Point of VIEW

ACS chemical biology

Inhibitor of Apoptosis (IAP) Proteins: Novel Insights into the Cancer-Relevant Targets for Cell Death Induction

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ABSTRACT Inhibitor of apoptosis (IAP) proteins are expressed at high levels in various cancers and block apoptosis at a key node. Therefore, there is currently much interest in IAP antagonists as cancer therapeutics. Structure-based development of selective IAP inhibitors now provides, for the first time, evidence that simultaneous inhibition of the c-IAP proteins and XIAP is necessary for efficient induction of cancer cell death by IAP antagonists.

Apoptosis is one of the most common forms of cell death in eukaryotes, and evasion of apoptosis is a hallmark of cancer. The inhibitor of apoptosis (IAP) family of proteins consists of eight human analogues, including cellular IAP1 (cIAP1), cellular IAP2 (cIAP2), and X-linked inhibitor of apoptosis (XIAP) (*1*). All IAP proteins contain at least one baculoviral IAP repeat (BIR) domain, a 70−80 amino acid long motif that relates to the initial discovery of these proteins in baculovirus, and may harbor one or more additional functional domains, for example, the really interesting new gene (RING) domain, which possesses E3 ubiquitin ligase activity, and the caspase activating and recruitment domain (CARD) domain (*1*).

In an article in this issue, Ndubaku *et al.* (*2*) developed IAP inhibitors with high affinities and selectivity for c-IAP1 over XIAP *via* rational structure-based design. The selectivity for c-IAP1 and c-IAP2 over XIAP is achieved by the following two elements: first, a methyl group serves to interact favorably with the residue Phe330 of the BIR3 domain of c-IAP1, while negatively with the Tyr324 residue of the BIR3 domain of XIAP; second, a pyrimidine ring in the P4 position of the compound causes electronic and steric repulsion with the BIR3 domain of XIAP, whereas it fits in the P4 pocket of the BIR3 domain of c-IAP. Interestingly, the pan-selective inhibitor, which simultaneously targets c-IAP1, c-IAP2, and XIAP, demonstrates in cell-based studies a significantly higher potency than the c-IAP-selective inhibitor to trigger apoptosis, to activate effector caspase-3 and -7, and to suppress long-term survival. Mechanistically, the induction of apoptosis was found to rely on TNFR1-mediated signaling and caspase activation in the case of both the pan-selective inhibitor and the c-IAP-selective IAP inhibitor. By comparison, pan-IAP- and c-IAP-selective inhibitors turn out to be similarly effective in causing degradation of c-IAPs as well as activation of canonical and non-canonical NF-κB signaling, resulting in comparable induction of NF-κB target gene expression. One can conclude from these results that neutralization of both XIAP and c-IAP proteins is necessary for potent induction of apoptosis and suppression of clonogenic growth, while antagonism of c-IAP proteins is sufficient to initiate proteasomal degradation of c-IAP proteins and NF-κB activation. The findings reflect the reported biological activities of XIAP, c-IAP1, and c-IAP2. To this end, it is in particular XIAP among the IAP family of proteins that exert an anti-apoptotic function *via* binding and inhibiting effector caspases such as caspase-3 and -7 (*3*). While c-IAP1 and c-IAP2 can bind to caspase-3 and -9, they have been reported not to inhibit these caspases (*4*). Recently, c-IAP1 and c-IAP2 have been shown to be involved in the control of caspase-8 activation upon TNFR1 stimulation and to play an important role in the regulation of

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Published online July 9, 2009
10.1021/cb900150g CCC: $40.75

