Chapter 4
Apoptotic Pathways in Tumor Progression and Therapy

Armelle Melet, Keli Song, Octavian Bucur, Zainab Jagani, Alexandra R. Grassian, and Roya Khosravi-Far*

Abstract Apoptosis is a cell suicide program that plays a critical role in development and tissue homeostasis. The ability of cancer cells to evade this programmed cell death (PCD) is a major characteristic that enables their uncontrolled growth. The efficiency of chemotherapy in killing such cells depends on the successful induction of apoptosis, since defects in apoptosis signaling are a major cause of drug resistance. Over the past decades, much progress has been made in our understanding of apoptotic signaling pathways and their dysregulation in cancer progression and therapy. These advances have provided new molecular targets for proapoptotic cancer therapies that have recently been used in drug development. While most of those therapies are still at the preclinical stage, some of them have shown much promise in the clinic. Here, we review our current knowledge of apoptosis regulation in cancer progression and therapy, as well as the new molecular targeted molecules that are being developed to reinstate cancer cell death.

Keywords apoptosis, cancer, therapy, inhibitors, signal transduction, oncogene, intrinsic, extrinsic

1 Introduction

Apoptosis, is an evolutionarily conserved mechanism for the selective removal of unwanted cells (Abe et al., 2000b; Degli Esposti, 1999; Lawen, 2003; Ozoren and El-Deiry, 2003; Peter and Krammer, 1998; Strasser et al., 2000; Thorburn, 2004). Regulation of apoptosis is critical for tissue homeostasis, therefore, its deregulation can lead to a variety of pathological conditions, including cancer. For this reason,

Armelle Melet, Keli Song, Octavian Bucur, Zainab Jagani, Alexandra R. Grassian, and Roya Khosravi-Far
Department of Pathology, Harvard Medical School, Beth Israel Deaconess Medical Center, 99 Brookline Avenue, Boston, MA 02215, USA
*To whom correspondence should be addressed: Tel.: (617) 667-8526; fax: (617) 667-3524; e-mail: rkhosrav@bidmc.harvard.edu

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inhibition of apoptosis or the promotion of resistance to apoptosis contributes to carcinogenesis and chemoresistance (Burns and el-Deiry, 2003; Daniel et al., 2001; Green and Evan, 2002; Ozoren and El-Deiry, 2003; Sheikh and Huang, 2004; Thompson, 1995; Zornig et al., 2001).

Apoptosis is primarily mediated through the activation of specific proteases called caspases (cysteinyl, aspartate-specific proteases) (Algeciras-Schimnich et al., 2002; Ozoren and El-Deiry, 2003; Salvesen and Dixit, 1997; Stegh and Peter, 2001; Thorburn, 2004). Caspases, which are effectors of PCD, cleave multiple substrates, leading to biochemical and morphological changes that are characteristic of suicidal cells (Abe et al., 2000b; Bouillet et al., 2000). Cells undergoing apoptosis undergo cell membrane remodeling and blebbing; the exposure of phosphatidylserine (PS) at the external surface of the cell; cell shrinkage with cytoskeletal rearrangements; nuclear condensation; and DNA fragmentation (Ashkenazi and Dixit, 1999; Green and Evan, 2002; Lawen, 2003; Peter and Krammer, 2003; Schulze-Osthoff et al., 1998; Thorburn, 2004). These morphological changes culminate in the formation of apoptotic bodies that are normally eliminated by phagocytosis (Geske and Gerschenson, 2001; Wallach, 1997).

In this chapter, we introduce the major apoptotic machinery and discuss some recent insights into the involvement of apoptosis in cancer progression, cancer therapy, and resistance to therapy.

2 Apoptotic Machinery

In mammals, the two major signaling systems that result in the activation of caspases and the consequent induction of apoptosis are the *extrinsic* death receptor pathway and the *intrinsic* mitochondrial pathway (Fig. 4.1) (Abe et al., 2000b; Ozoren and El-Deiry, 2003; Peter and Krammer, 2003; Strasser et al., 2000; Thorburn, 2004). In the past few years, increasing evidence indicates that the death receptor and mitochondrial pathways are not isolated systems. Instead, significant cross talks and "biofeedbacks" regulate the apoptotic machinery (Abe et al., 2000b; Li and Yuan, 1999; Reed, 2000; Zornig et al., 2001).

2.1 The Death Receptor Pathway of Apoptosis

The extrinsic apoptotic pathway is activated upon the binding of cytokine ligands (i.e., FasL, tumor necrosis factor [TNF], and TNF-related apoptosis-inducing ligand [TRAIL]) to members of the TNFα receptor superfamily, which are usually called the death receptors (i.e., Fas, also called CD95/Apo-1; TNF receptors; and TRAIL receptors) (Abe et al., 2000b; Ashkenazi and Dixit, 1999; Ozoren and El-Deiry, 2003; Peter and Krammer, 2003; Schulze-Osthoff et al., 1998; Thorburn, 2004). Death receptors contain an intracellular globular interaction domain known

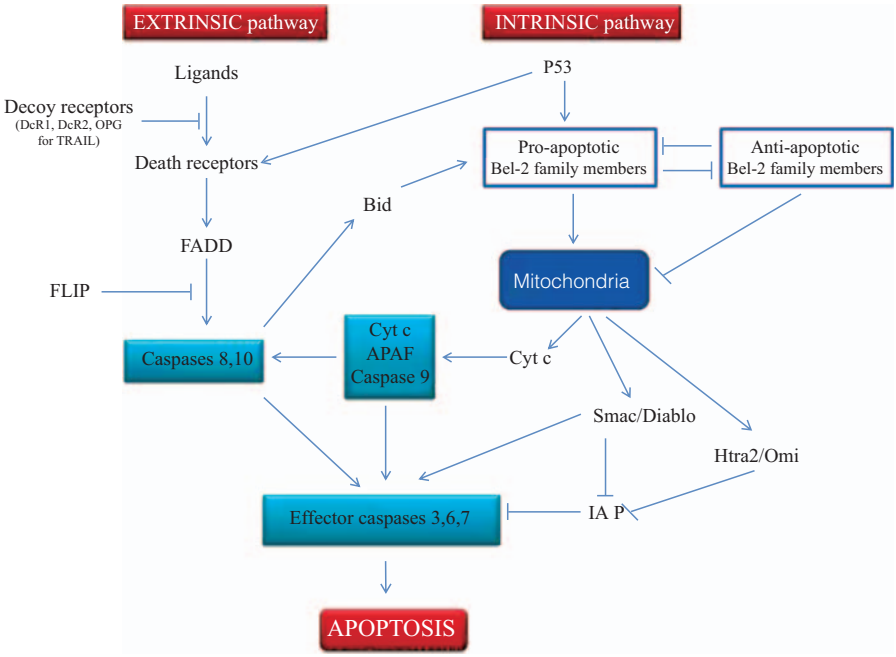

Fig. 4.1 Extrinsic and Intrinsic Apoptotic Machinery

as a death domain (DD). Upon ligand binding to their extracellular domains, death receptors aggregate at the cell surface and possibly form trimes. This results in the recruitment of adaptor molecules to the aggregated intracellular domains of the receptors. The Fas-associated death domain (FADD) is one of the major adaptors to be recruited to the death receptors. FADD possesses a DD that interacts either directly with the DD of death receptors, or indirectly through another adaptor molecule, TNF receptor-associated death domain (TRADD). FADD also contains a second protein interaction domain, known as the death effector domain (DED). This DED domain interacts with the DED of the weakly active zymogen pro-caspase-8, to form an intracellular multiprotein complex known as the death-inducing signaling complex (DISC) (Abe et al., 2000a; Ashkenazi and Dixit, 1998; Boatright et al., 2003; Cory and Adams, 2002; Wallach et al., 1999). Once formed, the DISC promotes the proximity-induced processing of caspase-8, which then proceeds to be further activated via an autoproteolysis mechanism (Salvesen and Dixit, 1999; Yang et al., 1998). Active caspase-8 subsequently activates executioner/effector caspases, such as caspase-3, leading to cell execution via degradation of the nucleus and other intracellular structures (Ashkenazi and Dixit, 1998; Cohen, 1997; Peter and Krammer, 2003; Scaffidi et al., 1998). This direct activation of caspase-dependent cell execution is thought to occur in certain cell types, including thymocytes, that

are classified as type I cells (Boatright et al., 2003; Ozoren and El-Deiry, 2002; Scaffidi et al., 1998). These cells are able to efficiently activate caspase-8 so that it can cleave and consequently activate its primary targets, the executioner caspases including caspase-3. This simplified pathway of type I cells plays an important role in the immune response that is involved in the deletion of transformed cells (Hickman, 2002; Zornig et al., 2001) and resembles the linear pathway of developmental cell death established in genetic studies of *Caenorhabditis elegans* (Horvitz, 1999; Vaux, 2002). Nonetheless, PCD in *C. elegans* is distinct in that Bcl-2/Ced-9 is unable to block caspase activation following death receptor stimulation in type I cells (Peter and Krammer, 2003; Scaffidi et al., 1999).

2.2 The Mitochondrial Pathway of Apoptosis

Mitochondria are thought to be the central organelles involved in mediating most apoptotic pathways in mammalian cells (Green and Kroemer, 2004; Kroemer, 2003; Newmeyer and Ferguson-Miller, 2003; Ravagnan et al., 2002; Sorice et al., 2004; Zamzami and Kroemer, 2001). Mitochondria are engaged via the intrinsic pathway of cell death, which can be initiated by a variety of stress stimuli, including ultraviolet (UV) radiation, γ-irradiation, heat, DNA damage, the actions of some oncoproteins and tumor suppressor genes, viral virulence factors, and most chemotherapeutic agents (Kroemer, 2003). These diverse forms of stress are sensed or decoded by multiple cytosolic or intraorganellar molecules which then transduce the signals to mitochondria, resulting in alterations in the permeability of the outer mitochondrial membrane (OM) (Esposti et al., 2003; Green and Kroemer, 2004; Kuwana et al., 2002; Newmeyer and Ferguson-Miller, 2003; Zamzami and Kroemer, 2001). This leads to increased permeability to apoptotic proteins that are normally trapped between the OM and the inner mitochondrial membrane (IM), thus enabling these proteins to escape the mitochondria and diffuse into the cytosol.

The release of apoptotic factors leads to apoptosome-mediated activation of caspases (Fig. 4.1). The apoptosome works like a large platform for recruiting and facilitating the self-activation of the apical caspase of the intrinsic pathway of apoptosis, pro-caspase-9 (Adams and Cory, 2002; Baliga and Kumar, 2003; Cain et al., 2002; Chinnaiyan, 1999; Hill et al., 2003; Salvesen and Renatus, 2002; Shi, 2002). The apoptosome promotes the local accumulation of zymogens that initiate an autocatalytic activation of caspase-9 in a manner similar to the activation of caspase-8 at the DISC (Adams and Cory, 2002; Baliga and Kumar, 2003; Cain et al., 2002; Chinnaiyan, 1999; Hill et al., 2003; Salvesen and Renatus, 2002; Shi, 2002). The apoptosome, however, requires additional regulatory factors to fully activate the caspase cascade. These factors include Smac/Diablo, a protein that interacts with several inhibitor of apoptosis proteins (IAPs) and displaces them from their inhibitory interaction with pro-caspase-9 and other caspases.(Adams and Cory, 2002; Baliga and Kumar, 2003; Cain et al., 2002; Shi, 2002).

The mitochondrial pathway can also be activated in response to death ligands. In type II cells, selective cleavage of Bid by caspase-8 has been found to connect upstream signals from the DISC to the mitochondria (Gross et al., 1999; Li et al., 1998; Luo et al., 1998). Furthermore, genetic ablation of Bid reduces Fas-induced hepatotoxicity and mitochondrial damage (Zinkel et al., 2003). The caspase-cleaved form of Bid, tBid, migrates to the OM, where it cooperates with other Bcl-2 family proteins, such as Bak or Bax, to induce the release of mitochondrial proteins into the cytosol (Wei et al., 2001b).

3 Oncogene-Induced Evasion of Apoptosis: A Mechanism for Tumor Progression

It has become clear that a fundamental property of cancer cells is their ability to evade the apoptotic cellular death program (Hanahan and Weinberg, 2000). This not only promotes their unchecked growth, but also suggests a mechanism whereby they can be controlled. Investigating the mechanisms underlying this resistance of tumor cells to apoptosis remains of significant interest, since a desired goal of anticancer therapies is to selectively unleash the apoptotic potential of tumor cells.

In the normal cellular context, proliferation and death programs are tightly linked. Given this, cells harboring a single oncogenic mutation driving proliferation undergo a protective growth inhibitory response, appropriately resulting in apoptosis of the pre-neoplastic cell. In contrast, such as in the progression of tumors, oncogenes overcome these protective cellular responses by taking advantage of cooperating, additional mutations in apoptosis signaling molecules, resulting in the abnormal proliferation and survival/antiapoptosis of the tumor cell (Lowe et al., 2004). In a classic example, overexpression of the wild-type c-Myc oncoprotein can induce apoptosis and sensitize cells towards a host of apoptotic stimuli in certain cell types (Pelengaris et al., 2002). However, several events, including inactivation of p53, overexpression of Bcl-2, and loss of Bim are able to cooperate with Myc in inducing tumorigenesis (Pelengaris et al., 2002). In another strategy for tumorigenicity, fusion proteins such as Bcr-Abl can simultaneously activate multiple pathways, including those involved in cellular proliferation and in the promotion of survival and suppression of apoptosis.

3.1 Myc

C-Myc is a proto-oncogene first identified as the cellular homologue of the oncogene found in the avian myelocytomatosis retrovirus (Gonda et al., 1982). The other two *Myc* genes in mammals are *MYCN* and *L-Myc*. Myc, which is a transcription factor, can both activate and repress target genes. Recent estimates suggest that *c-Myc* could regulate as many as 15% of genes in genomes from flies to humans

(Fernandez et al., 2003; Orian et al., 2003). These target genes are involved in diverse functions including cell proliferation, differentiation, cell adhesion, metabolism, DNA repair, and apoptosis (Dang, 1999; Oster et al., 2002). Myc expression and its activity in normal cells are tightly regulated. However, Myc overexpression has been found in up to 50% of all human cancers (Alitalo and Schwab, 1986; Pompetti et al., 1996). Myc is thought to contribute to tumorigenesis through unrestrained cellular growth and proliferation and also exerts its effects on cellular processes such as cellular adhesion, angiogenesis and genomic instability (Calvisi et al., 2004; Felsher and Bishop, 1999; Ingvarsson, 1990; Knies-Bamforth et al., 2004). In addition to its established roles in promoting cellular growth and proliferation, Myc was also found to be an inducer of apoptosis (Evan and Vousden, 2001; Evan et al., 1992). It has been reported that Myc potentiates apoptosis through both p53-dependent and p53-independent mechanisms (Sakamuro et al., 1995). Even though the mechanisms by which Myc protein drives such disparate functions are still not well understood, it has been suggested that the ability of Myc to sensitize cells to apoptosis could be an intrinsic property of cells. Abrogation of this proapoptotic property profoundly contributes to cancer progression (Sakamuro et al., 1995). Some principles have also emerged from studies in cell culture and animal models to explain how Myc can promote cancer growth while acting as an inducer of apoptosis.

