Reviewer’s report

Title: Maspin overexpression induces tumor cell apoptosis through the modulation of Bcl2 family proteins

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Reviewer: Charles Tannenbaum

Reviewer’s report:

General
In their manuscript entitled “Maspin overexpression induces tumor cell apoptosis through the modulation of Bcl-2 family proteins”, authors Zhang et al attempt to demonstrate that maspin mediates its proapoptotic effects by enhancing the apoptogenicity of stimuli that act through the mitochondrial apoptotic pathway. Specifically, the authors contend that maspin overexpression during serum starvation of cells increases Bcl-2 destabilization and enhances Bax accumulation. RNA protection analyses are provided, ostensibly to support the notion that maspin acts post-transcriptionally to mediate these effects, and an experiment performed in the presence or absence of cyclohexamide is presented to demonstrate that maspin inhibits the translation of a protein capable of degrading Bax.

The manuscript is in fact timely and of interest, since it is known that maspin can act as a tumor suppressor, but its mechanism of action is yet undefined at the molecular level. A role for maspin in mediating its effects by modulating members of the Bcl-2 family is supported by a very recent article (Mol. Cell. Biol 25:1737-1748, 2005) indicating that maspin translocates to the mitochondria and is linked to the opening of the permeability transition pore. In the manuscript now submitted to BMC, the authors clearly show that they have overexpressed maspin mRNA and protein in two clones and that the protein is expressed both cytoplasmically and on the cell membrane. The authors additionally demonstrate that maspin overexpression sensitizes the cells to staurosporin-induced apoptosis (Fig 2). In the most significant experiments performed, the authors show that there is decreased Bcl-2 protein in both maspin-overexpressing cell lines deprived of serum, as compared to the serum-deprived control cells. This correlated with an inverse relationship with the proapoptotic protein Bax, which was expressed at high levels in the serum-deprived, maspin-overexpressing cells—but interestingly—in only ONE of the staurosporin treated, maspin-expressing lines (why the discrepancy?).

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Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

Major Compulsory revisions:

1) The authors demonstrate that maspin overexpression sensitizes the cells to staurosporin-induced apoptosis (Fig 2). In the most significant experiments performed, the authors show that there is decreased Bcl-2 protein in both maspin-overexpressing cell lines deprived of serum, as compared to the serum-deprived control cells. This correlated with an inverse relationship with the proapoptotic protein Bax, which was expressed at high levels in the serum-deprived, maspin-overexpressing cells—but interestingly—in only ONE of the staurosporin treated, maspin-expressing lines (why the discrepancy?).

2) Staurosporin is purported to induce apoptosis of numerous cell types—even those that don’t overexpress maspin. Why is it that only the maspin overexpressing cells are stimulated by
staurosporin to release cytochrome c in the experiment depicted in Figure 6?

3) The authors never explain why the caspase 8 inhibitor should protect against staurosporin-induced apoptosis, which they acknowledge mediates apoptosis through the mitochondrial pathway.

4) Figure 5 purports to show that maspin does not regulate Bcl-related proteins at the transcriptional level, but the gel presented seems to contradict this notion: Maspin-overexpressing line 18 seems to express more bfl-1, Bcl-xl (L) and Bcl-xl (s) than the other lines, and line 16 appears to overexpress more "bad" protein, data which don’t seem to be reflected in the adjacent, averaged bar graph. So while Bax and Bcl-2 are not transcriptionally regulated by maspin, some of these other proteins that can potentially modulate apoptosis appear to be transcriptionally regulated. Do these proteins have any relevance to maspin-mediated effects?

5) Figure 6 shows that a 1h treatment with staurosporin (followed by 4 h w/o cyclohexamide) induces the synthesis of a protein that can degrade Bax, and ostensibly protect the cell from apoptosis. Maspin overexpressing cells, however, apparently prevent the action of this protease, protecting Bax, permitting cytochrome c release and inducing apoptosis. The authors never address the issue of why a mere 1h of staurosporin, followed by immediate harvest, reduces Bax expression in the maspin overexpressing cells, as compared to the similarly treated control cells.

6) The authors should expand their discussion to take into account the new findings reported in Mol. Cell. Biol 25:1737-1748, 2005, indicating that maspin translocates to the mitochondria and is linked to the opening of the permeability transition pore.

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

Discretionary Revisions (which the author can choose to ignore)

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article of importance in its field

Quality of written English: Not suitable for publication unless extensively edited

Statistical review: No

Declaration of competing interests:

I declare that I have no competing interests.