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REVIEW

IAP proteins as targets for drug development in oncology

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Abstract: The inhibitors of apoptosis (IAPs) constitute a family of proteins involved in the regulation of various cellular processes, including cell death, immune and inflammatory responses, cell proliferation, cell differentiation, and cell motility. There is accumulating evidence supporting IAP-targeting in tumors: IAPs regulate various cellular processes that contribute to tumor development, such as cell death, cell proliferation, and cell migration; their expression is increased in a number of human tumor samples, and IAP overexpression has been correlated with tumor growth, and poor prognosis or low response to treatment; and IAP expression can be rapidly induced in response to chemotherapy or radiotherapy because of the presence of an internal ribosome entry site (IRES)-dependent mechanism of translation initiation, which could contribute to resistance to antitumor therapy. The development of IAP antagonists is an important challenge and was subject to intense research over the past decade. Six molecules are currently in clinical trials. This review focuses on the role of IAPs in tumors and the development of IAP-targeting molecules for anticancer therapy.

Keywords: Smac mimetics, apoptosis, antitumor therapy

Introduction: IAP family of proteins

The inhibitors of apoptosis (IAPs) constitute a family of proteins highly conserved throughout evolution. IAPs were initially discovered in baculoviruses two decades ago,[1] as potent inhibitors of apoptosis in infected insect cells. The first human homologous neuronal apoptosis inhibitory protein (NAIP) and cellular IAP 1 and 2 (cIAP1 and cIAP2) were characterized 2 years later,[2,3] followed by X-chromosome linked IAP (XIAP),[4,5] survivin,[6] Apollon (also called BRUCE),[7] melanoma IAP (ML-IAP) (also called Livin),[8] and IAP-like protein 2 (ILP2).[9] The IAP family is defined by the presence of one to three conserved protein motifs named a baculoviral IAP repeat (BIR). Most of them form a surface hydrophobic groove that specifically binds a conserved tetrapeptide motif, called IAP binding motif (IBM), found in the active subunits of apoptotic protease caspase-3, -7, and -9 and in cellular IAP antagonists, such as the second mitochondria-derived activator of caspases (Smac) (also named direct IAP-binding protein with low isoelectric point (pI) [DIABLO])[10–13] and the high temperature requirement protein A2 (HtrA2)[12,14] (Figure 1). The first BIR of XIAP and cIAPs does not bind IBM but rather, the signaling molecule transforming growth factor beta (TGFβ)-activated kinase 1-binding protein 1 (TAB1)[15] or the tumor necrosis factor (TNF) receptor (TNFR) associated factors (TRAFs),[16–18] connecting XIAP and cIAPs with the TGF and TNF signaling pathways, respectively. In addition to the BIRs, cIAPs, XIAP, ML-IAP and ILP2 also possess a C-terminal RING (really interesting new gene)

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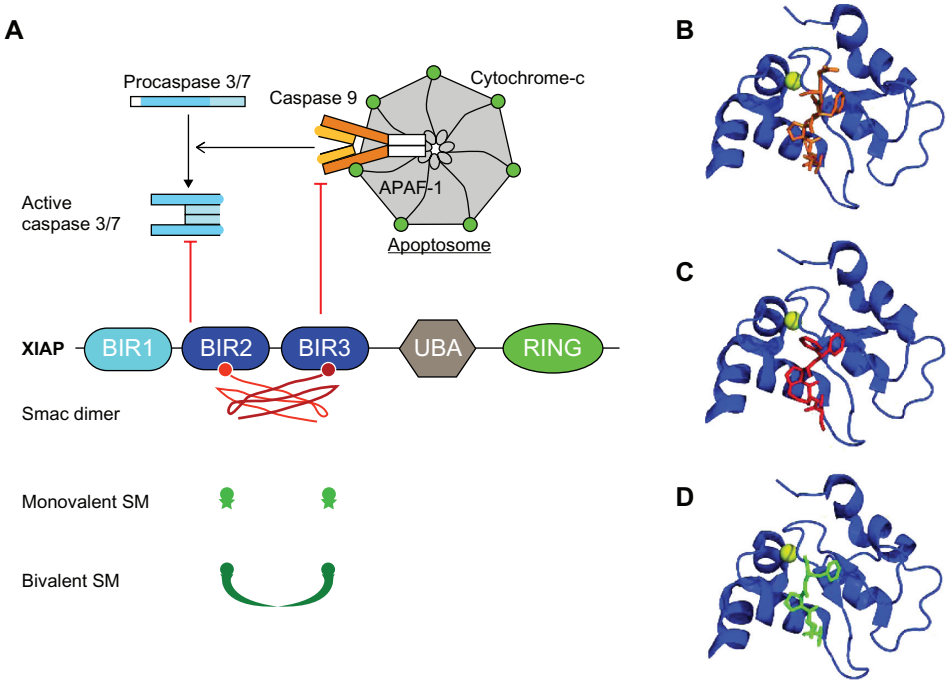

Figure 1 The inhibition of caspases by XIAP and the regulation by Smac and Smac mimetics.

Notes: (**A**) Among IAPs, XIAP is a potent caspase inhibitor. XIAP is composed of three BIR domains, one UBA domain (which binds ubiquitin chains), and one C-terminal RING domain, which confers to XIAP an E3-ubiquitin ligase activity. The first BIR (BIR1) can bind to TAB1, connecting XIAP to the TGFβ signaling pathway. The BIR2 and BIR3 contain a surface hydrophobic groove allowing the interaction with IBM found in caspase-3, -7, and -9 active subunits and in IAP antagonists, such as Smac or HtrA2. Moreover, the linker region upstream of BIR2 binds across the substrate binding pocket of caspase-3 and -7, and BIR3 binds the dimer interface of caspase-9, which hinder substrate accessibility and hide the catalytic residue of caspase. Smac is released from the mitochondria into the cytosol during apoptosis, after a maturation process that removes the N-terminal mitochondrial import signal and exposes the IBM to the N-extremity of the protein. Once cytosolic, Smac forms a symmetric dimer and binds the BIR2 and BIR3 IBM grooves of XIAP, preventing them from binding caspases. In a similar manner, monovalent and bivalent Smac mimetics efficiently bind the BIR2 and BIR3 surface hydrophobic grooves and abrogate XIAP-mediated caspase inhibition. (**B**–**D**) Comparison of the XIAP-BIR3 (blue) bound to the IBM of caspase-9 (ATPFQ) (orange) (pdb 1nw9): (**B**) The IBM (AVPI tetrapeptide) of Smac (red) (pdb 2opz); (**C**) The monovalent Smac mimetic SM-130 (green) (pdb 2 jk7); and (**D**) The BIR domains of IAPs are organized in four α-helices and three β-strand sheets maintained by a zinc ion (yellow). IBMs interact with the surface hydrophobic groove of BIRs (constructed using The PyMOL Molecular Graphics System, Version 1.5.0.4 Schrödinger, LLC, New York, NY, USA).