Modes of Myc dysregulation include chromosomal translocation and amplification, activation of upstream growth stimulatory signaling cascades, and increased protein stability (Oster et al., 2002). One of the important cellular processes caused by Myc dysregulation is genomic instability, which is prone to additional genomic mutations. Thus, activation of other oncogenes may follow in response to Myc deregulation. The antiapoptotic functions of some oncogenes can overcome the proapoptotic function of Myc. For example, in a conditional transgenic model of Myc-induced breast adenocarcinomas (Arvanitis and Felsher, 2006; Boxer et al., 2004; D'Cruz et al., 2001; Hutchinson and Muller, 2000), Myc inactivation results in tumor regression in about 50% of the tumors. Many of the tumors that initially regress subsequently relapse. Half of the tumors that do not regress and those that later relapse have active mutations in K-Ras or H-Ras. In those mice, mutant Ras appears to facilitate the ability of tumors to become independent of Myc.

Myc cooperation with other oncogenes is another important mechanism by which Myc promotes tumorigenesis. In mice, when the *C-Myc* transgene is coupled to the immunoglobulin heavy chain μ-enhancer, it leads to B-cell-specific overexpression of the *C-Myc* gene and development of lymphomas (Adams et al., 1985; Harris et al., 1988). This Eμ-Myc mouse is a model for the human disease Burkitt's lymphoma, where a reciprocal chromosomal translocation to the immunoglobulin locus leads to inappropriate expression of Myc in the B-cell compartment. The lymphomas that develop in the mouse model are consistently clonal, indicating that additional mutations are necessary to produce tumors. However, mice doubly transgenic for Eμ-Myc and Eμ-BCL2 mutations display a marked decrease in latency of disease, developing a leukemia of early progenitor cells (Strasser et al., 1990), rather than the lymphoma that develops with Eμ-Myc alone (Harris et al., 1988).

Studies in both cell culture and transgenic mice have shown that enforced Bcl2 expression was capable of blocking Myc-induced apoptosis and left the proliferation functions of Myc intact (Bissonnette et al., 1992; Fanidi et al., 1992; Letai et al., 2004).

Notably, the specific consequences of Myc inactivation appear to depend both on the type of cancer cells and the constellation of genetic events unique to a given tumor. Studies in conditional transgenic mouse model systems have shown that Myc inactivation results in the proliferation arrest, differentiation and/or apoptosis of tumor cells (Arvanitis and Felsher, 2006). Additionally, recent reports have suggested that targeted inactivation of Myc is a potential approach to cancer therapy, if used in conjunction with other anticancer treatments (Arvanitis and Felsher, 2006). To date, however, no drugs that target Myc have been identified for the treatment of humans with cancer (Arvanitis and Felsher, 2006).

3.2 Signaling Pathways Activated by Bcr-Abl and the Suppression of Apoptosis

The Bcr-Abl fusion protein activates multiple signaling pathways that lead to proliferation, reduced dependence on growth factors, apoptosis, and abnormal interactions with the extracellular matrix and stroma. Recent research suggests that one key mechanism by which Bcr-Abl facilitates the expansion of myeloid cells involves the suppression of apoptosis. Notably, the primary consequence of tyrosine kinase inhibition with imatinib in Bcr-Abl-transformed cells is the induction of apoptosis (Druker et al., 1996; Gambacorti-Passerini et al., 1997). Additionally, in growth factor-dependent hematopoietic cells, Bcr-Abl induces the survival and proliferation of cells that would otherwise undergo apoptotic cell death in response to growth factor withdrawal (Bedi et al., 1994). Furthermore, antisense oligonucleotide-mediated inhibition of Bcr-Abl expression in these transformed cells results in apoptosis without altering their cell cycle (Bedi et al., 1994). It has also been demonstrated that Bcr-Abl-positive cells are highly resistant to various apoptotic stimuli and become sensitized to drug treatment upon antisense inhibition of Bcr-Abl (McGahon et al., 1994). Additional evidence for the antiapoptotic effects of Bcr-Abl comes from experiments with temperature-sensitive Bcr-Abl kinase mutants, in which induction of Bcr-Abl kinase activity at the permissive temperature led to a significant decrease in apoptosis in the absence of growth factors (Carlesso et al., 1994; Kabarowski et al., 1994). In fact, studies in primary cells have revealed that chronic myelogenous leukemia (CML) progenitors show a normal proliferative response to growth factors and do not have a greater proliferative potential than normal progenitors (Emanuel et al., 1991). Furthermore, in the absence of serum and growth factors, neither normal nor CML progenitors proliferated, yet the latter maintained higher cell viability (Bedi et al., 1994).

As a result of its elevated tyrosine kinase activity, the Bcr-Abl fusion protein activates several signaling pathways, including Ras (Sawyers et al., 1995), PI3-K/Akt (Skorski et al., 1997; Varticovski et al., 1991), Stat (Carlesso et al., 1996; Ilaria and

Van Etten, 1996; Shuai et al., 1996), and NF-κB (Reuther et al., 1998) some of which may be crucial for its leukemogenic activity. In accordance with the ability of Bcr-Abl to substitute for the requirement of cytokines, many of these pathways are also activated by hematopoietic cytokines upon binding to their respective cytokine receptors. A functional consequence of the activation of these pathways involves changes in the activity and gene expression of key molecules, which have a direct impact on cellular survival, growth, and behavior. In particular, the Ras (Sawyers et al., 1995), PI3-K/Akt (Varticovski et al., 1991), Stat (Nieborowska-Skorska et al., 1999; Sillaber et al., 2000), NF-κB (Reuther et al., 1998), and FOXO (Ghaffari et al., 2003) pathways are capable of transmitting antiapoptotic signals, which could promote the evasion of Bcr-Abl-transformed cells from apoptosis. Thus, determining which of these antiapoptotic signals plays a role in Bcr-Abl-mediated evasion of apoptosis and promotion of leukemogenesis is of interest, especially since the cross talk between, and potential cooperation among, these pathways may be important in mediating the leukemogenic effects of Bcr-Abl.

4 Chemotherapeutic Drugs and Conventional Radiation-Induced Apoptosis in Tumor Cells

Aberrant cell proliferation, a major hallmark of cancer, has been exploited for anti-cancer drug development. Most existing chemotherapeutic drugs interfere with DNA synthesis and cell division, thereby preferentially killing rapidly dividing cells such as cancer cells (Schulze-Bergkamen and Krammer, 2004). These drugs include such diverse groups as antimetabolites, genotoxic/DNA-damaging agents (alkylating and intercalating agents, topoisomerase inhibitors) and mitotic inhibitors (vinca alkaloids and taxanes) (Luqmani, 2005). It is now well established that these cytotoxic agents exert their antitumor activity mainly through induction of apoptosis and that defects in apoptotic pathways can lead to treatment failure (Johnstone et al., 2002; Kaufmann and Earnshaw, 2000; Lowe and Lin, 2000; Mesner et al., 1997).

Apoptosis induced by chemotherapeutic drugs primarily involves the mitochondrial apoptotic pathway and, in some cases, the death receptor pathway and upregulation of death receptors and/or ligands (Bucur et al., 2006; Pommier et al., 2004). The relative contribution of each pathway to drug-induced apoptosis may depend on the cytotoxic drug, dose, kinetics, and cell type ((Fulda et al., 2001b), reviewed in (Debatin and Krammer, 2004)).

4.1 DNA-Damaging Agents and Induction of Apoptosis

Chemotherapeutic drugs damage DNA either directly or indirectly (antimetabolites) and subsequently initiate a DNA-damage response through both p53-dependent and p53-independent mechanisms (Waxman and Schwartz, 2003). Irradiation mainly induces direct DNA damage, but can also act indirectly, one example being

the modulation of the epigenetic effectors in distant bystander tissue in vivo. X-ray exposure to one part of the animal body induces DNA strand breaks and causes an increase in levels of Rad51 in unexposed bystander tissue (Koturbash et al., 2006).

Drug- and radiation-induced DNA damage is first sensed by DNA-binding factors such as Rad17-RFC and the Rad9-1-1 supercomplex, BRCA1 and the Ku subunit of DNA-PK. The DNA damage signal is then transduced by activation of the PI3K family members DNA-PK, ATM, and ATR which, in turn, phosphorylate effector kinases such as the Ser/Thr kinases Chk1 and Chk2 and the tyrosine kinase c-Abl. These activated kinases then phosphorylate their downstream targets including the transcription factors p53, p73, and E2F, resulting in the transactivation of numerous genes involved in DNA repair, cell cycle arrest, and apoptosis.

The tumor suppressor p53 plays a key role in cellular response to stress and DNA damage (Meek, 2004) and has been implicated frequently in drug-induced apoptosis (Blagosklonny, 2002). Following DNA damage, p53 is induced by phosphorylation via ATM and Chk2. Phosphorylation of p53 not only enhances its DNA binding and transcriptional activity, but also stabilizes the protein by inhibiting its MDM2-mediated ubiquitination and its subsequent proteasomal degradation. The resulting increase in protein stability ultimately enhances the transcription of p53 target genes. p53 was shown to activate the mitochondrial apoptotic pathway by upregulating proapoptotic genes such as Bax, Bid, Noxa, and Puma, and down-regulating antiapoptotic proteins such as Bcl-2 and Mcl-1 (Michalak et al., 2005; Schuler and Green, 2005; Yu and Zhang, 2005). In addition, p53 can activate the extrinsic pathway by upregulating death receptors such as Fas, DR4, and DR5, although this pathway alone seems insufficient to induce apoptosis in some cancer cells (Reinke and Lozano, 1997). Recent evidence also suggests that p53 exerts proapoptotic functions independent of transcription, by translocating to the mitochondrion (Erster and Moll, 2005; Marchenko et al., 2000) and binding to Bcl-2 (Mihara et al., 2003; Tomita et al., 2006) and Bcl-XL (Mihara et al., 2003; Xu et al., 2006).

DNA-damaging agents can also induce apoptosis through p53-independent pathways involving, for instance, the transcription factor E2F (Lin et al., 2001). E2F exerts important proapoptotic activity in p53-deficient cells through transactivation of proapoptotic genes such as *Apaf-1* (Furukawa et al., 2002; Moroni et al., 2001), the caspase proenzymes (Nahle et al., 2002), *p73* (Irwin et al., 2003; Seelan et al., 2002; Stiewe and Putzer, 2000; Wang and Ki, 2001), and through repression of *Mcl-1* (Croxton et al., 2002). In certain cell types, radiation treatment, when used alone, may activate death receptors to execute the apoptotic program (Gong and Almasan, 2000). Finally, DNA-damaging drugs can engage a stress response via the stress-activated protein kinase/JNK pathway to activate the AP-1 and NF-κB-dependent transcription of *FasL* (Herr and Debatin, 2001; Kasibhatla et al., 1998).

4.2 Targeting the Apoptotic Machinery Directly

Targeting Bcl-2 family of proteins, death receptors, IAPs, caspases, and p53 are discussed in Chapter 8.

4.3 Microtubule Inhibitors and Induction of Apoptosis

Like DNA-damaging agents, microtubule inhibitors also lead to the phosphorylation and stabilization of p53 as a mechanism for drug-induced apoptosis (Blagosklonny, 2002; Wang et al., 1999). However, in MCF-7 breast cancer cells, inactivation of p53 does not affect cellular sensitivity to paclitaxel killing. In those cells, p53 may act as a survival factor by blocking them in the G2/M phase, rather than serving as an apoptotic inducer. By contrast, the transcription factor FOXO3a has been shown to upregulate the proapoptotic Bcl-2 family member, Bim, and contribute to paclitaxel-induced cell death in MCF7 cells (Sunters et al., 2003). Similarly, another FOXO family member, FOXO1, has been implicated in drug-induced apoptosis through the transcriptional activation of the TNF-R1-associated protein TRADD (Rokudai et al., 2002).

4.4 Anticancer Therapeutics and Other Forms of Cell Death

In addition to classical apoptosis, anticancer drugs sometimes trigger autophagic and necrotic modes of cell death (Gozuacik and Kimchi, 2004; Kim et al., 2006; Kondo et al., 2005; Nelson and White, 2004). For instance, tamoxifen induces autophagic cell death in MCF-7 cells (Bursch et al., 1996). Similarly, the alkylating agent temozolomide kills malignant glioma cells through autophagy rather than apoptosis (Kanzawa et al., 2004). Some reports also indicate that paclitaxel and vinblastine induce both autophagic and apoptotic cell death (Broker et al., 2004; Hirsimaki and Hirsimaki, 1984). Necrotic cell death (Proskuryakov et al., 2003) has also been observed in vitro in resistant human ovarian carcinoma cells exposed to HPMA copolymer-bound doxorubicin (Demoy et al., 2000) and in vivo in p53/Bcl-2-deficient mice treated with DNA-alkylating agents (Zong et al., 2004). These alternative modes of cell death have only recently been identified and their respective importance and possible cross talk in drug cytotoxic action remain to be further defined. These forms of cell death together with mitotic catastrophe are further discussed in Chapter 3.

5 Mechanisms of Radiation and Drug Resistance

Drug resistance can be classified as nononcogenic (impaired drug-target interaction) and oncogenic (deregulation of apoptosis and the cell cycle). Principal mechanisms of nononcogenic resistance include increased drug membrane export involving the PgP protein product of the MDR gene; decreased drug activation; increased drug degradation; enhanced DNA repair; and mutations of drug targets (Longley and Johnston, 2005; Luqmani, 2005). In oncogenic resistance, the drug interacts with its target, but downstream pathways of apoptosis and the cell cycle are altered

(Longley and Johnston, 2005; Luqmani, 2005). Intrinsic or acquired oncogenic resistance can result from multiple mechanisms, as outlined below.

