Reviewer's report

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

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Reviewer: Aaron Spalding

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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. Currently CA 19-9 is commonly used to monitor response to treatment yet there are no predictive markers for this disease.

However, I have some concerns regarding the work and would call attention to the following:

Major Compulsory Revisions

The manuscript as written is hard to follow due to poor grammar. For example, in the abstract "MTT assay we determined the expression level of 14-3-3# and performed correlation analysis with clinical outcome and found that 14-3-3# protein level was increased significantly in about 71% (17 of 24) of human pancreatic cancer tissues and that the 14-3-3# protein level in the cancers correlates with lymph node metastasis and prognosis."

In the introduction "Unlike other solid tumors such as testicular cancer which can now be curable, more than 90% of pancreatic cancer patients die due to lack of response to therapy."

Sentences such as these need to be revised throughout.

Please explain how the normal tissue controls were obtained for the western blot analysis in figure 1. Were the normal tissues microdissected? Were they more than 5mm away from tumor?

Figure 3A is not the usual way to describe the effect of a variable on survival, that is why the Kaplan Meier curves are used as they take into account length of follow up. i.e. median survival is the gold standard endpoint. Just because the p value is 0.06 does not mean figure 3B is without value. Please remove 3A. Given the increased prevalence of lymph node metastasis of 14-3-3 expressing tumors, I would ask is there a difference in time to distant (non-lymphatic) metastasis between low and high expressing 14-3-3-sigma patients? This would perhaps be a replacement for figure 3A.

5-FU is also a common agent used in systemic treatment of pancreatic cancer patients. What is the effect in the MiaPaCa-2 wild type and ectopic 14-3-3-sigma
expressing cells on SRB assay with 5FU? Does ectopic expression change the IC50?

The data in figure 4b are puzzling. Why would one 60Gy fraction induce less cell death than one 10Gy fraction? It would seem that there is not a dose-response relationship. The answer may lie in the choice of assay, as SRB for radiation death has not been shown convincingly to adequately measure this endpoint. Similarly the results in figure 5b do not show much effect beyond 10Gy.

The clonogenic assays must be performed at 0,2,4,6,8, and 10 Gy for the miapac2 cells, as shown by others in the literature. Figure 5C is not interpretable, as the whole dose curve should be displayed, and REF (as shown below) needs determination.

For the clonogenic XRT experiments, a radiation enhancement factor can quantitate the degree of radioresistance for a given intervention. Please see for example:

Radiotherapy combined with gemcitabine and oxaliplatin in pancreatic cancer cells.


Please calculate the REF for the clonogenic survival curves.

Whether radiation therapy induces cell death by apoptosis or mitotic catastrophe is debatable as data exist to support both hypothesis. Likely this means both mechanisms play some role, depending on cell type and radiation dose. Please change the discussion in the discussion to illustrate this fact.

An asterisk should be added to all figures where calculated p values are significant.

Does shRNA knockdown of 14-3-3sigma in BxPC3 cells induce sensitivity to radiation or gemcitabine? If so, this would bolster the hypothesis of 14-3-3sigma as a predictive marker.

Minor Essential Revisions

change "This observation is consistent with a previous study by Hustinx et al. {Hustinx, 2005 #1529}." by entering the correct reference.

change "Pancreatic cancer was graded according to the most current AJCC Guidelines." to "Pancreatic cancer was staged according to the most current AJCC Guidelines."

Discretionary Revisions

**Level of interest:** An article whose findings are important to those with closely related research interests
Quality of written English: Needs some language corrections before being published

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:

I declare that I have no competing interests