Abbreviations: AVPI, Smac N-terminal tetrapeptide; BIR, baculoviral IAP repeat; HtrA2, high temperature requirement protein A2; IAPs, inhibitors of apoptosis; IBM, IAP binding motif; RING, really interesting new gene; SM, Smac mimetic; Smac, second mitochondria-derived activator of caspases; TAB1, TGFβ-activated kinase 1-binding protein 1; TGFβ, transforming growth factor beta; UBA, ubiquitin associated; XIAP, X-chromosome linked IAP; APAF-1, apoptotic peptidase activating factor.

domain conferring an E3 ligase activity in the ubiquitination or neddylation[19] reactions (for review,[20,21]).

Numerous partners of IAPs have been identified, including some caspases,[22–24] some signaling molecules,[25,26] some regulators of the NF-κB: nuclear factor of kappa-light polypeptide gene enhancer in B-cell activating pathways,[25] some regulators of the actin cytoskeleton,[27] and some transcriptional regulators.[28,29] Thus, although they were initially characterized as inhibitors of apoptosis, IAPs display additional nonapoptotic functions in the regulation of cell proliferation, cell division, cell differentiation, cell motility, and in proinflammatory and immune response (for review,[25,26]),which could contribute to oncogenesis.

Expression of IAPs in tumors

The expression of IAPs or cellular IAP antagonists such as Smac,[11] HtrA2, or the septin-like mitochondrial protein, ARTS,[30,31] were shown to be altered in a number of human tumor samples (Table 1). Overexpression of IAPs or downregulation of the cellular IAP antagonists have been correlated with advanced progressive disease, aggressiveness, and poor prognosis or low response to treatment (Supplementary Table S1). The alterations of IAP expression can be associated or not, with gene mutations. The *baculoviral IAP repeat containing protein (BIRC)2* and *BIRC3* genes, encoding cIAP1 and cIAP2, respectively, are located on chromosome 11q21-22, a region found amplified in human hepatocarcinoma,[32] mammary carcinoma,[33] medulloblastoma,[34] and in pancreatic,[35] cervical,[36] lung,[37] oral squamous cell,[38] and esophageal[39] carcinomas. Some (30%) mucosa-associated lymphoid tissue (MALT) lymphoma are associated with the chromosomal translocation t(11;18)(q21;q21) generating a chimeric protein composed of the N-terminal sequences of cIAP2 fused to the C-terminal sequence of MALT1.[17,40] Conversely, IAPs can also display antitumoral properties in lymphocytes. The *BIRC2* and/or

Table 1 Expression of IAPs and IAP antagonists in human tumors

IAPs and cellular IAP antagonists	Tumors
IAPs:	
XIAP overexpression	Acute myeloid leukemia,[138] B-cell chronic lymphocytic leukemia,[139,140] bladder carcinoma,[141] breast carcinoma,[142] cervical carcinoma,[143] colorectal cancer,[144,145] hepatocarcinoma,[146,147] melanoma,[148] non-small cell lung cancer,[149,150] ovarian cancer,[119] prostate carcinoma,[151,152] renal carcinoma,[153–155] thyroid carcinoma[156]
cIAP1 and cIAP2 overexpression (amplicon 11q21-22)	Cervical cancer,[36] esophageal squamous cell carcinoma,[39] hepatocarcinoma,[32] medulloblastoma,[34] non-small and small cell lung cancer,[37,150] oral squamous cell carcinoma,[38] pancreatic cancer[157]
cIAP1 overexpression independently from amplicon 11q21-22	B-cell chronic lymphocytic leukemia,[139,158,159] bladder carcinoma,[83] cervical carcinoma,[143,36] chronic lymphocytic leukemia,[139] colorectal cancer,[160] head and neck squamous cell carcinoma,[82] non-small and small cell lung cancer,[150]prostate carcinoma,[152] squamous carcinoma of tongue[161]
cIAP2 overexpression independently from amplicon 11q21-22	Breast cancer,[162] cervical carcinoma,[143] chronic lymphocytic leukemia,[139,159] colorectal carcinoma,[160] prostate carcinoma[152]
cIAP2/MALT chimeric protein t(11,18)(q21, q21)	MALT myeloma[163,164]
cIAP1/cIAP2 inactivation	Multiple myeloma[41,165]
ML-IAP overexpression	Acute myeloid leukemia,[166] childhood acute lymphoblastic leukemia,[167] bladder carcinoma,[168] colorectal carcinoma,[169] gastric cancer,[170] melanoma,[171] neuroblastoma,[172] osteosarcoma,[173] renal cell carcinoma,[174,175] testicular cancer[176]
cIAP antagonists:	
Smac downregulation	Acute myeloid leukemia,[177] bladder carcinoma,[178] breast carcinoma,[179] cervical carcinoma[180] chronic lymphocytic leukemia,[139] colorectal carcinoma,[181] endometrioid endometrial cancer,[182] esophageal carcinoma,[112] lung cancer,[183] rectal adenocarcinoma,[145]
Smac overexpression	Bladder cancer,[184] gastric adenocarcinoma,[185] renal adenocarcinoma,[154,176,186]
HtrA2 overexpression	Endometrial cancer,[187] ovarian cancer,[188] prostate carcinoma,[189,190] renal carcinoma,[186] stomach cancer,[191] thyroid cancer[192]
HtrA2 downregulation	Endometrial cancer,[187,193] ovarian cancer,[194] testicular cancer
ARTS overexpression	Astrocytoma[195]
ARTS downregulation	Acute myeloid leukemia[196]
Ratio IAP/IAP antagonists:	
Increased XIAP/Smac	Renal adenocarcinoma[154]
Reduced XIAP/Smac	Gastric carcinoma[185]
Increased cIAP1/HtrA2 and cIAP1/Smac	Chronic lymphocytic leukemia[139]

Abbreviations: ARTS, septin-like mitochondrial protein; cIAP, cellular IAP; HtrA2, high temperature requirement protein A2; IAPs, inhibitors of apoptosis; MALT, mucosa-associated lymphoid tissue; ML-IAP, melanoma IAP; Smac, second mitochondria-derived activator of caspases; XIAP, X-chromosome linked IAP.

BIRC3 genes were found to be mutated in some multiple myeloma samples,[41,42] and the *BIRC4* encoding XIAP in X-linked lymphoproliferative disease.[43] The expression and functions of the atypical IAP survivin in tumors, and the development of specific survivin-targeted therapy were recently reviewed by Coumar et al[44] and won't be discussed here.