5.1 Prosurvival Signaling (Mitogenic Kinases and NF-κB)

Mitogenic protein tyrosine kinases play a major role in drug resistance through their regulation of antiapoptotic signaling pathways (Blume-Jensen and Hunter, 2001). These include, for instance, members of the EGFR and Ras families, Bcr-Abl, and Akt. Overexpression of EGFR and Her-2 has been reported to increase resistance to chemotherapeutic drugs (Chevallier et al., 2004; Knuefermann et al., 2003; Mendelsohn and Fan, 1997; Nagane et al., 1998; Pegram et al., 1997). Activated Ras family members have also been shown to decrease cells' sensitivity to cytotoxic agents (Fan et al., 1997; Jansen et al., 1997). For example, several reports suggest that expression of Ras oncoproteins can contribute to cisplatin resistance by reducing drug uptake and increasing the degree of DNA repair (Dempke et al., 2000; Levy et al., 1994). Similarly, Bcr-Abl-expressing hematopoietic cell lines and various patient-derived CML cell lines are highly resistant to apoptotic induction by chemotherapy (Aichberger et al., 2005; Bedi et al., 1994; Cortez et al., 1996; Gesbert and Griffin, 2000; Keeshan et al., 2001; McGahon et al., 1994; Ray et al., 2004; Skorski, 2002; Underhill-Day et al., 2006).

The PI3K/Akt pathway, at the crossroads of multiple signaling networks, has been shown to be overactivated by upstream mitogenic kinases and oncogenic mutations in a wide range of tumors (Osaki et al., 2004). A number of studies have established that overexpression or activation of Akt increases chemoresistance both in cell lines (Page et al., 2000; Pommier et al., 2004) and in vivo (Kim et al., 2005; Martelli et al., 2005; McCormick, 2004; Wendel et al., 2004). Accordingly, inhibition of the PI3K/Akt pathway enhances the cytotoxic effects of a variety of chemotherapeutic agents (Hennessy et al., 2005; Nguyen et al., 2004; Nicholson et al., 2003; O'Gorman et al., 2000; Toretsky et al., 1999). The PTEN tumor suppressor is frequently mutated in human tumors. Loss of PTEN is associated with constitutive survival signaling through the PI3K/Akt pathway. Adenovirus-mediated expression of PTEN completely suppressed Akt activation in various cancer cell lines, such as the LNCaP prostate cancer cell line, and enhanced apoptosis induced by a broad range of apoptotic stimuli, including the chemotherapeutic agents mitoxantrone and etoposide, and death receptor-mediated treatments such as TRAIL, TNF-α, and agonistic antibodies against Fas (Yuan and Whang, 2002).

Finally, tumors with constitutive NF-κB activity are highly resistant to cytotoxic drugs (Arlt and Schafer, 2002; Baldwin, 2001). Accordingly, inhibition of NF-κB dramatically increases the sensitivity of these tumors to drugs by downregulation of antiapoptotic proteins (Nakanishi and Toi, 2005). Moreover, treatment with diverse cytotoxic drugs (including 5-FU, doxorubicin, paclitaxel, and cisplatin) can activate NF-κB, thereby blunting the ability of chemotherapy to induce cell death (Chuang et al., 2002).

5.2 Loss of p53 Function

Loss of p53 function is frequently encountered in human tumors and plays a critical role in resistance to chemotherapeutic drugs and conventional radiation (Levine et al., 2004; Lowe et al., 1994; Weller, 1998). Mechanisms responsible for p53 dysfunction include mutations or allelic loss in the p53 gene; upregulation of p53 inhibitors such as Mdm2; silencing of key p53 coactivators such as ARF; and altered upstream or downstream signaling (Vogelstein et al., 2000). For instance, lymphomas from mice deficient in p53 are markedly resistant to chemotherapy both in vitro and in vivo (Schmitt et al., 1999). p53 expression is predictive for response to chemotherapy in non-small-cell lung cancers (NSCLC) (Harada et al., 2003). Moreover, p53 mutations have been correlated with resistance to doxorubicin treatment and early relapse in patients with breast carcinomas (Aas et al., 1996). Cancers that retain wild-type p53 are more likely to respond to chemotherapy than other tumor types. However, many types of wt p53 tumors with defective apoptotic machinery do not undergo apoptosis despite genotoxic stress (Blagosklonny, 2001; Gudas et al., 1996).

5.3 Defective Apoptotic Machinery

5.3.1 Defective Mitochondrial Activation

The Bcl-2 protein family plays a pivotal role in the regulation of the mitochondrial apoptotic pathway and, consequently, its members serve as major regulators of tumor sensitivity to drugs (Kirkin et al., 2004; Kostanova-Poliakova and Sabova, 2005; Pommier et al., 2004). Overexpression of antiapoptotic Bcl-2 members such as Bcl-2, Bcl-XL, and Mcl-1, or deficiency of the proapoptotic members Bak and Bax, has been associated with drug resistance in cell lines, mouse models, and patients (Kirkin et al., 2004; Kostanova-Poliakova and Sabova, 2005; Pommier et al., 2004). Indeed, overexpression of Bcl-2 (Dole et al., 1994; Kamesaki et al., 1993; Miyashita and Reed, 1992; Walton et al., 1993) or Bcl-XL (Amundson et al., 2000) prevents apoptosis induced by most chemotherapeutic drugs in vitro. Some evidence also indicates similar effects with Mcl-1 overexpression (Song et al., 2005; Zhou et al., 1997). In concordance with overexpression data, downregulation of Bcl-XL or Bcl-2 has been shown to sensitize cancer cells to DNA damage-induced apoptosis. Fibroblasts deficient in both Bak and Bax are resistant to apoptosis induced by various agents (Wei et al., 2001a). While Bak deficiency renders Jurkat cells resistant to staurosporin, bleomycin, and cisplatin (Wang et al., 2001), loss of Bax expression is associated with acquired resistance to oxaliplatin (Gourdier et al., 2002) or resistance to 5-FU (Zhang et al., 2000) in colon carcinoma cell lines. Clinically, high expression of antiapoptotic Bcl-2 family members (Reed, 1996) and loss or inactivation of Bax (Ionov et al., 2000; Tai et al., 1998) has been

correlated with poor response to chemotherapy in some types of malignancy, but not all kinds of tumors.

5.3.2 Impaired Activation of the Death Receptor Pathway

Alterations in activation of the death receptor pathway are also implicated in chemoresistance. For instance, downregulation of Fas/CD95 in lymphoid and solid tumors is often associated with resistance to drug-induced cell death (Debatin and Krammer, 2004; Friesen et al., 1997; Fulda et al., 1998a; Fulda et al., 1998b). Direct downstream signaling molecules such as FADD and c-FLIP are also involved. Of note, absence or low expression levels of FADD in acute myeloid leukemia cells predicts resistance to chemotherapy and poor outcome (Tourneur et al., 2004). c-FLIP silencing dramatically sensitizes colorectal cancer cells to the chemotherapeutic agents 5-fluorouracil, oxaliplatin, and irinotecan (Longley et al., 2006). In addition, decoy receptors seem to be also implicated in resistance of cancer cells to different treatments, like Apo2L/TRAIL. This ligand has five receptors, two of which have cytoplasmic DDs (DR4 and DR5) and three of which act as "decoys" (DcR1, DcR2, and osteoprotegerin [OPG]). DcR1 and OPG lack a cytosolic region and DcR2 has a truncated, nonfunctional cytoplasmic DD (Almasan and Ashkenazi, 2003).

5.3.3 Deregulation of Caspase Activation

Both the death receptor and mitochondrial pathways lead to the activation of caspases, the final effectors of apoptotic cell death. Deregulation in the expression of caspases or their regulators (Apaf-1 and IAPs) has been observed in tumors. Although caspase mutations occur at low frequency, caspase expression and function appears to be impaired frequently by epigenetic mechanisms in cancer cells (Teitz et al., 2000). Caspase-8 expression was found to be inactivated by hypermethylation in varied resistant tumors, including childhood neuroblastoma, Ewing and malignant brain tumors and melanoma (Teitz et al., 2000). Importantly, restoration of caspase-8 expression by gene transfer or by demethylation treatment sensitizes resistant tumor cells to drug-induced apoptosis (Fulda et al., 2001a; Teitz et al., 2001). Downregulation of caspase-3 has been proposed as a possible mechanism for breast cancer chemoresistance. Doxorubicin-induced apoptosis was restored by reconstitution of caspase-3 expression in caspase-3-deficient MCF-7 breast cancer cells (Devarajan et al., 2002).

Inhibition of caspase activity by members of the IAP family is also involved in chemotherapy resistance in some tumors. The X-linked inhibitor of apoptosis protein (XIAP) is a factor in chemoresistance of human androgen-insensitive DU145 prostate cancer cells, as its inhibition induces apoptosis and enhances sensitivity to chemotherapy (Amantana et al., 2004). Overexpression of many IAPs has been reported in multidrug-resistant HL-60 leukemia cells (Notarbartolo et al.,

2002). Survivin expression has been shown to inhibit paclitaxel-induced apoptosis in HeLa cells (Giodini et al., 2002) and to correlate with paclitaxel resistance in ovarian cancer (Zaffaroni et al., 2002). As a final example, loss of *Apaf-1* has been associated with chemoresistance in melanoma cells (Soengas et al., 2001).

6 Strategies to Overcome Chemotherapeutic Resistance

Conventional drugs target cancer cells preferentially, but not exclusively. As a result, they also kill high-proliferating normal cells from bone marrow and the gut, causing unwanted side effects. Moreover, the efficacy of therapy is limited by innate or acquired resistance to such agents. New targeted cancer therapies, though, aim at using drugs that interfere with specific defects of cancer cells to improve selectivity. Current therapies include the use of monoclonal antibodies, small molecules, RNAi, and adenovirus-based gene therapy. These methods are currently being developed and studied for use alone or in combination with conventional drugs to overcome resistance (Fesik, 2005). The major proapoptotic targeted therapies are outlined below.

6.1 Inhibition of Mitotic Kinases (RTK, Ras, Akt, and mTOR)

Targeting the mitotic kinases that are involved in the survival of cancer cells has become a potential strategy for the induction of apoptosis either as a single treatment or in combination with traditional therapies. Two major approaches are being considered for targeting these kinases: small-molecule inhibitors and blocking monoclonal antibodies.

Tyrosine kinase inhibitors have been designed to compete with and prevent the binding of ATP to the tyrosine kinase domain. One of the greatest advances in molecular targeted therapy in cancer involves the treatment of CML with a small-molecule inhibitor of the Bcr-Abl oncogenic kinase called imatinib-mesylate (Gleevec) (Deininger et al., 2005; Druker et al., 1996). Imatinib leads to unprecedented responses in the chronic phase, with 80% of newly diagnosed patients achieving complete hematological remission. Imatinib has been shown to eradicate Bcr-Abl-positive leukemia cells through the induction of apoptotic (le Coutre et al., 1999) or nonapoptotic caspase-independent cell death (Okada et al., 2004). Since imatinib also inhibits other kinases such as c-Kit and PDGFR, its application has been broadened to other types of cancer such as c-Kit-positive gastrointestinal stromal tumors (GIST) (Dagher et al., 2002). Other successful examples of targeted small molecules include two EGFR/HER1 tyrosine kinase inhibitors, gefitinib (Iressa), and erlotinib (Tarceva), both of which have recently been approved by the Food and Drug Administration (FDA) for the treatment of NSCLC (De Marinis et al., 2006).

Humanized monoclonal antibodies targeting the EGFR superfamily have also been developed to bind the extracellular domain of these receptors competitively and thus prevent tyrosine kinase activation. Trastuzumab (herceptin), a monoclonal antibody against the extracellular domain of Her-2, is a prime example (Emens, 2005). The drug, which is approved for the treatment of breast cancers overexpressing Her-2, is best used in combination with paclitaxel for first-line therapy, but may also be used as a single agent as second- and third-line therapy.

As Ras mutations have been found in a great majority of carcinomas, targeting of Ras or downstream effector pathways of Ras has been of great interest (Khosravi-Far et al., 1998; Khosravi-Far and Der, 1994; Wennerberg et al., 2005). Farnesyl transferase inhibitors have potently inhibited Ras in preclinical studies, but have exhibited rather disappointing results in clinics so far (Appels et al., 2005). Chemical inhibitors of the PI3K/Akt pathway have a potential use as suppressors of tumor growth and inducers of apoptosis (Hennessy et al., 2005). Although inhibition of the PI3K family members has been shown to inhibit growth of both cancer cells in vitro and tumors in animal models, these compounds so far lack selectivity. By contrast, rapamycin and its analogues, which inhibit the Akt downstream substrate mTOR, slow the growth of tumors in animal models without displaying significant toxicity (Morgensztern and McLeod, 2005) (Dudkin et al., 2001; Eng et al., 1984). These compounds are currently in clinical trials for the treatment of breast, colon, and lung cancers.

6.2 Targeting of Transcription Factors

Several transcription factors, including p53, members of the FOXO superfamily, and NF-κB, are involved in drug-induced cellular response and have therefore emerged as attractive targets for new apoptosis-inducing therapies (Kim et al., 2003). Restoring p53 activity in tumor cells has a therapeutic potential because p53 loss or dysfunction in many tumors is a major cause of drug resistance. Different approaches to restore p53 function include gene transfer of wt p53, chemical restoration of wt p53 activity, and inhibition of Mdm2–p53 interaction (Blagosklonny, 2002). Many clinical trials employing wt p53 gene transfer are ongoing in different types of p53-deficient cancers. p53 activity can also be restored by small molecules that modify mutant p53 back to wt (Bykov et al., 2003; Foster et al., 1999). CP-31398, a styrylquinazoline, restores a wt DNA-binding conformation to mutant p53 and is capable of suppressing tumor growth in vitro and in vivo (Luu et al., 2002). Blocking the interaction of Mdm2–p53 in order to inhibit p53 degradation has also been considered as a valuable strategy for cancer therapy. A small molecule inhibiting the p53 pocket of Mdm2 (IC50 = 100 nM) was recently discovered within a series of *cis*-imidazoline analogues called the nutlins (Vassilev et al., 2004). Dose-dependent antiproliferative and cytotoxic activities of nutlins were shown to be dependent on the p53 status of tumor cell lines. Nutlins inhibited the growth of tumors in xenograft models without causing

significant toxicities (Vassilev et al., 2004). These results emphasize that small molecule inhibitors of Mdm2 could be valuable anticancer agents, especially for tumors retaining wt p53 but overexpressing Mdm2.

Recently, Hu et al. (2004) demonstrated that FOXO3a is inactivated by IKKβ in two thirds of breast cancer patients studied and that the presence of active FOXO3a correlates with improved patient survival. Additionally, FOXO3a has also been shown to be involved in paclitaxel-induced apoptosis in MCF-7 breast cancer cells. Notably, the FOXO family of transcription factors have been shown to regulate expression of proapoptotic genes such as Fas (Suhara et al., 2002), TRAIL (Ghaffari et al., 2003; Modur et al., 2002), and Bim (Gilley et al., 2003; Stahl et al., 2002). Taken together, these studies suggest that downregulation of FOXO transcription factors may be a key mechanism in tumorigenesis. As a proof of concept, chemical library screening identified a series of compounds that could target FOXO1 to the nucleus and that restored the induction of apoptosis in PTEN-null cells (Kau et al., 2003; Wang and El-Deiry, 2004).

