoprotection from p53-dependent apoptosis: potential clinical applications of the p53 inhibitors. Biochem. Pharmacol. *62*, 657–667.

Kondo, S., Shinomura, Y., Miyazaki, Y., Kiyohara, T., Tsutsui, S., Kitamura, S., Nagasawa, Y., Nakahara, M., Kanayama, S., and Matsuzawa, Y. (2000). Mutations of the bak gene in human gastric and colorectal cancers. Cancer Res. *60*, 4328–4330.

Krek, W., Xu, G., and Livingston, D.M. (1995). Cyclin A-kinase regulation of E2F–1 DNA binding function underlies suppression of an S phase checkpoint. Cell *83*, 1149–1158.

Leone, G., Mele, L., Pulsoni, A., Equitani, F., and Pagano, L. (1999). The incidence of secondary leukemias. Haematologica *84*, 937–945.

Liu, R., Page, C., Beidler, D.R., Wicha, M.S., and Nunez, G. (1999). Overexpression of Bcl-x(L) promotes chemotherapy resistance of mammary tumors in a syngeneic mouse model. Am. J. Pathol. *155*, 1861–1867.

Los, M., Wesselborg, S., and Schulze-Osthoff, K. (1999). The role of caspases in development, immunity, and apoptotic signal transduction: lessons from knockout mice. Immunity *10*, 629–639.

Lowe, S.W., and Lin, A.W. (2000). Apoptosis in cancer. Carcinogenesis *21*, 485–495.

Marks, P.A., Richon, V.M., Breslow, R., and Rifkind, R.A. (2001). Histone deacetylase inhibitors as new cancer drugs. Curr. Opin. Oncol. *13*, 477–483.

Martinou, J.C., and Green, D.R. (2001). Breaking the mitochondrial barrier. Nat. Rev. Mol. Cell. Biol. *1*, 63–67.

Moroni, M.C., Hickman, E.S., Denchi, E.L., Caprara, G., Colli, E., Cecconi, F., Muller, H., and Helin, K. (2001). Apaf-1 is a transcriptional target for E2F and p53. Nat. Cell Biol. *3*, 552–558.

Muschen, M., Warskulat, U., and Beckmann, M.W. (2000). Defining CD95 as a tumor suppressor gene. J. Mol. Med. *78*, 312–325.

Ng, S.S.W., Tsao, M.S., Chow, S., and Hedley, D.W. (2000). Inhibition of phosphatidylinositide 3-kinase enhances gemcitabine-induced apoptosis in human pancreatic cancer cells. Cancer Res. *60*, 5451–5455.

Omer, C.A., Chen, Z., Diehl, R.E., Conner, M.W., Chen, H.Y., Trumbauer, M.E., Gopal-Truter, S., Seeburger, G., Bhimnathwala, H., Abrams, M.T., et al. (2000). Mouse mammary tumor virus-Ki-rasB transgenic mice develop mammary carcinomas that can be growth-inhibited by a farnesyl:protein transferase inhibitor. Cancer Res. *60*, 2680–2688.

Paulsen, M., and Ferguson-Smith, A.C. (2001). DNA methylation in genomic imprinting, development, and disease. J. Pathol. *195*, 97–110.

Rampino, N., Yamamoto, H., Ionov, Y., Li, Y., Sawai, H., Reed, J.C., and Perucho, M. (1997). Somatic frameshift mutations in the BAX gene in colon cancers of the microsatellite mutator phenotype. Science *275*, 967–969.

Reed, J.C. (1999). Dysregulation of apoptosis in cancer. J. Clin. Oncol. *17*, 2941–2953.

Robertson, K.D., and Jones, P.A. (1998). The human ARF cell cycle regulatory gene promoter is a CpG island which can be silenced by DNA methylation and down-regulated by wild-type p53. Mol. Cell. Biol. *18*, 6457–6473.

Rosen, D., Li, J.H., Keidar, S., Markon, I., Orda, R., and Berke, G. (2000). Tumor immunity in perforin-deficient mice: a role for CD95 (Fas/APO-1). J. Immunol. *164*, 3229–3235.

Roymans, D., and Slegers, H. (2001). Phosphatidylinositol 3-kinases in tumor progression. Eur. J. Biochem. *268*, 487–498.

Ryan, K.M., Ernst, M.K., Rice, N.R., and Vousden, K.H. (2000). Role of NF-kappaB in p53-mediated programmed cell death. Nature *404*, 892–897.