Role of IAPs in cancer

IAPs as apoptotic regulators

IAPs were first characterized as inhibitors of apoptosis because of their ability to bind caspases. Indeed, cIAPs, XIAP and ML-IAP can bind caspase-3, -7, and -9 via the BIRs[10,11,45,46] and can induce their ubiquitination or neddylation via the RING domain.[19,22–24] The influence of the ubiquitination is still not very well established, triggering degradative or nondegradative consequences,[22–24] while the neddylation of caspase-7, by XIAP, inhibits its activity.[19] In addition, XIAP is able to directly inhibit the enzymatic activity of caspases (Figure 1). The XIAP BIR3 binds the dimer interface of caspase-9, and the linker region upstream of BIR2 binds across the substrate binding pocket of caspase-3 and -7, which hinder substrate accessibility and hide the catalytic residue of caspases.[47–49] The capacity of XIAP to inhibit caspase activity could account for the resistance of cancer cells to antitumor therapy. Indeed, DNA-damaging treatments, such as ionizing irradiations, induce a translational upregulation of XIAP as a consequence of the presence of an internal ribosome entry site (IRES)-dependent translation mechanism, which results in the resistance of carcinoma cells to radiation-induced apoptosis.[50,51]

IAPs can also inhibit cell death at an earlier step, preventing the assembly of caspase-8- or -10-activating platforms. Caspase-8 and -10 are initiator caspases recruited

by the adaptor Fas-associated death domain protein (FADD) in multiprotein complexes, which provide the proximity required for caspase homodimerization and self-activation (for review,[52]). These molecular platforms are assembled either in response to the engagement of death receptor from the TNFR superfamily (in which case, these are referred to as death-inducing signaling complex [DISC] and complex II)[52,53] or in response to genotoxic stress, tumor necrosis factor-like weak inducer of apoptosis (TWEAK) engagement, or toll-like receptor (TLR) 3 stimulation (in which case, they are referred to as Ripoptosome).[54,55] Complex II[50,51] and Ripoptosome[52,53] share, in addition to the caspase and the adaptor FADD, the serine/threonine kinase receptor interacting protein (RIP) (Figure 2). cIAPs and XIAP are potent regulators of proteins from the RIP family, catalyzing the conjugation of ubiquitin chains that control either protein degradation or signal transduction pathways[56–62] (Figure 2). In the absence of cIAPs, non-ubiquitinated RIP1 promotes (through its kinase

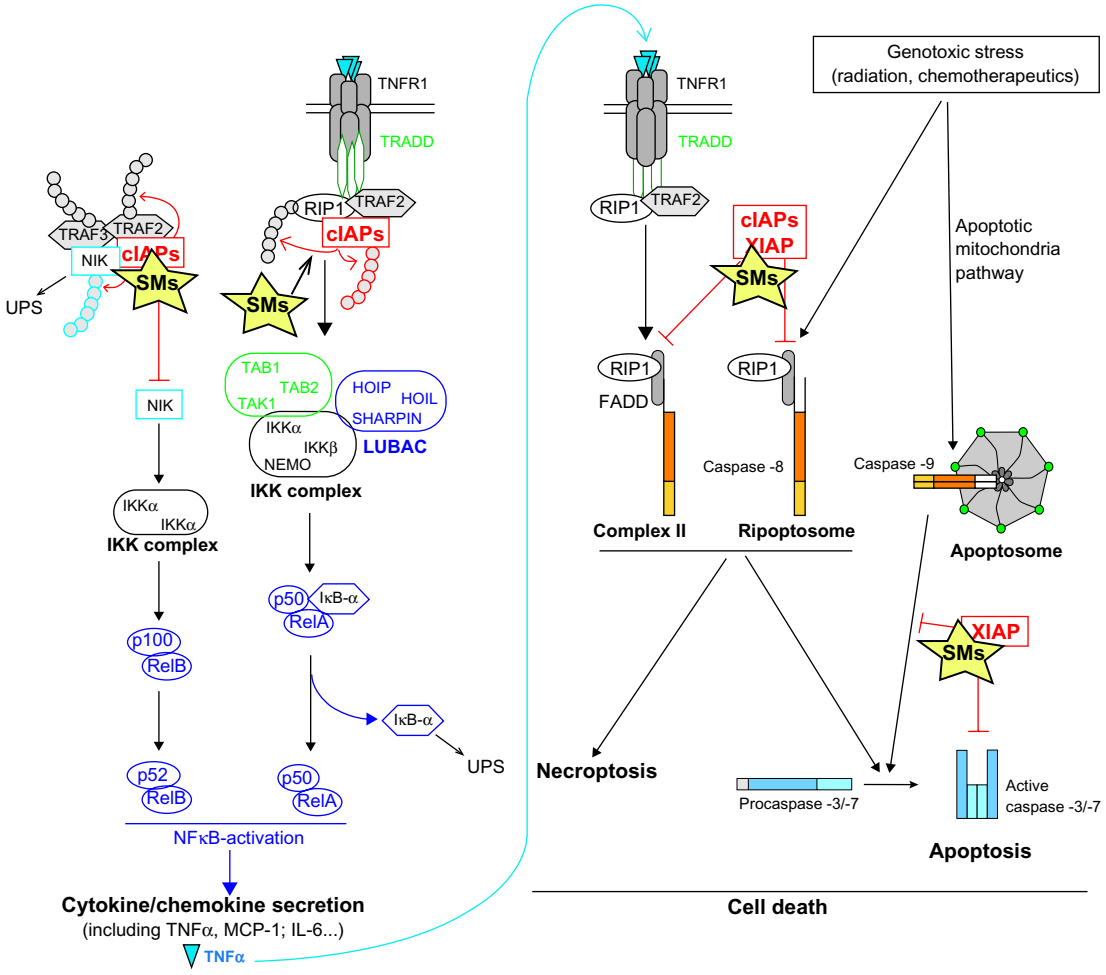

Figure 2 Mechanisms of action of Smac mimetics.

Notes: IAPs are important regulators of NF-κB-activating signaling pathways. Upon TNFα stimulation, cIAPs and RIP1 are recruited to TNFR1 via the adaptors TRADD and TRAF2. cIAPs trigger self-ubiquitination and ubiquitination of RIP1. These ubiquitin chains serve as a scaffold for the recruitment of IKK, TAK, and LUBAC. Once activated, IKK complex triggers phosphorylation of IκBα, which is then degraded by the UPS and releases NF-κB dimer, which promotes the transcription of target genes. In the noncanonical pathway of NF-κB activation, cIAPs promote the degradative ubiquitination of NIK and associated TRAF proteins and prevent the activation the IKK complex required for transcription factor activation. On the other hand, cIAPs and XIAP prevent the assembly of RIP1-containing initiator caspase-activating complexes, named complex-II and Ripoptosome; and XIAP inhibits, through a direct interaction via BIR2 and BIR3, the activity of caspase-3, -7, and -9. SMs bind to the BIR domains of cIAPs and stimulate their E3-ubiquitine ligase activity. This results first, in the ubiquitination of RIP1, leading to the canonical NF-κB activation, and second, in the rapid autoubiquitination and subsequent proteasome-mediated degradation of cIAPs. Depletion of cIAPs releases NIK, resulting in the noncanonical activation of NF-κB, and NF-κB target gene expression, including TNFα, MCP-1, and IL-6. TNFα engages TNFR1 via an autocrine pathway. In the absence of cIAP1, stimulation of TNFR1 triggers the assembly of the secondary RIP1-containing cytoplasmic complex (complex II), leading to cell death. SM-mediated IAP depletion can also favor the formation of the Ripoptosome, leading to cell death.