Finally, an NF-κB inductive response to cytotoxic drugs can be targeted through its physiological inhibitor, IκB. Indeed, adenovirus-based inhibition of NF-κB elicited by gene delivery of an IκB superrepressor abrogates chemoresistance in some types of tumors such as androgen-independent prostate cancer cells and in glioma-derived cell lines (Orlowski and Baldwin, 2002). Targeting the IKK kinases that phosphorylate and promote the proteasomal degradation of IκB could be another approach, which is all the more appealing since FOXO3a is also regulated by IKKs in breast cancer cells. Moreover, conditional knockout of IKKβ in intestinal epithelial cells impedes irradiation-induced NF-κB activation and promotes the activation of p53 and apoptosis in those cells (Egan et al., 2004). Thus, as suggested by Finnberg and El-Deiry (2004), direct activation of FOXO3a, inhibition of NF-κB, and indirect activation of p53 by targeting IKKs could be an effective multifaceted anticancer therapy to inhibit cellular proliferation and promote cell death by multiple signaling pathways.

6.3 Direct Targeting of the Apoptotic Machinery

6.3.1 Activators of the Intrinsic/Mitochondrial Pathway

The mitochondria, as a major cell death checkpoint, constitute a prominent target for new anticancer therapies. The mitochondrial pathway can be selectively targeted by gene delivery of proapoptotic proteins such as Apaf-1 (Perkins et al., 2000) or Bax (Kagawa et al., 2000; Kaliberov et al., 2002). Alternatively, overexpressed antiapoptotic proteins such as Bcl-2, Bcl-XL, and XIAP can be downregulated. An antisense oligo against BCl-2, oblimersen, sensitizes patient-derived malignant melanoma cells to apoptosis induced by dacarbazine (Jansen et al., 2000) and has been recently approved by FDA for use in combination with this drug in advanced melanoma (Kim et al., 2004; Klasa et al., 2002). Phase II/III clinical trials are being

carried out to assess the benefits of oblimersen in combination with conventional drugs in Acute myelogenous leukemia (AML) and Non-small Cell Lung Cancer (NSCLC). Varied designer ligands (peptidomimetics or organic small molecules) that bind to the BH3-binding pocket of BCL-2 and Bcl-XL have been shown to induce apoptosis in vitro (Yin et al., 2005; Degterev et al., 2001; Enyedy et al., 2001; Kutzki et al., 2002; Tzung et al., 2001; Walensky et al., 2004; Wang et al., 2000). To date, a stapled BH3 peptide has been reported to inhibit the growth of leukemia xenografts (Walensky et al., 2004) and a small-molecule inhibitor of the Bcl-2 family members, ABT-737 (Abbot Laboratories), has been shown to induce regression of solid tumors in vivo (Oltersdorf et al., 2005). Moreover, a dual Bcl-2/BclXL antagonist (GX15-070, GeminX Biotechnology) entered clinical trials last year.

Inhibition of XIAP and other IAPs is another mechanism considered to induce apoptosis in cancer cells (Huang et al., 2004; Schimmer et al., 2006). Knockdown of XIAP by antisense oligos or RNAi induces apoptosis in cancer cells (Adams, 2003; Lima et al., 2004; McManus et al., 2004). Peptidic and nonpeptidic inhibitors of XIAPs have also been reported (Huang et al., 2004; Schimmer et al., 2006). In particular, cell-permeable Smac peptidomimetics that inhibit IAPs potently induce caspase activation and apoptosis in cancer cells and inhibit tumor growth in xenograft mouse models (Fulda et al., 2002).

Finally, drugs which act directly on mitochondrial components are also being developed to enforce cell death in tumor cells in which upstream apoptotic pathways are disabled (reviewed in Dias and Bailly, 2005; Bouchier-Hayes et al., 2005; Costantini et al., 2000; Debatin et al., 2002). Betulinic acid, a natural pentacyclic triterpenoid which acts via the permeabilization transition pore, has been shown to exert antitumor effects against neuroblastodermal and malignant head and neck tumors irrespective of their p53 status (Fulda and Debatin, 2000; Pisha et al., 1995).

6.3.2 Activators of the Extrinsic/Death Receptors Pathway

There is significant interest in targeting the extrinsic pathway to circumvent drug resistance, since chemorefractory cells tend to have dysfunctional p53 and defects in their intrinsic pathway. Death receptor ligands such as Fas, TNF, and TRAIL can be strong inducers of apoptosis in tumor cells in vitro. Among these ligands, TRAIL emerges as the most promising antitumor agent due to its lack of toxicity (Abe et al., 2000b; Yagita et al., 2004). Unlike Fas and TNF, recombinant TRAIL induces tumor regression in preclinical models with little toxicity to normal tissues (Ashkenazi et al., 1999; Walczak et al., 1999) and is currently in phase I clinical trials for the treatment of solid tumors. Agonistic antibodies against DR4 and DR5 also induce apoptosis in cancer cells, but not in normal cells and slow the growth of tumors in xenograft tumor models with no apparent systemic toxicity (Yagita et al., 2004). Phases I and II clinical trials have been initiated for an agonistic antibody targeting DR4 and phase II clinical trials are ongoing for an antibody that targets DR5.

6.4 General Inhibitors (Proteasome, Hsp90, and HDAC)

Targeting more general cellular components such as the 26S proteasome (Adams, 2004), the molecular chaperone protein Hsp90 (Whitesell and Lindquist, 2005), and histone deacetylases (HDAC) (Minucci and Pelicci, 2006; Yoo and Jones, 2006) has led to some surprising success in specific anticancer therapy. These therapies, while not intended to induce apoptosis, preferentially kill cancer cells by exploiting their greater dependence on the targeted cellular processes than their normal counterparts.

The proteasome inhibitor Velcade has been approved for treatment of multiple myeloma and is under evaluation as a single agent or in combination chemotherapy for the treatment of other hematopoietic and solid cancers (Adams and Kauffman, 2004). Preclinical studies demonstrate that proteasome inhibition by Velcade potentiates the activity of other cancer therapeutics, in part by downregulating chemoresistance pathways such as NF-κB and by inducing proapoptotic proteins such as p53 or FOXO3a (Fujita et al., 2005; Ghaffari et al., 2003).

Hsp90 is a molecular chaperone protein required for the stability and function of multiple mutated chimeric and overexpressed signaling proteins. Hsp90 inhibitors have shown promising antitumor activity in preclinical model systems (Banerji et al., 2005) and a 17-AAG compound has reached phase II clinical trials (Heath et al., 2005).

HDAC inhibitors are novel anticancer agents in clinical development that target the family of HDAC enzymes responsible for deacetylating core nucleosomal histones and other proteins. The precise mechanisms resulting in the antiproliferative biological effects of these agents are not fully understood. Nevertheless, a phase I clinical trial of suberoylanilide hydroxamic acid (SAHA) has shown that it is well tolerated, and has antitumor activity in both solid and hematological tumors (Kelly et al., 2005).

6.5 Combined Treatment as a Strategy to Overcome Resistance to Conventional Radiation and Chemotherapeutic Drugs

Most chemotherapeutic agents utilize the apoptotic pathway to induce cancer cell death, as does radiation. To overcome resistance to apoptosis, combination therapies involving two or more treatments can be used. The efficiency is usually highest when these treatments act on different signaling pathways. Targeting apoptosis through both the intrinsic and extrinsic pathways has been shown to be a good strategy. For example, joint activation of the intrinsic pathway (via the Bcl-2 family of proteins) and the extrinsic pathway (using different ligands like Apo2L/TRAIL) has a synergistic effect in prostate cancer cell lines (Almasan and Ashkenazi, 2003). Also, resistance to death receptor-induced cell death (as in resistance to Apo2L/TRAIL treatment) can be overcome by using a variety of therapeutic strategies,

like the activation of the intrinsic pathway, inhibition of survival factors, metabolic inhibition (blocking protein synthesis), proteasome inhibition (bortezomib), and others (Bucur et al., 2006).

6.5.1 Conclusions and Future Directions

Apoptosis and its deregulation in cancer has been an intensive field of research over the past decades. A deeper understanding of the mechanisms involved in evasion of cancer cells from apoptosis, and its link to drug resistance, has enabled the recent development of molecular targeted proapoptotic therapies. These therapies have produced significant results in cancer treatment and, in the case of Gleevec in CML, have even exceeded expectations. However, as for conventional drugs, resistance has subsequently emerged, even to single targeted agents. To avoid resistance, combination therapies involving both an apoptosis inducer and a conventional drug appear to be the best approach to date, but different strategies can be also used.

When apoptosis is impaired, resistance can often be overcome by targeting both the extrinsic and intrinsic pathways of apoptosis. In addition, alternative modes of cell death, such as autophagy, mitotic catastrophe, or necrosis, might be activated. Involvement of these different types of cell death in drug-induced cytotoxicity raises the possibility of using these newly identified cellular pathways instead to treat chemoresistant cancers. A deeper understanding of these alternative modes of cell death and identification of the interplay and molecular switches between apoptosis–autophagy and necrosis might provide new therapeutic targets for cancer therapy.

Finally, it is becoming clearer that tumor cells are not homogeneous and that neither most conventional drugs nor even targeted agents such as Gleevec can eliminate the cancer stem cells from which the disease arises (Bhatia et al., 2003). Relapses could occur in part due to the failure of current therapies to target this specific and original cancer cell population. Therefore, future directions of cancer research must better decipher the different modes of cell death and their potential application in attacking cancer stem cells.

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References

Aas, T., Borresen, A. L., Geisler, S., Smith-Sorensen, B., Johnsen, H., Varhaug, J. E., Akslen, L. A., and Lonning, P. E. (1996). Specific P53 mutations are associated with de novo resistance to doxorubicin in breast cancer patients. Nat Med 2, 811–814.

Abe, K., Kurakin, A., Mohseni-Maybodi, M., Kay, B., and Khosravi-Far, R. (2000a). The complexity of TNF-related apoptosis-inducing ligand. Ann NY Acad Sci, 926, 52–63.

Abe, K., Kurakin, A., Mohseni-Maybodi, M., Kay, B., and Khosravi-Far, R. (2000b). The complexity of TNF-related apoptosis-inducing ligand. Ann N Y Acad Sci 926, 52–63.

Adams, J. (2003). The proteasome: structure, function, and role in the cell. Cancer Treat Rev 29 (Suppl 1), 3–9.

Adams, J. (2004). The proteasome: a suitable antineoplastic target. Nat Rev Cancer 4, 349–360.
Adams, J. and Kauffman, M. (2004). Development of the proteasome inhibitor Velcade (Bortezomib). Cancer Invest 22, 304–311.
Adams, J. M. and Cory, S. (2002). Apoptosomes: engines for caspase activation. Curr Opin Cell Biol 14, 715–720.
Adams, J. M., Harris, A. W., Pinkert, C. A., Corcoran, L. M., Alexander, W. S., Cory, S., Palmiter, R. D., and Brinster, R. L. (1985). The c-myc oncogene driven by immunoglobulin enhancers induces lymphoid malignancy in transgenic mice. Nature 318, 533–538.
Aichberger, K. J., Mayerhofer, M., Krauth, M. T., Skvara, H., Florian, S., Sonneck, K., Akgul, C., Derdak, S., Pickl, W. F., Wacheck, V., et al. (2005). Identification of mcl-1 as a BCR/ABL-dependent target in chronic myeloid leukemia (CML): evidence for cooperative antileukemic effects of imatinib and mcl-1 antisense oligonucleotides. Blood 105, 3303–3311.
Algeciras-Schimnich, A., Shen, L., Barnhart, B. C., Murmann, A. E., Burkhardt, J. K., and Peter, M. E. (2002). Molecular ordering of the initial signaling events of CD95. Mol Cell Biol 22, 207–220.
Alitalo, K. and Schwab, M. (1986). Oncogene amplification in tumor cells. Advances in Cancer Research 47, 235–281.
Almasan, A. and Ashkenazi, A. (2003). Apo2L/TRAIL: apoptosis signaling, biology, and potential for cancer therapy. Cytokine Growth Factor Rev 14, 337–348.
Amantana, A., London, C. A., Iversen, P. L., and Devi, G. R. (2004). X-linked inhibitor of apoptosis protein inhibition induces apoptosis and enhances chemotherapy sensitivity in human prostate cancer cells. Mol Cancer Ther 3, 699–707.
Amundson, S. A., Myers, T. G., Scudiero, D., Kitada, S., Reed, J. C., and Fornace, A. J., Jr. (2000). An informatics approach identifying markers of chemosensitivity in human cancer cell lines. Cancer Res 60, 6101–6110.
Appels, N. M., Beijnen, J. H., and Schellens, J. H. (2005). Development of farnesyl transferase inhibitors: a review. Oncologist 10, 565–578.
Arlt, A. and Schafer, H. (2002). NFkappaB-dependent chemoresistance in solid tumors. Int J Clin Pharmacol Ther 40, 336–347.
Arvanitis, C. and Felsher, D. W. (2006). Conditional transgenic models define how MYC initiates and maintains tumorigenesis. Semin Cancer Biol 16, 313–317.
Ashkenazi, A. and Dixit, V. M. (1998). Death receptors: signaling and modulation. Science 281, 1305–1308.
Ashkenazi, A. and Dixit, V. M. (1999). Apoptosis control by death and decoy receptors. Curr Opin Cell Biol 11, 255–260.
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.
Baldwin, A. S. (2001). Control of oncogenesis and cancer therapy resistance by the transcription factor NF-kappaB. J Clin Invest 107, 241–246.
Baliga, B. and Kumar, S. (2003). Apaf-1/cytochrome c apoptosome: an essential initiator of caspase activation or just a sideshow? Cell Death Differ 10, 16–18.
Banerji, U., O'Donnell, A., Scurr, M., Pacey, S., Stapleton, S., Asad, Y., Simmons, L., Maloney, A., Raynaud, F., Campbell, M., et al. (2005). Phase I pharmacokinetic and pharmacodynamic study of 17-allylamino, 17-demethoxygeldanamycin in patients with advanced malignancies. J Clin Oncol 23, 4152–4161.
Bedi, A., Zehnbauer, B. A., Barber, J. P., Sharkis, S. J., and Jones, R. J. (1994). Inhibition of apoptosis by Bcr-Abl in chronic myeloid leukemia. Blood 83, 2038–2044.
Bhatia, R., Holtz, M., Niu, N., Gray, R., Snyder, D. S., Sawyers, C. L., Arber, D. A., Slovak, M. L., and Forman, S. J. (2003). Persistence of malignant hematopoietic progenitors in chronic myelogenous leukemia patients in complete cytogenetic remission following imatinib mesylate treatment. Blood 101, 4701–4707 (epub 2003 Feb 4706).
Bissonnette, R. P., Echeverri, F., Mahboubi, A., and Green, D. R. (1992). Apoptotic cell death induced by c-myc is inhibited by bcl-2. Nature 359, 552–554.
Blagosklonny, M. V. (2001). Paradox of Bcl-2 (and p53): why may apoptosis-regulating proteins be irrelevant to cell death? Bioessays 23, 947–953.

