Author's response to reviews

Title: Smac Mimetic-Derived Augmentation of Chemotherapeutic Response in Experimental Pancreatic Cancer

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Author's response to reviews:

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Re: Revised manuscript submission, MS: 5449271380908824

Dear Ms. Judith Gorton:

Thank you for the re-review of the revised manuscript “Smac Mimetic-Derived Augmentation of Chemotherapeutic Response in Experimental Pancreatic Cancer” by Awasthi et al., submitted earlier for publication in BMC Cancer. We would like to thank the reviewer for his insightful and detailed comments.

We have made required changes (highlighted) in the revised manuscript according to the reviewer’s suggestions. All comments made by the reviewer have been addressed as stated in detail as follows:

Reviewer Comment 1:

Response to the major comment 1 was only verbally explained although the comment requests to repeat the study since the mouse number was too small as an in vivo study in general. However, the author’s explanation is rational and acceptable since this experiment suggests that only the combination treatment of JP and GEM shrunk tumor size. However, authors should be aware that the weakness of the small number is clearly evident in the difference between the treatment of Gem in this study (figure 5) and the survival study in the figure 6A; the treatment Gem alone in the study of figure 5 did not show statistical significance, but in the survival study Gem treatment extended the survival time approximately 10 days. Therefore, this issue may be appropriate to insert in the discussion.
Response:
We thank the reviewer for the comment on our response that “the author’s explanation is rational and acceptable”. We agree with the reviewer’s comment that the differences in results obtained by Gem alone treatment in tumor growth inhibition and survival experiment primarily is related to the small number of mice in tumor growth inhibition experiment. As suggested by the reviewer, we have now included this statement in the discussion section (please see page 14).

Reviewer Comment 2:
They have added loading controls in the figures 2B and 4. However, pictures of these loading controls (alpha tubulin) appear to have derived from different gel electrophoreses since band shapes of the PARPS and alpha tubulin are quite different. The principle of the loading controls must be obtained from the same gel electrophoresis sample by re-blotting. This is not only embarrassing for the authors but the journal as well. This experiment needs to be redone.

Response:
Reviewer’s comment is correct that the loading controls were run on a separate gel. We agree with the reviewer that principally loading control should be probed from the same blot, however, under certain circumstances running loading control on separate gel might be necessary, and in fact acceptable. We ran a separate gel for loading control for the following reasons:

1. We used PARP polyclonal antibody that recognizes 116-kDa full-length PARP and 89-kDa C-terminal cleaved PARP and 24-kDa N-terminal cleaved PARP. Therefore cutting the blot to probe with different antibodies was not possible. Also, this antibody binds to several non-specific targets in the range of 30 to 60 kDa (please see original blot) which prohibited us to reprobe with any of the control housekeeping alpha-tubulin/beta-actin/GAPDH antibodies.

2. To clearly observe the PARP cleavage, we have to load approximately 50 µg total cell lysate in each well. Since control housekeeping proteins are generally highly expressed proteins 50 µg total lysate gives highly saturated bands that render it difficult to see small changes and quantitation by densitometry.

We believe that in the present study running a separate gel for loading control is acceptable for the following reasons:

1. We have taken care to load equal amount of protein in each lane by analyzing protein concentration of each sample by BSA assay.

2. Loading control Western blot analysis was performed on a separate gel but at the same time and in parallel with PARP cleavage analysis.

3. An advantage in using this antibody is that it clearly demonstrates apoptosis by increase in cleaved PARP band with concomitant decrease in full-length PARP. This minimizes the absolute importance of loading control on same gel.

4. Please see figure 1 of following reference from BMC Cancer to justify acceptability of loading control on separate gel: Nagel S, Venturini L, Przybylski

We have now mentioned in the figure legends of Figure 2B and Figure 4 that the expression of leading control was analyzed on a separate gel (please see page 19 and 20).

If the reviewer or editorial board thinks that loading control on separate gel is not acceptable, we will be happy to redo these experiments using an antibody that only recognizes cleaved PARP and then re-probe with alpha-tubulin antibody. In this case we will greatly appreciate an extension for resubmitting revised manuscript.

We hope that the revised manuscript now meets all requirements for successful acceptance, and we believe that the readers of BMC Cancer will find this a very useful contribution.

Thank you very much for your interest.

Sincerely yours

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