Ryan, K.M., Phillips, A.C., and Vousden, K.H. (2001). Regulation and function of p53 tumor suppressor protein. Curr. Opin. Cell Biol. *13*, 332–337.

Sasaki, H., Sheng, Y., Kotsuji, F., and Tsang, B.K. (2000). Down-regulation of X-linked inhibitor of apoptosis protein induces apoptosis in chemoresistant human ovarian cancer cells. Cancer Res. *60*, 5659–5666.

Schmitt, C.A., McCurrach, M.E., de Stanchina, E., Wallace-Brodeur, R.R., and Lowe, S.W. (1999). INK4a/ARF mutations accelerate lymphomagenesis and promote chemoresistance by disabling p53. Genes Dev. *13*, 2670–2677.

Schmitt, C.A., Rosenthal, C.T., and Lowe, S.W. (2000). Genetic analysis of chemoresistance in primary murine lymphomas. Nat. Med. *6*, 1029–1035.

Sherr, C.J., and Weber, J.D. (2000). The ARF/p53 pathway. Curr. Opin. Genet. Dev. *10*, 94–99.

Shin, M.S., Kim, H.S., Lee, S.H., Park, W.S., Kim, S.Y., Park, J.Y., Lee, J.H., Lee, S.K., Lee, S.N., Jung, S.S., et al. (2001). Mutations of tumor necrosis factor-related apoptosis-inducing ligand receptor 1 (TRAIL-R1) and receptor 2 (TRAIL-R2) genes in metastatic breast cancers. Cancer Res. *61*, 4942–4946.

Sierra, A., Castellsague, X., Escobedo, A., Lloveras, B., Garcia-Ramirez, M., Moreno, A., and Fabra, A. (2000). Bcl-2 with loss of apoptosis allows accumulation of genetic alterations: a pathway to metastatic progression in human breast cancer. Int. J. Cancer *89*, 142–147.

Soengas, M.S., Alarcon, R.M., Yoshida, H., Giaccia, A.J., Hakem, R., Mak, T.W., and Lowe, S.W. (1999). Apaf-1 and caspase-9 in p53-dependent apoptosis and tumor inhibition. Science *284*, 156–159.

Soengas, M.S., Capodieci, P., Polsky, D., Mora, J., Esteller, M., Opitz-Araya, X., McCombie, R., Herman, J.G., Gerald, W.L., Lazebnik, Y.A., et al. (2001). Inactivation of the apoptosis effector Apaf-1 in malignant melanoma. Nature *409*, 207–211.

Sorlie, T., Perou, C.M., Tibshirani, R., Aas, T., Geisler, S., Johnsen, H., Hastie, T., Eisen, M.B., van de Rijn, M., Jeffrey, S.S., et al. (2001). Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc. Natl. Acad. Sci. USA *98*, 10869–10874.

Stambolic, V., MacPherson, D., Sas, D., Lin, Y., Snow, B., Jang, Y., Benchimol, S., and Mak, T.W. (2001). Regulation of PTEN transcription by p53. Mol. Cell *8*, 317–325.

Straus, S.E., Jaffe, E.S., Puck, J.M., Dale, J.K., Elkon, K.B., Rosen-Wolff, A., Peters, A.M., Sneller, M.C., Hallahan, C.W., Wang, J., et al. (2001). The development of lymphomas in families with autoimmune lymphoproliferative syndrome with germline Fas mutations and defective lymphocyte apoptosis. Blood *98*, 194–200.

Suzuki, Y., Imai, Y., Nakayama, H., Takahashi, K., Takio, K., and Takahashi, R. (2001). A serine protease, Htra2, is released from the mitochondria and interacts with xiap, inducing cell death. Mol. Cell *8*, 613–621.

Swisher, S.G., Roth, J.A., Nemunaitis, J., Lawrence, D.D., Kemp, B.L., Carrasco, C.H., Connors, D.G., El-Naggar, A.K., Fossella, F., Glisson, B.S., et al. (1999). Adenovirus-mediated p53 gene transfer in advanced non-small-cell lung cancer. J. Natl. Cancer Inst. *91*, 763–771.

Tai, Y.T., Strobel, T., Kufe, D., and Cannistra, S.A. (1999). In vivo cytotoxicity of ovarian cancer cells through tumor-selective expression of the BAX gene. Cancer Res. *59*, 2121–2126.