Blagosklonny, M. V. (2002). P53: an ubiquitous target of anticancer drugs. Int J Cancer 98, 161–166.

Blume-Jensen, P. and Hunter, T. (2001). Oncogenic kinase signalling. Nature 411, 355–365.

Boatright, K. M., Renatus, M., Scott, F. L., Sperandio, S., Shin, H., Pedersen, I. M., Ricci, J. E., Edris, W. A., Sutherlin, D. P., Green, D. R., and Salvesen, G. S. (2003). A unified model for apical caspase activation. Mol Cell 11, 529–541.

Bouchier-Hayes, L., Lartigue, L., and Newmeyer, D. D. (2005). Mitochondria: pharmacological manipulation of cell death. J Clin Invest 115, 2640–2647.

Bouillet, P., Huang, D. C., O'Reilly, L. A., Puthalakath, H., O'Connor, L., Cory, S., Adams, J. M., and Strasser, A. (2000). The role of the pro-apoptotic Bcl-2 family member bim in physiological cell death. Ann N Y Acad Sci 926, 83–89.

Boxer, R. B., Jang, J. W., Sintasath, L., and Chodosh, L. A. (2004). Lack of sustained regression of c-MYC-induced mammary adenocarcinomas following brief or prolonged MYC inactivation. Cancer Cell 6, 577–586.

Broker, L. E., Huisman, C., Span, S. W., Rodriguez, J. A., Kruyt, F. A., and Giaccone, G. (2004). Cathepsin B mediates caspase-independent cell death induced by microtubule stabilizing agents in non-small cell lung cancer cells. Cancer Res 64, 27–30.

Bucur, O., Ray, S., Bucur, M. C., and Almasan, A. (2006). APO2 ligand/tumor necrosis factor-related apoptosis-inducing ligand in prostate cancer therapy. Front Biosci 11, 1549–1568.

Burns, T. F. and el-Deiry, W. S. (2003). Cell death signaling in maligancy. Cancer Treat Res 115, 319–343.

Bursch, W., Ellinger, A., Kienzl, H., Torok, L., Pandey, S., Sikorska, M., Walker, R., and Hermann, R. S. (1996). Active cell death induced by the anti-estrogens tamoxifen and ICI 164 384 in human mammary carcinoma cells (MCF-7) in culture: the role of autophagy. Carcinogenesis 17, 1595–1607.

Bykov, V. J., Selivanova, G., and Wiman, K. G. (2003). Small molecules that reactivate mutant p53. Eur J Cancer 39, 1828–1834.

Cain, K., Bratton, S. B., and Cohen, G. M. (2002). The Apaf-1 apoptosome: a large caspase-activating complex. Biochimie 84, 203–214.

Calvisi, D. F., Ladu, S., Factor, V. M., and Thorgeirsson, S. S. (2004). Activation of beta-catenin provides proliferative and invasive advantages in c-myc/TGF-alpha hepatocarcinogenesis promoted by phenobarbital. Carcinogenesis 25, 901–908.

Carlesso, N., Frank, D. A., and Griffin, J. D. (1996). Tyrosyl phosphorylation and DNA binding activity of signal transducers and activators of transcription (STAT) proteins in hematopoietic cell lines transformed by Bcr/Abl. J Exp Med 183, 811–820.

Carlesso, N., Griffin, J. D., and Druker, B. J. (1994). Use of a temperature-sensitive mutant to define the biological effects of the p210Bcr-Abl tyrosine kinase on proliferation of a factor-dependent murine myeloid cell line. Oncogene 9, 149–156.

Chevallier, P., Robillard, N., Wuilleme-Toumi, S., Mechinaud, F., Harousseau, J. L., and Avet-Loiseau, H. (2004). Overexpression of Her2/neu is observed in one third of adult acute lymphoblastic leukemia patients and is associated with chemoresistance in these patients. Haematologica 89, 1399–1401.

Chinnaiyan, A. M. (1999). The apoptosome: heart and soul of the cell death machine. Neoplasia 1, 5–15.

Chuang, S. E., Yeh, P. Y., Lu, Y. S., Lai, G. M., Liao, C. M., Gao, M., and Cheng, A. L. (2002). Basal levels and patterns of anticancer drug-induced activation of nuclear factor-kappaB (NF-kappaB), and its attenuation by tamoxifen, dexamethasone, and curcumin in carcinoma cells. Biochem Pharmacol 63, 1709–1716.

Cohen, G. M. (1997). Caspases: the executioners of apoptosis. Biochem J 326 (Pt 1), 1–16.

Cortez, D., Stoica, G., Pierce, J. H., and Pendergast, A. M. (1996). The Bcr-Abl tyrosine kinase inhibits apoptosis by activating a Ras-dependent signaling pathway. Oncogene 13, 2589–2594.

Cory, S. and Adams, J. M. (2002). The Bcl2 family: regulators of the cellular life-or-death switch. Nat Rev Cancer 2, 647–656.

Costantini, P., Jacotot, E., Decaudin, D., and Kroemer, G. (2000). Mitochondrion as a novel target of anticancer chemotherapy. J Natl Cancer Inst 92, 1042–1053.

Croxton, R., Ma, Y., Song, L., Haura, E. B., and Cress, W. D. (2002). Direct repression of the Mcl-1 promoter by E2F1. Oncogene 21, 1359–1369.

D'Cruz, C. M., Gunther, E. J., Boxer, R. B., Hartman, J. L., Sintasath, L., Moody, S. E., Cox, J. D., Ha, S. I., Belka, G. K., Golant, A., et al. (2001). c-MYC induces mammary tumorigenesis by means of a preferred pathway involving spontaneous Kras2 mutations. Nature Medicine 7, 235–239.

Dagher, R., Cohen, M., Williams, G., Rothmann, M., Gobburu, J., Robbie, G., Rahman, A., Chen, G., Staten, A., Griebel, D., and Pazdur, R. (2002). Approval summary: imatinib mesylate in the treatment of metastatic and/or unresectable malignant gastrointestinal stromal tumors. Clin Cancer Res 8, 3034–3038.

Dang, C. V. (1999). c-Myc target genes involved in cell growth, apoptosis, and metabolism. Mol Cell Biol 19, 1–11.

Daniel, P. T., Wieder, T., Sturm, I., and Schulze-Osthoff, K. (2001). The kiss of death: promises and failures of death receptors and ligands in cancer therapy. Leukemia 15, 1022–1032.

De Marinis, F., De Santis, S., and De Petris, L. (2006). Second-line chemotherapy for non-small cell lung cancer. Ann Oncol 17 (Suppl 5), v68–v71.

Debatin, K. M. and Krammer, P. H. (2004). Death receptors in chemotherapy and cancer. Oncogene 23, 2950–2966.

Debatin, K. M., Poncet, D., and Kroemer, G. (2002). Chemotherapy: targeting the mitochondrial cell death pathway. Oncogene 21, 8786–8803.

Degli Esposti, M. (1999). To die or not to die–the quest of the TRAIL receptors. J Leukoc Biol 65, 535–542.

Degterev, A., Lugovskoy, A., Cardone, M., Mulley, B., Wagner, G., Mitchison, T., and Yuan, J. (2001). Identification of small-molecule inhibitors of interaction between the BH3 domain and Bcl-xL. Nat Cell Biol 3, 173–182.

Deininger, M., Buchdunger, E., and Druker, B. J. (2005). The development of imatinib as a therapeutic agent for chronic myeloid leukemia. Blood 105, 2640–2653 (epub 2004 Dec 2623).

Demoy, M., Minko, T., Kopeckova, P., and Kopecek, J. (2000). Time- and concentration-dependent apoptosis and necrosis induced by free and HPMA copolymer-bound doxorubicin in human ovarian carcinoma cells. J Control Release 69, 185–196.

Dempke, W., Voigt, W., Grothey, A., Hill, B. T., and Schmoll, H. J. (2000). Cisplatin resistance and oncogenes – a review. Anticancer Drugs 11, 225–236.

Devarajan, E., Sahin, A. A., Chen, J. S., Krishnamurthy, R. R., Aggarwal, N., Brun, A. M., Sapino, A., Zhang, F., Sharma, D., Yang, X. H., et al. (2002). Down-regulation of caspase 3 in breast cancer: a possible mechanism for chemoresistance. Oncogene 21, 8843–8851.

Dias, N. and Bailly, C. (2005). Drugs targeting mitochondrial functions to control tumor cell growth. Biochem Pharmacol 70, 1–12.

Dole, M., Nunez, G., Merchant, A. K., Maybaum, J., Rode, C. K., Bloch, C. A., and Castle, V. P. (1994). Bcl-2 inhibits chemotherapy-induced apoptosis in neuroblastoma. Cancer Res 54, 3253–3259.

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

Dudkin, L., Dilling, M. B., Cheshire, P. J., Harwood, F. C., Hollingshead, M., Arbuck, S. G., Travis, R., Sausville, E. A., and Houghton, P. J. (2001). Biochemical correlates of mTOR inhibition by the rapamycin ester CCI-779 and tumor growth inhibition. Clin Cancer Res 7, 1758–1764.

Egan, L. J., Eckmann, L., Greten, F. R., Chae, S., Li, Z. W., Myhre, G. M., Robine, S., Karin, M., and Kagnoff, M. F. (2004). IkappaB-kinasebeta-dependent NF-kappaB activation provides radioprotection to the intestinal epithelium. Proc Natl Acad Sci USA 101, 2452–2457.

Emanuel, P. D., Bates, L. J., Castleberry, R. P., Gualtieri, R. J., and Zuckerman, K. S. (1991). Selective hypersensitivity to granulocyte-macrophage colony-stimulating factor by juvenile chronic myeloid leukemia hematopoietic progenitors. Blood 77, 925–929.

Emens, L. A. (2005). Trastuzumab: targeted therapy for the management of HER-2/neu-overexpressing metastatic breast cancer. Am J Ther 12, 243–253.

Eng, C. P., Sehgal, S. N., and Vezina, C. (1984). Activity of rapamycin (AY-22,989) against transplanted tumors. J Antibiot (Tokyo) 37, 1231–1237.

Enyedy, I. J., Ling, Y., Nacro, K., Tomita, Y., Wu, X., Cao, Y., Guo, R., Li, B., Zhu, X., Huang, Y., et al. (2001). Discovery of small-molecule inhibitors of Bcl-2 through structure-based computer screening. J Med Chem 44, 4313–4324.

Erster, S. and Moll, U. M. (2005). Stress-induced p53 runs a transcription-independent death program. Biochem Biophys Res Commun 331, 843–850.

Esposti, M. D., Cristea, I. M., Gaskell, S. J., Nakao, Y., and Dive, C. (2003). Proapoptotic Bid binds to monolysocardiolipin, a new molecular connection between mitochondrial membranes and cell death. Cell Death Differ 10, 1300–1309.

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

Evan, G. I., Wyllie, A. H., Gilbert, C. S., Littlewood, T. D., Land, H., Brooks, M., Waters, C. M., Penn, L. Z., and Hancock, D. C. (1992). Induction of apoptosis in fibroblasts by c-myc protein. Cell 69, 119–128.

Fan, J., Banerjee, D., Stambrook, P. J., and Bertino, J. R. (1997). Modulation of cytotoxicity of chemotherapeutic drugs by activated H-ras. Biochem Pharmacol 53, 1203–1209.

Fanidi, A., Harrington, E. A., and Evan, G. I. (1992). Cooperative interaction between c-myc and bcl-2 proto-oncogenes. Nature 359, 554–556.

Felsher, D. W. and Bishop, J. M. (1999). Transient excess of MYC activity can elicit genomic instability and tumorigenesis. Proc Natl Acad Sci USA 96, 3940–3944.

Fernandez, P. C., Frank, S. R., Wang, L., Schroeder, M., Liu, S., Greene, J., Cocito, A., and Amati, B. (2003). Genomic targets of the human c-Myc protein. Genes Dev 17, 1115–1129.

Fesik, S. W. (2005). Promoting apoptosis as a strategy for cancer drug discovery. Nat Rev Cancer 5, 876–885.

Finnberg, N. and El-Deiry, W. S. (2004). Activating FOXO3a, NF-kappaB and p53 by targeting IKKs: an effective multi-faceted targeting of the tumor-cell phenotype? Cancer Biol Ther 3, 614–616 (epub 2004 July 2024).

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.

Friesen, C., Fulda, S., and Debatin, K. M. (1997). Deficient activation of the CD95 (APO-1/Fas) system in drug-resistant cells. Leukemia 11, 1833–1841.

Fujita, T., Washio, K., Takabatake, D., Takahashi, H., Yoshitomi, S., Tsukuda, K., Ishibe, Y., Ogasawara, Y., Doihara, H., and Shimizu, N. (2005). Proteasome inhibitors can alter the signaling pathways and attenuate the P-glycoprotein-mediated multidrug resistance. Int J Cancer 117, 670–682.

Fulda, S. and Debatin, K. M. (2000). Betulinic acid induces apoptosis through a direct effect on mitochondria in neuroectodermal tumors. Med Pediatr Oncol 35, 616–618.

Fulda, S., Friesen, C., and Debatin, K. M. (1998a). Molecular determinants of apoptosis induced by cytotoxic drugs. Klin Padiatr 210, 148–152.

Fulda, S., Los, M., Friesen, C., and Debatin, K. M. (1998b). Chemosensitivity of solid tumor cells in vitro is related to activation of the CD95 system. Int J Cancer 76, 105–114.

Fulda, S., Kufer, M. U., Meyer, E., van Valen, F., Dockhorn-Dworniczak, B., and Debatin, K. M. (2001a). Sensitization for death receptor- or drug-induced apoptosis by re-expression of caspase-8 through demethylation or gene transfer. Oncogene 20, 5865–5877.

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

Fulda, S., Wick, W., Weller, M., and Debatin, K. M. (2002). Smac agonists sensitize for Apo2L/TRAIL- or anticancer drug-induced apoptosis and induce regression of malignant glioma in vivo. Nat Med 8, 808–815 (epub 2002 July 2015).

Furukawa, Y., Nishimura, N., Satoh, M., Endo, H., Iwase, S., Yamada, H., Matsuda, M., Kano, Y., and Nakamura, M. (2002). Apaf-1 is a mediator of E2F-1-induced apoptosis. J Biol Chem 277, 39760–39768 (epub 32002 July 39730).

