Author's response to reviews

Title: SFRP1 reduction results in an increased sensitivity to TGF-beta signaling

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Version: 2 Date: 13 January 2011

Author's response to reviews: see over
Dear Dr. Norton,

I have revised the manuscript entitled “SFRP1 reduction results in an increased sensitivity to TGF-β signaling.” I would like the manuscript to be re-considered for publication in *BMC Cancer*.

This manuscript was written to determine whether the TGF-β signaling pathway is augmented when a key Wnt signaling antagonist, Secreted Frizzled Related Protein-1, is down-regulated. The findings presented in this primary research article are significant because we show that TGF-β sensitivity can be partially ameliorated by blocking the expression of ZEB2, a key transcription factor that regulates the progression of tumor cell metastasis, and therefore, ZEB2 may be an excellent anti-cancer therapeutic target.

I have included a point by point description of how I dealt with the concerns of the reviewers. I have copied and pasted the reviewer’s comments verbatim. Below (in bold) I addressed how I amended the manuscript accordingly.

Referee #1
1. The authors present data indicating that loss of SFRP1 enhances TGF-β signaling however there is no experimental data to indicate that increased Wnt signaling (which one would predict is the result of SFRP repression) enhances TGF-β response. It would be of great interest to know if hyperactivation of Wnt signaling could enhance TGF-β signaling independent of SFRP loss. The authors should address this issue in the discussion section.

   **This topic has been addressed in the discussion.** Our published data describing hyperactivation of Wnt signaling in TERT-siSFRP1 is now referenced. Additionally, two reviews that describe the cross-talk between Wnt and TGF-β are now included in the discussion. Finally, 2 primary research papers that provide experimental evidence of Wnt-mediated TGF-β signaling are referenced and one of the articles alludes to the mechanisms by which this occurs.

2. The authors correctly state that TGF-β has divergent effects on mammary gland development and breast cancer initiation or progression. What I found thought provoking in this work was the distinct possibility that Wnt signaling status may predict TGF-β activity. The authors should address this issue in the discussion section.

   **This is a keen observation and we agree that Wnt hyperactivation may indeed yield the observed results. Therefore, we address this topic in the discussion section.**

3. The authors present compelling data to indicate that Zeb2 expression correlates with increased TGF-β signaling. Given the role of TGF-β in tumor progression it would be of significant interest to know if Zeb2 expression correlates with prognosis or outcome in breast cancer. This can be accomplished by mining publicly
available data sets.

We did access the pubmed GEO database to determine if microarray data from other researchers provided any indication that ZEB2 expression is associated with poor clinical outcome. Our search did not unveil any prevalent findings, however, our literature search yielded a research article which addresses this reviewer’s question. Therefore, we inserted the following sentence “Elloul et. al. [41] analyzed the expression of ZEB2 as well as other transcriptional repressors of E-cadherin in ovarian and breast carcinoma effusions and revealed that elevated mRNA levels of ZEB2 is a predictor of poor survival [41].”


Referee #1

1. The authors used TGF-β to treat cell and analyze the role of SFRP in TGF-β-mediated signaling. Therefore, I am curios about that only TGF-β-Smad pathway influenced by SFRP, but not other the complex signal pathways triggered by TGF-β. The 76N TERT cell line is a non-malignant immortalized mammary epithelial cell line. Could TERT-siSFRP1 cells induce tumor in mouse model?

These experiments are ongoing in conjunction with a separate manuscript that is in preparation. Therefore, we allude to the fact that these are important experiments and that we are currently conducting such *in vivo* studies in the conclusion section of this manuscript.

We hope that we have met the reviewers concerns suitably. Thank you for your consideration.

Sincerely,

Kelly J. Gauger, Ph.D.