Abbreviations: BIR, baculoviral IAP repeat; cIAP, cellular IAP; IκBα, nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha; IAPs, inhibitors of apoptosis; IL, interleukin; IKK, IκB kinase complex; LUBAC, linear ubiquitin chain assembly complex; MCP-1, monocyte chemoattractant protein; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NIK, NF-κB-inducing kinase; RIP1, receptor interacting protein 1; SM, Smac mimetic; Smac, second mitochondria-derived activator of caspases; TAK, TGFβ-activated kinase; TGFβ, transforming growth factor beta; TNF, tumor necrosis factor; TNFR1, tumor necrosis factor receptor 1; TRADD, TNFR1-associated death domain; TRAF, TNFR associated factor; UBA, ubiquitin proteasome system; XIAP, X-chromosome linked IAP; TAB,TAK1-binding partners; HOIL, heme-oxidized IRP2 ligase-1; HOIP, HOIL-1L-interaction protein; NEMO, nuclear factor-κB (NF-κB) essential modulator; UPS, ubiquitin-proteasome system.

activity) the assembly of the caspase-activating platforms that leads to cell death[56,62] (Figure 2). Thus, cIAPs inhibit RIP1-containing caspase-activating platform assembly, either by promoting the ubiquitin-proteasome-mediated degradation of the components of the Ripoptosome[54] or by inducing a nondegradative ubiquitination of RIP1, which inhibits the cell death complex assembly and promotes survival-signaling pathway transduction.[56,59,63]

IAPs as cell-signaling regulators

The role of IAPs in the regulation of the NF-κB-activating signaling pathways is well documented (for review,[25,26]). NF-κB is a transcription factor induced by the stimulation of antigen or cytokine receptors, by the recognition of microbiological patterns by the TLRs, the nucleotide-binding oligomerization domain-containing proteins (NODs), or the NOD-like receptors (NLRs), or in response to intracellular injuries, such as DNA damage or reactive oxygen species. NF-κB contributes to the adaptive response of cells, by mediating the expression of the proinflammatory molecules that counter microbial invasion and by promoting the expression of genes involved in cell survival, cell differentiation, and cell proliferation.[64] The transcription factor consists of heterodimers formed by one Rel subunit (RelA [also called p65], RelB, or c-Rel) and one NF-κB subunit (the p50 subunit of NF-κB1 or the p52 subunit of NF-κB2). In resting cells, the p50/RelA dimer is sequestered into the cytoplasm by the inhibitor of κB (IκB) proteins. Upon stimulation of the cell surface or intracellular receptors, or DNA damage, p50/RelA is released as a consequence of the degradation of NF-kappa-B inhibitor alpha (IκB-α) and translocated into the nucleus to stimulate proinflammatory gene transcription (Figure 2). Degradation of IκB-α requires its phosphorylation by the IκB kinase (IKK) complex, which is activated by ubiquitination by the linear ubiquitin chain assembly complex (LUBAC) and by phosphorylation by TGFβ-activated protein kinase 1 (TAK1)[64] (Figure 2). cIAPs and XIAP promote the steric proximity of TAK1, LUBAC, and IKK complex. In the TNF-R1-signaling pathway, cIAPs are recruited along with RIP1 to the receptor[61] and trigger self-ubiquitination and the nondegradative polyubiquitination of RIP1[56,57,66] (Figure 2), and in NOD2-mediated inflammatory signaling, XIAP and cIAPs mediate the conjugation of ubiquitin chains to RIP2.[67–69] These ubiquitin chains serve as a scaffold for the recruitment and activation of the signaling complexes leading to IKK activation[56,61,68,70] (Figure 2). cIAPs can also modulate NF-κB activation by catalyzing the monoubiquitination of the IKK component NF-κB essential modulator (NEMO), which is required for IKK activation,[71,72] and XIAP promotes the activation of TAK1 and the steric proximity of TAK1 and IKK complex[71] during TGFβ and myelin basic protein (MBP) receptor signaling, or in response to DNA damage.[15,71,73–75] A second NF-κB-activating signaling pathway, named the noncanonical pathway, involves NF-κB-inducing kinase (NIK), which catalyzes the phosphorylation of IKKα. In turn, IKKα induces the phosphorylation of the p100 NF-κB2 precursor, leading to its proteolytic activation into active p52 NF-κB2 (Figure 2). cIAPs prevent the noncanonical activation of NF-κB by mediating the ubiquitination and the proteasomal-mediated degradation of NIK[70,76–79] (Figure 2). Mutations in cIAP-encoding genes leading to NIK stabilization and chronic NF-kB activation could facilitate B cell malignancy and lymphomagenesis, as observed in some multiple myelomas that harbor mutations in the cIAP1- or cIAP2-encoding genes[41,42] and as observed in MALT lymphoma that is associated with a chromosomal translocation t(11;18)(q21;q21), generating a chimeric protein composed of the N-terminal sequence of cIAP2 fused to the C-terminal sequence of MALT1.[17,40,80]

Cell proliferation and migration

cIAPs are positive regulators of cell proliferation, a function correlated with the nuclear localization of the proteins.[29,81] Interestingly, the nuclear expression of cIAP1 has been associated with advanced disease stages and poor patient prognosis in human cervical and esophageal squamous cell carcinomas and bladder cancers[36,82,83] (Supplementary Table S1). The influence of IAPs on cell proliferation can be explained by their capacity to stimulate the activity of the c-Myc and E2F1 transcription factors, which are important regulators of cell cycle progression and cell proliferation with oncogenic properties.[28,29] IAPs have also been involved in the regulation of the invasive properties of mammalian cancer cells, as recently reviewed.[84]

Targeting IAPs in cancer therapy

Targeting IAPs in tumors is an important challenge and several strategies have been explored, including the use of antisense oligonucleotides and antagonist molecules. A synthetic antisense oligonucleotide to XIAP, named AEG35156, was developed by Aegera Therapeutics Inc (Montreal, QC, Canada).[85] It demonstrated promising efficiency in the preclinical studies. It induced a decrease of XIAP expression in tumor cell lines and tumor xenograft models, and sensitized cells to various standard chemotherapeutic agents and Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand

(TRAIL) receptor agonists.[86] AEG35156 entered into clinical trials (http://www.clinicaltrials.gov/) in 2005, and to date, ten Phase 1,2, or 1/2 clinical trials have been completed in solid tumors and in acute myeloid leukemia (AML) (Table 2) (for review,[86,87]). In the trials, AEG35156 appeared to accumulate in the liver and to have efficiently downregulated XIAP messenger ribonucleic acid (mRNA) in peripheral blood mononuclear cells and hepatocytes. AEG35156 is generally well tolerated except when administered in repeated high doses. Promising results were obtained with AEG35156 used as a single agent in solid tumors[88] and in combination with cytarabine and idarubicin in AML[89] in the Phase 1 studies, but it failed to show any significant antitumoral activity in the randomized Phase 2 studies in pancreatic adenocarcinoma, when combined with Gemcitabine,[90] or in AML, when it was given in combination with cytarabine and idarubicin.[91]

The structural characterization of the interaction of XIAP with caspases, or with Smac, or the drosophila Smac homologs has provided very potent tools for the design of synthetic IAP antagonists aiming to inhibit the capacity of XIAP to neutralize caspases.[11,92–94] The surface hydrophobic groove of IAP BIRs binds the IBM found in the N-terminal of the active subunits of caspase-3, -7, and -9 and exposed by activating proteolytic processing.[10,11] Cellular IAP antagonists also own an IBM.[10–13] During the apoptotic process, the Smac IBM is exposed as a consequence of the cleavage of the mitochondria-targeting signal, and matured Smac is released from the mitochondria into the cytosol.[10–12] The tetrapeptide Ala-Val-Pro-Ile (AVPI) IBM motif of Smac inserts into the XIAP BIR2 and BIR3-caspase interaction pocket and abrogates XIAP-mediated caspase inhibition[93,95,96] (Figure 1). The Smac N-terminal peptide was also derived to produce cell permeable peptides and was shown to mimic the activity of Smac and to sensitize human cancer cell lines to diverse chemotherapeutic agents, including etoposide, teniposide, cisplatin, paclitaxel, 7-ethyl-10-hydroxycamptothecin (SN-38), and TRAIL agonists.[97–100] In xenograft models, a Smac-derived peptide, made permeable by linking to the shuttle peptide trans-activation of transcription (TAT) from HIV, enhanced the antitumoral effect of TRAIL in glioma,[99] and a polyarginine-conjugated Smac peptide was shown to sensitize non-small cell lung carcinoma cells to cisplatin,[98] with little toxicity to normal tissues. The pharmacological

Table 2 AEG35156 XIAP antisense oligonucleotide in clinical trials (http://www.clinicaltrials.gov/)

Phase	Drug combination	Start date	Condition	Observations	Ref
Phase I	Docetaxel	07/2006	Adult solid tumor		
Phase I/2	Cytarabine and idarubicin	08/2006	Refractory or relapsed acute myeloid leukemia (24 patients)	• Generally well tolerated • Toxicity included two cases of neuropathy in patients having received multiple AEG35316 doses • Achieved target knockdown • Clinical evidence of antitumoral activity	89 197
Phase I	Docetaxel	09/2006	Adult solid tumor		
Phase I	Single agent	10/2006	Advanced cancer (22 patients)	• Well tolerated • Evidence of efficiency (decreased XIAP mRNA in peripheral blood mononuclear cells) • Clinical evidence of antitumoral activity in patients with refractory lymphoma, melanoma, and breast cancer	88
Phase I/2	Gemcitabine	11/2007	Metastatic pancreatic adenocarcinoma (14 patients)	• Toxicities include neutropenia, thrombocytopenia, peripheral neuropathy, fatigue, ascites, and nausea/vomiting • Failed to show significant antitumoral activity	90
Phase I/2	Paclitaxel	11/2007	Mammary carcinoma		
Phase I/2	Carboplatin and paclitaxel	11/2007	Non-small cell lung carcinoma		
Phase I/2	Single agent	10/2008	Refractory chronic lymphocytic leukemia and indolent B-cell lymphomas		
Phase I/2	Sorafenib	04/2009	Advanced hepatocellular carcinoma		
Phase 2	Cytarabine and idarubicin	11/2009	Refractory or relapsed acute myeloid leukemia (27 patients)	• Well tolerated • Did not improve rates of remission	91

Abbreviations: RNA, ribonucleic acid; XIAP, X-chromosome linked IAP; IAPs, inhibitors of apoptosis; mRNA, messenger RNA.

properties of such Smac-derived peptides were not good enough to merit consideration of these molecules as therapeutic agents; however, they provided the bases for the structure-based design of IAP antagonists named Smac mimetics (SMs). Several approaches were used, including the screening of peptide or peptidomimetic libraries,[101,102] and the structure-based design of conformationally constrained SMs[103,104] (Figure 3). Considerable efforts were invested to improve the affinity of the compounds to the IAP BIR domains, to improve their ability to antagonize IAPs,[104–107] to improve cellular delivery and activity (ie, their capacity to induce apoptosis or to sensitize to apoptotic agents), and to improve their in vivo stability and bioavailability. The preclinical assays demonstrated their capacity to inhibit tumor growth in multiple solid tumors,[102,107,108] acute lymphoblastic leukemia (ALL),[108] and multiple myeloma[109] xenograft models and to sensitize cells to TRAIL, proteasome inhibitors, B-cell lymphoma protein 2 (Bcl-2) family-targeting compounds, and more conventional therapeutic agents, such as radiation, melphalan, or cisplatin.[103,109–114] Importantly, these compounds were well tolerated by animals and did not display toxicity against normal lymphocytes and bone marrow stromal cells[109] or normal mammary epithelial cells.[115] The analysis of binding affinity revealed that similarly to Smac,[93,95,96] SMs can bind to XIAP-BIR2, preventing XIAP-caspase-7 and -3 binding, and to XIAP-BIR3, abrogating the XIAP-mediated inhibition of caspase-9. Structural and biochemical studies of the apoptotic activity of Smac cellular protein revealed, first, that it forms a symmetric dimer;[93,94] second, that