Gambacorti-Passerini, C., le Coutre, P., Mologni, L., Fanelli, M., Bertazzoli, C., Marchesi, E., Di Nicola, M., Biondi, A., Corneo, G. M., Belotti, D., et al. (1997). Inhibition of the ABL kinase activity blocks the proliferation of BCR/ABL + leukemic cells and induces apoptosis. Blood Cells Mol Dis 23, 380–394.

Gesbert, F. and Griffin, J. D. (2000). Bcr/Abl activates transcription of the Bcl-X gene through STAT5. Blood 96, 2269–2276.

Geske, F. J. and Gerschenson, L. E. (2001). The biology of apoptosis. Hum Pathol 32, 1029–1038.

Ghaffari, S., Jagani, Z., Kitidis, C., Lodish, H. F., and Khosravi-Far, R. (2003). Cytokines and Bcr-Abl mediate suppression of TRAIL-induced apoptosis through inhibition of forkhead FOXO3a transcription factor. Proc Natl Acad Sci USA 100, 6523–6528.

Gilley, J., Coffer, P. J., and Ham, J. (2003). FOXO transcription factors directly activate bim gene expression and promote apoptosis in sympathetic neurons. J Cell Biol 162, 613–622.

Giodini, A., Kallio, M. J., Wall, N. R., Gorbsky, G. J., Tognin, S., Marchisio, P. C., Symons, M., and Altieri, D. C. (2002). Regulation of microtubule stability and mitotic progression by survivin. Cancer Res 62, 2462–2467.

Gonda, T. J., Sheiness, D. K., and Bishop, J. M. (1982). Transcripts from the cellular homologs of retroviral oncogenes: distribution among chicken tissues. Mol Cell Biol 2, 617–624.

Gong, B. and Almasan, A. (2000). Genomic organization and transcriptional regulation of human Apo2/TRAIL gene. Biochem Biophys Res Commun 278, 747–752.

Gourdier, I., Del Rio, M., Crabbe, L., Candeil, L., Copois, V., Ychou, M., Auffray, C., Martineau, P., Mechti, N., Pommier, Y., and Pau, B. (2002). Drug specific resistance to oxaliplatin is associated with apoptosis defect in a cellular model of colon carcinoma. FEBS Lett 529, 232–236.

Gozuacik, D. and Kimchi, A. (2004). Autophagy as a cell death and tumor suppressor mechanism. Oncogene 23, 2891–2906.

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

Green, D. R. and Kroemer, G. (2004). The pathophysiology of mitochondrial cell death. Science 305, 626–629.

Gross, A., Yin, X. M., Wang, K., Wei, M. C., Jockel, J., Milliman, C., Erdjument-Bromage, H., Tempst, P., and Korsmeyer, S. J. (1999). Caspase cleaved BID targets mitochondria and is required for cytochrome c release, while BCL-XL prevents this release but not tumor necrosis factor-R1/Fas death. J Biol Chem 274, 1156–1163.

Gudas, J. M., Nguyen, H., Li, T., Sadzewicz, L., Robey, R., Wosikowski, K., and Cowan, K. H. (1996). Drug-resistant breast cancer cells frequently retain expression of a functional wild-type p53 protein. Carcinogenesis 17, 1417–1427.

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

Harada, T., Ogura, S., Yamazaki, K., Kinoshita, I., Itoh, T., Isobe, H., Yamashiro, K., Dosaka-Akita, H. and Nishimura, M. (2003). Predictive value of expression of P53, Bcl-2 and lung resistance-related protein for response to chemotherapy in non-small cell lung cancers. Cancer Sci 94, 394–399.

Harris, A. W., Pinkert, C. A., Crawford, M., Langdon, W. Y., Brinster, R. L., and Adams, J. M. (1988). The E mu-myc transgenic mouse. A model for high-incidence spontaneous lymphoma and leukemia of early B cells. J Exp Med 167, 353–371.

Heath, E. I., Gaskins, M., Pitot, H. C., Pili, R., Tan, W., Marschke, R., Liu, G., Hillman, D., Sarkar, F., Sheng, S., et al. (2005). A phase II trial of 17-allylamino-17- demethoxygeldanamycin in patients with hormone-refractory metastatic prostate cancer. Clin Prostate Cancer 4, 138–141.

Hennessy, B. T., Smith, D. L., Ram, P. T., Lu, Y., and Mills, G. B. (2005). Exploiting the PI3K/AKT pathway for cancer drug discovery. Nat Rev Drug Discov 4, 988–1004.

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

Hickman, J. A. (2002). Apoptosis and tumourigenesis. Curr Opin Genet Dev 12, 67–72.

Hill, M. M., Adrain, C., and Martin, S. J. (2003). Portrait of a killer: the mitochondrial apoptosome emerges from the shadows. Mol Interv 3, 19–26.

Hirsimaki, Y. and Hirsimaki, P. (1984). Vinblastine-induced autophagocytosis: the effect of disorganization of microfilaments by cytochalasin B. Exp Mol Pathol 40, 61–69.

Horvitz, H. R. (1999). Genetic control of programmed cell death in the nematode *Caenorhabditis elegans*. Cancer Res 59, 1701s–1706s.

Hu, M. C., Lee, D. F., Xia, W., Golfman, L. S., Ou-Yang, F., Yang, J. Y., Zou, Y., Bao, S., Hanada, N., Saso, H., et al. (2004). IkappaB kinase promotes tumorigenesis through inhibition of forkhead FOXO3a. Cell 117, 225–237.

Huang, Y., Lu, M., and Wu, H. (2004). Antagonizing XIAP-mediated caspase-3 inhibition. Achilles' heel of cancers? Cancer Cell 5, 1–2.

Hutchinson, J. N. and Muller, W. J. (2000). Transgenic mouse models of human breast cancer. Oncogene 19, 6130–6137.

Ilaria, R. L., Jr. and Van Etten, R. A. (1996). P210 and P190(BCR/ABL) induce the tyrosine phosphorylation and DNA binding activity of multiple specific STAT family members. J Biol Chem 271, 31704–31710.

Ingvarsson, S. (1990). The myc gene family proteins and their role in transformation and differentiation. Semin Cancer Biol 1, 359–369.

Ionov, Y., Yamamoto, H., Krajewski, S., Reed, J. C., and Perucho, M. (2000). Mutational inactivation of the proapoptotic gene BAX confers selective advantage during tumor clonal evolution. Proc Natl Acad Sci USA 97, 10872–10877.

Irwin, M. S., Kondo, K., Marin, M. C., Cheng, L. S., Hahn, W. C., and Kaelin, W. G., Jr. (2003). Chemosensitivity linked to p73 function. Cancer Cell 3, 403–410.

Jansen, B., Schlagbauer-Wadl, H., Eichler, H. G., Wolff, K., van Elsas, A., Schrier, P. I., and Pehamberger, H. (1997). Activated N-ras contributes to the chemoresistance of human melanoma in severe combined immunodeficiency (SCID) mice by blocking apoptosis. Cancer Res 57, 362–365.

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.

Johnstone, R. W., Ruefli, A. A., and Lowe, S. W. (2002). Apoptosis: a link between cancer genetics and chemotherapy. Cell 108, 153–164.

Kabarowski, J. H., Allen, P. B., and Wiedemann, L. M. (1994). A temperature sensitive p210 Bcr-Abl mutant defines the primary consequences of Bcr-Abl tyrosine kinase expression in growth factor dependent cells. EMBO J 13, 5887–5895.

Kagawa, S., Gu, J., Swisher, S. G., Ji, L., Roth, J. A., Lai, D., Stephens, L. C., and Fang, B. (2000). Antitumor effect of adenovirus-mediated Bax gene transfer on p53-sensitive and p53-resistant cancer lines. Cancer Res 60, 1157–1161.

Kaliberov, S. A., Buchsbaum, D. J., Gillespie, G. Y., Curiel, D. T., Arafat, W. O., Carpenter, M., and Stackhouse, M. A. (2002). Adenovirus-mediated transfer of BAX driven by the vascular endothelial growth factor promoter induces apoptosis in lung cancer cells. Mol Ther 6, 190–198.

Kamesaki, S., Kamesaki, H., Jorgensen, T. J., Tanizawa, A., Pommier, Y., and Cossman, J. (1993). bcl-2 protein inhibits etoposide-induced apoptosis through its effects on events subsequent to topoisomerase II-induced DNA strand breaks and their repair. Cancer Res 53, 4251–4256.

Kanzawa, T., Germano, I. M., Komata, T., Ito, H., Kondo, Y., and Kondo, S. (2004). Role of autophagy in temozolomide-induced cytotoxicity for malignant glioma cells. Cell Death Differ 11, 448–457.

Kasibhatla, S., Brunner, T., Genestier, L., Echeverri, F., Mahboubi, A., and Green, D. R. (1998). DNA damaging agents induce expression of Fas ligand and subsequent apoptosis in T lymphocytes via the activation of NF-kappa B and AP-1. Mol Cell 1, 543–551.

Kau, T. R., Schroeder, F., Ramaswamy, S., Wojciechowski, C. L., Zhao, J. J., Roberts, T. M., Clardy, J., Sellers, W. R., and Silver, P. A. (2003). A chemical genetic screen identifies inhibitors of regulated nuclear export of a Forkhead transcription factor in PTEN-deficient tumor cells. Cancer cell 4, 463–476.

Kaufmann, S. H. and Earnshaw, W. C. (2000). Induction of apoptosis by cancer chemotherapy. Exp Cell Res 256, 42–49.

Keeshan, K., Mills, K. I., Cotter, T. G., and McKenna, S. L. (2001). Elevated Bcr-Abl expression levels are sufficient for a haematopoietic cell line to acquire a drug-resistant phenotype. Leukemia 15, 1823–1833.

Kelly, W. K., O'Connor, O. A., Krug, L. M., Chiao, J. H., Heaney, M., Curley, T., MacGregore-Cortelli, B., Tong, W., Secrist, J. P., Schwartz, L., et al. (2005). Phase I study of an oral histone deacetylase inhibitor, suberoylanilide hydroxamic acid, in patients with advanced cancer. J Clin Oncol 23, 3923–3931 (epub 2005 May 3916).

Khosravi-Far, R., Campbell, S., Rossman, K. L., and Der, C. J. (1998). Increasing complexity of Ras signal transduction: involvement of Rho family proteins. Adv Cancer Res 72, 57–107.

Khosravi-Far, R. and Der, C. J. (1994). The Ras signal transduction pathway. Cancer Metastasis Rev 13, 67–89.

Kim, D., Dan, H. C., Park, S., Yang, L., Liu, Q., Kaneko, S., Ning, J., He, L., Yang, H., Sun, M., et al. (2005). AKT/PKB signaling mechanisms in cancer and chemoresistance. Front Biosci 10, 975–987 (print 2005 Jan 2001).

Kim, R., Emi, M., Tanabe, K., and Toge, T. (2004). Therapeutic potential of antisense Bcl-2 as a chemosensitizer for cancer therapy. Cancer 101, 2491–2502.

Kim, R., Emi, M., Tanabe, K., Uchida, Y., and Arihiro, K. (2006). The role of apoptotic or nonapoptotic cell death in determining cellular response to anticancer treatment. Eur J Surg Oncol 20, 20.

Kim, R., Tanabe, K., Emi, M., Uchida, Y., Inoue, H., and Toge, T. (2003). Inducing cancer cell death by targeting transcription factors. Anticancer Drugs 14, 3–11.

Kirkin, V., Joos, S., and Zornig, M. (2004). The role of Bcl-2 family members in tumorigenesis. Biochim Biophys Acta 1644, 229–249.

Klasa, R. J., Gillum, A. M., Klem, R. E., and Frankel, S. R. (2002). Oblimersen Bcl-2 antisense: facilitating apoptosis in anticancer treatment. Antisense Nucleic Acid Drug Dev 12, 193–213.

Knies-Bamforth, U. E., Fox, S. B., Poulsom, R., Evan, G. I., and Harris, A. L. (2004). c-Myc interacts with hypoxia to induce angiogenesis in vivo by a vascular endothelial growth factor-dependent mechanism. Cancer Res 64, 6563–6570.

Knuefermann, C., Lu, Y., Liu, B., Jin, W., Liang, K., Wu, L., Schmidt, M., Mills, G. B., Mendelsohn, J., and Fan, Z. (2003). HER2/PI-3K/Akt activation leads to a multidrug resistance in human breast adenocarcinoma cells. Oncogene 22, 3205–3212.

Kondo, Y., Kanzawa, T., Sawaya, R., and Kondo, S. (2005). The role of autophagy in cancer development and response to therapy. Nat Rev Cancer 5, 726–734.

Kostanova-Poliakova, D. and Sabova, L. (2005). Anti-apoptotic proteins-targets for chemosensitization of tumor cells and cancer treatment. Neoplasma 52, 441–449.

Koturbash, I., Rugo, R. E., Hendricks, C. A., Loree, J., Thibault, B., Kutanzi, K., Pogribny, I., Yanch, J. C., Engelward, B. P., and Kovalchuk, O. (2006). Irradiation induces DNA damage and modulates epigenetic effectors in distant bystander tissue in vivo. Oncogene 25, 4267–4275.

Kroemer, G. (2003). Mitochondrial control of apoptosis: an introduction. Biochem Biophys Res Commun 304, 433–435.

Kutzki, O., Park, H. S., Ernst, J. T., Orner, B. P., Yin, H., and Hamilton, A. D. (2002). Development of a potent Bcl-x(L) antagonist based on alpha-helix mimicry. J Am Chem Soc 124, 11838–11839.

