Drugs of the Future 2005, 30(7): 707-723

CCC: 0377-8282/2005
DOI: 10.1358/dof.2005.30.07.915915

Review Article

Apoptosis-based therapies for hematological malignancies

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CONTENTS

Abstract

Apoptosis, or programmed cell death, is central to the development and homeostasis of the hemopoietic system. Dysregulation of apoptosis plays an important role in the development of a variety of human pathologies, including cancer, autoimmune diseases and neurodegenerative disorders. Studies carried out in recent years have shown that leukemia, lymphoma and multiple myeloma cells invariably have abnormalities in one or more apoptotic pathways, determining a survival advantage of these cells over their normal counterparts. Furthermore, abnormalities in the apoptotic response also play a pivotal role in the development of drug resistance by leukemia/lymphoma cells. Tremendous progress in elucidating the structure and function of the core components of the apoptosis machinery have led to the identification of many molecular apoptotic targets for the development of new drugs targeting antiapoptotic molecules abnormally expressed or dysregulated in cancer cells. In this review, we describe some of the drug discovery targets thus far identified within the core apoptotic machinery, the corresponding drugs that have been developed, their effects on leukemia/lymphoma cells and their potential impact on the therapy of these diseases.

Introduction

Chemotherapy, surgical resection and radiotherapy represent the standard therapeutic approaches in the treatment of cancer. These therapeutic strategies, however, are often not curative and associated with considerable secondary and toxic effects, and frequently result in the selection of highly malignant treatment-resistant tumor cells. Given these limitations, there is a need for the development of alternative therapeutic strategies based on new drugs endowed with different mechanisms of action, specifically targeting tumor cells and with more acceptable toxicity profiles. In this context, particularly promising is the area of biological therapies based on agents able to selectively kill tumor cells while sparing normal cells, displaying limited *in vivo* toxicity, and able to bypass or circumvent acquired tumor resistance to conventional treatments. Some of these new therapeutic approaches derive from a better understanding of the mechanisms that regulate apoptosis in normal cells and of the abnormalities of these mechanisms existing in cancer cells.

Apoptotic processes play a key role in the control of tissue homeostasis, particularly in tissues associated with rapid renewal, such as hemopoietic tissue. Studies carried out during the last 20 years have dramatically improved our understanding of the role of apoptotic mechanisms in the control of the differentiation, proliferation and survival of normal and leukemia cells. The understanding of the apoptotic machinery at the cellular, molecular and structural level and of abnormalities in leukemia and lymphoma cells has led to the identification of several apoptotic molecules as potential targets for the development of new therapies for leukemia and lymphoma. In this review, the main potential drug targets thus far identified within the core apoptotic machinery are outlined. In parallel, efforts to translate basic knowledge about these targets into new therapies for leukemia and lymphoma are described in detail.

Apoptosis and cancer

Defects in DNA repair lead to genomic instability and predispose to cancer development. This genetic instabili-

ty represents the mechanism by which normal cells can accumulate a sufficient number of mutations to become malignant (1). The cells, however, possess important mechanisms of protection against this genomic instability, mainly orchestrated by the tumor suppressor protein p53, which acts as “a guardian of the genome” in protecting cells against cancer (*i.e.*, by inducing the death of cells that have accumulated genetic defects) (2). p53 is the most frequently mutated gene in human cancers and its inactivation by mutation certainly plays an important role in tumor development (2). There is now evidence that, in addition to p53 inactivation, tumor cells exhibit multiple defects in cell death pathways. Resistance to cell death, and particularly to apoptotic cell death, plays a key role both in tumor development and in the mechanism of resistance to anticancer drugs (3). Resistance to programmed cell death plays a major role in several pathogenetic mechanisms of tumors, allowing tumor cells to abnormally survive beyond their normal lifetime, reducing the need for exogenous growth factors, providing protection from hypoxia and oxidative stress, increasing the time and therefore the opportunity for the development of additional genetic abnormalities altering cell proliferation, interfering with cell differentiation, promoting angiogenesis and increasing cell motility and invasiveness during tumor progression.

The understanding of the mechanisms responsible for the resistance of tumor cells to programmed cell death is of fundamental importance not only for a better understanding of cancer biology, but also for the development of new cancer therapies focused on devising ways to overcome this resistance and to induce apoptosis of cancer cells.

Apoptotic pathways

The most common and well-defined form of programmed cell death is represented by apoptosis, a physiological process of cellular suicide required for the maintenance of cell homeostasis during embryonic development and for the differentiation and function of hematopoietic and lymphoid cells.

A common feature of the apoptotic process is the constant involvement of caspases, a family of intracellular cysteine proteases (cysteine aspartyl-specific proteases). These enzymes are present as inactive zymogens in all animal cells, but can be triggered to assume an active state, usually through their proteolytic processing at conserved aspartic acid residues. During activation, procaspase is cleaved to generate large and small subunits; the active enzymes are heterotetramers composed of two large and two small subunits. It is important to note that active caspases cleave their substrates at the Asp residues and they are also activated by proteolytic cleavage at Asp residues. This unique property implies that these enzymes form part of proteolytic cascades, where caspases activate themselves and each other.

Based on their level of action, caspases are distinguished as “initiator” caspases (which include caspase-2, -8, -9 and -10) and “effector” caspases (caspase-3, -6 and -7); the first act at the origin of the apoptotic process,

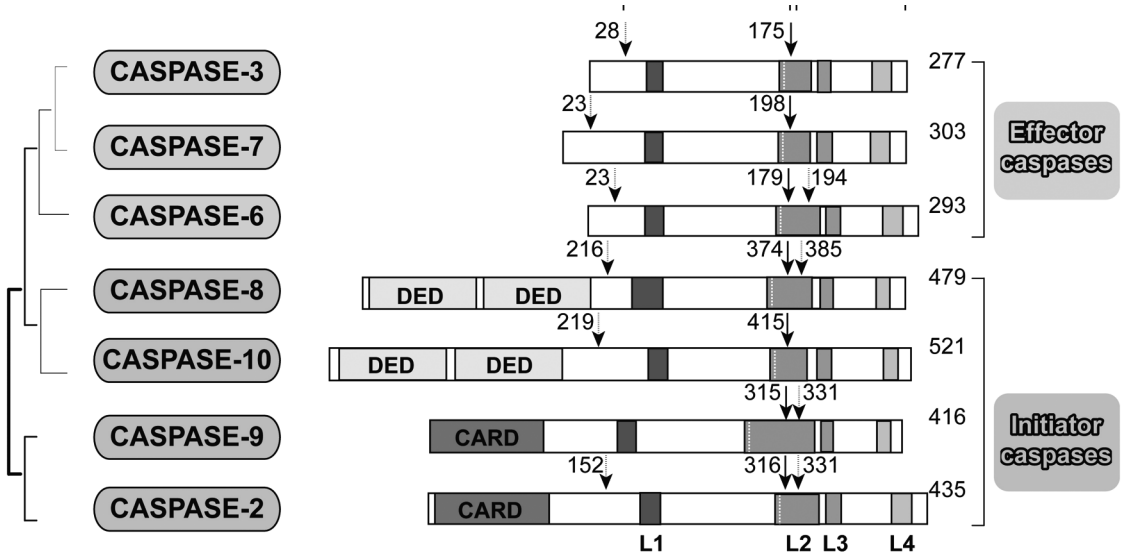

Fig. 1. Schematic representation of the main caspases, subdivided into “initiator” (yellow) and “effector” (green) caspases. The site of interchain activation cleavage is indicated by a black arrow (this site is located between the small p10 and the large p20 subunits). Additional sites of cleavage are outlined by broken arrows. The prodomain region of “effector” caspases contains homotypic interaction motifs, represented by the caspase recruitment domain (CARD) and the death effector domain (DED). Four catalytic loops, indicated as L1, L2, L3 and L4, contribute to the formation of the catalytic groove essential for enzyme activity. At the beginning of loop 2, a white line indicates the presence of a catalytic Cys residue.

