Antagonizing XIAP-mediated caspase-3 inhibition: Achilles' heel of cancers?

In this issue of *Cancer Cell*, Schimmer et al. report the identification of small molecule antagonists of XIAP that overcome its inhibition of caspase-3. It was remarkable that the compounds directly induced cell death in tumor cells while having little toxicity on normal cells. This suggests that caspases are already activated in tumor cells, which is different from the caspase activation status in normal mammalian cells. In comparison with Smac peptides targeting XIAP-mediated caspase-9 inhibition, which do not directly induce cell death, it appears that liberating downstream caspases rather than upstream caspases may be a preferred strategy for cancer drug discovery.

Ever since the discovery of caspases as major executioners of apoptotic cell death, scientists have been contemplating whether caspases may be exploited to kill tumor cells. However, caspases are tightly regulated in many different ways. For example, they are synthesized as zymogens that require proteolytic processing and/or dimerization for activation. Upstream caspases are activated by apoptotic signaling cascades, which in turn proteolytically activate downstream caspases for the actual cellular destruction. In addition, once processed, caspases are subject to inhibition by endogenous inhibitors of apoptosis (IAPs). These and other complexities of caspase regulation make it difficult to identify specific and effective targets that are different between normal and tumor cells for inducing caspase activity differentially and potently.

To this rescue, X chromosome-linked IAP (XIAP), the best studied member of the IAP family, is differentially upregulated in many forms of human cancers, suggesting its potential as an anticancer target. To prevent cell death, XIAP directly binds to and inhibits the upstream caspase, caspase-9, and downstream caspases, caspase-3 and -7 (Figure 1). By doing so, it acts as a brake on caspase-mediated cellular destruction Structural and biochemical studies have shown that the linker proceeding the BIR2 domain of XIAP directly blocks the active sites of caspase-3 and caspase-7, while the BIR3 domain inhibits caspase-9 activity by sterically hindering its dimerization (Liston et al., 2003).

In the report by Schimmer et al. (2004), a caspase-3 derepression assay was used to screen a total of eleven mixture-based small molecule combinatorial libraries for compounds that relieve XIAP-mediated caspase-3 inhibition. At least eight active compounds were identified from a chemical library of 89,856 unique polyphenylureas. These compounds specifically reversed XIAP-mediated inhibition of caspase-3 and caspase-7, but not of caspase-9, and were shown to interact with the linker-BIR2 region of XIAP.

What was remarkable is that as single agents, these polyphenylurea compounds induced apoptosis of a broad range of solid tumor and leukemia cell lines in vitro, suppressed clonogenic survival of tumor cells, and were effective in tumor xenograft models. This is the first case in which reagents aiming to relieve caspase inhibition induced tumor cell death directly, in addition to their potentiation of cell death by chemotherapeutic agents and/or death-inducing cytokines. The kinetics of killing were rapid and the efficacy of killing compared favorably with two currently used anticancer drugs, etoposide and doxorubicin. In contrast, relatively little toxicity was exerted on normal cells.

These findings are highly significant not only in cancer treatment, but also in our understanding of differential caspase regulation in tumor and normal cells. The effectiveness of XIAP antagonists to directly induce tumor cell death suggests that caspases are already proteolytically processed in tumor cells but are kept in check by upregulated IAPs. This status of caspase activation in tumor cells is similar to the *Drosophila* cell death system, in which caspases are active but counterinhibited by IAPs, and relief of IAP-mediated caspase inhibition triggers cell death. Consistent with this analysis, recent data showed evidence of processed caspase-3 in tumor cell lines and tumor tissues (Yang et al., 2003a). For normal mammalian cells, however, it appears that the stimulation of caspase processing and activation by apoptotic

