Molecular Pathways

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 thezymogens of initiator caspases (caspase-8 or -9), which, in turn, cleave and activate thezymogens 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,
Fig. 1. 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. Broadly divided, there are two major apoptotic pathways. The extrinsic pathway is a sensor for extracellular signals, mainly emitted by immune-effector cells including cytolytic T lymphocytes (CTL) and natural killer (NK) cells, and ensures tissue homeostasis and immune surveillance. Immune-effector cells eliminate target cells either by engaging the death receptors (TNF membrane-receptor family members) and activating the initiator caspase, caspase-8, through formation of the death-inducing signaling complex (DISC), or by calcium-dependent exocytosis of granules containing perforin and granzymes. The intrinsic pathway senses intracellular damage and promotes the release of mitochondrial proapoptotic factors like cytochrome c, and activates the initiator caspase, caspase-9. Although activated by different cues, these initiator proteases (caspase-8, granzyme B, and caspase-9) cleave and activate the zymogens of executioner caspases like caspase-3 and -7. However, the initial processing of executioner procaspases by initiator caspases alone is insufficient to induce the level of executioner caspase activity required for apoptosis. To reach the critical level of executioner caspases, the release of additional proapoptotic factors, such as SMAC or Omi from the mitochondria, is necessary to displace XIAP from partially processed caspases, thus completing caspase auto-activation.

Impact of XIAP on Tumor Chemoresistance

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/HIAP-2/hMIIH/BIRC2, cIAP-2/HiAP-1/hMIIHC/BIRC3, XIAP/hILP/MIHA/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...
and ionizing radiation, and a variety of chemotherapeutic agents (23–31).

Second, elevated XIAP expression was reported in a variety of human cancers (5), and was found to be associated with adverse tumor histology and decreased patient survival (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. Specifically, small molecules targeting XIAP, which were primarily designed to relieve XIAP-mediated caspase inhibition or specific down-regulation of XIAP expression by RNA interference or antisense oligonucleotides, induced cell death directly or synergistically with chemotherapeutic agents in tumor cell lines, in tumor xenograft models and in clinical phase I–II trials (17–19).

These observations clearly supported a role of XIAP in apoptosis resistance and suggested that elevated expression 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 chemotherapeutic agents; however, a rapidly growing body of evidence argues against this.

First, our view of XIAP as a chemoresistance factor is derived from structural and biochemical studies employing 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 biological systems, XIAP-mediated anti-apoptosis seems to be a highly trigger-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 inhibited 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. Although 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 overexpressing 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, vinblastine, and vincristine, routinely used in the treatment of cancer. Even more surprising, recent work showed that overexpression of XIAP induces apoptosis by promoting mitochondrial outer membrane permeabilization (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. Apparently, overexpression of XIAP in nonmalignant tissues interferes with certain death-signaling cascades. For example, 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 resistance to brain injury caused by transient forebrain ischemia after occlusion of the middle cerebral artery (42), protecting the neonatal brain against hypoxia ischemia (43). Overexpression of XIAP in nigrostriatal dopaminergic neurons promoted resistance to the damaging effects of the dopaminergic neurotoxin MPTP (44); and overexpression 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 produced inconsistent results. Whereas in patients with leukemia (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 activity 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 re-activate 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 cIAP1 and cIAP2 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 obvious that by targeting XIAP, the apoptotic threshold to an array of chemotherapeutics can be lowered; but, a complete restoration of the apoptotic machinery by XIAP targeting 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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