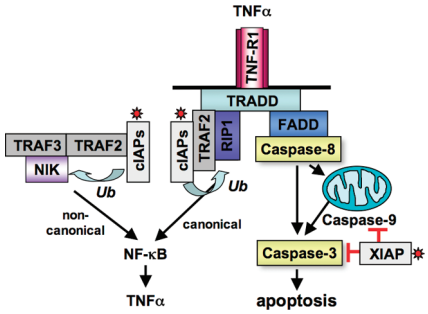

Figure 1. Regulation of apoptosis and NF-κB signaling by IAP proteins and IAP antagonists. Neutralization of XIAP-imposed inhibition of caspase-3 and -9 by IAP antagonists promotes caspase-dependent apoptosis. c-IAPs regulate activation of canonical and non-canonical NF-κB pathways. In the canonical pathway, TRAF2 and RIP1 are substrates for c-IAPs-mediated ubiquitination and proteasomal degradation. In the non-canonical pathway, c-IAPs ubiquitinate NIK and cause its proteasomal degradation, thus preventing processing of p100 and NF-κB activation. IAP antagonists trigger activation of the canonical NF-κB pathway *via* autoubiquitination and proteasomal degradation of c-IAPs, while stimulation of non-canonical NF-κB signaling is initiated by stabilization of NIK. This leads to induction of NF-κB target genes such as TNFα, which in turn can engage TNFR1-mediated caspase-8 activation and apoptosis in an autocrine/paracrine manner. Stars marks IAP antagonists and their targets. Please see text for more details.

canonical and non-canonical NF-κB signaling, at least in part due to their auto- and heteroubiquitination activity (Figure 1) (*5*–*9*).

The novelty of this study in particular resides in the demonstration that concomitant targeting of several IAP proteins by small molecule inhibitors, namely, XIAP, c-IAP1, and c-IAP2, is superior over selective antagonism of c-IAP proteins for inducing apoptotic cell death in cancer cells. Since small molecule IAP inhibitors that were disclosed so far generally show broad activity against several IAPs (*1*), the relative importance of c-IAP over XIAP antagonism for apoptosis induction in cancer cells has remained obscure. The development of compounds that specifically target individual IAP proteins has now provided the tools to address this important question.

The implications of this study are manifold. From the perspective of a chemist, the identification of relevant structural elements within XIAP and c-IAPs that can be exploited for the design of IAP-selective compounds will likely stimulate the design of a series of novel, selective IAP antagonists. From a biologist's point of view, the demonstration that targeting of c-IAPs as well as XIAP is required for effective induction of apoptosis and, importantly, also for potent suppression of long-term survival has important implications for the development of experimental strategies to target IAP proteins in human cancers. Beyond cancer, these inhibitors present valuable tools to dissect the contribution of individual IAP proteins in other physiological or pathological conditions, for example, in innate immunity signaling (*10*).

The work by Ndubaku *et al.* also raises a number of questions. First and foremost, it will be critical for the future clinical development of IAP inhibitors as cancer therapeutics to explore whether the higher antitumor activity of pan-IAP antagonists is also associated with higher toxicities on non-malignant normal cells, which may limit their clinical application. Will it be possible to maximize the potency of IAP antagonists, for example, through the design of broad-range inhibitors, without concomitantly increasing the toxicity on normal cells? Second, are pan-IAP antagonists also more potent compared to selective inhibitors in sensitizing cancer cells to the induction of apoptosis, for example, *via* chemotherapy or treatment with death receptor ligands such as TRAIL? Another question relates to possible cell-type-specific differences in the contribution of individual IAP proteins to the regulation of apoptosis and other signaling pathways, as key experiments in the present study were performed in a few cell lines. Furthermore, while the importance of c-IAPs over XIAP with respect to apoptosis induction, c-IAP stability, and NF-κB signaling has been examined in the study by Ndubaku *et al.*, the specific contribution of c-IAP1 compared to that of c-IAP2 has not been addressed, as the c-IAP-selective antagonist targets both c-IAP1 and c-IAP2. Finally, one might also envision circumstances where more selective inhibitors may offer advantages over pan-IAP antagonists, for example, in cancers with preferential upregulation of one of the IAP proteins.

By demonstrating that concomitant antagonism of the c-IAP proteins and XIAP is necessary for efficient induction of cancer cell death by IAP antagonists, the present study is expected to stimulate future work on the development of small molecule inhibitors to neutralize the IAP family of proteins and their therapeutic targeting in human cancers.

Acknowledgment: Work in the author's laboratory is supported by grants from the Deutsche Forschungsgemeinschaft, the Bundesministerium für Forschung und Technologie, the Deutsche Krebshilfe, the EU (ApopTrain, APO-SYS), the Wilhelm Sander Stiftung, the Else-Kröner-Fresenius Stiftung, the Novartis Stiftung für therapeutische Forschung, and IAP6/18.

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