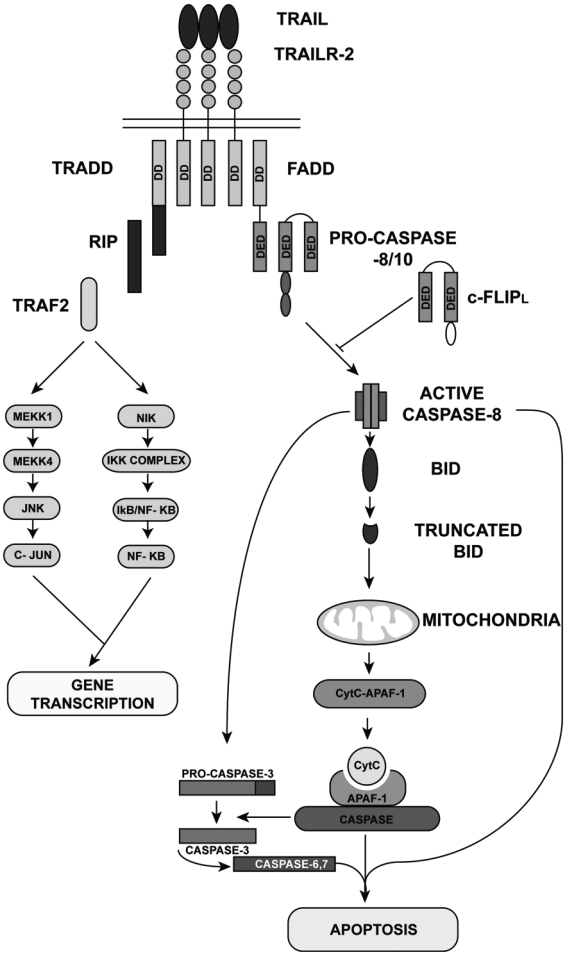

Fig. 2. Outline of the extrinsic apoptotic pathway. This pathway is activated when death ligands such as FasL, TRAIL or TNF-α bind their cognate receptors (Fas, TRAIL-R1/TRAIL-R2 and TNF-R1, respectively). The initial step is represented by the progressive recruitment first of FADD (Fas-associated protein with death domain) and then of procaspase-8, with formation of the DISC (death-induced signaling complex). Caspase-8 is then activated and in turn determines the activation of "effector" caspases, which culminates in cell death.

while the latter act at the late steps of the apoptotic process (Fig. 1). Once activated, the effector caspases are responsible for the proteolytic cleavage of a broad spectrum of cellular proteins, which ultimately culminates in cell death. The "initiator" caspases are structurally characterized by an extended *N*-terminal region, comprising one or two adaptor domains that are essential for their function; in contrast, the "effector" caspases contain 20-30 amino acid residues in their prodomain region.

There are two pathways by which caspase activation is induced: the extrinsic and the intrinsic apoptotic pathways (Figs. 2 and 3). Both pathways converge at the activation of effector caspases but require different initiation caspases to start the process. The extrinsic pathway is activated by the engagement of death receptors on the cell membrane. Binding of ligands such as FasL, TNF and TRAIL (tumor necrosis factor-related apoptosis-inducing ligand) to their respective membrane receptors Fas, TNF-R and TRAIL-Rs induces the formation of the oligomeric death-induced signaling complex (DISC) (Fig. 2). DISC in turn promotes caspase-8 recruitment and a cascade of caspase enzyme activation that culminates in cell death. The activation of caspase-8 is antagonized by cellular FLICE (FADD-like interleukin-1β-converting enzyme)-inhibitory protein (c-FLIP), an enzymatically inactive relative of caspase-8 and -10 that binds to DISC. The knockdown of c-FLIP augments DISC recruitment, activation and processing of caspase-8, thereby enhancing effector caspase stimulation and apoptosis.

The intrinsic pathway is triggered by various extracellular and intracellular stresses, including growth factor deprivation, DNA damage, oncogene induction, hypoxia and cytotoxic drugs (Fig. 3). Cellular signals originated by various mechanisms by these different stresses converge on a cellular target represented by mitochondria. A series of biochemical events is then induced that leads to damage of the mitochondrial membrane, the release of cytochrome *c* and other proapoptotic molecules, with the consequent formation of the apoptosome, a large molecular complex formed by cytochrome *c*, apoptotic protease-activating factor 1 (APAF1) and caspase-9, and caspase activation (Fig. 3). The permeabilization of the outer mitochondrial membrane is inhibited by antiapoptotic molecules pertaining to the Bcl-2 family.

The intrinsic apoptosis pathway is controlled by other mitochondrial proteins. Thus, apoptosis-inducing-factor (AIF) and endonuclease G may induce cell death independently of caspase activation. Furthermore, Smac (second mitochondria-derived activator of caspase)/Diablo (direct IAP-binding protein with low pI) and HtrA2 (high temperature requirement protein A2) promote caspase activation by countering the activity of proteins acting as inhibitors of apoptosis (IAP); these proteins exert an inhibitory effect on caspase activation. The extrinsic pathway can crosstalk with the intrinsic pathway through the caspase-8-mediated cleavage of Bid, which then triggers the release of mitochondrial proteins.

Death ligands and death receptors

There is growing interest in the development of therapeutic strategies that kill cancer cells via activation of the extrinsic apoptotic pathway. Some members of the TNF family directly trigger apoptosis and their activation could be used to induce the killing of tumor cells. Three ligands (TNF-α, FasL and TRAIL) of the TNF family and their four corresponding receptors (TNF-R1, Fas and TRAIL-R1/TRAIL-R2) have been considered for their potential use as anticancer therapeutics. The strategy for development of potential drugs consists either in the synthesis of recombinant ligands or in the production of agonist monoclonal antibodies which bind to death membrane recep-

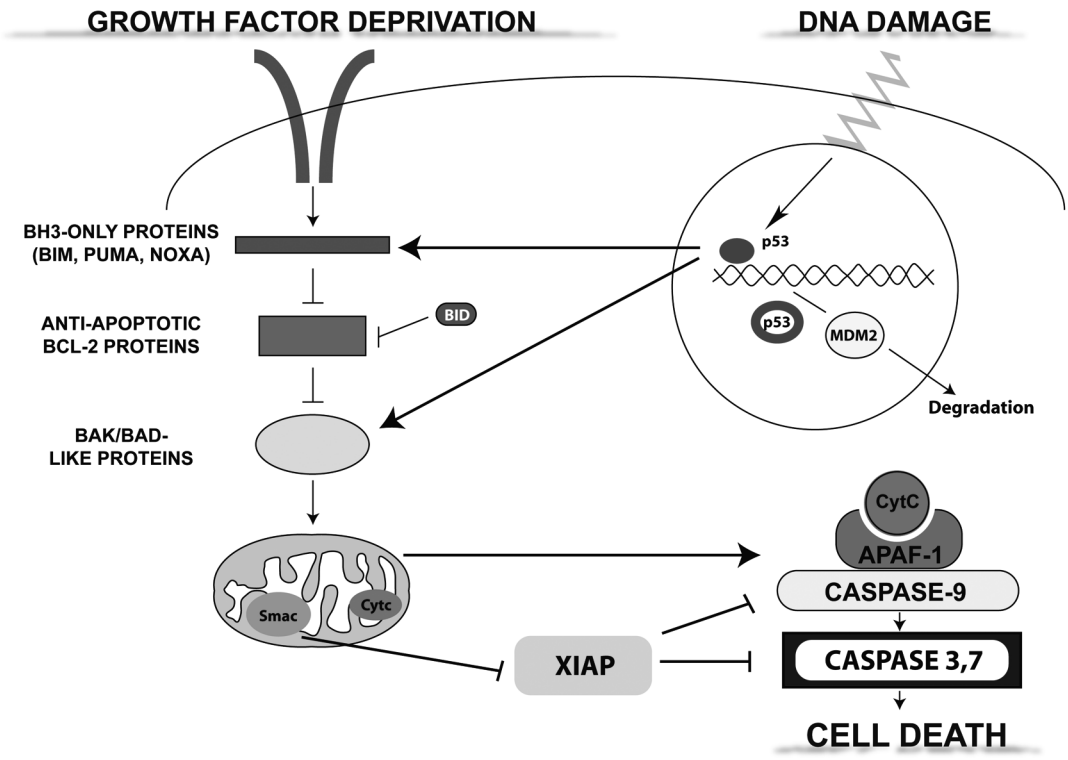

Fig. 3. Outline of the intrinsic apoptotic pathway. This pathway is triggered by different types of stress conditions, including growth factor deprivation, DNA damage and other cytotoxic stimuli. Death stimuli activate BH3-only proteins, which initiate apoptosis by a mechanism requiring Bax/Bak; these two proapoptotic proteins determine the damage of the outer mitochondrial membrane, resulting in the release of cytochrome *c* (CytC) and other mitochondrial apoptogenic factors. The release of CytC in the cytoplasm determines APAF1 (apoptotic protease-activating factor 1)-mediated activation of caspase-9, which in turn induces the activation of effector caspases (caspase-3 and -7). The intrinsic apoptotic pathway may be inhibited at two levels: at the level of the activity of BH3-only proteins by the anti-apoptotic Bcl-2 members, or at the level of caspases by XIAP (X-linked inhibitor of apoptosis proteins).

tors and then induce cell death by activating the apoptotic machinery.