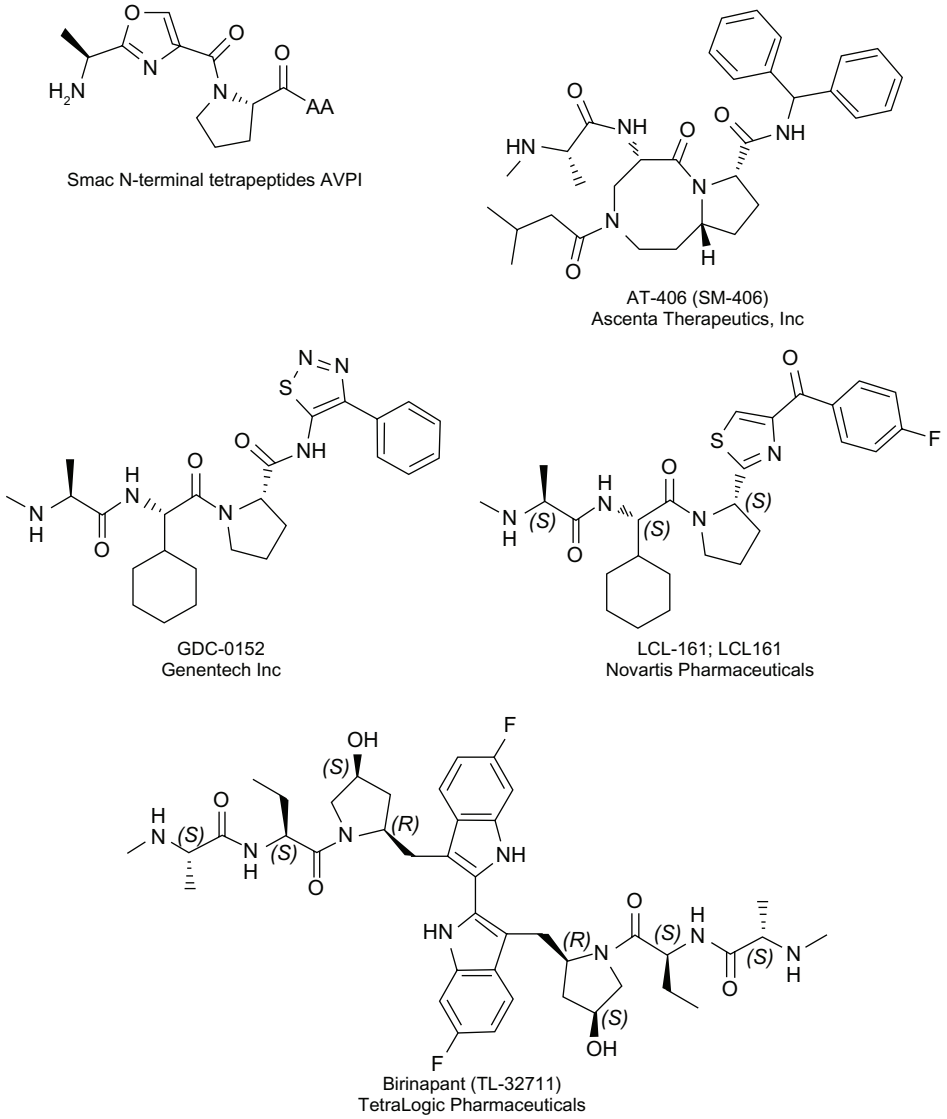

Figure 3 Structure of the Smac N-terminal tetrapeptide (AVPI) and SMs used in clinical trials.
Notes: AT-406: CAS RN 1071992-99-8; GDC-0152: CAS RN 873652-48-3; LCL161: CAS RN 1005342-46-0; and Birinapant: CAS RN 1260251-31-7.
Abbreviations: CAS RN, CAS Registry Number®; SM, Smac mimetic; Smac, second mitochondria-derived activator of caspases.

Table 3 SMs in clinical trials

Compound	Structure	Preclinical assays in animals	Clinical trial	Conditions
AT-406 (SM-406 – Debio 1143) Ascenta therapeutics/ debiopharm SA	Monovalent	• Inhibited tumor growth and sensitized cells to carboplatin in ovarian cancer xenograft model[119] • Inhibited tumor growth in breast tumor xenograft model with no sign of toxicity[120] • Increased chemo- and radiotherapy sensibility in head and neck squamous cell carcinoma tumor xenograft model[221]	Phase I Phase I	Advanced solid tumors and lymphoma Combination with daunorubicin and cytarabine in patients with poor-risk acute myelogenous leukemia
Birinapant (TL-32711) TetraLogic pharmaceuticals	Bivalent	• Tumor growth arrest or inhibition in patient-derived primary pancreatic cancer explant model[199] • Remission in acute lymphoblastic leukemia xenograft models[200]	Phase I	Refractory solid tumors or lymphoma ➔ Well tolerated with no dose limiting toxicities, potent and sustained target inhibition, apoptotic pathway activation in tumor and antitumoral activity in colon cancer and melanoma[203]
		• Delayed the tumor growth and increases survival in combination with ionizing radiation in a glioblastoma multiform model in mice[201]	Phase I/2	Combination chemotherapy (doxorubicin, paclitaxel, carboplatin, gemcitabine, irinotecan, docetaxel) in advanced and metastatic solid tumors
		• Inhibited tumor growth in combination with the immunomodulatory agents IFNα or GM-CSF in a kidney carcinoma xenograft model[202]	Phase I/2 Phase I Phase 2 Phase I/2	Acute myelogenous leukemia, myelodysplastic syndrome and acute lymphoblastic leukemia Combination with gemcitabine in patients with advanced solid tumor Advanced ovarian, fallopian tube and peritoneal cancers Combination with 5-azacytidine in myelodysplastic syndrome
GDC-0917 Genentech	Monovalent		Phase I	Refractory solid tumors or lymphoma
GDC-0152 Genentech	Monovalent	• Inhibits tumor growth in breast cancer xenograft without affecting normal mammary epithelial cells[204] • Induces an increased systemic level of cytokines and chemokines (TNFα and MCP-1), a systemic inflammatory response and hepatic injury when IV administered in dogs;[205] such effects were not observed in human[133]	Phase I	Locally advanced or metastatic solid malignancies, or non-Hodgkin's lymphoma without leukemic phase ➔ Well tolerated, no signs of a systemic inflammatory response
HGS1029 (AEG-40826) Human Genome Sciences	Bivalent		Phase I Phase I	Advanced solid tumors and refractory lymphoid malignancies Relapsed or refractory lymphoid malignancies
LCL161 Novartis pharmaceuticals	Monovalent	• Delays tumor growth in multiple solid tumor xenograft models as a single agent but is ineffective in acute lymphoblastic leukemia xenograft models[108] • Antitumor activity in combination with chemotherapy against a range of solid tumors including primary models of breast cancer (Novartis website)* • Inhibits tumor growth in combination with a Bcl-2 inhibitor in hepatocellular carcinoma xenograft models[110] • Inhibits tumor growth and prolongs survival in combination with adeno-associated virus bacteriophage-TNFα in melanoma xenograft models[111]	Phase I Phase I Phase 2	Solid tumors ➔ Well tolerated[135] Combination with weekly paclitaxel in patients with advanced solid tumor Combination with weekly paclitaxel in patients with breast cancer

Note: *Novartis website: http://www.novartisoncology.us/research/pipeline/lcl161.jsp

