X-linked Inhibitor of Apoptosis: A Chemoresistance Factor or a Hollow Promise

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Abstract

The X-linked inhibitor of apoptosis (XIAP) is the only cellular protein that has evolved to potently inhibit the enzymatic activity of mammalian caspases and promotes resistance to apoptosis. Given its role in apoptosis and its frequently elevated expression in malignant cells, XIAP has garnered the most attention as a promising therapeutic target in cancer to overcome drug resistance. Accordingly, XIAP is thought to render tumor cells resistant to chemotherapy through its ability to inhibit caspases, and it is on this basis that XIAP has been proposed as an important adverse biomarker for chemoresistance in cancer patients. Here, the current understanding of the role of XIAP in cancer is reviewed. Further, the notion is explored that the elevated XIAP expression frequently observed in malignant tissues is, at least, not exclusively responsible for the resistance of tumor cells to conventional therapeutic treatment; rather, the function of XIAP seems to be conducive to the process of malignant transformation and/or progression.

Background

Disease progression due to chemoresistance is a frequent complication in cancer treatment. Although various forms of chemotherapy have diverse biochemical targets, it is generally believed that chemotherapy kills cancer cells by induction of a final common pathway that leads to apoptosis. Indeed, a variety of studies have shown that apoptosis is a frequent outcome of effective therapy. Conversely, when apoptosis is inhibited, damaged cells that ordinarily would be eliminated, instead, accumulate and form aggressive tumors. Thus, understanding the mechanism of apoptotic malfunction in chemoresistant tumors may help to advance cancer drug development and, ultimately, improve patient outcome (1).

In particular, alterations in the expression of components of the apoptotic machinery contribute to the chemoresistance phenotype of many tumors. Molecular screening programs and in vitro analyses of putative factors, combined with a better understanding of drug action, have helped to identify potential biomarkers for chemoresistance. It is anticipated that, on the basis of these biomarkers, the most suitable therapeutic option for individual patients can be selected to further improve clinical outcome.

Apoptosis is brought about by a family of proteases known as the caspases, the activity of which is responsible for the organized destruction of the cell. The regulatory mechanisms of caspase cascade activation require, in general, activation of the zymogens of initiator caspases (caspase-8 or -9), which, in turn, cleave and activate the zymogens of executioner caspases (caspases-3 and -7). The activated executioner caspases are responsible for the majority of proteolytic events that ultimately result in the destruction of the cell. Each step of the apoptotic signaling cascade is under stringent control. Apoptotic signaling can either be regulated at the apical point of the apoptotic cascade by controlling the translation of death-inducing signals into proteolytic activation of caspases, or more critically, by direct modulation of the proteolytic activity of caspases. The latter involves the direct interaction of caspases with the X-linked inhibitor of apoptosis protein (XIAP; ref. 2).

XIAP is the only cellular protein that has evolved to potently inhibit the enzymatic activity of mammalian caspases at both the initiation phase (caspase-9) and the execution phase (caspase-3 and -7) of apoptosis (Fig. 1; refs. 3, 4). Given its role in apoptosis and its frequently elevated expression in malignant cells, XIAP has garnered the most attention as a promising therapeutic target in cancer (5). At odds with these findings, however, XIAP expression in some tumors has been shown to be associated with a favorable clinical outcome, and accumulating evidence suggests additional cellular functions for XIAP other than the regulation of apoptosis. In view of these findings,
even its name “X-linked inhibitor of apoptosis” has been called into question, as it becomes increasingly clear that this does not reflect the true nature of XIAP’s normal biological activities (6).

Discovery of the mammalian cellular inhibitors of apoptosis
The baculovirus inhibitors of apoptosis proteins (IAP) Cp-IAP and Op-IAP were the first members of IAP family...
to be identified in 1993, on the basis of their ability to functionally complement the cell-death inhibitor p35 in mutant viruses (lacking p35; refs. 7, 8). These IAPs were originally shown to block apoptosis induced by the RNA synthesis inhibitors actinomycin D, amanitin, 5,6-dichloro-1-11-d-ribofuranosylbenzimidazole (DRB), or Autographa californica nuclear polyhedrosis virus (AcMNPV) infection. The common structural feature of these IAPs is a motif termed the baculovirus IAP repeat (BIR), which is required for their cytoprotective function. Subsequently, proteins containing BIR domains have been identified in a wide range of eukaryotic species, including yeast, nematode, fruit fly, and several mammalian species, including mice, rats, chickens, pigs, and humans (9). The first human IAP, the neuronal apoptosis inhibitory protein (NAIP), also called BIRC1, was found to be involved in the neurodegenerative disorder spinal muscular atrophy (SMA; ref. 10), in which it blocks cell death in response to treatment with menadione and tumor necrosis factor (TNF; ref. 11). Further human IAPs, including cIAP-1/2, hIAP-2/hIAP-BIRC2, cIAP-2/HIAP-1/hIAPC/BIRC3, XIAP/hIAP/MIIA/BIRC4, survivin/BIRC5, and ML-IAP, were subsequently identified by DNA database searches and examined for their anti-apoptotic capacity (4).

