X-Linked Inhibitor of Apoptosis Antagonism: Strategies in Cancer Treatment

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Background

X-linked inhibitor of apoptosis (XIAP), first identified in 1996 (1), is a member of the IAP family of endogenous caspase inhibitors that blocks the execution of apoptosis. The inhibitory function of XIAP can be reversed by its antagonists, whereas its abundance can be regulated in a proteasome-dependent manner. In less than a decade, great strides made in understanding basic XIAP function has translated into clinical research targeting this IAP in cancer malignancy (2).

Apoptosis pathways and XIAP

Apoptosis is controlled by the activation of a zymogen caspase cascade and the inhibition of the resulting active proteases (3). Initiation of apoptosis occurs by signals from two distinct but convergent pathways: the extrinsic death receptor pathway and intrinsic mitochondrial apoptosome pathway (Fig. 1). These two pathways consist of largely distinct molecular interactions and use different upstream, initiator caspases that are often interconnected at numerous steps to ultimately converge at the level of downstream effector caspase activation (4). As the only known endogenous proteins that function as direct, physiologic repressors of both initiator and effector caspases (5, 6), the IAPs play a pivotal role in the regulation of the apoptotic cascade, representing an important survival factor in cancer cells.

XIAP (hILP/birc4) is arguably the most potent IAP with respect to its antiapoptotic functions; therefore, it is not surprising that XIAP is also one of the better studied and understood IAPs. XIAP is a multifaceted molecule composed of conserved RING and baculovirus IAP repeat (BIR) zinc finger domains, all under the translational control of a stress-regulated internal ribosome entry site (7). The antiapoptotic property of XIAP has been attributed to direct inhibition and proteasome-dependent degradation of caspases, as well as the activation nuclear factor-κB signaling pathways. Although XIAP was initially described as an activator of c-Jun NH2-terminal kinase survival pathway, it was subsequently found that depending on cell type and the initial signal, XIAP may either activate or repress c-Jun NH2-terminal kinase activation (8, 9).

XIAP protein possesses three characteristic NH2-terminal 70- to 80-amino-acid BIR domains and a COOH-terminal RING zinc finger domain. BIR1 of XIAP contains an Akt phosphorylation site at residue Ser87 that is involved in protein stabilization (10). The anti-caspase activities of XIAP can be ascribed to BIR domains and their linker regions: BIR3 is an inhibitor to the initiator caspase-9 and the linker preceding BIR2 functions as an inhibitor to effector caspase-3 and caspase-7 (6). The COOH-terminal RING domain of XIAP is an E3 ubiquitin ligase capable of recruiting target proteins to a complex containing an E2 enzyme for ubiquitin-conjugation and proteasomal degradation (11). XIAP can trigger the ubiquitination of caspase-3 and caspase-7 (12, 13), suggesting that targeting of caspases to the proteasome may be another antiapoptotic mechanisms of the IAPs. Interestingly, E3 auto-ubiquitination function of XIAP can be turned on by the binding of antagonists, such as Smac3, a splice variant of Smac with exon 4 deleted (14).

XIAP and cancer: regulation, antagonists, and proteasome

XIAP is frequently overexpressed in NCI 60 cell line panel of cancer cells and in cancer tissues compared with normal tissues (15, 16). Notably, a strong positive correlation exists between the levels of XIAP and caspase-3 in breast, colon, and pancreatic cancers (17, 18), suggesting that the down-regulation of XIAP might release caspase-3 inhibition and promote the execution of apoptosis in cancer cells.

The therapeutic potential of XIAP suppression in cancer treatment has encouraged research into identifying and characterizing mechanisms that regulate XIAP abundance as well as antagonists that block XIAP functions. In addition to the E3 ligase of XIAP promoting its own destruction (19), proteasome-dependent degradation of XIAP can be mediated by E3 ligases of other IAPs, such as cIAP1 and cIAP2 (20). Conversely, formation of another IAP-XIAP complex, involving survivin that lacks a E3 ligase RING domain, promotes increased XIAP stability against proteasome-mediated degradation and synergistic inhibition of apoptosis (21). Moreover, upon phosphorylation at Ser87, XIAP is protected from ubiquitination and degradation in response to the chemotherapeutic agent cisplatin (10).
The caspase inhibition function of the BIR domains can be reversed by negative regulators, primarily mitochondrial apoptogenic factors, such as Smac/DIABLO (22, 23) and possibly Omi/HtrA2 (24), but also other factors, such as cytoplasmic/endoplasmic reticulum protein eRF3 (25) and the candidate tumor suppressor XIAP-associated factor 1 (26). Caspase activation as a result of displacement from XIAP by Smac, Omi, and eRF3 is mediated by the IAP-binding motif, a conserved tetrapeptide sequence (e.g., AVPI) exposed at the NH2 terminus after proteolytic cleavage (22, 23). Smac was the earliest identified XIAP antagonist and has become the basis for many studies that examine the potential of suppressing XIAP in cancer treatment. Recent studies have reported that small-molecule mimics of Smac are effective agents in relieving XIAP-mediated suppression of caspase activity to promote the regression of established tumors in xenograft models in mice (27, 28).