The first of these molecules to be studied for its potential use as an anticancer drug was TNF-α. The demonstration that TNF-α selectively kills tumor cells but not normal cells raised hopes in terms of its potential use for cancer treatment (4). Unfortunately, the marked proinflammatory effects of TNF-α precluded its systemic administration.

Subsequent studies were focused on the other two molecules, FasL and TRAIL. At variance with TNF-α, these cytokines do not have proinflammatory effects and are suitable as potential anticancer drugs. Unfortunately, agonist antibodies triggering Fas activation are highly hepatotoxic, inducing death in mice (5). In contrast, TRAIL and agonist anti-TRAIL-R1/TRAIL-R2 antibodies appear to be well tolerated *in vivo*. In this context, initial studies have shown that TRAIL/Apo-2L exhibits potent antitumor activity and induces minimal cytotoxic effects in human tumor xenograft models in immunodeficient mice (6). However, the *in vivo* half-life of the TRAIL ligand was very short (< 4 min), suggesting that agonist anti-TRAIL-R1 or anti-TRAIL-R2 antibodies might have a better pharmacological impact due to their prolonged *in vivo* half-life (7, 8). Another additional advantage of agonist TRAIL-R1 and TRAIL-R2 antibodies is that, in contrast to the TRAIL ligand, they do not bind to the TRAIL decoy receptors TRAIL-R3 and TRAIL-R4, often present on the membranes of tumor cells.

Two anti-TRAIL-R antibodies have been developed for clinical use. One of these is called HGS-ETR1 (Human Genome Sciences) and is a fully human agonist antibody with high affinity and specificity for TRAIL-R1 (9). This antibody induces killing of tumor cell lines through activation of both extrinsic and intrinsic apoptotic pathways. Importantly, HGS-ETR1 was shown to have a long half-life *in vivo* (7-9 days in mice) and suppressed the growth of several tumors in xenograft models in athymic mice (6). Finally, HGS-ETR1 potentiated the antitumor efficacy of several chemotherapeutic drugs (9). These observations clearly indicate that HGS-ETR1 has significant potential as a cancer therapeutic agent. The HGS-ETR1 antibody was evaluated in a phase I/II clinical trial in patients with advanced solid or hematological tumors, revealing little toxicity (10).

A second fully human antibody to TRAIL-R2, KMTR2, was recently reported (11). This antibody, through its

binding to TRAIL-R2, induces the tumor cell death and no crosslinking is required for induction of cell apoptosis (11).

The use of fusion proteins comprised of recombinant human TRAIL fused to a monoclonal antibody against a membrane antigen can be used to induce target antigen-restricted apoptosis. An example of such a fusion protein is scFvCD7:sTRAIL, comprised of soluble TRAIL genetically linked to an scFv antibody fragment specific for the T-cell surface antigen CD7; this fusion protein induces cytotoxicity in primary acute T-lymphoblastic leukemia cells and potentiates the cytotoxic effect of the antitumor drug vincristine (12). Additional examples of fusion proteins are scFv425:sTRAIL (comprised of the EGFR-blocking antibody fragment scFv425 genetically fused to soluble TRAIL) (13) or scFvEGP2:sTRAIL (comprised of the anti-pancarcinoma-associated antigen EGP2 genetically fused to soluble TRAIL) (14).

Intracellular modifiers of the extrinsic pathway

Certain intracellular molecules modulate the cellular sensitivity to death ligands; among them, a prominent role is played by FADD (Fas-associated protein with death domain), c-FLIP and the nuclear transcription factor NF-κB. These molecules are now considered important targets for anticancer therapy, as outlined below.

FADD

FADD is a key adaptor molecule transmitting the death signal mediated by death receptors. The FADD protein is present both in the nucleus and in the cytoplasm; the role of cytoplasmic FADD in cell death signaling is well established, while the role of nuclear FADD is unclear (15). FADD is the main signal transducing intermediate adaptor molecule of several death receptors, including Fas, TNF-R1, death receptor 3 (DR3), TRAIL-R1 and TRAIL-R2. The cytoplasmic tails of these receptors possess a death domain (DD) able, once the receptors are activated, to recruit the DD of FADD (Fig. 4). The function of FADD in death receptor signaling is inhibited by c-FLIP, which through its DD is able to bind to the DD of FADD, thus preventing its recruitment by activated death receptors (Fig. 4).

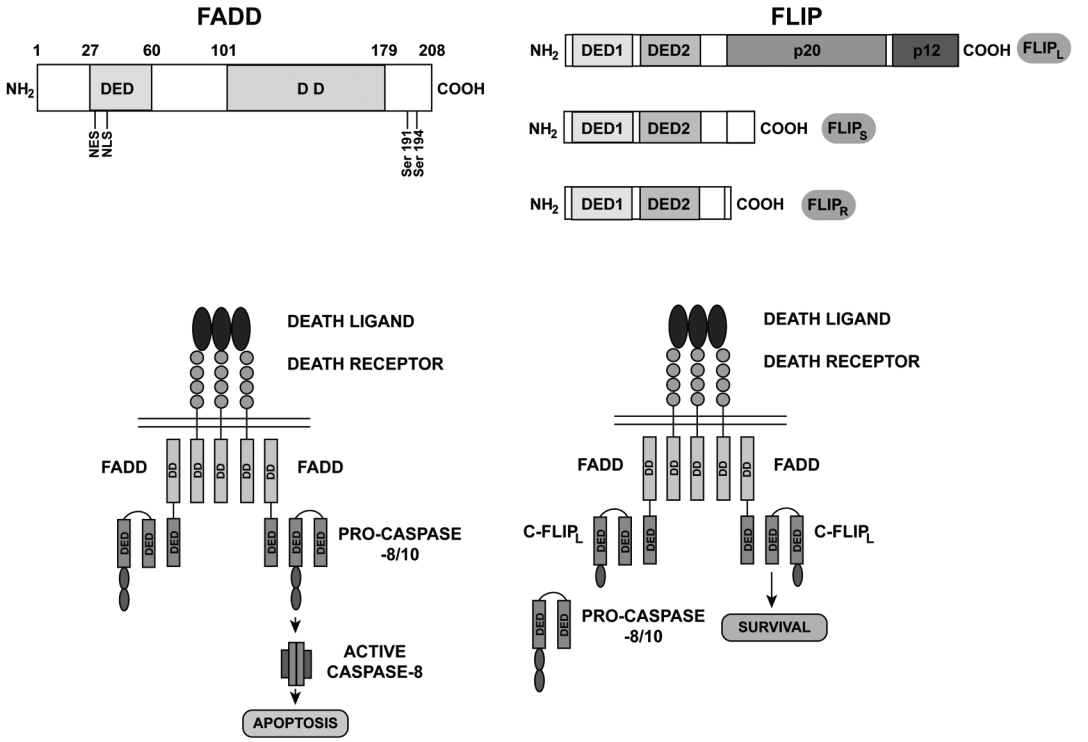

Fig. 4. Schematic representation of the structure of FADD (Fas-associated protein with death domain) and FLIP (FLICE-inhibitory protein) proteins. Left, top: the structure of FADD protein, with two boxes indicating one death effector domain (DED) and one death domain (DD), is shown. The numbers indicate amino acid residues. Within the DED domain, the export sequence (NES) and nuclear localization sequence (NLS) have been identified; they determine the cellular localization of FADD either in the cytoplasm or in the nucleus. In the COOH-terminal sites, two serine residues (Ser^{191} and Ser^{194}), essential for FADD function, are indicated. Right, top: the structure of $FLIP_L$, $FLIP_S$ and $FLIP_R$, with their structural domains, is shown. Left, bottom: signaling along the extrinsic apoptotic pathway based on FADD and caspase-8 recruitment and activation, with the consequent induction of the effector apoptotic machinery. Right, bottom: Blockade of apoptotic signaling along the extrinsic pathway where c-FLIP interacts with FADD, hampering subsequent caspase-8 recruitment and activation.

