Reviewer's report

Title: Inducing Apoptosis Effects and Its Molecular Mechanisms of BAK Gene Over-expression on Gastric cancer Cells

Version: 1 Date: 15 April 2004

Reviewer: Steven Grant

Reviewer's report:

General

In this manuscript, the authors examine the effects of transfecting gastric carcinoma cells with a plasmid encoding the pro-apoptotic protein Bak. They report that cells transfected with Bak exhibit diminished proliferative capacity, cell cycle arrest in G1, and enhanced apoptosis, manifested by morphological features, TUNEL positivity, and increased caspase-3 activation. The authors conclude that transfection of gastric cancer cells with Bak inhibitors their proliferation and increases their susceptibility to apoptosis. They also raise the possibility that transfection of tumor cells with Bak may represent a possible therapeutic strategy.

The results presented appear, for the most part, credible if somewhat as expected. The availability of a gastric cancer line overexpressing Bak could be a useful experimental tool. Having said this, the manuscript provides relatively few insights into the mechanism by which ectopic expression of Bax inhibits growth or triggers apoptosis. The following issues need to be addressed.

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

1. It is unclear from the results shown whether the diminished proliferation of cells transfected with Bak results from G1 arrest, induction of apoptosis, or a combination of the two. For example, the MTT assay reflects both growth inhibition and cell death. Some attempt should be made to resolve this issue.
2. Figure 3: The increase in Bak protein levels seems to be much more than three-fold. A tubulin or actin control is necessary to ensure equivalent loading and transfer.
3. Figure 4: The significance (or lack thereof) of differences for early time points (e.g., 1 or 3 days) is not described.
4. Figure 6: Histograms: Why is there no sub-diploid population corresponding to the apoptotic cell fraction?
5. The authors make a leap of faith to tie together Bak overexpression with activation of the effector caspase caspase-3. It would be useful to show increased mitochondrial injury (e.g., loss of mitochondrial membrane potential or cytochrome c release) in Bak overexpressors. Alternatively, enhanced activation/cleavage of procaspase-9 could be shown.

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

1. Abstract: Here and elsewhere, the authors use the term improved when increased should be substituted.
2. The authors refer to the cells as partial, when they presumably mean parietal

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 whose findings are important to those with closely related research interests

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

**Statistical review:** No

**Declaration of competing interests:**

none