Programmed cell death, or apoptosis, is a genetically regulated process that plays an important role in development and homeostasis in vertebrates and invertebrates. Inhibition of apoptosis enhances the survival of cancer cells and facilitates their escape from immune surveillance and cytotoxic therapies. Among the principal molecules contributing to this phenomenon are the inhibitor of apoptosis (IAP) proteins, a family of antiapoptotic regulators that block cell death in response to diverse stimuli through interactions with inducers and effectors of apoptosis. IAP proteins are expressed in the majority of human malignancies at elevated levels and play an active role in promoting tumor maintenance through the inhibition of cellular death and participation in signaling pathways associated with malignancies. Here, we discuss the role of IAP proteins in cancer and options for targeting IAP proteins for therapeutic intervention.

**Abstract**

Apoptosis is a cell suicide process with a major role in development and homeostasis in vertebrates and invertebrates. Inhibition of apoptosis enhances the survival of cancer cells and facilitates their escape from immune surveillance and cytotoxic therapies. Among the principal molecules contributing to this phenomenon are the inhibitor of apoptosis (IAP) proteins, a family of antiapoptotic regulators that block cell death in response to diverse stimuli through interactions with inducers and effectors of apoptosis. IAP proteins are expressed in the majority of human malignancies at elevated levels and play an active role in promoting tumor maintenance through the inhibition of cellular death and participation in signaling pathways associated with malignancies. Here, we discuss the role of IAP proteins in cancer and options for targeting IAP proteins for therapeutic intervention.

**Programmed cell death, or apoptosis, is a genetically regulated process that plays an important role in development and homeostasis in metazoans (1). Abnormalities in apoptosis have been linked to a variety of human diseases, including cancer, neurodegeneration, and autoimmune disorders (2, 3). There are two well-characterized apoptotic pathways, one initiated through the engagement of cell surface death receptors by their specific ligands (4) and the other triggered by changes in internal cellular integrity (Fig. 1; refs. 5, 6). Both pathways converge, resulting in activation of caspases (cysteine-specific proteases) responsible for the execution of the apoptotic process (Fig. 1; ref. 7).

Inhibitor of apoptosis (IAP) proteins are major regulators of apoptosis due, in part, to their ability to inhibit caspases (8, 9). Originally identified in baculoviruses, IAP proteins have been discovered in both invertebrates and vertebrates. Human IAP family members include X-chromosome–linked IAP (XIAP, also known as hIAP, MIHA, and BIRC4), cellular IAP 1 (c-IAP1, also known as HIAP2, MIHB, and BIRC2), c-IAP2 (also known as HIAP1, MIHC, and BIRC3), neuronal apoptosis inhibitory protein (also known as BIRC1), survivin (also known as TIAP and BIRC5), Apollon (also known as Bruce and BIRC6), melanoma IAP (ML-IAP, also known as KA, livin, and BIRC7), and IAP-like protein 2 (also known as BIRC8; reviewed in refs. 9, 10). All IAP proteins contain one to three baculovirus IAP repeat (BIR) domains that are required for antiapoptotic activity, and most of them also possess a carboxyl-terminal RING domain (9). Some IAP proteins, like c-IAP1 and c-IAP2, possess a caspase recruitment domain (11). XIAP is the best-described IAP and possibly the most potent suppressor of apoptosis (12). It is unique among IAP proteins because of its ability to directly bind to and inhibit activated caspase-3, caspase-7, and caspase-9 (13). Structure-function analysis of XIAP showed that it uses different BIR domains for inhibition of distinct classes of caspases; the second BIR domain together with the immediately preceding linker region binds and inhibits caspase-3 and caspase-7, whereas the third BIR domain specifically inhibits caspase-9 (9). The XIAP-mediated inhibition of these caspases is antagonized by the mitochondrial protein Smac (second mitochondrial activator of caspases)/DIABLO (direct IAP binding protein with low isoelectric point), which is released into the cytoplasm in response to proapoptotic stimuli (14, 15). The proapoptotic function of Smac/DIABLO is dependent on a conserved four-residue IAP protein-interaction motif (A-V-P-I) found at the amino-terminus of the mature, posttranslationally processed protein (14, 15). This IAP protein interaction motif binds to a surface groove on the BIR domains of the IAP proteins (16–19). The Smac-binding groove of XIAP-BIR3 also makes critical contacts with an IAP protein-interaction motif located at the amino terminus of the small subunit of processed caspase-9 (20, 21).