Studies in FADD$^{-/-}$ mice suggest that this protein could exert an additional role in the control of cell proliferation. Studies in animal models of tumor development have shown that FADD may act as a tumor suppressor (reviewed in 15). This interpretation is supported by recent studies showing that about two-thirds of acute myeloid leukemia (AML) blasts show low or absent FADD protein. The absence of FADD protein in AML is associated with a poor prognosis (16). The absent/low FADD protein in AML may be one of the factors responsible for the resistance of AML blasts to Fas- and TRAIL-mediated apoptosis. In fact, the large majority of AML patients are resistant to Fas-mediated cell death despite expressing the Fas receptor (17) and/or FasL (18). Similarly, AML patients often express TRAIL-R1 or TRAIL-R2 and membrane-bound TRAIL, but are systematically resistant to TRAIL-mediated apoptosis (19). The absence of FADD expression may confer to AML blasts resistance to immune-mediated attack and to some chemotherapeutic drugs, such as anthracyclines and etoposide, which are known to enhance TRAIL-R2 expression and sensitivity to TRAIL- and Fas-mediated apoptosis (20). These observations suggest that FADD could represent an important target for the development of antileukemia therapies aiming to restore normal FADD expression in these cells.

A recent study (21) suggested that inhibition of FADD could be of value in the therapy of myelodysplastic syndrome (MDS). MDS is characterized by peripheral blood cytopenia and by increased FADD and caspase-8 activity. The transduction of a dominant-negative form of FADD in CD34^{+} MDS cells restored normal erythroid differentiation (21). In addition to transduction of a FADD dominant-negative mutant, other strategies could be used to inhibit FADD, including short interfering RNA (siRNA) (22), non-peptide compounds that block caspase-8 activation in the DISC (23) and peptides that competitively inhibit the binding of FADD to procaspase-8 (24).

FLIP

The death effector domains (DEDs) present in caspase-8 and FADD are responsible for their reciprocal interaction, with the consequent formation of death receptor signaling complexes. However, other DED-containing proteins such as FLIP (also known as Flame, Casper or Usurpin) exert a negative role in the control of death receptor signaling. In fact, the FLIP protein exhibits extensive homology with procaspase-8 and -10, containing two *N*-terminal DEDs followed by a pseudocaspase domain that lacks critical residues required for protease activity, including the catalytic cysteine (Fig. 4) (25, 26). FLIP associates with procaspase-8 and competes with procaspase-8 and -10 for binding to FADD and, through this mechanism, inhibits the assembly of the machinery required for death receptor signaling.

At the mRNA level, c-FLIP exists as multiple splice variants, whereas at the protein level, three forms (c-FLIP$_L$, c-FLIP$_S$ and c-FLIP$_R$) have been detected so far (Fig. 4) (27). All three FLIPs contain two NH_2-terminus DED domains, essential for their biological activity. The *C*-terminus of c-FLIP$_L$ consists of two inactive caspase-like domains (p20 and p12) (Fig. 4). The short *C*-terminus of both c-FLIP$_S$ and c-FLIP$_R$ shows no homology with procaspase-8 or -10. c-FLIP$_L$ and c-FLIP$_S$ are widely expressed in various tissues, while c-FLIP$_R$ expression appears to be limited to B- and T-lymphoid cells.

c-FLIP$_S$ and c-FLIP$_R$ block caspase activation at the level of the DISC. In contrast, the role of c-FLIP$_L$ is controversial in that some reports characterize c-FLIP$_L$ as an antiapoptotic molecule acting like c-FLIP$_S$, while other reports ascribe proapoptotic functions to c-FLIP$_L$, referring to its role in the autocatalytic activation of procaspase-8 at the level of the DISC. However, a recent report based on the selective knockdown of c-FLIP$_L$ provided definitive evidence that the long variant of c-FLIP functions primarily as an inhibitor of death receptor-mediated apoptosis (28).

Many tumors have been reported to express inappropriately high levels of FLIP, rendering them resistant to apoptosis induced by FasL-expressing cytolytic T-lymphocytes (29). Furthermore, FLIP-mediated resistance to FasL and TRAIL may even allow tumor cells to tolerate FasL and TRAIL expression, using these death ligands to trigger apoptosis of immune cells (30).

Overexpression of c-FLIP occurs frequently in a variety of B-cell malignancies, some forms of AML and chronic lymphocytic leukemia (CLL). In particular, the presence of elevated c-FLIP levels is significantly correlated with a poor prognosis in Burkitt's lymphoma (31). In contrast, in large B-cell lymphomas the expression level of c-FLIP does not represent a prognostic factor, while the presence of inhibitors of the caspase-9-mediated pathway is associated with a negative outcome (32).

Although no compound able to directly inhibit c-FLIP has been described, several experimental agents were reported to decrease c-FLIP expression and seemingly to predispose through this mechanism to apoptosis. One of these agents is the synthetic triterpenoid CDDO (2-cyano-3,12-dioxooleana-1,9-dien-28-oic acid) and its derivative CDDO-Im. Both CDDO and CDDO-Im have been shown to inhibit cellular proliferation and induce apoptosis of leukemia (33, 34), multiple myeloma (35), CLL (36) and many solid tumors. The mechanisms of the proapoptotic effects induced by CDDO and CDDO-Im are complex and have been defined only in part. One of the main effects induced by CDDO is the ubiquitination and proteasome-dependent destruction of FLIP in cultured cancer cells (37). In many tumor cell types, CDDO and CDDO-Im downregulate c-FLIP and upregulate TRAIL-R1 and TRAIL-R2, rendering them sensitive to TRAIL. CDDO and CDDO-Im are well tolerated in mice when administered alone or in combination with TRAIL (38) and CDDO is now under investigation in phase I clinical studies.

The IKK NF-κB system

The NF-κB proteins constitute a small group of closely related transcription factors. This family of transcription factors is composed of five members: Rel, RelA, RelB, NF-κB1 and NF-κB2. A common feature of all five proteins is the presence of a Rel homology domain (RHD) that contains a nuclear localization sequence (NLS) and is involved in dimerization, sequence-specific DNA binding and interaction with the inhibitory IκB proteins (39). The NLS is masked in unstimulated cells by the binding of IκB proteins, which act as specific NF-κB inhibitors. As a consequence of this inhibitory effect of IκB factors, NF-κB dimers are retained in the cytoplasm. IκB proteins include: IκBα, IκBβ and IκBγ. The common structural features of these proteins consist of 6-7 ankyrin repeats that mediate binding to the RHD (39).

Three major signaling pathways induce activation of NF-κB, with its concomitant translocation from the cytoplasm to the nucleus. Proinflammatory cytokines acting through the TNF-R and the IL-1R induce activation of the of the IκB kinase (IKK) complex, consisting of a trimer formed by the interaction of the three IκB proteins. The mechanism of NF-κB activation involves both phosphorylation and ubiquitination of the IκB proteins: IκB proteins are first phosphorylated on Ser^{32} and Ser^{36} and then polyubiquitinated. Following polyubiquitination, IκB undergoes rapid degradation by the the 26S proteasome and the liberated NF-κB dimers translocate to the nucleus, where they act as modulators of the transcription of a specific set of genes (39). The second pathway, also called the *alternative pathway*, is Nemo (NF-κB essential modulator) (IKKγ)-independent and is triggered by cytokines such as lymphotoxin-β, B-cell-activating factor (BAFF), the CD40 ligand, human T-cell leukemia virus and Epstein-Barr virus. This signaling pathway is based on the recruitment of TRAF (tumor necrosis factor receptor-associated factors) proteins to the cell membrane and activation of NF-κB-inducing kinase (NIK), which activates IKKα; activated IKKα in turn phosphorylates p100, with its subsequent ubiquitination and cleavage to generate the NF-κB protein p52, which then forms a heterodimer with RelB and translocates to the cell nucleus (40). The third activating pathway is also called the *atypical pathway* because, in contrast to the first two pathways, it is IKK-independent and is triggered by DNA damage caused by various agents such as UV radiation or the chemotherapeutic agent doxorubicin. UV radiation induces IκBα phosphorylation at the level of serine residues in the *C*-terminus and its subsequent degradation via the proteasome, while oxidative stress elicits IκBα phosphorylation at the level of tyrosine residues (Tyr^{42}) (40).

NF-κB is known to inhibit apoptosis through the induction of antiapoptotic proteins and/or the suppression of proapoptotic proteins. Constitutive NF-κB activation is frequently observed in many cancers and protects tumor cells from apoptotic stimuli, including apoptosis induced by chemotherapeutic drugs. Intriguingly, many anticancer agents stimulate NF-κB activity, which could in turn contribute to the development of chemoresistance.

The constitutive NF-κB activation observed in cancer cells can be caused by mutations affecting the genes encoding NF-κB or IκB, or by uncontrolled IKK stimulation (41, 42). The genetic alterations are represented by chromosomal aberrations involving the genes encoding NF-κB1, NF-κB2, Rel and RelA, which are found in many solid and hemopoietic cancers. On the other hand, the constitutively high level of NF-κB is due in the majority of cases to the constitutive activation of upstream kinases or, less frequently, to mutations inactivating IκBs (41, 42). Constitutive IKK activity is observed in Hodgkin's disease and childhood acute lymphoblastic leukemia (ALL) (43).