Clinical-Translational Advances

The on-going validation of XIAP as a genuine anticancer target has been paralleled by strategies that aim to antagonize this IAP (Fig. 2). These different approaches are in various stages of development, from preclinical to a phase I clinical trial. A brief detailing of these efforts offers us a glimpse of what can be anticipated in the coming years.

Inducers of mRNA degradation. Currently, the sole strategy being evaluated in the clinic is with AEG35156, a second-generation antisense targeting the coding region of XIAP (2). Antisense or RNA interference approaches allow the design of specific drugs to target XIAP mRNA and induce their degradation through RnaseH or RISC complexes. Relatively soon after the discovery of XIAP, functional studies showed that overexpression of this IAP was highly cytoprotective (29). The converse, however, that XIAP down-regulation in a cancer cell would lead either to outright cell death, or at the least, sensitization to apoptosis induction by cytotoxic agents, took many more years to firmly establish. This question has been positively answered, initially with full-length antisense followed by antisense oligonucleotides, then short hairpin RNA or small interfering RNA vectors, and most recently, by other gene ablation “knock-out” approaches (2). For example, a recent publication by Ravi et al. (30) shows that HCT116 human colon carcinoma xenografts lacking XIAP are highly sensitive to immune attack in mice compared with cells either containing XIAP or lacking other apoptotic pathway genes.

Advances in second-generation chemistry have made antisense a tractable route to therapeutic development. Indeed, a XIAP antisense compound (AEG35156) has been in the clinic for the past 2 years (2). The much anticipated results from these and future trials will establish if XIAP is a bona fide drug target, or not, and perhaps lead the way forward to the development of novel small-molecule anti-XIAP compounds.

Inhibitors of translation. XIAP protein levels are primarily influenced through translation control and protein stability, mediated by the ubiquitin-proteasome pathways. The XIAP mRNA transcript is quite stable, almost ubiquitous and unchanging. Thus, one strategic approach is to develop inhibitors of XIAP translation to block the expression of XIAP.

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Fig. 1. XIAP is central to the regulation of apoptosis. Pathways triggered by death receptor (e.g., tumor necrosis factor – related apoptosis-inducing ligand mediated) and mitochondria-mediated pathway converge at the execution phase of apoptosis. The caspase-binding and caspase-inhibiting activity of XIAP resides in the NH2-terminal BIR domains that specifically binds and inhibits the initiator caspase-9 as well as the effector caspase-3. The anti-caspase activity of XIAP can be blocked by Smac/DIABLO (and also Omi and XAF1). The E3 ubiquitin ligase activity of XIAP is involved in autoubiquitination and self-destruction as well as the removal of caspase-3 and Smac.

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CCR Molecular Pathways

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in cancer cells under stress. This approach has merit, as searches for inhibitors of the Hepatitis C viral internal ribosome entry site continue, although without success to date. A greater understanding of these internal ribosome entry site–based translational control mechanisms is needed to identify truly tractable targets. Nonetheless, a recent report by Yan et al. (31) identify the mammalian target of rapamycin inhibitor rapamycin as preferentially and significantly inhibiting the translation of XIAP as substantiated by a decreased polyosomal association of the XIAP mRNA. Such studies hold promise that specific and useful small-molecule inhibitors can be found. An alternative to the small-molecule approach is the use of a morpholino antisense oligonucleotide targeting the ATG (32) or a second-generation antisense targeting the region just upstream of the ATG (33). In addition, small interfering RNA targeting the 3′ untranslated region can potentially interfere with translation or deadenylate and destabilize the message (34, 35).