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

Lawen, A. (2003). Apoptosis-an introduction. Bioessays 25, 888–896.
le Coutre, P., Mologni, L., Cleris, L., Marchesi, E., Buchdunger, E., Giardini, R., Formelli, F., and Gambacorti-Passerini, C. (1999). In vivo eradication of human BCR/ABL-positive leukemia cells with an ABL kinase inhibitor. J Natl Cancer Inst 91, 163–168.
Letai, A., Sorcinelli, M. D., Beard, C., and Korsmeyer, S. J. (2004). Antiapoptotic BCL-2 is required for maintenance of a model leukemia. Cancer Cell 6, 241–249.
Levine, A. J., Finlay, C. A., and Hinds, P. W. (2004). P53 is a tumor suppressor gene. Cell 116, S67–S69, 61 p following S69.
Levy, E., Baroche, C., Barret, J. M., Alapetite, C., Salles, B., Averbeck, D., and Moustacchi, E. (1994). Activated ras oncogene and specifically acquired resistance to cisplatin in human mammary epithelial cells: induction of DNA cross-links and their repair. Carcinogenesis 15, 845–850.
Li, H. and Yuan, J. (1999). Deciphering the pathways of life and death. Curr Opin Cell Biol 11, 261–266.
Li, H., Zhu, H., Xu, C. J., and Yuan, J. (1998). Cleavage of BID by caspase 8 mediates the mitochondrial damage in the Fas pathway of apoptosis. Cell 94, 491–501.
Lima, R. T., Martins, L. M., Guimaraes, J. E., Sambade, C., and Vasconcelos, M. H. (2004). Specific downregulation of bcl-2 and xIAP by RNAi enhances the effects of chemotherapeutic agents in MCF-7 human breast cancer cells. Cancer Gene Ther 11, 309–316.
Lin, W. C., Lin, F. T., and Nevins, J. R. (2001). Selective induction of E2F1 in response to DNA damage, mediated by ATM-dependent phosphorylation. Genes Dev 15, 1833–1844.
Longley, D. B. and Johnston, P. G. (2005). Molecular mechanisms of drug resistance. J Pathol 205, 275–292.
Longley, D. B., Wilson, T. R., McEwan, M., Allen, W. L., McDermott, U., Galligan, L., and Johnston, P. G. (2006). c-FLIP inhibits chemotherapy-induced colorectal cancer cell death. Oncogene 25, 838–848.
Lowe, S. W., Bodis, S., McClatchey, A., Remington, L., Ruley, H. E., Fisher, D. E., Housman, D. E., and Jacks, T. (1994). p53 status and the efficacy of cancer therapy in vivo. Science 266, 807–810.
Lowe, S. W., Cepero, E., and Evan, G. (2004). Intrinsic tumour suppression. Nature 432, 307–315.
Lowe, S. W. and Lin, A. W. (2000). Apoptosis in cancer. Carcinogenesis 21, 485–495.
Luo, X., Budihardjo, I., Zou, H., Slaughter, C., and Wang, X. (1998). Bid, a Bcl2 interacting protein, mediates cytochrome c release from mitochondria in response to activation of cell surface death receptors. Cell 94, 481–490.
Luqmani, Y. A. (2005). Mechanisms of drug resistance in cancer chemotherapy. Med Princ Pract 14, 35–48.
Luu, Y., Bush, J., Cheung, K. J., Jr., and Li, G. (2002). The p53 stabilizing compound CP-31398 induces apoptosis by activating the intrinsic Bax/mitochondrial/caspase-9 pathway. Exp Cell Res 276, 214–222.
Marchenko, N. D., Zaika, A., and Moll, U. M. (2000). Death signal-induced localization of p53 protein to mitochondria. A potential role in apoptotic signaling. J Biol Chem 275, 16202–16212.
Martelli, A. M., Tabellini, G., Bortul, R., Tazzari, P. L., Cappellini, A., Billi, A. M., and Cocco, L. (2005). Involvement of the phosphoinositide 3-kinase/Akt signaling pathway in the resistance to therapeutic treatments of human leukemias. Histol Histopathol 20, 239–252.
McCormick, F. (2004). Cancer: survival pathways meet their end. Nature 428, 267–269.
McGahon, A., Bissonnette, R., Schmitt, M., Cotter, K. M., Green, D. R., and Cotter, T. G. (1994). Bcr-Abl maintains resistance of chronic myelogenous leukemia cells to apoptotic cell death. Blood 83, 1179–1187.
McManus, D. C., Lefebvre, C. A., Cherton-Horvat, G., St-Jean, M., Kandimalla, E. R., Agrawal, S., Morris, S. J., Durkin, J. P., and Lacasse, E. C. (2004). Loss of XIAP protein expression by RNAi and antisense approaches sensitizes cancer cells to functionally diverse chemotherapeutics. Oncogene 23, 8105–8117.

Meek, D. W. (2004). The p53 response to DNA damage. DNA Repair (Amst) 3, 1049–1056.
Mendelsohn, J. and Fan, Z. (1997). Epidermal growth factor receptor family and chemosensitization. J Natl Cancer Inst 89, 341–343.
Mesner, P. W., Jr., Budihardjo, II, and Kaufmann, S. H. (1997). Chemotherapy-induced apoptosis. Adv Pharmacol 41, 461–499.
Michalak, E., Villunger, A., Erlacher, M., and Strasser, A. (2005). Death squads enlisted by the tumour suppressor p53. Biochem Biophys Res Commun 331, 786–798.
Mihara, M., Erster, S., Zaika, A., Petrenko, O., Chittenden, T., Pancoska, P., and Moll, U. M. (2003). p53 has a direct apoptogenic role at the mitochondria. Mol Cell 11, 577–590.
Minucci, S. and Pelicci, P. G. (2006). Histone deacetylase inhibitors and the promise of epigenetic (and more) treatments for cancer. Nat Rev Cancer 6, 38–51.
Miyashita, T. and Reed, J. C. (1992). bcl-2 gene transfer increases relative resistance of S49.1 and WEHI7.2 lymphoid cells to cell death and DNA fragmentation induced by glucocorticoids and multiple chemotherapeutic drugs. Cancer Res 52, 5407–5411.
Modur, V., Nagarajan, R., Evers, B. M., and Milbrandt, J. (2002). FOXO proteins regulate tumor necrosis factor-related apoptosis inducing ligand expression. Implications for PTEN mutation in prostate cancer. J Biol Chem 277, 47928–47937.
Morgensztern, D. and McLeod, H. L. (2005). PI3K/Akt/mTOR pathway as a target for cancer therapy. Anticancer Drugs 16, 797–803.
Moroni, M. C., Hickman, E. S., Lazzerini Denchi, E., 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.
Nagane, M., Levitzki, A., Gazit, A., Cavenee, W. K., and Huang, H. J. (1998). Drug resistance of human glioblastoma cells conferred by a tumor-specific mutant epidermal growth factor receptor through modulation of Bcl-XL and caspase-3-like proteases. Proc Natl Acad Sci USA95, 5724–5729.
Nahle, Z., Polakoff, J., Davuluri, R. V., McCurrach, M. E., Jacobson, M. D., Narita, M., Zhang, M. Q., Lazebnik, Y., Bar-Sagi, D., and Lowe, S. W. (2002). Direct coupling of the cell cycle and cell death machinery by E2F. Nat Cell Biol 4, 859–864.
Nakanishi, C. and Toi, M. (2005). Nuclear factor-kappaB inhibitors as sensitizers to anticancer drugs. Nat Rev Cancer 5, 297–309.
Nelson, D. A. and White, E. (2004). Exploiting different ways to die. Genes Dev 18, 1223–1226.
Newmeyer, D. D. and Ferguson-Miller, S. (2003). Mitochondria: releasing power for life and unleashing the machineries of death. Cell 112, 481–490.
Nguyen, D. M., Chen, G. A., Reddy, R., Tsai, W., Schrump, W. D., Cole, G., Jr., and Schrump, D. S. (2004). Potentiation of paclitaxel cytotoxicity in lung and esophageal cancer cells by pharmacologic inhibition of the phosphoinositide 3-kinase/protein kinase B (Akt)-mediated signaling pathway. J Thorac Cardiovasc Surg 127, 365–375.
Nicholson, K. M., Quinn, D. M., Kellett, G. L., and Warr, J. R. (2003). LY294002, an inhibitor of phosphatidylinositol-3-kinase, causes preferential induction of apoptosis in human multidrug resistant cells. Cancer Lett 190, 31–36.
Nieborowska-Skorska, M., Wasik, M. A., Slupianek, A., Salomoni, P., Kitamura, T., Calabretta, B., and Skorski, T. (1999). Signal transducer and activator of transcription (STAT)5 activation by BCR/ABL is dependent on intact Src homology (SH)3 and SH2 domains of BCR/ABL and is required for leukemogenesis. J Exp Med 189, 1229–1242.
Notarbartolo, M., Cervello, M., Dusonchet, L., Cusimano, A., and D'Alessandro, N. (2002). Resistance to diverse apoptotic triggers in multidrug resistant HL60 cells and its possible relationship to the expression of P-glycoprotein, Fas and of the novel anti-apoptosis factors IAP (inhibitory of apoptosis proteins). Cancer Lett 180, 91–101.
O'Gorman, D. M., McKenna, S. L., McGahon, A. J., Knox, K. A., and Cotter, T. G. (2000). Sensitisation of HL60 human leukaemic cells to cytotoxic drug-induced apoptosis by inhibition of PI3-kinase survival signals. Leukemia 14, 602–611.
Okada, M., Adachi, S., Imai, T., Watanabe, K., Toyokuni, S. Y., Ueno, M., Zervos, A. S., Kroemer, G., and Nakahata, T. (2004). A novel mechanism for imatinib mesylate-induced cell death of

Bcr-Abl-positive human leukemic cells: caspase-independent, necrosis-like programmed cell death mediated by serine protease activity. Blood 103, 2299–2307 (epub 2003 Nov 2226).
Oltersdorf, T., Elmore, S. W., Shoemaker, A. R., Armstrong, R. C., Augeri, D. J., Belli, B. A., Bruncko, M., Deckwerth, T. L., Dinges, J., Hajduk, P. J., et al. (2005). An inhibitor of Bcl-2 family proteins induces regression of solid tumours. Nature 435, 677–681 (epub 2005 May 2015).
Orian, A., van Steensel, B., Delrow, J., Bussemaker, H. J., Li, L., Sawado, T., Williams, E., Loo, L. W., Cowley, S. M., Yost, C., et al. (2003). Genomic binding by the Drosophila Myc, Max, Mad/Mnt transcription factor network. Genes Dev 17, 1101–1114.
Orlowski, R. Z. and Baldwin, A. S., Jr. (2002). NF-kappaB as a therapeutic target in cancer. Trends Mol Med 8, 385–389.
Osaki, M., Oshimura, M., and Ito, H. (2004). PI3K-Akt pathway: its functions and alterations in human cancer. Apoptosis 9, 667–676.
Oster, S. K., Ho, C. S., Soucie, E. L., and Penn, L. Z. (2002). The myc oncogene: MarvelouslY Complex. Adv Cancer Res 84, 81–154.
Ozoren, N. and El-Deiry, W. S. (2002). Defining characteristics of Types I and II apoptotic cells in response to TRAIL. Neoplasia 4, 551–557.
Ozoren, N. and El-Deiry, W. S. (2003). Cell surface death receptor signaling in normal and cancer cells. Semin Cancer Biol 13, 135–147.
Page, C., Lin, H. J., Jin, Y., Castle, V. P., Nunez, G., Huang, M., and Lin, J. (2000). Overexpression of Akt/AKT can modulate chemotherapy-induced apoptosis. Anticancer Res 20, 407–416.
Pegram, M. D., Finn, R. S., Arzoo, K., Beryt, M., Pietras, R. J., and Slamon, D. J. (1997). The effect of HER-2/neu overexpression on chemotherapeutic drug sensitivity in human breast and ovarian cancer cells. Oncogene 15, 537–547.
Pelengaris, S., Khan, M., and Evan, G. (2002). c-MYC: more than just a matter of life and death. Nat Rev Cancer 2, 764–776.
Perkins, C. L., Fang, G., Kim, C. N., and Bhalla, K. N. (2000). The role of Apaf-1, caspase-9, and bid proteins in etoposide- or paclitaxel-induced mitochondrial events during apoptosis. Cancer Res 60, 1645–1653.
Peter, M. E. and Krammer, P. H. (1998). Mechanisms of CD95 (APO-1/Fas)-mediated apoptosis. Curr Opin Immunol 10, 545–551.
Peter, M. E. and Krammer, P. H. (2003). The CD95(APO-1/Fas) DISC and beyond. Cell Death Differ 10, 26–35.
Pisha, E., Chai, H., Lee, I. S., Chagwedera, T. E., Farnsworth, N. R., Cordell, G. A., Beecher, C. W., Fong, H. H., Kinghorn, A. D., Brown, D. M., and et al. (1995). Discovery of betulinic acid as a selective inhibitor of human melanoma that functions by induction of apoptosis. Nat Med 1, 1046–1051.
Pommier, Y., Sordet, O., Antony, S., Hayward, R. L., and Kohn, K. W. (2004). Apoptosis defects and chemotherapy resistance: molecular interaction maps and networks. Oncogene 23, 2934–2949.
Pompetti, F., Rizzo, P., Simon, R. M., Freidlin, B., Mew, D. J., Pass, H. I., Picci, P., Levine, A. S., and Carbone, M. (1996). Oncogene alterations in primary, recurrent, and metastatic human bone tumors. J Cell Biochem 63, 37–50.
Proskuryakov, S. Y., Konoplyannikov, A. G., and Gabai, V. L. (2003). Necrosis: a specific form of programmed cell death? Exp Cell Res 283, 1–16.
Ravagnan, L., Roumier, T., and Kroemer, G. (2002). Mitochondria, the killer organelles and their weapons. J Cell Physiol 192, 131–137.
Ray, S., Lu, Y., Kaufmann, S. H., Gustafson, W. C., Karp, J. E., Boldogh, I., Fields, A. P., and Brasier, A. R. (2004). Genomic mechanisms of p210Bcr-Abl signaling: induction of heat shock protein 70 through the GATA response element confers resistance to paclitaxel-induced apoptosis. J Biol Chem 279, 35604–35615 (epub 32004 May 35621).
Reed, J. C. (1996). Mechanisms of Bcl-2 family protein function and dysfunction in health and disease. Behring Inst Mitt, 72–100.
Reed, J. C. (2000). Mechanisms of apoptosis. Am J Pathol 157, 1415–1430.