Particularly interesting are the findings in AML regarding NF-κB. Initial studies have documented an increased IKKβ activity associated with activated NF-κB in AML blasts (44). Importantly, constitutively active NF-κB was also observed in leukemic stem cells, *i.e.,* leukemia cells that are able to initiate and maintain the leukemic process (45). Various inhibitors of NF-κB, including resveratrol (46), AS-602868 (47) and parthenolide (48), induce apoptosis of AML blasts and potentiate the cytotoxic effects of antileukemic drugs. Interestingly, NF-κB inhibitors are able to induce the death of leukemic stem cells while sparing normal stem cells/progenitors (48).

The biological role of NF-κB as a positive modulator of the antiapoptotic machinery and its increased and constitutive activation in many cancers have stimulated the search for compounds able to inhibit this transcription factor and to act as anticancer drugs. *In vivo* animal models of different types of tumors have clearly shown that NF-κB inhibition increases the efficacy of anticancer drugs (reviewed in 49). Several drugs able to inhibit NF-κB have been described. None of these agents, however, is specific for NF-κB and in the majority of cases their toxicity has yet to be evaluated. The only NF-κB inhibitor approved for clinical use is the proteasome inhibitor PS-341 (bortezomib, Velcade®). This drug inhibits NF-κB activity by blocking the degradation of IκB (50). However, this inhibition has effects on the stability of many cellular proteins, suggesting that the antitumor activity of proteasome inhibitors may only in part be related to inhibition of NF-κB activity. Interestingly, the proteasome inhibitor also induces a marked decline in c-$FLIP_L$ levels in many tumor cells, rendering them sensitive to the death ligand TRAIL.

PS-341 was tested for efficacy and safety in several hematological diseases. A randomized study in relapsed multiple myeloma patients clearly showed a better survival in those assigned to receive PS-341 compared to patients receiving high-dose dexamethasone (51). Two phase II studies demonstrated that PS-341 was well tolerated and had significant activity in patients with certain subtypes of non-Hodgkin's lymphomas (52, 53). Finally, phase I studies indicated promising antitumor activity for PS-341 administered with pegylated liposomal doxorubicin in relapsed advanced AML patients (54).

Two NF-κB inhibitors, Bay-11-7082 and Bay-11-7085, inhibit IκBα phosphorylation, thereby blocking proteaso-

mal degradation of IκBα (55). These two compounds were shown to induce *in vitro* apoptosis of colon cancer, leukemia, T-cell leukemia and multiple myeloma. *In vivo* studies using an ovarian cancer model showed that pretreatment with these NF-κB inhibitors potentiated the response of tumor cells to cisplatin. These studies also demonstrated that these compounds were well tolerated in rats at up to 20 mg/kg/day (55).

Parthenolide, the active ingredient in the herb feverfew which is frequently used in the treatment of migraine, inhibits the activity of the IKK complex and increases the sensitivity of various tumor cells to cytotoxic drugs at low concentrations.

CHS-828, a pyridylcyanoguanidine under evaluation as an anticancer agent in phase I studies, also acts as an inhibitor of NF-κB activity (56) and increases the sensitivity of tumor cells to cytotoxic agents.

Flavopiridol, a synthetic flavone, induces apoptosis of leukemia cells through a mechanism involving mitochondrial damage (57). This drug was evaluated in phase I clinical studies (58, 59). Flavopiridol exerts various pharmacological activities, including the inhibition of cyclin-dependent kinases (CDKs) and the blockade of NF-κB nuclear translocation (60).

Dehydroxymethylepoxyquinomicin (DHMEQ) is another NF-κB inhibitor that blocks the nuclear translocation of this transcription factor when it is activated (61). *In vivo* experiments in nude mice transplanted with human tumors provided evidence that this inhibitor is particularly active against hormone-refractory prostate cancer cells (61).

NF-κB activity may also be inhibited by dietary agents present in relatively high concentrations in some foods. This is the case of genistein, a natural isoflavonoid found in soybean products, which was shown to sensitize pancreatic cancer cells to standard chemotherapeutic agents (62).

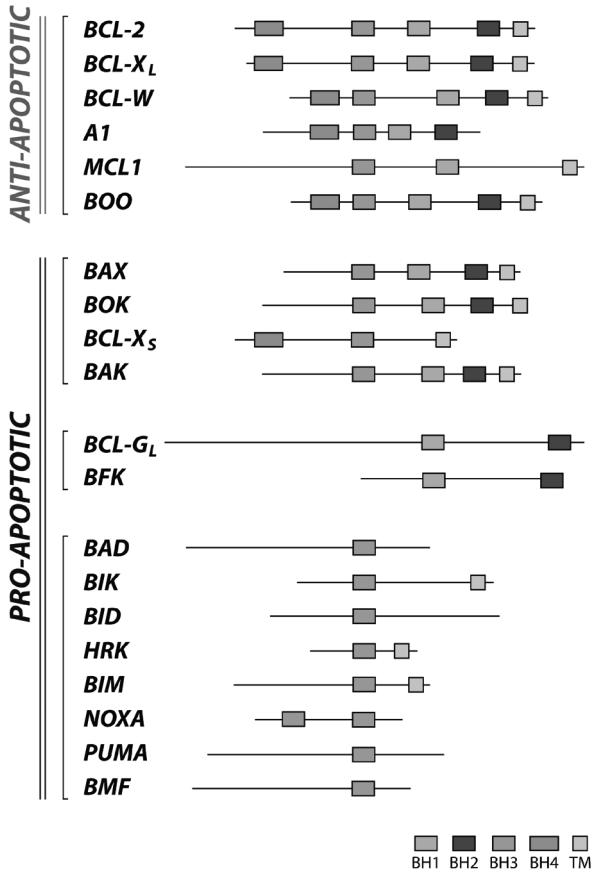

Fig. 5. Schematic representation of the mammalian Bcl-2 family members. According to their function, Bcl-2 family members are subdivided into two groups: antiapoptotic and proapoptotic. The antiapoptotic members each have two or four Bcl-2 homology (BH) domains. The proapoptotic members of the Bcl-2 family can be further subdivided into two groups: 1) the Bax/Bak-like proteins; and 2) the BH3-only proteins. The BH domains are indicated as colored boxes (BH1, BH2, BH3 and BH4). TM indicates the presence of a transmembrane domain anchoring this protein to the membrane of mitochondria.

The Bcl-2 family proteins

In humans, 26 members of the B-cell lymphoma 2 (*BCL2*) gene family have been identified. Bcl-2 family proteins are conserved during metazoan evolution, with homologues found in vertebrate and invertebrate animal species. The Bcl-2 family members are key regulators of the intrinsic apoptotic pathway and of mitochondrial integrity. The ratio between proapoptotic and antiapoptotic Bcl-2 family proteins is a major determinant of the sensitivity or resistance of cells to different kinds of apoptotic stimuli, including growth factor deprivation, hypoxia, radiation, oxidants and anticancer drugs. The proteins of the Bcl-2 family are characterized by the presence of Bcl-2 homology (BH) domains: BH1, BH2, BH3 and BH4.

Bcl-2 family members can be divided into two groups according to their function in apoptosis: antiapoptotic and proapoptotic proteins. The proapoptotic Bcl-2 family members can be further subdivided into two groups according to their structure/function: 1) proapoptotic Bax/Bak-like proteins (Bax, Bok, Bcl-X_S, Bak); and 2) proapoptotic BH3-only proteins (Bad, Bik, Bid, Bim, Hrk, Noxa, Puma and Bmf) (Fig. 5) (63). The prosurvival Bcl-2 group comprises Bcl-2, Bcl-X_L, Bcl-W, A1, Mcl1 and Boo (63). Bcl-2, Bcl-X_L, Bak and several other members of the Bcl-2 family have a hydrophobic stretch of amino acids near the outer mitochondrial membrane (Fig. 5). In contrast, other Bcl-2 family members, such as Bid and Bad, lack these membrane-anchoring domains, but target mitochondria in response to specific stimuli.

The antiapoptotic Bcl-2 family members exert a protective effect against apoptosis in several cell types. Gene knockout experiments have shown that Bcl-2 is required for the survival of mature B- and T-cells, Mcl1 for the survival of T-cells and hemopoietic stem cells/progenitor cells, and Bcl-X_L for the survival of erythroid cells and neurons.