Interactions with the corresponding groove on the surface of XIAP-BIR2 also contribute substantially to inhibition of caspase-3 and caspase-7 (22).

Other human IAP proteins, such as c-IAP1, c-IAP2, and ML-IAP, are not potent physiologic inhibitors of caspases (23–25). Instead, c-IAP1, c-IAP2, and ML-IAP may function by binding mature Smac and sequestering it from XIAP, thus facilitating XIAP-mediated inhibition of caspases (23, 24). The c-IAP1 and c-IAP2 proteins were originally identified through their ability to interact with tumor necrosis factor receptor–associated factor 2 (TRAF2; ref. 27). Through TRAF2 interactions, c-IAP1 and c-IAP2 are recruited to TNFR1- and TNFR2-associated complexes where they regulate receptor-mediated apoptosis (28, 29). XIAP, c-IAP1, c-IAP2, and ML-IAP are also RING domain–containing ubiquitin ligation capable of promoting ubiquitination and proteasomal degradation of...
caspases, TRAF2, and several other of their binding partners (reviewed in ref. 30). Survivin, the smallest human IAP protein with a single BIR domain, is associated with polymerized microtubules through its coiled-coil domain. Although the mechanistic aspects of its antiapoptotic activity remain somewhat controversial, survivin is essential for cell division (reviewed in ref. 31).

**IAP Proteins in Human Malignancies**

Overexpression of IAP proteins has been shown to confer protection against a number of proapoptotic stimuli in a variety of solid tumors and hematologic malignancies (32–35). Studies that have examined the prognostic significance of IAP protein expression indicated potential links to poor prognosis (36–39). There is also a large body of data demonstrating elevated expression of IAP proteins (particularly for XIAP, c-IAP1, and c-IAP2) in almost all human malignancies (38–40). XIAP is a ubiquitously expressed protein that plays a critical role in resistance to chemotherapeutic agents and other proapoptotic stimuli such as Apo2L/tumor necrosis factor-related apoptosis-inducing ligand (41–43). Survivin expression has a prominent cancer bias because it is undetectable in most adult tissues but is expressed at high levels in a majority of human tumors (reviewed in ref. 44). ML-IAP also has a tumor-exclusive expression pattern with the highest levels detected in melanomas (45, 46). In addition, c-IAP1 is a target of genetic amplification and c-IAP2 undergoes genetic translocation that fuses its BIR domains with MALT1 (mucosa-associated lymphoid tissue protein; refs. 47, 48, and reviewed in ref. 49). These genetic modifications seem to be correlated with resistance to antitumor agents and activation of prosurvival and inflammatory pathways (47–49).

Besides acting as direct inhibitors of apoptotic pathways, IAP proteins have also been implicated in activation of signal transduction pathways associated with malignancy, including activation of c-Jun-NH₂-kinase 1, nuclear factor-κB, transforming growth factor-β, and phosphatidylinositol 3-kinase/Akt, thus expanding their participation in tumor homeostasis (50–54). The functional importance of IAP proteins in progression and resistance of various malignancies has been tested through the employment of antisense oligonucleotide or RNA interference technologies (34). Thus, down-regulation of XIAP leads to induction of apoptosis and sensitization of tumor cells to death induced by γ-irradiation and chemotherapeutics, both in vitro and in vivo (55–58). Similarly, small interfering RNA (siRNA) – or antisense oligo-mediated suppression of c-IAP1, ML-IAP, or survivin protein levels leads to direct stimulation of cell death and greater sensitivity to apoptosis induced by death receptors or chemotherapeutic agents (44, 59–62).

