Author's response to reviews

Title: BAK Overexpression Mediates p53-independent Apoptosis Inducing Effects on Human Gastric Cancer Cells

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PDF covering letter
Dear editors:

Please find our revised manuscript (1320209177310605). The following are our point-by-point responses to reviewer’s comments. We appreciate your kind help and further consideration of this manuscript. If this manuscript needs more revision, please let us know.

Thank you very much.

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

1. One major concern is that the entire work was done with one cell line. Perhaps the authors have some experimental information on other gastric cancer cell lines, which would be pertinent to include here. Also the authors should speculate more on the possible role of Bak in cancer and its possible future applications as therapy.

Response: In fact, we have investigated the BAK overexpression effects on both wild-type p53 (MKN-45) and mutant-type p53 (MKN-28) gastric cancer cells. We found the apoptosis inducing effects was p53-independent. In this revised manuscript, we have provided the corresponding data about NKN-28 cells. And we speculated more on the possible role of Bak in cancer therapy in the “DISCUSSION” part (page 9-10). Besides, we converted the title of this manuscript into “BAK Overexpression Mediates p53-independent Apoptosis Inducing Effects on Human Gastric Cancer Cells”.

2. 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.

Response: MTT assay reflects cellular growth status. In this research, we demonstrated BAK overexpression could lead to cellular growth inhibition, which resulted from both cell cycle arrest and induction of apoptosis.
3. 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.

Response: A α-Tubulin control for western Blotting was used in our research. In this revised manuscript, we have provided their relative figures (page 15).

4. Figure 4: The significance (or lack thereof) of differences for early time points (e.g., 1 or 3 days) is not described.

Response: Cellular growth curve and MTT assay detected the same thing---cell growth. We have removed the data of cellular growth curve in this revised manuscript.

5. Figure 6: Histograms: Why is there no sub-diploid population corresponding to the apoptotic cell fraction?

Response: We have investigated the cell cycle arrest effects of BAK overexpression for at least 3 times. As a matter of fact, we have observed sub-G1 peak, corresponding to the apoptotic cell fraction, in BAK overexpressed cancer cells. In this revised manuscript, we have added their figures in page 16.

6. 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.

Response: It has been demonstrated by other reports, BAK overexpression could lead to mitochondrial injury, including cytochrome c release and loss of mitochondrial membrane potential. We have stated these finding in “DISCUSSION” part. However, it is unclear whether BAK overexpression results in caspase-3 activation, which is a key step during apoptotic process. So, in this manuscript we focused on the caspase-3 assay. Besides, we have added the Western Blotting assay of caspase-3 expression in page 18.

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. The beginning of the Results section (1. Identification of eukaryotic…) contains experimental details that belong to the Materials and Methods, and not to Results. This should be corrected.

Response: It has been corrected (page 4).
2. Figures 1, 2 and 3, which are controls of the system used, should be combined into one figure with three parts A, B and C.

Response: Figures 1, 2 and 3 have been combined into one figure with part A, B and C. (page 15)

3. Figures 4 and 5 really represent two different methods of measuring the same thing (cellular growth), and one of them should be removed (perhaps Fig. 4). Fig. 5 should then be combined with Figure 6, which analyzes the cell cycle, also an indication of cell division.

Response: Figure 4 was removed. The data about MTT assay and flow cytometry has been combined into one figure.

4. pg. 8, first line: the time after which the apoptosis rate is 4.7% should be indicated.

Response: It has been indicated (page 7).

5. The Introduction section is very short and the beginning of the Discussion reads more as an introduction to the paper. This should be re-written and some information stated in the Introduction.

Response: Introduction has been re-written and added some information (page 3).

6. There are no Figure legends on this manuscript. This section must be included since it is crucial for understanding the figures

Response: Figure legends have been described in this revised manuscript (page 15-page 18).

7. The manuscript contains several important language mistakes and should be corrected by someone familiar with English writing before publication.

Response: Prof. Dechun Li (Johns Hopkins University School of Medicine, USA) has corrected the written English of this manuscript.

8. Abstract: Here and elsewhere, the authors use the term improved when increased should be substituted.

Response: It has been substituted.
9. The authors refer to the cells as partial, when they presumably mean parietal

**Response:** It has been substituted