Reinke, V. and Lozano, G. (1997). Differential activation of p53 targets in cells treated with ultraviolet radiation that undergo both apoptosis and growth arrest. Radiat Res 148, 115–122.
Reuther, J. Y., Reuther, G. W., Cortez, D., Pendergast, A. M., and Baldwin, A. S., Jr. (1998). A requirement for NF-kappaB activation in Bcr-Abl-mediated transformation. Genes Dev 12, 968–981.
Rokudai, S., Fujita, N., Kitahara, O., Nakamura, Y., and Tsuruo, T. (2002). Involvement of FKHR-dependent TRADD expression in chemotherapeutic drug-induced apoptosis. Mol Cell Biol 22, 8695–8708.
Sakamuro, D., Eviner, V., Elliott, K. J., Showe, L., White, E., and Prendergast, G. C. (1995). c-Myc induces apoptosis in epithelial cells by both p53-dependent and p53-independent mechanisms. Oncogene 11, 2411–2418.
Salvesen, G. S. and Dixit, V. M. (1997). Caspases: intracellular signaling by proteolysis. Cell 91, 443–446.
Salvesen, G. S. and Dixit, V. M. (1999). Caspase activation: the induced-proximity model. Proc Natl Acad Sci USA 96, 10964–10967.
Salvesen, G. S. and Renatus, M. (2002). Apoptosome: the seven-spoked death machine. Dev Cell 2, 256–257.
Sawyers, C. L., McLaughlin, J., and Witte, O. N. (1995). Genetic requirement for Ras in the transformation of fibroblasts and hematopoietic cells by the Bcr-Abl oncogene. J Exp Med 181, 307–313.
Scaffidi, C., Fulda, S., Srinivasan, A., Friesen, C., Li, F., Tomaselli, K. J., Debatin, K. M., Krammer, P. H., and Peter, M. E. (1998). Two CD95 (APO-1/Fas) signaling pathways. EMBO J 17, 1675–1687.
Scaffidi, C., Kirchhoff, S., Krammer, P. H., and Peter, M. E. (1999). Apoptosis signaling in lymphocytes. Curr Opin Immunol 11, 277–285.
Schimmer, A. D., Dalili, S., Batey, R. A., and Riedl, S. J. (2006). Targeting XIAP for the treatment of malignancy. Cell Death Differ 13, 179–188.
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.
Schuler, M. and Green, D. R. (2005). Transcription, apoptosis and p53: catch-22. Trends Genet 21, 182–187.
Schulze-Bergkamen, H., and Krammer, P. H. (2004). Apoptosis in cancer – implications for therapy. Semin Oncol 31, 90–119.
Schulze-Osthoff, K., Ferrari, D., Los, M., Wesselborg, S., and Peter, M. E. (1998). Apoptosis signaling by death receptors. Eur J Biochem 254, 439–459.
Seelan, R. S., Irwin, M., van der Stoop, P., Qian, C., Kaelin, W. G., Jr., and Liu, W. (2002). The human p73 promoter: characterization and identification of functional E2F binding sites. Neoplasia 4, 195–203.
Sheikh, M. S. and Huang, Y. (2004). Death receptors as targets of cancer therapeutics. Curr Cancer Drug Targets 4, 97–104.
Shi, Y. (2002). Apoptosome: the cellular engine for the activation of caspase-9. Structure (Camb) 10, 285–288.
Shuai, K., Halpern, J., ten Hoeve, J., Rao, X., and Sawyers, C. L. (1996). Constitutive activation of STAT5 by the Bcr-Abl oncogene in chronic myelogenous leukemia. Oncogene 13, 247–254.
Sillaber, C., Gesbert, F., Frank, D. A., Sattler, M., and Griffin, J. D. (2000). STAT5 activation contributes to growth and viability in Bcr/Abl-transformed cells. Blood 95, 2118–2125.
Skorski, T. (2002). BCR/ABL regulates response to DNA damage: the role in resistance to genotoxic treatment and in genomic instability. Oncogene 21, 8591–8604.
Skorski, T., Bellacosa, A., Nieborowska-Skorska, M., Majewski, M., Martinez, R., Choi, J. K., Trotta, R., Wlodarski, P., Perrotti, D., Chan, T. O., et al. (1997). Transformation of hematopoietic cells by BCR/ABL requires activation of a PI-3k/Akt-dependent pathway. EMBO J 16, 6151–6161.

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.

Song, L., Coppola, D., Livingston, S., Cress, D., and Haura, E. B. (2005). Mcl-1 regulates survival and sensitivity to diverse apoptotic stimuli in human non-small cell lung cancer cells. Cancer Biol Ther 4, 267–276 (epub 2005 Mar 2020).

Sorice, M., Circella, A., Cristea, I. M., Garofalo, T., Renzo, L. D., Alessandri, C., Valesini, G., and Esposti, M. D. (2004). Cardiolipin and its metabolites move from mitochondria to other cellular membranes during death receptor-mediated apoptosis. Cell Death Differ 11, 1133–1145.

Stahl, M., Dijkers, P. F., Kops, G. J., Lens, S. M., Coffer, P. J., Burgering, B. M., and Medema, R. H. (2002). The forkhead transcription factor FoxO regulates transcription of p27Kip1 and Bim in response to IL-2. J Immunol 168, 5024–5031.

Stegh, A. H. and Peter, M. E. (2001). Apoptosis and caspases. Cardiol Clin 19, 13–29.

Stiewe, T. and Putzer, B. M. (2000). Role of the p53-homologue p73 in E2F1-induced apoptosis. Nat Genet 26, 464–469.

Strasser, A., Harris, A. W., Bath, M. L., and Cory, S. (1990). Novel primitive lymphoid tumours induced in transgenic mice by cooperation between myc and bcl-2. Nature 348, 331–333.

Strasser, A., O'Connor, L., and Dixit, V. M. (2000). Apoptosis signaling. Annu Rev Biochem 69, 217–245.

Suhara, T., Kim, H. S., Kirshenbaum, L. A., and Walsh, K. (2002). Suppression of Akt signaling induces Fas ligand expression: involvement of caspase and Jun kinase activation in Akt-mediated Fas ligand regulation. Molecular and Cellular Biology 22, 680–691.

Sunters, A., Fernandez de Mattos, S., Stahl, M., Brosens, J. J., Zoumpoulidou, G., Saunders, C. A., Coffer, P. J., Medema, R. H., Coombes, R. C., and Lam, E. W. (2003). FoxO3a transcriptional regulation of Bim controls apoptosis in paclitaxel-treated breast cancer cell lines. J Biol Chem 278, 49795–49805 (epub 42003 Oct 49793).

Tai, Y. T., Lee, S., Niloff, E., Weisman, C., Strobel, T., and Cannistra, S. A. (1998). BAX protein expression and clinical outcome in epithelial ovarian cancer. J Clin Oncol 16, 2583–2590.

Teitz, T., Lahti, J. M., and Kidd, V. J. (2001). Aggressive childhood neuroblastomas do not express caspase-8: an important component of programmed cell death. J Mol Med 79, 428–436.

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.

Thompson, C. B. (1995). Apoptosis in the pathogenesis and treatment of disease. Science 267, 1456–1462.

Thorburn, A. (2004). Death receptor-induced cell killing. Cell Signal 16, 139–144.

Tomita, Y., Marchenko, N., Erster, S., Nemajerova, A., Dehner, A., Klein, C., Pan, H., Kessler, H., Pancoska, P., and Moll, U. M. (2006). WTp53 but not tumor-derived mutants bind to BCL2 via the DNA binding domain and induce mitochondrial permeabilization. J Biol Chem 26, 26.

Toretsky, J. A., Thakar, M., Eskenazi, A. E., and Frantz, C. N. (1999). Phosphoinositide 3-hydroxide kinase blockade enhances apoptosis in the Ewing's sarcoma family of tumors. Cancer Res 59, 5745–5750.

Tourneur, L., Delluc, S., Levy, V., Valensi, F., Radford-Weiss, I., Legrand, O., Vargaftig, J., Boix, C., Macintyre, E. A., Varet, B., et al. (2004). Absence or low expression of fas-associated protein with death domain in acute myeloid leukemia cells predicts resistance to chemotherapy and poor outcome. Cancer Res 64, 8101–8108.

Tzung, S. P., Kim, K. M., Basanez, G., Giedt, C. D., Simon, J., Zimmerberg, J., Zhang, K. Y., and Hockenbery, D. M. (2001). Antimycin A mimics a cell-death-inducing Bcl-2 homology domain 3. Nat Cell Biol 3, 183–191.

Underhill-Day, N., Pierce, A., Thompson, S. E., Xenaki, D., Whetton, A. D., and Owen-Lynch, P. J. (2006). Role of the C-terminal actin binding domain in BCR/ABL-mediated survival and drug resistance. Br J Haematol 132, 774–783.

Varticovski, L., Daley, G. Q., Jackson, P., Baltimore, D., and Cantley, L. C. (1991). Activation of phosphatidylinositol 3-kinase in cells expressing abl oncogene variants. Mol Cell Biol 11, 1107–1113.

Vassilev, L. T., Vu, B. T., Graves, B., Carvajal, D., Podlaski, F., Filipovic, Z., Kong, N., Kammlott, U., Lukacs, C., Klein, C., et al. (2004). In vivo activation of the p53 pathway by small-molecule antagonists of MDM2. Science 303, 844–848 (epub 2004 Jan 2002).

Vaux, D. L. (2002). Apoptosis timeline. Cell Death Differ 9, 349–354.

Vaux, D. L. and Korsmeyer, S. J. (1999). Cell death in development. Cell 96, 245–254.

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

Walczak, H., Miller, R. E., Ariail, K., Gliniak, B., Griffith, T. S., Kubin, M., Chin, W., Jones, J., Woodward, A., Le, T., et al. (1999). Tumoricidal activity of tumor necrosis factor-related apoptosis-inducing ligand in vivo. Nature medicine 5, 157–163.

Walensky, L. D., Kung, A. L., Escher, I., Malia, T. J., Barbuto, S., Wright, R. D., Wagner, G., Verdine, G. L., and Korsmeyer, S. J. (2004). Activation of apoptosis in vivo by a hydrocarbon-stapled BH3 helix. Science 305, 1466–1470.

Wallach, D. (1997). Apoptosis. Placing death under control. Nature 388, 123, 125–126.

Wallach, D., Varfolomeev, E. E., Malinin, N. L., Goltsev, Y. V., Kovalenko, A. V., and Boldin, M. P. (1999). Tumor necrosis factor receptor and Fas signaling mechanisms. Annu Rev Immunol 17, 331–367.

Walton, M. I., Whysong, D., O'Connor, P. M., Hockenbery, D., Korsmeyer, S. J., and Kohn, K. W. (1993). Constitutive expression of human Bcl-2 modulates nitrogen mustard and camptothecin induced apoptosis. Cancer Res 53, 1853–1861.

Wang, G. Q., Gastman, B. R., Wieckowski, E., Goldstein, L. A., Gambotto, A., Kim, T. H., Fang, B., Rabinovitz, A., Yin, X. M., and Rabinowich, H. (2001). A role for mitochondrial Bak in apoptotic response to anticancer drugs. J Biol Chem 276, 34307–34317 (epub 32001 July 34310).

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, J. Y. and Ki, S. W. (2001). Choosing between growth arrest and apoptosis through the retinoblastoma tumour suppressor protein, Abl and p73. Biochem Soc Trans 29, 666–673.

Wang, L. G., Liu, X. M., Kreis, W., and Budman, D. R. (1999). The effect of antimicrotubule agents on signal transduction pathways of apoptosis: a review. Cancer Chemother Pharmacol 44, 355–361.

Wang, W. and El-Deiry, W. S. (2004). Targeting FOXO kills two birds with one stone. Chem Biol 11, 16–18.

Waxman, D. J. and Schwartz, P. S. (2003). Harnessing apoptosis for improved anticancer gene therapy. Cancer Res 63, 8563–8572.

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. (2001a). Proapoptotic BAX and BAK: a requisite gateway to mitochondrial dysfunction and death. Science 292, 727–730.

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. (2001b). Proapoptotic BAX and BAK: a requisite gateway to mitochondrial dysfunction and death. Science 292, 727–730.

Weller, M. (1998). Predicting response to cancer chemotherapy: the role of p53. Cell Tissue Res 292, 435–445.

Wendel, H. G., De Stanchina, E., Fridman, J. S., Malina, A., Ray, S., Kogan, S., Cordon-Cardo, C., Pelletier, J., and Lowe, S. W. (2004). Survival signalling by Akt and eIF4E in oncogenesis and cancer therapy. Nature 428, 332–337.

Wennerberg, K., Rossman, K. L., and Der, C. J. (2005). The Ras superfamily at a glance. J Cell Sci 118, 843–846.

Whitesell, L. and Lindquist, S. L. (2005). HSP90 and the chaperoning of cancer. Nat Rev Cancer 5, 761–772.

Xu, H., Tai, J., Ye, H., Kang, C. B., and Yoon, H. S. (2006). The N-terminal domain of tumor suppressor p53 is involved in the molecular interaction with the anti-apoptotic protein Bcl-Xl. Biochem Biophys Res Commun 341, 938–944 (epub 2006 Jan 2023).

Yagita, H., Takeda, K., Hayakawa, Y., Smyth, M. J., and Okumura, K. (2004). TRAIL and its receptors as targets for cancer therapy. Cancer Sci 95, 777–783.

Yang, X., Chang, H. Y., and Baltimore, D. (1998). Autoproteolytic activation of pro-caspases by oligomerization. Mol Cell 1, 319–325.

Yin, H., Lee, G. I., Sedey, K. A., Kutzki, O., Park, H. S., Orner, B. P., Ernst, J. T., Wang, H. G., Sebti, S. M., and Hamilton, A. D. (2005). Terphenyl-Based Bak BH3 alpha-helical proteomimetics as low-molecular-weight antagonists of Bcl-xL. J Am Chem Soc 127, 10191–10196.

Yoo, C. B. and Jones, P. A. (2006). Epigenetic therapy of cancer: past, present and future. Nat Rev Drug Discov 5, 37–50.

Yu, J. and Zhang, L. (2005). The transcriptional targets of p53 in apoptosis control. Biochem Biophys Res Commun 331, 851–858.

Yuan, X. J. and Whang, Y. E. (2002). PTEN sensitizes prostate cancer cells to death receptor-mediated and drug-induced apoptosis through a FADD-dependent pathway. Oncogene 21, 319–327.

Zaffaroni, N., Pennati, M., Colella, G., Perego, P., Supino, R., Gatti, L., Pilotti, S., Zunino, F., and Daidone, M. G. (2002). Expression of the anti-apoptotic gene survivin correlates with taxol resistance in human ovarian cancer. Cell Mol Life Sci 59, 1406–1412.

Zamzami, N. and Kroemer, G. (2001). The mitochondrion in apoptosis: how Pandora's box opens. Nat Rev Mol Cell Biol 2, 67–71.

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.

Zhou, P., Qian, L., Kozopas, K. M., and Craig, R. W. (1997). Mcl-1, a Bcl-2 family member, delays the death of hematopoietic cells under a variety of apoptosis-inducing conditions. Blood 89, 630–643.

Zinkel, S. S., Ong, C. C., Ferguson, D. O., Iwasaki, H., Akashi, K., Bronson, R. T., Kutok, J. L., Alt, F. W., and Korsmeyer, S. J. (2003). Proapoptotic BID is required for myeloid homeostasis and tumor suppression. Genes Dev 17, 229–239.

Zong, W. X., Ditsworth, D., Bauer, D. E., Wang, Z. Q., and Thompson, C. B. (2004). Alkylating DNA damage stimulates a regulated form of necrotic cell death. Genes Dev 18, 1272–1282 (epub 2004 May 1214).

Zornig, M., Hueber, A., Baum, W., and Evan, G. (2001). Apoptosis regulators and their role in tumorigenesis. Biochim Biophys Acta 1551, F1–F37.