To the editor:

Survivin does not inhibit caspase-3 activity

Conway et al recently reported that alternatively spliced forms of mouse survivin exhibit different antiapoptotic properties. This was inferred from inhibition of recombinant caspase-3 catalytic activity using a standard chromogenic assay in the presence of increasing concentrations of mammalian-expressed murine survivin isoforms of 140, 121, and 40 amino acids, respectively. If confirmed, the ability of survivin to inhibit caspase-3 activity would have a major impact on targeting this ubiquitous cytoprotection pathway in cancer and during cell cycle progression. The data of survivin inhibition of caspase-3 activity presented by Conway et al raise serious concerns of specificity. First, the experiments contained no controls with genuine inhibitors of caspase-3, including DEVD-CHO or another IAP family protein with well-documented anti–caspase-3 activity (ie, XIAP). Second, from the data presented it was impossible to derive an inhibition constant (K_i) of the cleavage reaction, which is indispensable to quantitatively characterize potential caspase inhibitors. We have now reinvestigated the data presented by Conway et al and attempted to reproduce their caspase-3 inhibition experiments using mouse or human survivin proteins. Mouse recombinant survivin was expressed, purified to homogeneity, and properly folded by 1D-NMR analysis. Concentrations of recombinant mouse survivin up to 80 μmol/L failed to decrease purified, recombinant caspase-3 activity using a peptide substrate cleavage assay similar to that reported by Conway et al (Figure 1B). In contrast, 0.1 μmol/L XIAP completely inhibited caspase-3 activity, in agreement with previous data. In an attempt to reproduce exactly the mammalian cell expression approach used by Conway et al, we immunopurified native survivin from Jurkat T cells. Eluted fractions contained a single 16.5-kd survivin band by immunoblotting with an antibody to survivin (Figure 1C). But increasing concentrations of native, immunopurified survivin did not affect caspase-3 catalytic activity, as determined by substrate peptide cleavage (Figure 1D).

In conclusion, our data strongly argue against any role of survivin in directly inhibiting caspase-3 activity, at variance with the preliminary work of Conway et al. These discrepancies cannot be ascribed to species specificity, protein purification, or differences in experimental protocol. Moreover, available structural data demonstrate that a linker region upstream of the second baculovirus IAP repeat is required for docking IAP proteins (ie, XIAP) to active caspase-3. This linker region is absent in survivin, thus further weakening the hypothesis proposed by Conway et al. Therefore, the general conclusion of Conway et al that alternatively spliced isoforms of survivin have different antiapoptotic functions is not experimentally substantiated. The means by which survivin participates in the apoptosis balance in cancer and during cell cycle progression require further investigation.

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References

Figure 1. Survivin does not inhibit caspase-3 activity. (A) SDS gel electrophoresis of purified mouse recombinant survivin. (B) Effect of murine survivin or human XIAP on caspase-3 activity by hydrolysis of the fluorogenic substrate, DEVD-AMC. (C) Western blotting of immunoaffinity-purified human survivin isolated from Jurkat T cells by chromatography on α-survivin-sepharose. (D) Effect of native human survivin on caspase-3 activity by hydrolysis of the fluorogenic substrate, DEVD-AMC. W, whole cell extracts.
Response:

**Evidence that survivin inhibits caspase-3 activity**

Earlier this year, we provided a definitive report indicating that there exist at least 3 murine survivin mRNA variants, each encoding a distinct protein. We demonstrated, with appropriate controls, that both survivin140 and survivin121 are able to inhibit caspase-3 activity, while survivin120 does not.

In contrast to the claims of Altieri’s group in their letter, several reports support our finding that survivin interferes with caspase-3 activity. These include the following: Reed’s group reported that “survivin was able to substantially reduce caspase activity, as measured by cleavage of a tetrapeptide substrate, AspGluValAspaminofluorocoumarin. Similar results were obtained in intact cells when Survivin was overexpressed.” Altieri’s group noted in their Nature paper that “like other IAP proteins, survivin inhibits the terminal effectors caspase-3 and caspase-7.” Kobayashi et al demonstrated that overexpression of murine survivin in Rat1 cells inhibited caspase-induced cell death and also that a purified GST fusion protein encoding murine survivin could bind directly to caspase-3. This group further transfected Jurkat cells with epitope-tagged survivin and showed by immunoprecipitation with anti–caspase-3 antibodies that survivin “can bind efficiently to processed caspase 3.” In their Nature Cell Biology paper, Altieri’s group once again noted that survivin regulates apoptosis via caspase-3: “Expression of survivin (C84A) or survivin antisense cDNA also resulted in increased activity of the apoptosis effector caspase-3, as judged by hydrolysis of the fluorogenic caspase-3 substrate.”

Altieri’s group also performed studies on cultured endothelial cells and reported that “[r]ecombinant expression of green fluorescent protein survivin in endothelial cells reduced caspase-3 activity and counteracted apoptosis induced by tumor necrosis factor.” And finally, a recent report confirms that the down-regulation of survivin mRNA levels by an antisense approach results in increased caspase-3 activity.

We note that many of the data indicating that survivin interferes with caspase-3 activity predate our own and, in several cases, have actually been provided by Altieri’s group. We are not in a position to assess fully the experiments described by Altieri’s group and would point out only that they are not identical to our own, in contrast to what that group claims. In addition, we would add that we have observed that the activity of recombinant survivin is highly dependent on the method chosen for its synthesis and purification. Based on our experiments and the current published data, we believe that specific forms of survivin do inhibit caspase-3 activity. Nevertheless, we agree that the means by which survivin participates in the apoptosis balance requires further investigation, and we hypothesize that the alternatively spliced isoforms of survivin may modulate apoptosis differentially.

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To the editor:

**Another possible mechanism of resistance to STI571**

Le Coutre et al report the characterization of a cell line (LAMA48R) that expresses the oncogenic tyrosine kinase Bcr/Abl but is resistant to the tyrosine kinase inhibitor STI571. Those authors argue that this resistance is solely due to overexpression of the Bcr/Abl protein as a result of gene amplification.

An alternative explanation for this data is that some fraction of the Bcr/Abl in the resistant cell line has developed partial resistance to STI571 through a point mutation in the ATP binding region. To test this hypothesis, le Coutre et al amplified a BCR/ABL fragment from both cell lines, sequenced the product, and compared it to the known nucleotide sequence. For this comparison, those authors chose a stretch of 87 nucleotides (29 amino acids) that they refer to as the “ATP binding domain” of BCR/ABL. How this stretch of sequence was chosen is unclear, but what is certain is that it does not represent even the major fraction of the ATP binding region of BCR/ABL. The residues that directly contact ATP in a typical protein kinase span a region of 130 to 160 amino acids in primary sequence. Even this subset does not include many residues that do not directly contact ATP, but which when mutated could readily have structural effects that influence inhibitor sensitivity.

Resistance of Bcr/Abl to STI571 would presumably be indicated by reduced sensitivity to the drug in an in vitro kinase assay. Le Coutre et al found that both cell lines display sensitivity to STI571 but that “[d]iffering from Western blot experiments, the inhibition of kinase activity [in vitro] was never complete, even at 10 μM [concentration of STI571].” This result is inconsistent with the reported potency of STI571 against the Abl kinase in vitro (IC50 = 0.038 μM) and suggests...
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