**Disruptors of caspase-3 binding.** Because the major antiapoptotic action of XIAP is through inhibition of caspase-3 and caspase-7 (via the BIR1-2 linker) and caspase-9 (via BIR3), chemical screen of compounds that disrupt the interaction between caspase-3 and XIAP have been undertaken. One such approach has led to the identification of a novel class of polyphenylurea compounds that antagonizes the ability of XIAP to inhibit caspase-3 (36, 37). The best candidates in this class (e.g., antagonist 1396-34; ref. 36) show a good therapeutic index with apoptosis induction in a select group of cancer cell lines. Furthermore, antitumor effects in vivo have been shown (36). Although therapeutic compounds that disrupt protein–protein interactions may never, in fact, translate to the clinic, they represent important proof-of-concept for the testing of many important questions regarding XIAP function, biology, and antagonism.

**Disruptors of caspase-9 binding (Smac peptidomimetics).** The discovery of the second mitochondrial activator of caspases, Smac/DIABLO (the first activator being cytochrome c) as an antagonist of XIAP showed the importance of this particular pathway in programmed cell death (22, 23). Smac is also the "competence to die" factor required for the death of postmitotic cells for which cytochrome c, by itself, is insufficient (38). Cocrystal structures of XIAP and Smac revealed that a simple IAP-binding motif of four NH2-terminal amino acid residues, AVPI, was sufficient to bind to a conserved groove on XIAP BIR3, a site that was also shown to be important for caspase-9 inhibition (39). Simple tetrapeptides consisting of natural or non-natural amino acids, built around this IAP-binding motif sequence, were capable of displacing caspase-9 from XIAP and of activating the caspase cascade (27), a finding that provided the rationale for the design of small-molecule peptidomimetics as specific drug candidates. This class of Smac mimetics has drawn the most interest thus far, with many development efforts under way (27, 28). It remains to be proven if compounds that selectively disrupt protein–protein interactions can translate successfully to the clinic and then to the market. However, advances being made with other disruptor compounds, such as Nutlin-3 that targets p53-MDM2 interaction (40), however, offer promise that such approaches are viable.

**Destabilizers of XIAP protein half-life.** The various Akt kinase inhibitors under development may derive their benefit in part by destabilizing XIAP, leaving the hypophosphorylated XIAP protein more susceptible to ubiquitination-mediated degradation (10). Alternatively, compounds that directly modulate the ubiquitin-proteasomal pathways may be useful, although it is unclear how E3 ligase inhibitors, for example, may affect cancer cell viability. Presumably, they would block the antiapoptotic action of XIAP RING domain that would function to ubiquinate key targets, such as caspase-3 and Smac, and potentially also destroy the ability of the cell to remove XIAP when cell death is induced. Nevertheless, the identification and characterization of E3 ligase inhibitors remains crucial to the understanding of apoptosis inhibition in cancer and to the development of novel therapeutic strategies aimed at overcoming apoptotic resistance.

**Nonspecific inhibitors: XIAP loss, cause, or consequence?** All compounds that claim to be XIAP antagonists are not necessarily direct XIAP inhibitors. For example, phenoxodiol may represent one such compound (41) for which no evidence exists for a specific mechanism of XIAP antagonism. Like so many other cytotoxic compounds, phenoxodiol treatment of
cancer cells results in the loss of XIAP protein. Thus, it is difficult to discern if phenoxodiol treatment results in the direct loss of XIAP or if XIAP protein reduction is an indirect consequence of death. Most likely, the effects of these nonspecific inhibitors of XIAP are mediated through some other targets or pathways that ultimately trigger cell death and the degradation of XIAP.

Gene therapy, peptide, and protein therapy. Other forms of therapy, different than small molecules and antisense oligonucleotides, may be available in the future. These forms would include gene therapy approaches at delivering XIAP antagonists, such as Smac or XIAP-associated factor 1, to tumor cells. Peptide or protein therapy approaches using transducing constructs involving AVP1 or processed Smac polypeptides have already shown merit in preclinical models of cancer. For example, mice bearing gliomas in the central nervous system were cured with a coadministration of Tat-Smac peptides and tumor necrosis factor–related apoptosis-inducing ligand but were not by either agent alone (42). Surely, many other forms of therapy may be envisaged in the future, for which we are only just beginning to understand.

The Path Forward

Over the last decade, numerous studies have revealed that XIAP is a central regulator of caspase activity and a crucial factor in the execution of cell death. Various strategies are under development seeking to take advantage of the key role of XIAP in programmed cell death. First and foremost, the current approach using XIAP antisense has offered an informative and quick route to the clinic. Hopefully, these initial trials will usher in a new era of therapeutics aimed at inducing cell death in a malignant population of cells while sparing normal cells. These and other specific XIAP antagonists could be used alone, or in combination with a conventional chemotherapy or radiotherapy. Clearly, we will enhance our understanding of cancer treatment in the clinic through the chemical modulation of XIAP and the associated apoptosis pathways.

References


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