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**Fig. 1.** The intrinsic and extrinsic apoptotic pathways. The intrinsic cell death pathway is initiated by stimuli such as irradiation, treatment with chemotherapeutic agents, or growth factor withdrawal. Subsequent activation of proapoptotic BH3-only members of the Bcl-2 family neutralizes the antiapoptotic proteins Bcl-2, Bcl-xL, and Mcl-1, leading to disruption of mitochondrial membrane potential and the release of cytochrome c and Smac from the mitochondria into the cytoplasm. These events culminate in Apaf-1–mediated activation of caspase-9, subsequent activation of the effector caspase-3 and caspase-7, and ultimately death of the cell. The extrinsic apoptotic pathway is triggered when death receptors, such as Fas, DR5, or TNF receptor 1, are engaged by their respective ligands, resulting in recruitment of the adaptor protein FADD and caspase-8. This leads to activation of caspase-8 and subsequent activation of the effector caspase-3 and caspase-7. IAP proteins represent the ultimate line of defense against cellular suicide as they inhibit caspase-3, caspase-7, and caspase-9 (XIAP). IAP proteins also prevent the endogenous antagonist Smac from blocking caspase-inhibitory activity of XIAP (ML-IAP, c-IAP1, and c-IAP2). Smac mimetics bind IAP proteins and abrogate their inhibitory activity by disrupting critical IAP-caspase and IAP-Smac interactions.
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High expression in cancer tissues, together with functional importance in tumor maintenance and therapeutic resistance, makes IAP proteins attractive targets for anticancer therapeutic intervention (40). The most appealing strategy involves reagents that mimic the amino-terminus of the endogenous IAP protein antagonist Smac and thus interfere with critical IAP to caspase and IAP to Smac interactions (34, 63). Indeed, Smac-derived peptides and Smac mimetics have been shown to stimulate cell death and sensitize a number of tumor cell lines to apoptosis induced by a variety of proapoptotic agents (Table 1; refs. 26, 64–68). Even more impressive has been reported success with treating malignant glioma, breast cancer, non–small cell lung cancer, and multiple myeloma models in vivo with Smac-based peptides and Smac mimetics (69–72). At the time of writing, a small-molecule IAP antagonist that binds selectively to the BIR domains of XIAP, cIAP-1, cIAP-2, and ML-IAP and antagonizes their interactions with proapoptotic proteins such as caspase-9 and Smac (73) is in preparation for phase I clinical testing. Binding of this molecule to IAP proteins in vitro induces apoptosis, as measured by caspase-3 and caspase-7 activation and cell viability assays, in a subset of cancer cell lines (73). This, together with demonstrated preclinical efficacy in human tumor xenograft mouse models of breast cancer, colon cancer, and melanoma, suggests that it may be very useful for treatment of cancer (73). Parallel efforts aimed at disrupting exclusively the XIAP-BIR2 interaction with caspase-3 have also led to the discovery of nonpeptidic small-molecule inhibitors that show efficacy in vitro and in vivo (74–76).

An alternative strategy to block the action of IAP proteins involves antisense oligonucleotides that down-regulate IAP protein levels by targeting their native mRNAs. Although siRNA-mediated suppression of protein expression for multiple IAP proteins has shown significant effects on the viability of tumor cells, most preclinical studies have concentrated on XIAP and survivin (77–80). This is a reasonable choice because XIAP seems to be the strongest antiapoptotic family member, whereas survivin, on the other hand, is not susceptible to the Smac-based targeting approach because of its structural properties (81). Indeed, XIAP and survivin antisense oligonucleotides cause induction of apoptosis and combine with irradiation and chemotherapeutics to induce significant cell death in vitro and tumor growth inhibition in vivo (55, 77). This strategy is currently the most advanced modality of targeting IAP proteins in cancer as phase I/II clinical trials are under way for targeting XIAP (AEG-35156, Aegera Therapeutics, Inc.) and survivin (LY-2181308, ISIS Pharmaceuticals, Inc., and Eli Lilly & Company; refs. 78, 82). In addition, the compound YM-155 (Astellas Pharma, Inc.), an inhibitor that targets survivin expression, has entered phase II trials in the United States and Europe.

Finally, several reports have identified ML-IAP- and survivinspecific antibodies in the serum of breast cancer, lung cancer, colorectal cancer, and melanoma patients, indicating that these IAP proteins may serve as major tumor-associated antigens (83–87). Thus, ML-IAP and survivin are potentially suitable targets for cancer immunotherapy through antigen-based vaccination (88).

Overall, the ability of IAP proteins to act as inhibitors of apoptosis induced by the extrinsic and intrinsic apoptosis pathways, together with their prominent expression in human malignancies, makes them interesting targets for therapeutic intervention. Equally important, the feasibility of targeting the IAP proteins to disrupt their interactions with proapoptotic proteins, such as caspases and Smac, has been shown. We predict that during the next decade, we will witness clinical justification of this therapeutic approach that will significantly benefit cancer patients.

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References


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