Takakura, M., Kyo, S., Sowa, Y., Wang, Z., Yatabe, N., Maida, Y., Tanaka, M., and Inoue, M. (2001). Telomerase activation by histone deacetylase inhibitor in normal cells. Nucleic Acids Res. *29*, 3006–3011.

Takeda, K., Hayakawa, Y., Smyth, M.J., Kayagaki, N., Yamaguchi, N., Kakuta, S., Iwakura, Y., Yagita, H., and Okumura, K. (2001). Involvement of tumor necrosis factor-related apoptosis-inducing ligand in surveillance of tumor metastasis by liver natural killer cells. Nat. Med. *7*, 94–100.

Tamm, I., Dorken, B., and Hartmann, G. (2001). Antisense therapy in oncology: new hope for an old idea? Lancet *358*, 489–497.

Teitz, T., Wei, T., Valentine, M.B., Vanin, E.F., Grenet, J., Valentine, V.A., Behm, F.G., Look, A.T., Lahti, J.M., and Kidd, V.J. (2000). Caspase 8 is deleted or silenced preferentially in childhood neuroblastomas with amplification of MYCN. Nat. Med. *6*, 529–535.

Tepper, C.G., and Seldin, M.F. (1999). Modulation of caspase-8 and FLICE-inhibitory protein expression as a potential mechanism of Epstein-Barr virus tumorigenesis in Burkitt's lymphoma. Blood *94*, 1727–1737.

Trapani, J.A., Davis, J., Sutton, V.R., and Smyth, M.J. (2000). Proapoptotic functions of cytotoxic lymphocyte granule constituents in vitro and in vivo. Curr. Opin. Immunol. *12*, 323–329.

Vogelstein, B., Lane, D., and Levine, A.J. (2000). Surfing the p53 network. Nature *408*, 307–310.

Volm, M. (1998). Multidrug resistance and its reversal. Anticancer Res. *18*, 2905–2917.

Walczak, H., Bouchon, A., Stahl, H., and Krammer, P.H. (2000). Tumor necrosis factor-related apoptosis-inducing ligand retains its apoptosis-inducing capacity on Bcl-2- or Bcl-xL-overexpressing chemotherapy-resistant tumor cells. Cancer Res. *60*, 3051–3057.

Walker, A., Taylor, S.T., Hickman, J.A., and Dive, C. (1997). Germinal center-derived signals act with Bcl-2 to decrease apoptosis and increase clonogenicity of drug-treated human B lymphoma cells. Cancer Res. *57*, 1939–1945.

Wallace-Brodeur, R.R., and Lowe, S.W. (1999). Clinical implications of p53 mutations. Cell. Mol. Life Sci. *55*, 64–75.

Wang, J.L., Liu, D., Zhang, Z.J., Shan, S., Han, X., Srinivasula, S.M., Croce, C.M., Alnemri, E.S., and Huang, Z. (2000). Structure-based discovery of an organic compound that binds Bcl-2 protein and induces apoptosis of tumor cells. Proc. Natl. Acad. Sci. USA *97*, 7124–7129.

Wang, X. (2001). The expanding role of mitochondria in apoptosis. Genes Dev. *15*, 2922–2933.

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

Wei, M.C., Zong, W.X., Cheng, E.H., Lindsten, T., Panoutsakopoulou, V., Ross, A.J., Roth, K.A., MacGregor, G.R., Thompson, C.B., and Korsmeyer, S.J. (2001). Proapoptotic BAX and BAK: a requisite gateway to mitochondrial dysfunction and death. Science *292*, 727–730.

Wolf, B.B., Schuler, M., Li, W., Eggers-Sedlet, B., Lee, W., Tailor, P., Fitzgerald, P., Mills, G.B., and Green, D.R. (2001). Defective cytochrome c-dependent caspase activation in ovarian cancer cell lines due to diminished or absent apoptotic protease activating factor-1 activity. J. Biol. Chem. *276*, 34244–34251.

Wu, Y., Mehew, J.W., Heckman, C.A., Arcinas, M., and Boxer, L.M. (2001). Negative regulation of bcl-2 expression by p53 in hematopoietic cells. Oncogene *20*, 240–251.

Yang, A., and McKeon, F. (2000). p63 and p73: p53 mimics, menaces and more. Nat. Rev. Mol. Cell. Biol. *1*, 199–207.

Zhang, L., Yu, J., Park, B.H., Kinzler, K.W., and Vogelstein, B. (2000). Role of BAX in the apoptotic response to anticancer agents. Science *290*, 989–992.