The proapoptotic Bax/Bak-like proteins contain two to three BH domains. When overexpressed, these proteins induce cell death. Gene knockout studies have shown that Bax/Bak are essential for the apoptotic response of the cells to growth factor deprivation or to some cytotoxic drugs (64). The activation of Bax/Bak represents a key event in the commitment of a cell to apoptosis. Bax and Bak act by promoting the release of cytochrome *c* and other apoptogenic proteins from mitochondria and by controlling the activation of initiator caspases.

The proapoptotic BH3-only proteins share with the other members of the Bcl-2 family proteins only the BH3 domain. These proteins play an essential role in the initiation of stress-induced apoptosis. The BH3-only proteins are able to bind with high affinity to antiapoptotic Bcl-2 proteins through an interaction involving the BH3 domain on the BH3-only protein and a hydrophobic cleft on the surface of antiapoptotic Bcl-2 proteins (65). Overexpressed BH3-only proteins require the presence of Bax/Bak to induce apoptosis, indicating that they act upstream of Bax/Bak (66, 67). Two BH3-only proteins are able to bind to Bax/Bak, in addition to Bcl-2 antiapoptotic proteins.

Three different models have been proposed to explain how BH3-only, Bax/Bak and antiapoptotic Bcl-2 proteins interact to control cell death (63, 68, 69). The first model proposes that BH3-only proteins induce apoptosis through their direct binding to Bax/Bak-like proteins and that antiapoptotic Bcl-2 proteins promote cell survival by inactivating BH3-only proteins. The second model suggests that antiapoptotic Bcl-2 proteins maintain the Bax/Bak protein in an inactive state through direct binding; following an apoptotic stimulus, BH3-only proteins are activated, bind to antiapoptotic Bcl-2 proteins and liberate Bax/Bak proteins. The third model proposes in addition to antiapoptotic Bcl-2 proteins, a caspase activator; binding could be disrupted by apoptotic stimuli activating BH3-only and Bax/Bak proteins.

An increased expression of antiapoptotic Bcl-2 proteins or a decreased expression of proapoptotic Bcl-2 family proteins has been observed in some hematological neoplasias. A prototype of this type of tumor is low-grade follicular non-Hodgkin's lymphoma, where markedly increased levels of Bcl-2 protein are observed very frequently (80-90%) as a consequence of the t(14;18) translocation involving the *BCL2* gene (70). In CLL, three abnormalities in the expression of Bcl-2 family proteins have been reported: an increased Bcl-2 expression due to gene promoter hypomethylation; a decreased Bax expression, frequently associated with point mutations in the promoter region; and an increase in Mcl1 protein levels (occurring in about 50% of patients), frequently associated with mutations in the *MCL1* gene promoter (71). Abnormally elevated levels of Bcl-2 protein are observed in about 35-40% of patients with diffuse large cell lymphomas. Bcl-2 is clearly expressed in about 25% of Hodgkin's lymphoma patients and, in these patients, Bcl-2 expression is associated with a poor outcome (72).

These observations indicate that Bcl-2 may represent an important molecular target in the treatment of several hematological diseases. Three different strategies have been attempted (73): 1) inhibition of *BCL2* gene transcription; 2) induction of *BCL2* mRNA degradation by antisense oligonucleotides; and 3) inhibition of protein function by small-molecule drugs.

Some drugs have been reported to decrease Bcl-2 expression through a transcriptional mechanism, such as histone deacetylase inhibitors. These drugs are entering clinical trials and exhibit some antilymphoma and antileukemia activity. These agents, however, are not specific for the *BCL2* gene and affect the expression of many other genes.

In contrast to the above-mentioned agents, antisense oligonucleotides targeting *BCL2* mRNA specifically affect Bcl-2 expression. G3139 (oblimersen sodium) is an antisense oligonucleotide developed for clinical studies. It acts by forming a double strand with the first 18 nucleotides within the reading frame of *BCL2* mRNA; the hybrid is recognized and degraded by ribonuclease H (74). G3139 would be expected to reduce *BCL2* mRNA and protein levels in cancer cells *in vivo*, an effect which should consistently increase the sensitivity of these cells to cytotoxic chemotherapy. However, control studies carried out in patients undergoing therapy with G3139 showed that only some of them exhibited the expected decrease in *BCL2* mRNA/protein, while in the other patients the drug did not induce the expected effect. Initial phase I studies provided some evidence for antitumor activity against non-Hodgkin's lymphoma. Presently, G3139 is under clinical evaluation in phase III studies in CLL, AML and multiple myeloma administered together with cytotoxic chemotherapy. It is also under evaluation in some solid tumors, including prostate, breast and small cell lung cancer.

The third category of anti-Bcl-2 drugs is represented by small chemical compounds directly binding to this protein and acting as inhibitors of its activity. These compounds may be subdivided according to their origin into molecules obtained by chemical synthesis and naturally occurring substances.

The ideal compound pertaining to this category of molecules should possess two important properties: potent inhibitory activity against antiapoptotic Bcl-2 proteins and low toxicity *in vivo*. One example of a naturally occurring compound is gossypol, a natural polyphenol present in cottonseed (Fig. 6A), which inhibits Bcl-2 and Bcl-X_L activity by binding to a hydrophobic pocket present at the level of the BH3 domain. In spite of these favorable properties, however, gossypol displayed pronounced toxicity *in vivo* which precluded its further clinical development. A derivative of gossypol, apogossypol, devoid of reactive aldheyde residues, has been synthesized and is under investigation for possible clinical development (Fig. 6A). Other examples of naturally occurring substances inhibiting antiapoptotic Bcl-2 proteins are certain thiaflavins and epigallocatechins present in elevated concentrations in black and green tea, respectively.

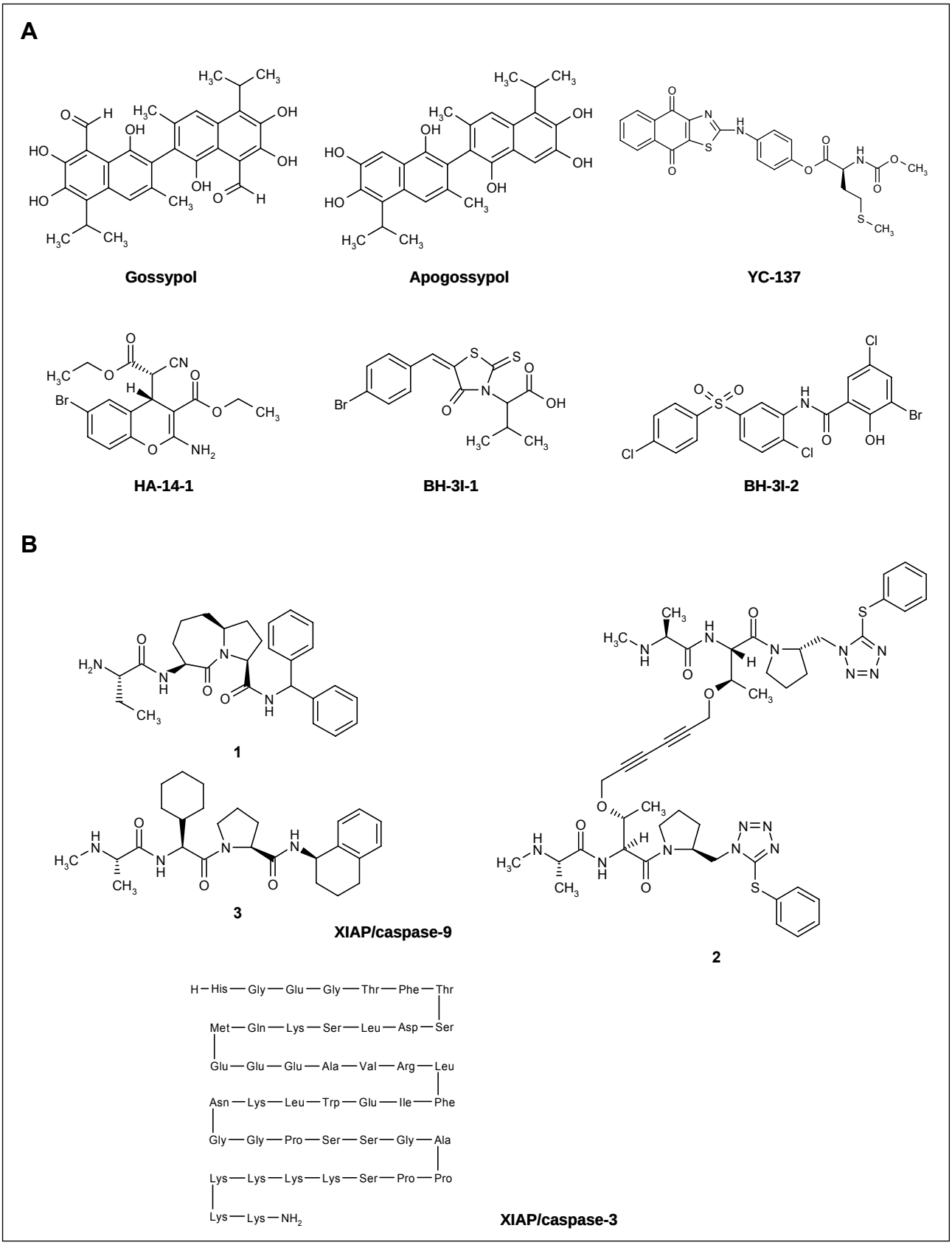

Fig. 6. **A**. The structures of nonpeptide antagonists of Bcl-2 and Bcl-X_L are shown. **B**. The structures of nonpeptide inhibitors of apoptosis proteins (IAP) are shown.