Several IAP family members have been shown to regulate apoptosis in response to various cellular assaults, and it was assumed that direct caspase inhibition is an important conserved function of most family members. However, detailed biochemical and structural studies have mapped the elements of IAPs required for caspase inhibition (4), showing that these elements are not conserved among IAPs, and suggesting that XIAP is probably the only bona fide cellular caspase inhibitor.

The most intensively studied IAP family member, XIAP, is a 57-kDa protein that seems to be ubiquitously expressed in adult and fetal tissues (12). XIAP consists of three BIR domains (BIR 1-3), a RING-finger domain conferring ubiquitin protein ligase (E3) activity, and a recently discovered evolutionarily conserved ubiquitin-binding (UBA) domain (13, 14). XIAP overexpression blocks apoptosis, and subsequent biochemical investigations identified the BIR2 and BIR3 domains as the responsible caspase-inhibitory elements of XIAP (3, 4, 15). Both BIR domains use a two-site binding mechanism for potent caspase inhibition. One of these sites, defined as the IAP-binding motif (IBM)-interacting groove, is a conserved surface groove found in BIR2 and BIR3 of XIAP.

On activation, caspases (caspase-3, caspase-7, and caspase-9) are proteolytically processed, as a result of either autocatalytic processing or the activity of upstream proteases. The newly generated amino terminus of the small subunit of caspases constitutes an IBM, which is bound by the BIR domains of XIAP. The inhibitory action of XIAP, in turn, is regulated by several mitochondrial IBM-containing proteins, including second mitochondria-derived activator of caspases/direct IAP binding protein with low pi (SMAC/DIABLO) and OMI/HTRA2 (16). In intact cells, SMAC and OMI are mitochondrial proteins that are released after mitochondrial outer membrane permeabilization during apoptosis. Once in the cytosol, they bind to XIAP and relieve caspases.

**Clinical-Translational Advances**

From the discovery of XIAP in the second half of the 1990s, research on this unique IAP has been exponential, giving us a detailed structural and mechanistic view of its function. As a result, XIAP has been considered a promising therapeutic target in mammalian cancer, and research efforts have lately been focusing on the development of drugs targeting XIAP (IAP inhibitors), as a new way to counteract cancer and overcome drug resistance (5, 17). Two broad approaches have been taken to develop clinical inhibitors of XIAP, including antisense oligonucleotides diminishing XIAP expression and small molecule inhibitors antagonizing XIAP function (caspase binding and/or inhibition), which are currently being evaluated in preclinical and clinical phase I-II studies (18). In particular, the feasibility of disrupting IAP protein interactions with SMAC-derived peptidomimetic compounds (SMAC mimetics) has been broadly established in recent years (more than 50 patents and patent applications pertaining to IAP antagonists have been published over the past 10 years; ref. 19). Four such compounds have entered or been approved to enter human clinical trials, which will hopefully allow the utility of this potential therapeutic approach to be evaluated in cancer patients.

A crucial step in validating a cancer biomarker is to show, by robust statistical analyses, that the expression level of the target influences patient outcomes (20). In the case of XIAP, studies assessing its prognostic or predictive importance in tumor patients have been inconsistent for different malignancies, so that its value as a cancer biomarker remains unclear.

**Impact of X-linked inhibitor of apoptosis protein on chemoresistance**

*X-linked inhibitor of apoptosis protein and chemoresistance, the convergence.* On the basis of its ability to inhibit caspases, XIAP has been described repeatedly as a chemoresistance factor in mammalian cancer. Indeed, this is supported by several lines of evidence.

First, the initial investigations addressing the anti-apoptotic activity of XIAP showed that XIAP overexpression conferred resistance to a variety of cytotoxic insults, including serum withdrawal (11), Sindbis virus–induced cell death (21), overexpression of proapoptotic molecules such as caspase-1 (15), Bax (3), and caspase-2 or -3, as well as γ-irradiation (22). In the following years, accumulating evidence showed that XIAP inhibits cell death induced by death receptors, including CD95, TNF, and TNF-related apoptosis-inducing ligand (TRAIL), granzyme B, anoikis, UV
Impact of XIAP on Tumor Chemoresistance

and ionizing radiation, and a variety of chemotherapeutic agents (23–31).