Abbreviations: GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN, interferon; IV, intravenous; TNF, tumor necrosis factor; MCP-1, monocyte chemoattractant protein; Smac, second mitochondria-derived activator of caspases; SMs, Smac mimetics; Bcl-2, B-cell lymphoma 2.

dimerization is essential for Smac function;[93,116] and third, that the capacity of Smac to abrogate XIAP-mediated caspase inhibition required the binding to both BIR2 and BIR3.[95] Overall, these observations support the conclusion that compounds targeting both BIR domains could be more efficient as XIAP antagonists and lead to the development of bivalent small molecules containing two Smac AVPI IBM motif mimetics.[117] As expected, these compounds appeared to be more potent than their monovalent counterparts, in antagonizing XIAP and in activating caspases.[104,117,118] Like the monovalent versions, the bivalent molecules either inhibited tumor growth or sensitized cells to both conventional and nonconventional anticancer therapies in the preclinical assays and did not display toxicity to normal human primary cells;[104,117–121] however, unlike the monovalent molecules, the bivalent SMs are not orally bioavailable. To date, more than 50 applications for patents related to IAP antagonists have been filed (for review,[122]), and six SMs have entered human clinical trials (http://www.clinicaltrials.gov/) for the treatment of cancer (described in Table 3).

Mechanisms of action of SMs

As expected, SMs abrogate XIAP-mediated caspase inhibition and therefore increase caspase-3 and -7 activities (Figure 1). However, in addition to binding XIAP BIRs, SMs also bind the BIR domains of ML-IAP, cIAP1 and cIAP2.[56,104,105,115,118] SMs stimulate the E3-ubiquitine ligase activity of cIAPs, which results in the ubiquitination of RIP1, leading in turn, to canonical NF-κB activation and the rapid autoubiquitination and subsequent proteasome-mediated degradation of cIAPs[123–126] (Figure 2). Depletion of cIAPs abolishes the cIAP-mediated ubiquitination and degradation of NIK and induces canonical activation of NF-κB. In turn, NF-κB induces the expression of proinflammatory cytokines and chemokines, including TNFα, which can trigger cell death by an autocrine pathway.[66,125–127] Furthermore, depletion of cIAPs favors the assembly of the RIP1-containing cytoplasmic cell death complexes, such as complex II and Ripoptosome, resulting in cell death in some sensitive cancer cells, or in the sensitization to TNFα or DNA-damaging chemotherapeutic agents[54,55] (Figure 2). SMs exert their activity through XIAP and cIAPs and both effects are required for their maximal antitumoral activity.[128–130] Indeed, IAP antagonists displaying a high and selective affinity for cIAPs over XIAP appeared less potent than pan-IAP antagonists in promoting cancer cell death[129] and in sensitizing cancer cells to TRAIL.[131]

As a consequence of cIAP degradation and NF-κB activation, the administration of SMs such as LCL161, GDC-0152, and HGS1029, resulted in the upregulation of cytokines and chemokines,[132–134] including TNFα, monocyte chemoattractant protein (MCP)-1, interleukin (IL)-7, IL-6, and interferon (IFN)γ.[134] MCP-1 was used as a clinical biomarker for SMs efficiency in clinical programs.[133,135] The analysis of the proinflammatory characteristics of cellular Smac-induced cell death suggests that the proinflammatory response elicited by SMs could activate the adaptive antitumor immune response in cancers.[136] In dogs, intravenous (IV) administration of GDC-0152 induced an acute systemic inflammatory response with lung and hepatic injury, which are consistent with TNF-α mediated toxicity;[134] however, a similar TNF-α-driven inflammatory response was not observed in humans.[133] Although the first clinical trials did not reveal extensive toxicity of SMs when orally or intravenously administered, additional analysis of the consequences of cytokine and chemokine secretion are required. Because osteoclast differentiation and function are stimulated by activation of the noncanonical NF-κB pathway and because osteoclasts are susceptible to TNF-mediated death, Yang et al analyzed the influence of SMs on bone metastasis and demonstrated that SMs stimulated osteoporosis and specifically enhanced metastasis in bone.[137]

Conclusion

SMs are a very promising new class of anticancer therapeutics. Results from preclinical studies have demonstrated an acceptable safety profile and some signs of antitumoral activity, in their use as a single agent or in combination with conventional or nonconventional therapies, such as dead receptor agonists, Bcl-2, or kinase-targeting therapies. The first clinical trials demonstrated a good tolerance and target inhibition. Ongoing and future clinical trials will determine the safety, appropriate indications, and drugs combinations. It will be important to determine the level and the site of production of TNFα and other cytokines and the consequences of cytokine production for tumoral and non-tumoral cells. Since IAPs are involved in the regulation of various cellular functions, it will be interesting to target specific IAP functions in order to limit possible adverse impacts. The consequences of SMs on the immune system in vivo and the use of cIAPs as potential therapeutic targets for inflammatory or immune disorders are still important questions that need to be addressed.

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Disclosure

The authors report no conflicts of interest in this work.

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Supplementary material

Table S1 Role of IAPs in cancer

	Cohort	Observations
XIAP overexpression		
AML	92[1]	Associated with poor cytogenetics[1]
	78[2]	Inversely correlated with overall survival[1,2]
		Correlated with sensitivity to anticancer drugs (cytarabine)[2]
BCLL	100[3]	Correlated with Ki-67 proliferation index and progressive disease; inverse correlation with overall survival[3]
	301[4]	Associated with poor clinical outcome[4]
Bladder carcinoma	176[5]	Independent prognostic factor for early recurrence of invasive cancers
		Correlated with poor differentiation
		Inversely correlated with recurrence-free survival
Breast carcinoma	102[6]	Nuclear expression
		Independent negative prognostic factor for overall survival
Cervical carcinoma	77[7]	
Colorectal cancer	96[8]	Cytoplasmic expression
		Independent negative prognostic factor
		Correlated with tumor dedifferentiation, invasion, stage, and lower disease-free and overall survival[8]
	38[9]	Correlated with resistance to irradiation[9]
Hepatocellular carcinoma	69[10]	Associated with shorter survival[10,11] and increased risk of relapse and metastasis[11]
	192[11]	The cytoplasmic expression is an independent negative prognostic factor[11]
Melanoma	55[12]	Correlated with advanced tumor stage and inversely correlated with patient survival
NSCLC	144[13]	Mainly expressed in the cytoplasm
		Independent positive prognostic factor for survival in resected patients
		Correlated with longer overall survival
		Inversely correlated with proliferation Ki-67 proliferation index
	55[14]	Cytoplasmic expression
		No correlation with chemotherapy or radiotherapy
Ovarian cancer		AT-406-induced apoptosis is correlated with its ability to downregulate XIAP expression[15]
Prostate carcinoma	226,[16] 691[17]	Deregulation of XIAP occurs early in the pathogenesis of prostate cancer[17]
		Independent predictor of tumor recurrence[16]
Renal carcinoma	145[18]	Independent negative prognostic factor[18]
	66[19]	Correlated with tumor grade and advanced tumor stage[18,19,20]
	109[20]	Inversely correlated with patient survival[18,19,20]
Thyroid carcinoma	72[21]	
cIAP1 and cIAP2 overexpression associated with amplicon 11q21-22		
Cervical cancer	70[22]	Nuclear expression correlated with low overall survival
ESC	42[23]	Correlated with resistance to cisplatin/campthotecin
Hepatocarcinoma	25[24]	
Mammary carcinoma	[25]	
Medulloblastoma	17[26]	
NSCLC and SCLC	25[27]	
	55[14]	Cytoplasmic expression
		No correlation with chemotherapy or radiotherapy[14]
Pancreatic cancer	22,[28] 33[29]	Inversely correlated with patient survival
cIAP1 overexpression independent from 11q21-22 amplicon		
AML		Associated with resistance to several anticancer drugs[2]
B-cell CLL	22,[30] 30[31]	Correlated with resistance to irradiation[30]
		No correlation with fludarabine sensitivity[31]
Bladder cancer	102[32]	Nuclear expression correlated with proliferation index (Ki-67), tumor stage, and grade
		Inversely correlated with overall survival and recurrence free-survival
Cervical carcinoma	70[22]	Nuclear expression
		Correlated with the resistance to irradiation
		Inversely correlated with overall survival and recurrence-free survival
CLL	100[3]	Correlated with advanced tumor stage
Colorectal cancer	46[33]	Nuclear expression