Other chemical inhibitors of antiapoptotic Bcl-2 proteins have been obtained either using a computational modeling approach (examples of these type of compounds are HA-14-1 and YC-137) or a BH3 displacement assay (BH-3I-1 and BH-3I-2) (Fig. 6A). In this context, the technique known as SAR by NMR (structure-activity relationships by nuclear magnetic resonance) appears to be particularly powerful and allows the identification of compounds able to bind to a domain of a given protein (75). This approach allowed the identification of a compound, ABT-737 (Fig. 6A), that acts as a very potent inhibitor of the antiapoptotic Bcl-2 proteins Bcl-2, Bcl-X_L and Bcl-W (76). This compound exerts potent antitumor activity *in vitro* and *in vivo* against a wide spectrum of tumor cell types, including primary lymphoma cells (76). ABT-737 synergizes with antitumor chemotherapeutics and radiation and was well tolerated *in vivo* by experimental animals.

Recent studies suggest an alternative potential means to decrease Bcl-2 expression. This approach is based on the finding that TR3, an orphan member of the retinoid/steroid family of nuclear receptors, may translocate from the nucleus to the cytoplasm, where it binds a regulatory domain within the Bcl-2 protein and accumulates in the mitochondria. The binding of TR3 to Bcl-2 determines a conformational change in the Bcl-2 protein, converting it from a protector to a killer (77). The process of translocation of TR3 from the nucleus to the cytoplasm may be potentiated by analogues of the retinoid AHPN (adamantylnaphthalenecarboxylic acid), which have shown antileukemic activity *in vitro* and in animal models (78).

Convergence point of the apoptotic pathways

As mentioned before, the extrinsic and intrinsic pathways converge at the point of the activation of effector caspases, such as caspase-3 and -7. The activity of these terminal steps of apoptosis is controlled by a family of proteins known as inhibitors of apoptosis proteins (IAPs); these proteins bind and inhibit caspase-3, -7 and -9, but not caspase-8.

Eight human IAP family members have been identified; these proteins are characterized by the presence of one, two or three baculovirus IAP repeats (BIRs), a zinc-binding domain composed of about 70 amino acids. The BIR domain is essential for IAP function. In addition to the BIR domain, some IAP family members also possess a CARD (caspase activation recruiting domain) or a ring finger.

The IAP proteins can be divided into three classes according to their structural features and particularly to the presence of a ring finger and to the homology of the

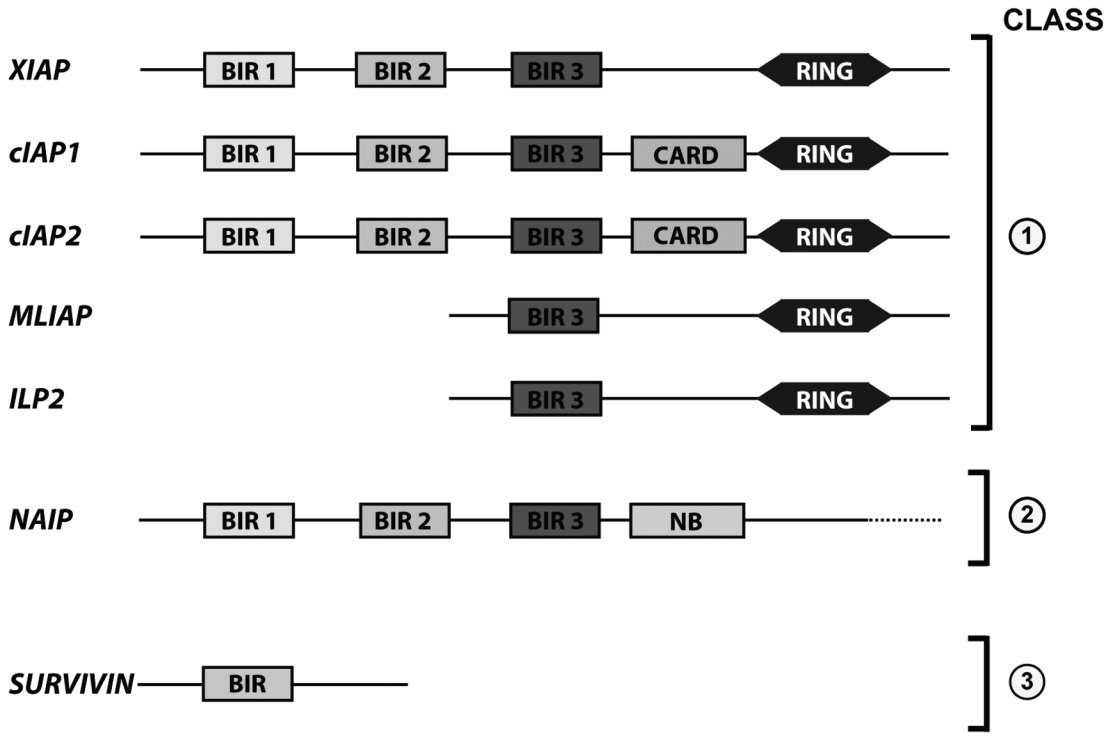

Fig. 7. Schematic representation of the IAP (inhibitors of apoptosis proteins) family proteins. Eight human IAP family members (seven are shown) have been identified according to the presence of shared BIR (baculovirus IAP repeat) domains. IAP proteins are subdivided into three classes according to the absence or the presence of a ring finger and the homology of their BIR domains (numbered from 1 to 3). c-IAP1 and c-IAP2 also possess a caspase activation recruiting domain (CARD). Finally, the class II member NAIP (neuronal apoptosis inhibitor protein) also possesses a nucleotide-binding domain indicated as NB.

BIR domains (Fig. 7) (79). Class I members comprise XIAP (X-linked inhibitor of apoptosis protein), comprised of three BIR domains and one ring finger, IAP1 and IAP2, homologues of XIAP containing three BIR domains, one ring finger and one CARD domain, ILP2 (IAP-like protein 2) and MLIAP (melanoma inhibitor of apoptosis protein), comprised of only one BIR3 domain and one ring finger ring (Fig. 7).

The prototype of class I IAPs is XIAP; this IAP protein inhibits caspase-3 and -7 through the BIR2 domain and caspase-9 through the BIR3 domain. These findings were of fundamental importance for the development of inhibitors of XIAP selectively targeting the caspase-interacting domains of this IAP protein. MLIAP, another member of the class I IAPs, contains a single BIR and inhibits caspase-9, but not caspase-3 and -7 (Fig. 7). The mechanism of caspase inhibition induced by survivin, a member of class III IAPs, is peculiar in that it inhibits caspase-9, but not caspase-3 and -7; this inhibition requires the presence of a cofactor, HBXIP (hepatitis B X-interacting protein), and the complex survivin/HBXIP acts as an inhibitor of caspase-9 (Fig. 7) (80).

In addition to their role in the control of apoptosis, IAPs play a role in cell cycle control (particularly XIAP and survivin) and also in cell signaling (XIAP may activate NF-κB). Overexpression of IAP family members often occurs in cancer. Survivin is expressed in many types of cancer (81), and also in fetal tissues, but it is largely absent in adult tissues (82). Transcriptosome analysis by microarray clearly showed that survivin is among the most tumor-specific genes thus far identified (83). Several studies have documented that the level of survivin expression in several tumor cell types represents a factor of negative prognosis, and survivin detection in some biological fluids (*e.g.*, in the urine) may be used for diagnostic purposes (reviewed in 79).