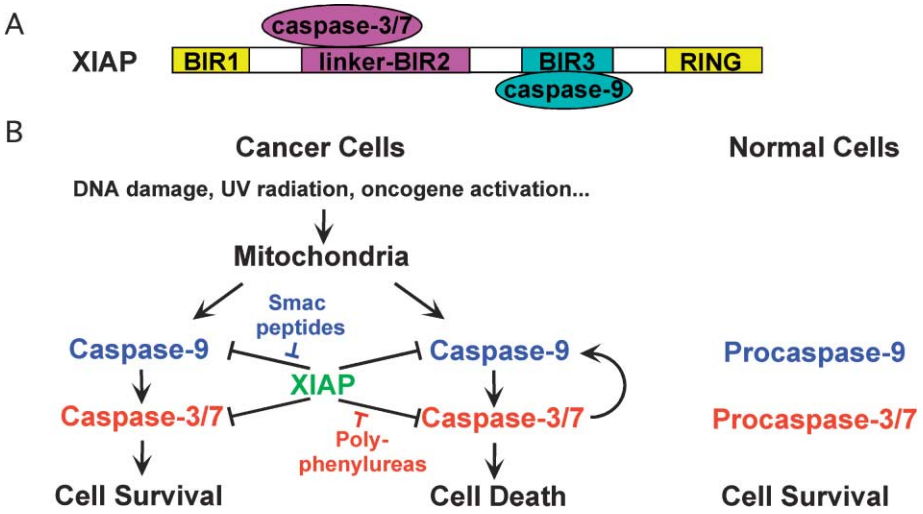

Figure 1. XIAP, caspases, and XIAP antagonists

A: Domain organization of XIAP, showing the regions responsible for caspase-3/7 and caspase-9 inhibition.

B: Differential caspase activation status in cancer and normal cells and effects of different XIAP antagonists, polyphenylureas, and Smac peptides.

stimuli, either through the mitochondrial pathway or the death receptor pathway, is the major trigger of cell death.

Therefore, there is a fundamental difference in the state of caspase activation between normal and tumor cells, which was exploited for the anticancer potency of these polyphenylurea XIAP antagonists. This difference may be rationalized by considering the presence of many more drives for caspase activation in tumor cells in comparison with normal cells (Figure 1).

However, this analysis does not explain why previously designed XIAP antagonists, which mimic the actions of some endogenous IAP antagonists, did not directly induce but only synergize tumor cell death when used together with chemotherapeutic agents (Fulda et al., 2002; Yang et al., 2003b). There are a number of endogenous IAP antagonists in mammals, including Smac/DIABLO, HtrA2/Omi, and XAF1 (Liston et al., 2003). Because both Smac and HtrA2 contain an N-terminal IAP binding motif for interacting with the BIR2 and BIR3 domains of XIAP, short cell permeable Smac peptides have been designed to relieve XIAP-mediated caspase inhibition. The Smac peptides/BIR3 interaction sterically excludes the caspase-9/BIR3 interaction to release caspase-9. On the other hand, the Smac peptides cannot remove caspase-3 from the grip of XIAP because they do not interact with the linker region of XIAP. In the context of the full-length Smac dimer, it is the simultaneous interaction with both the BIR2 and BIR3 domains of XIAP that creates the steric hindrance for the neighboring linker to release caspase-3 and -7 (Huang et al., 2003).

So, which aspect of the polyphenylurea compounds makes them capable of direct cell killing? Is it possible that the compounds, which have higher potency in comparison with the Smac peptides, simply relieve more caspases from XIAP inhibition or have a broader effect on other IAP family members to induce significant cell death? Is it possible that the compounds possess other activities, in addition to their role in antagonizing XIAP, such as stimulating the activity of the apoptosome in a way analogous to the small molecule PETCM (Jiang et al., 2003), or inhibiting more recently described NF-κB activation by XIAP (Hofer-Warbinek et al., 2000), or mimicking the action of another endogenous IAP antagonist XAF1 (Liston et al., 2003)?

Among various possibilities, it is perhaps the most intellectually satisfying to hypothesize that the differences in cell death induction between the polyphenylurea compounds and Smac peptides are due to their effects on different caspases (Figure 1). While the relief of caspase-3 and -7 from XIAP inhibition by the polyphenylurea compounds acts at the very distal end of the caspase cascade to induce direct cell death, the relief of caspase-9 inhibition by the Smac peptides does not necessarily stimulate the activity of downstream caspases if XIAP is still around to inhibit them. Furthermore, it appears that downstream caspase activation exerts a positive feedback for upstream caspase activation, because caspase-9 cleavage by caspase-3 has been shown to stimulate the apoptosome and caspase-9 activity (Zou et al., 2003), and the loss of caspase-3 inhibition has been shown to compromise the ability of XIAP to maintain inhibition of caspase-9 within the apoptosome (Bratton et al., 2002).

Interestingly, a completely different family of chemicals was identified from a similar high-throughput in vitro casapse-3 derepression assay (Wu et al., 2003). In a cellular assay, these compounds were able to synergize with death receptor stimulation to bypass the apoptosis block resulting from the loss of the pro-apoptotic Bcl-2 family member Bax. However, these compounds have not been tested against a broad array of tumor cells, and it would be important to determine if they too can induce direct cell death.

In any case, the current study appears to have revealed that there is a fundamental difference in the state of caspase activation in tumor and normal cells, and that relieving downstream rather than upstream caspases from XIAP inhibition is a preferred strategy for effective anticancer therapy. Therefore, removing inhibition of caspase-3 and -7 at the very distal end of the apoptotic cascade may be aiming at an Achilles' heel of cancers.

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