(*Continued*)

Table S1 (*Continued*)

	Cohort	Observations
HNSCC	55[34]	Nuclear expression correlated with metastasis, advanced stage, and poor patient prognosis
NSCLC and SCLC	55[14]	Nuclear expression
		No correlation with chemotherapy or radiotherapy
Prostate carcinoma	691[17]	Inversely correlated with refractory disease
Squamous carcinoma of tongue	75[35]	Nuclear and cytoplasmic expression Correlated with metastasis
cIAP1/cIAP2 inactivation		
Multiple myeloma	155[36,37]	
c-IAP1/ HtrA2		
c-IAP1/Smac DIABLO		
CLL	100[3]	Correlated with a better response to treatment (cladribine, cyclophosphamide, fludarabine)
c-IAP2 overexpression independent of t(11q21)		
Breast cancer	144[38]	
Cervical carcinoma	77[7]	
CLL	100[3]	Associated with progressive disease
	30[31]	Cytoplasmic expression
		No correlation with fludarabine sensitivity
Colorectal cancer	46[33]	Cytoplasmic expression
Prostate carcinoma	691[17]	Deregulation of c-IAP2 occurs early in the pathogenesis of prostate cancer
		Correlated with tumor stage and with refractory disease
c-IAP2/MALT chimeric protein t(11,18)(q21, q21)		
MALT myeloma	5[39,40]	
ML-IAP overexpression		
AML	34[41]	Inversely correlated with overall survival
Adults ALL	34[41]	Inverse correlation with relapse-free survival and overall survival
Childhood ALL	222[42]	Correlated with relapse-free survival
Bladder cancer	30[43]	Correlated with relapse-free survival
Colorectal cancer		Correlated with resistance to etoposide, vincristine, 5-fluorouracil[44]
Gastric cancer	40[45]	Correlated with metastasis and dedifferentiation
Melanoma	27[46]	Resistance to etoposide
Neuroblastoma	68[47]	Associated with *MYCN* amplification → inversely correlated with patient survival
Osteosarcoma	29[48]	Nuclear expression: inverse correlation with overall survival
Renal cell carcinoma	152,[49] 204[50]	Nuclear expression[50]
Testicular cancer	131[51]	Correlated with dedifferentiation
Smac downregulation		
AML	71[52]	Correlated with response to chemotherapy
Bladder cancer	173[53] (serum)	Inversely correlated with advanced tumor stage and tumor grade
Breast cancer	62[54]	Inversely correlated with tumor stage
CLL	100[3]	Inversely correlated with advanced tumor stage
Cervical carcinoma	86[55]	Inversely correlated with local recurrence
Colorectal carcinoma	121[56]	Inversely correlated with metastasis and advanced tumor stage
		Correlated with patient survival
Endometrioid endometrial cancer	76[57]	Inversely correlated with tumor grade and correlated with longer disease-specific survival
Esophageal carcinoma	86[58]	Inversely correlated with chemoresistance
Lung cancer	88[59]	Inversely correlated with advanced tumor stage
Rectal adenocarcinoma	38[9]	Correlated with resistance to irradiation
Smac overexpression		
Bladder cancer	75[60]	Correlated with postoperative recurrence-free period
Gastric adenocarcinoma	46[61]	Correlated with advanced tumor stage
Renal carcinoma	66,[19] 85[62]	Correlated with advanced tumor stage
XIAP/ Smac		
Gastric adenocarcinoma	46[61]	Low XIAP/ Smac ratio
Renal carcinoma	66[19]	High XIAP/ Smac ratio is correlated with advanced tumor stage

(*Continued*)

Table S1 (*Continued*)

	Cohort	Observations
HtrA2 overexpression		
Endometrial cancer	139[63]	Nuclear HtrA2 expression is elevated in poorly differentiated and lymph node metastatic cancer Nuclear HtrA2 expression is an independent prognostic factor for endometrial cancer progression-free survival
Ovarian cancer	[64]	Cytoplasmic HtrA2 expression increased in cisplatin-resistant cells
Prostate carcinoma	105,[65] 61[66]	Correlated with tumor grade and dedifferentiation[65]
Renal carcinoma	85[62]	Correlated with recurrence-free and tumor-specific survival
Stomach cancer	60[67]	
Thyroid cancer	[68]	
HtrA2 downregulation		
Endometrial cancer	124[69]	
Ovarian cancer	79[70]	
ARTS overexpression		
Astrocytoma	72[71]	Correlation with tumor grade and higher rate of apoptosis
ARTS downregulation		
AML	[72]	Resistance to 5-azacytidine

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; ARTS, septin-like mitochondrial protein; BCLL, B-cell chronic lymphocytic leukemia; cIAP1, cellular inhibitors of apoptosis; CLL, chronic lymphocytic leukemia; DIABLO, IAP-binding protein with low pI; ESC, esophageal squamous cell carcinoma; HNSCC, head and neck squamous cell carcinomas; HtrA2, high temperature requirement protein A2; IAP, inhibitors of apoptosis; NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer; MALT, mucosa-associated lymphoid tissue; ML-IAP, melanoma IAP; SLL, small lymphocytic lymphoma; Smac, second mitochondria-derived activator of caspases; XIAP, X-chromosome linked IAP; pI, isoelectric point.

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