Second, elevated XIAP expression was reported in a
diversity of human cancers (5), and was found to be associated
with adverse tumor histology and decreased patient sur-
vival (32–34).

Finally, one of the central pieces of evidence validating
XIAP as a chemoresistance factor was the observation that
XIAP targeting markedly enhanced the cytotoxic activity of
different cytostatic drugs in various tumor types. Specif-
ically, small molecules targeting XIAP, which were primarily
designed to relieve XIAP-mediated caspase inhibition or
specific down-regulation of XIAP expression by RNA inter-
fERENCE or antisense oligonucleotides, induced cell death
directly or synergistically with chemotherapeutic agents
in tumor cell lines, in tumor xenograft models and in clin-
ical phase I-II trials (17–19).

These observations clearly supported a role of XIAP in
apoptosis resistance and suggested that elevated expres-
sion of XIAP was responsible for tumor chemoresistance.

X-linked inhibitor of apoptosis protein and chemoresistance,
the divergence. Early studies investigated the role of XIAP
in mammalian cancer, and the initial presumption was
that XIAP renders tumor cells resistant toward chemother-
apeutic agents; however, a rapidly growing body of evidence
disagrees against this.

First, our view of XIAP as a chemoresistance factor is
derived from structural and biochemical studies employ-
ing cell-free systems and recombinant proteins, yeast, and
transient-overexpression studies of XIAP at levels far in
excess of physiological concentrations (6). Furthermore,
as is frequently the case with in vitro recapitulation of bi-
ological systems, XIAP-mediated anti-apoptosis seems to be
a highly trigger- and cell type–specific phenomenon;
what was observed in one system did not always hold
true for another. For example, XIAP overexpression in
one study induced resistance against serum withdrawal
but not staurosporine (11); in another study, it inhibited
γ-irradiation– but not serum withdrawal–induced cell
death (22). Others showed that XIAP overexpression in-
hibited cell death mediated by overexpression of caspase-
1 but not overexpression of FADD (15), blockade of
CD95– but not etoposide-induced cell death (35), and
inhibition of UV-induced but not serum withdrawal–
induced cell death (28).

Clearly, these initial studies do not reflect the impact
of elevated long-term expression of XIAP in malignant
tissues under physiological conditions. Indeed, these
studies were largely focused on investigating the role of
elevated XIAP expression on tumor chemoresistance. Al-
though XIAP is a prosurvival and/or antideath factor, few
published studies are dedicated to the analysis of cell
lines stably expressing exogenous XIAP to resolve this
issue (35–39). Indeed, we and others (35, 39) have shown
that, in long-term expression systems stably overexpres-
sing XIAP at concentrations comparable to that in tumor
cells (two to five fold), XIAP does not provide protection
against cytostatic agents, such as doxorubicin, etoposide,
mitoxantrone, vincristine, and vincristine, routinely used
in the treatment of cancer. Even more surprising, recent
work showed that overexpression of XIAP induces apo-
ptosis by promoting mitochondrial outer membrane per-
meabilization (40). Furthermore, data obtained from the
few in vivo animal models for studying the physiologic
relevance of elevated XIAP expression in cancer failed to
support a role of XIAP in tumor chemoresistance. Ap-
parently, overexpression of XIAP in nonmalignant tissues
interferes with certain death-signaling cascades. For ex-
ample, T-cell–specific overexpression of XIAP resulted in the
accumulation of thymocytes and/or T cells in primary
and secondary lymphoid tissue and resistance to anti-
Fas– and/or dexamethasone-induced apoptosis (41); and
neuronal overexpression of XIAP promoted resis-
tance to brain injury caused by transient forebrain isch-
emia after occlusion of the middle cerebral artery (42),
protecting the neonatal brain against hypoxia ischemia
(43). Overexpression of XIAP in nigrostriatal dopaminer-
gic neurons promoted resistance to the damaging effects
of the dopaminergic neurotoxin MPTP (44); and overexp-
ression of XIAP slowed age-related hearing loss in
C57BL/6 mice (45).

