Author’s response to reviews

Title: Role of 14-3-3sigma in poor prognosis and in radiation and drug resistance of human pancreatic cancers

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Author’s response to reviews:

September 2, 2010

Dear Dr. Stoeltzing,

Thank you for your email dated August 12, 2010 regarding our manuscript entitled “Role of 14-3-3sigma in poor prognosis and in drug and radiation resistance of human pancreatic cancers”. We have carefully read the reviewer’s critic and made appropriate revision accordingly. Our point-to-point responses to the reviewer’s comments are appended. With the revision and additional data, we believe that this manuscript is now in a publishable format for BMC Cancer.

Thank you very much for your time concerning this manuscript. We are looking forward to your favorable decision.

Sincerely yours,

Jian-Ting Zhang, Ph.D.
Professor of Pharmacology and Toxicology
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Point by Point Responses to Reviewer’s Comments

Responses to the first reviewer’s critic.
1. This reviewer thought that the data are interesting, convincing and clear although several publications already demonstrated the over-expression of 14-3-3sigma in PDAC.

Response: It is true that 14-3-3sigma over-expression in PDAC has been shown in the past. However, these past studies mostly addressed 14-3-3sigma at its
mRNA level. Our study made further advancement to address 14-3-3sigma protein level in PDAC.

2. This reviewer thought that there is a need to provide deeper insights into the molecular mechanisms how 14-3-3sigma is regulating processes like apoptosis resistance or the G2/M-phase of the cell cycle in PDAC cells.

Response: We agree that detailed mechanism studies are needed. However, we believe that these detailed studies are out of the scope of this manuscript. We are currently pursuing these studies which will take a while before we can complete the study. Nevertheless, in the revision we added a new experiment by including an experiment using a stable pancreatic cancer cell line with 14-3-3sigma knockdown and confirmed that 14-3-3sigma over-expression causes radiation and drug resistance (Fig. 5).

Responses to the second reviewer’s critic

1. This reviewer stated that “Overall these data are thought-provoking and contribute to our understanding of the inherent therapeutic resistance observed in pancreatic cancer. These studies indicate 14-3-3sigma could potentially be a prognostic and predictive marker for pancreatic cancer.”

Response: Thank you.

2. This reviewer found a few grammatic errors and typos.

Response: We apologize for these errors and have changed all of them throughout the manuscript.

3. This reviewer questioned if the normal tissues microdissected and if they were more than 5mm away from tumor.

Response: The normal tissues were not microdissected. Instead, the normal tissues were the ones that were from the farthest point (at least 20 mm) away from lesion (tumor). This statement was added in the revision (page 4, 2nd paragraph).

4. This reviewer thought that “Figure 3A is not the usual way to describe the effect of a variable on survival” and suggested to delete it.

Response: Fig. 3A has been deleted.

5. This reviewer questioned if there is a difference in time to distant (non-lymphatic) metastasis between low and high expressing 14-3-3-sigma patients

Response: The patient record does not have this information for this retrospective study.

6. This reviewer questioned if ectopic 14-3-3-sigma expression affect responses to 5FU.

Response: We have not yet performed any studies with 5-FU but plans to do these studies with 5-FU in the future for continuation of this project.
7. This reviewer thought that the data in figure 4b are puzzling which might be due to the assay used.
Response: We apologize for the confusion and agree that the assay may cause the puzzling results at high dose. To eliminate the confusion, we have revised Fig. 4B to show only without and with 10 Gy radiation treatment. Since this data is only to show the correlation of 14-3-3-sigma expression with treatment responses in two different cell lines and considering that many other data later show the effect of 14-3-3-sigma on treatment responses, we believe that the data shown in Fig. 4B is sufficient. The text in results and figure legends has also been corrected accordingly.

8. This reviewer suggested to perform clonogenic assays at higher doses instead of the data shown in Fig. 5C and perhaps to perform an analysis of REF.
Response: As suggested by the reviewer, we performed additional studies and found that at 5 Gy (see new Fig. 5C) majority of PaCa-2 cells died. With the dose response curve, we were able to generate an REF of 0.79, consistent with radio resistance induced by 14-3-3-sigma over-expression. Statements on REF in materials and methods (page 6, 2nd paragraph) and results (page 9, 2nd paragraph) have been added.

9. This reviewer suggested adding a discussion on mitotic catastrophe in addition to apoptosis.
Response: In the revision, we have modified the discussion to reflect this issue (page 14, 2nd paragraph).

10. This reviewer suggested adding asterisks to figures where calculated p values are significant.
Response: asterisks are added to the figures during the revision.

11. This reviewer questioned if shRNA knockdown of 14-3-3-sigma in BxPC3 cells induce sensitivity to radiation or gemcitabine and stated “If so, this would bolster the hypothesis of 14-3-3-sigma as a predictive marker.”
Response: we have done the knockdown study and the results are positive (Fig. 5). Thus, it further confirms that 14-3-3-sigma may be used as a predictive marker. Methods (page 5, 4th paragraph) and results (page 9, 4th paragraph) have also been modified accordingly.

12. This reviewer found an error on the citation {Hustinx, 2005 #1529}.
Response: This error has been corrected.

13. This reviewer suggested to change "Pancreatic cancer was graded according to the most current AJCC Guidelines." to "Pancreatic cancer was staged according to the most current AJCC Guidelines."
Response: In the revision, this statement has been changed as suggested (page 4, 2nd paragraph).