As for other steps in the apoptotic cascade, the activity of IAPs is regulated by a series of small proteins that physiologically act as IAP inhibitors. These IAP-binding proteins were initially reported in *Drosophila melanogaster* and homologues were subsequently described in mammalian cells. In humans, four IAP-binding proteins have been identified: Smac/Diablo, HtrA2, Smacβ and XAF1 (XIAP-associated factor 1). Smac and HtrA2 are mitochondrial proteins that are released along with cytochrome *c* during apoptotic damage of mitochondria; once released in the cytosol, these proteins are activated by cleavage and in their active form bind IAP proteins, thus preventing their IAP-inhibitory activity on caspase activation (84). The IAP-inhibitory function of the Smac family of proteins depends on the NH_2-terminal sequence; peptides corresponding to the 7 NH_2-terminal amino acids are able to bind XIAP. In particular, it was shown that the NH_2-terminus of Smac/Diablo binds the BIR3 domain of XIAP, c-IAP1 and c-IAP2 proteins. Smacβ is a naturally occurring alternate splicing form of Smac lacking the mitochondrial targeting sequence. Although Smacβ is unable to interact with IAPs, it exerts proapoptotic activity through an unknown mechanism. XAF1 is another IAP inhibitor localized in the nucleus, where it inhibits XIAP.

Since IAPs are preferentially expressed in cancer cells, where they are one of the factors involved in the survival advantage of tumor cells, they represent potential therapeutic targets. The possible strategies involve the development of drugs that inhibit either the expression or the function of IAPs and mimic the function of Smac/Diablo family proteins. Three different strategies have been used to develop IAP inhibitors. The first approach consisted of the development of Smac-like molecules mimicking the inhibitory effects of IAPs elicited by Smac/Diablo family proteins based on the ability of Smac/Diablo peptides to bind to the BIR3 pocket of XIAP. The use of fluorescence polarization assays allowed the identification of small molecules able to compete with Smac peptides for binding to the BIR3 pocket (85). These peptides, however, were only poorly characterized for their ability to induce tumor cell death.

The second strategy consisted of the identification of XIAP-binding peptides using phage display. This strategy allowed the identification of peptides that bind selectively to the BIR2 domain of XIAP and are able to induce leukemia cell death (86).

The third strategy consisted of screening a large number of compounds using a biochemical assay (reversion of XIAP-mediated repression of recombinant caspase-3) for their capacity to inhibit XIAP. Using this approach, several small molecules and peptides that inhibit XIAP have been identified (Fig. 6B). Several of these compounds induce apoptosis of cancer cells and may represent antitumor drug candidates. These compounds have been characterized on the basis of their ability to bind either BIR3 or BIR2 on the XIAP molecule. Two BIR3 ligands identified using this approach did not stimulate apoptosis on their own, but greatly potentiated apoptosis in the presence of cytotoxic drugs (Fig. 6B, compound **1**) (87) or TRAIL (Fig. 6B, compound **2**) (88). A third ligand, moreover, was able to induce caspase activation without the need for additional stimuli (Fig. 6B, compound **3**) (89).

The use of a biochemical derepression assay based on the known property of XIAP to directly inhibit caspase-3 and screening combinatorial libraries of chemical compounds allowed the identification of active pharmacophores. Among them, compounds containing a polyphenylurea pharmacophore were particularly active. Particularly interesting are the properties of compound 1396-12 (90), an XIAP inhibitor that induces direct apoptosis in cell lines and primary leukemia blasts and sensitizes cells to chemotherapy-induced apoptosis (Fig. 6B) (90). Furthermore, compound 1396-12 inhibits XIAP by binding to its BIR2 domain at a site distinct from the binding pocket of the endogenous XIAP inhibitor Smac (91). Compound 1396-12 directly induced apoptosis in AML patient samples at low micromolar concentrations through a biochemical mechanism involving caspase-3 and -7 activation (92).

As mentioned above, the caspase inhibitor survivin is preferentially expressed in tumor *versus* normal tissues, being undetectable in the large majority of normal tissues, with the exception of thymocytes and hemopoietic progenitors. In contrast, survivin is overexpressed in the large majority of cancers and is the fourth most highly expressed protein in human cancer tissue compared to normal tissue (93). These features indicate that survivin may be eligible to serve as a widely expressed tumor antigen target for therapeutic purposes.

Two different therapeutic approaches involving survivin are under development. The first is based on the use of survivin antisense oligonucleotides. In experimental models, these oligonucleotides have been shown to be able to reduce survivin expression in tumor cells (lung cancer, gastric cancer, lymphoma), resulting in apoptosis of these cells; in contrast, no toxicity to normal cells was observed (94, 95). A remarkable antitumor effect was also elicited by adenoviral delivery of a nonphosphorylatable dominant-negative variant of survivin (96). Clinical-grade survivin antisense oligonucleotides are currently under development by Isis Pharmaceuticals and Lilly and are expected to enter phase I studies shortly.

A second strategy for survivin inhibition consists of the development of ribozymes directed against different portions of survivin mRNA; treatment with these agents resulted in decreased survivin expression and increased sensitivity of various types of cancer cells (melanoma, prostate cancer, breast cancer) to cytotoxic antitumor drugs (97).

A third strategy consists of the development of RNAi using specific siRNAs. Treatment of cancer cells with these siRNAs produced increased sensitivity to various proapoptotic agents, including cytotoxic drugs and death ligands (98).

A fourth strategy focuses on developing chemical inhibitors of survivin. Recently, a cell-permeable peptidomimetic molecule, shepherdin, modeled on the binding interface between the molecular chaperone heat shock protein Hsp90 and survivin, was developed (99). The development of this molecule was based on the observation that the interaction between survivin and Hsp90 was necessary for survivin stability (100). It is therefore expected that an agent able to disrupt this interaction would destabilize survivin, with its subsequent degradation. Systemic administration of shepherdin was well tolerated and inhibited human tumors in mice without toxicity (99). Another compound, tetra-*O*-methylnorihydroguaiaretic acid, was shown to induce a decrease of survivin in tumor cells, but its effect was not specific for this protein (101).

Histone deacetylase inhibitors as modulators of the apoptotic response of cancer cells

The structural modification of histones is regulated by acetylation/deacetylation of the *N*-terminal tail, which is crucial in modulating gene expression because it affects the interaction of DNA with transcription-regulatory complexes. The balance between the acetylated and deacetylated conditions of histones is regulated by two different sets of enzymes: acetyltransferases and histone deacetylases (HDACs). HDACs primarily act by removing acetyl groups at the level of lysine residues; these free lysine residues attach firmly to the phosphate backbone of the DNA, thus preventing transcription.

Acetylation/deacetylation of histones is deregulated in cancer cells, with consequent changes in the gene expression pattern. Furthermore, studies in certain leukemias have shown that the formation of complexes between HDACs and fusion proteins (such as AML-1/ETO or PML/RARα) in leukemia cells as a consequence of chromosomal translocations plays a key role in the blockade of cell differentiation observed in these cells. These observations have prompted the development of agents that inhibit HDACs.

HDAC inhibitors block histone deacetylation, causing cell cycle arrest, differentiation and/or apoptosis of many tumors (reviewed in 102). Many pharmacological agents able to inhibit HDACs have been isolated. According to their structure, these agents may be classified as: 1) carboxylates (short fatty acids); 2) hydroxamates; 3) electrophilic ketones (epoxides); 4) cyclic peptides; 5) benzamides; or 6) hybrid compounds (103). Some of these agents are under investigation in phase I/II clinical studies.

Numerous studies carried out with these compounds in leukemia cell lines provided evidence that HDAC inhibitors are effective in inducing cell cycle arrest, apoptosis, anti-angiogenesis and differentiation (reviewed in 104). Concerning the effects on apoptosis, HDAC inhibitors trigger the intrinsic apoptotic pathway and sensitize tumor cells to death ligands that initiate the extrinsic pathway of apoptosis (104). As for the effects on the extrinsic pathway, recent studies indicate that HDAC inhibitors upregulate TRAIL-R1 and TRAIL-R2 expression on leukemia cells (105) and sensitize leukemia blasts to TRAIL-mediated apoptosis (106, 107). Furthermore, HDAC inhibitors upregulate TRAIL-R1 and TRAIL-R2 in primary CLL cells and sensitize these cells to TRAIL-mediated apoptosis selectively via TRAIL-R1 (108).

In addition to these proapoptotic effects, HDAC inhibitors also stimulate NF-κB activity through a molecular mechanism directly related to the physiology of NF-kappaB activation. In fact, NF-κB, when inactive, forms a molecular complex with HDAC1 and p300; HDAC inhibitors destabilize this repressor complex (109). The induction of NF-κB activity in leukemia cells elicited by HDAC inhibitors may be detrimental to their proapoptotic effects; in fact, co-treatment with an NF-κB inhibitor and an HDAC inhibitor greatly potentiates the effect of the latter compound on the induction of apoptosis in leukemia cells (110).

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