Second, studies assessing the prognostic or predictive
importance of XIAP in patients with malignancy pro-
duced inconsistent results. Whereas in patients with leu-
kemia (46) and cervical cancer (47), XIAP expression did
not correlate with apoptotic and proliferative parameters,
disease stage, or patient survival, several recent reports
completely at odds with the presumed anti-apoptotic ac-
tivity of XIAP showed a direct association between XIAP
expression and favorable clinical outcome in patients
with radically resected non–small cell lung carcinoma
(48), prostate cancer (49), and mouse prostate cancer
model (50).

Finally, XIAP antagonists were intended to de-repress
XIAP-mediated inhibition of caspase activity and to reac-
tivate apoptosis in cancer cells. However, several groups
have quite unexpectedly shown that the cytotoxic activity
of these XIAP-antagonizing compounds in cancer cells is
actually a consequence of casPA1 and casPA2 degradation
and alteration of TNF-mediated signaling (NFκB activity),
rather than being due to functional XIAP inhibition (51–
56). Further striking evidence showed that the enhanced
chemosusceptibility induced by these compounds also
occurred in a TNF-α–dependent manner (57, 58). How-
XIAP is involved in TNF-induced signaling is a matter
of controversy, and other evidence suggests that XIAP is
a central determinant of apoptosis induced by other TNF-
receptor family members, including TRAIL (59–62) and
CD95 (59, 63). Together, these data implicate XIAP as
an important factor in controlling receptor-induced cell-
death cascades rather than as a chemoresistance factor.
Furthermore, in view of previous data, it becomes obvi-
ous that by targeting XIAP, the apoptotic threshold to an
array of chemotherapeutics can be lowered; but, a com-
plete restoration of the apoptotic machinery by XIAP tar-
geting has not been shown or achieved.
Overall, these findings suggest that alternative mechanisms of apoptosis resistance, beyond elevated XIAP expression, are responsible for chemoresistance in tumors. The defective mitochondrial apoptotic pathway is one of the key characteristics of chemoresistant tumor cells, resulting in the failure to release mitochondrial cytochrome c/SMAC and impaired caspase activation. Accordingly, XIAP targeting reproduces the effect of cytosolic SMAC, and thus only in part, restores the mitochondrial proapoptotic function “the regulation of caspase activation,” but not its initiation. In contrast to chemotherapeutic drugs, death receptor–induced apoptosis does not primarily involve mitochondrial cytochrome c to initiate the proteolytic activity of caspases. Under these conditions, XIAP targeting results in complete reactivation of the apoptotic machinery, regardless of mitochondrial functional state.

X-linked inhibitor of apoptosis protein, more than a chemoresistance factor

One important point should be considered in trying to reconcile the observed discrepancies of the role and the impact of XIAP in cancer. Although early studies mainly dealt with the characterization of XIAP in apoptosis, recent evidence suggests that XIAP has important roles in a diverse set of nonapoptotic signaling pathways, including NF-κB, MAP kinase, and the ubiquitin proteosome pathways, and in modulating a variety of cellular functions including immune regulation, cell division and differentiation, cell migration, morphogenesis, and heavy metal metabolism (6), which may, in fact, prove to be more important during normal physiologic processes than previously anticipated. Thus, alterations in expression of XIAP result in cellular malfunctions in response to several intrinsic and environmental cues, which can also contribute to disease progression. In particular, XIAP seems to be a potent regulator of lymphocyte homeostasis, as supported by the finding that XIAP deficiency in humans causes an X-linked lymphoproliferative syndrome (64, 65). Whether the anti-apoptotic function of XIAP or its contribution to nonapoptotic signaling cascades have an impact on lymphocyte homeostasis is still not known. Presumably, elevated XIAP expression alters intra- and intercellular cross-talk, resulting in malfunction of surveillance mechanisms governing normal cell proliferation and tissue homeostasis, which culminate in malignant progression. This notion is in line with the observations that XIAP is an important factor in controlling TNF receptor family–induced apoptotic and nonapoptotic signaling, which physiologically ensures tissue homeostasis.

Conclusion

Taken together, elevated XIAP expression in malignant tissues is, at least, not exclusively responsible for the resistance of tumor cells to conventional therapeutic treatments, rather, it seems to be conducive to the process of malignant transformation and/or progression. Furthermore, although elevated XIAP expression has been found to be tightly associated with the malignant phenotype, the underlying molecular mechanisms giving rise to XIAP accumulation in malignant tissues is still unresolved. The challenge now is to unravel the molecular mechanisms supporting XIAP accumulation in malignant cells and to establish appropriate animal models to address the physiologic role of elevated XIAP expression during the course of malignant transformation.

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