Author's response to reviews

Title: Frizzled-8 Receptor is Activated by the Wnt-2 Ligand in Non-Small Cell Lung Cancer

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Version: 2 Date: 29 April 2013

Author's response to reviews: see over
April, 29, 2013

Dear Editor-in-Chief,

Thank you for your review of our manuscript entitled “Frizzled-8 Receptor is Activated by the Wnt-2 Ligand in Non-Small Cell Lung Cancer”.

Attached please find our revised manuscript and our response to comments. We have done extensive revisions to address the reviewer’s comments. We thank the reviewer because her comments make our manuscript a better one. We have revised our manuscript according to her suggestions and answered point by point to each comment.

Sincerely,

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Reviewer 2

Title: Frizzled-8 Receptor is Activated by the Wnt-2 Ligand in Non-Small Cell Lung Cancer

Version: 1 Date: 3 April 2013

Reviewer: Sarah J Freemantle

Reviewer's report:

In this paper 50 lung tumors and normal adjacent tissues are analyzed for Wnt2 and Fzd8 levels. Wnt2 was higher in tumors than normal tissue for 70% of samples and Fzd8 was higher in 42% (21 samples). Of the 21 samples with elevated Fzd8 19 also had elevated Wnt2. Previously this group and others had reported that Wnt2 was elevated in lung and other tumors but the Frizzled receptors that this Wnt activates were not known.

They then tested a group of frizzled receptors in a transcription assay to see which ones became activated with Wnt2. Fzd8 gave the highest level of induction with Fzd9 slightly behind and Fzd7 giving a moderate activation. Frizzled receptors 1-5 and 10 gave no activation.

They designed a dominant negative Wnt2 (dnWnt2) that could inhibit Wnt2 activation of Fzd8. When this was stably expressed in the A549 lung cells it inhibited growth, colony formation and xenograft growth in nude mice.

It is becoming clear that Wnt deregulation is important in lung tumorigenesis but due to the complexity of the Wnt pathway and the lack of good pharmacological tools, research in this area is lagging behind that of other tumorigenic pathways. This is an interesting paper that seeks to further our understanding of which pathway components represent potential therapeutic targets.

Major revisions:

1) The order of the figures doesn’t make sense. The levels of Fzd8 in tumor samples are presented before the panel of Fzd receptors is analyzed. Figures 1 and 2 should be reversed.

Answer: Done as suggested. We have reversed Figures 1 and 2, and also modified text on p11 and p21.

2) Several times the authors state that the inhibition of cell growth and tumor growth by the dnWnt2 is specifically due to the Fzd8 interaction. There is no direct data to prove this. While it is true that Wnt2 can activate Fzd8, the growth and antitumor effects could be due to inhibition of any of the Fzd or other Wnt receptors.
Answer:
Done as suggested. We have modified all the Fzd8 statements on p2, p5, p14, p15, and p16.

3) In the key receptor experiment (Fig2) identifying Fzd8 as the receptor that is most strongly activated by Wnt2 it was somewhat surprising that no activation of the other Fzds was seen. The same experiment was done in the same cell line, 293T, but using Wnt3a (Liu et al 2005 MCB 25:p3475) and it could activate Fzd1/2/5/7/8. The specificity of Wnt2 for Fzd8 would have been strengthened enormously if the Wnt3a had been tested side by side with the Wnt2 in this assay. You could then also use the Wnt3a to determine if dnhWnt2 is specific to inhibiting Wnt2 activity or if it can block other Wnts. This would strengthen this paper.

Answer:
1) In addition to Fzd8 which showed most activity as a Wnt2 receptor, we have also identified Fzd9 and Fzd7 (to a lesser extent) as additional receptor for Wnt2 (Fig 2). This Fzd9 finding is consistent with the results of a previous publication (Karasawa T. et al., J Biol Chem, 2002, 277(40): p37479-86). The statement was addressed on p.15-line4.

2) There are at least four other receptors for Wnt3a, and Fzd5 appears to be the most active one (Liu et al 2005 MCB 25:p3475). It is unlikely that dnhWNT2 will completely block Wnt3a activity. We have added the discussion (p15) and this reference to the manuscript (Ref. 50).

3) To date, the role of Wnt3a in carcinogenesis is not clear. For instance, exogenous Wnt3a promoted tumor growth in one human tumor cell line but inhibited tumor growth in another human tumor cell line (Green JL. Et al., PNAS, 2013, April). Moreover, Wnt3a decreased the proliferation of B16 melanoma cells (Chien et al., PNAS, 2009, 106:1193-8). Thus, Wnt3a appears to have both functions as oncogene and tumor suppressor gene depending on the specific cancer cell lines. We believe that the role of Wnt3a in lung cancer cells needs to be clarified first (e.g., oncogene, tumor suppressor gene, or both). Our goal of this study is to test if the dnhWNT2 inhibits the growth of lung cancer cells expressing Wnt2. We have added the discussion (p15) and these references to the manuscript (Ref. 51, 52).

Minor revisions.

1) In figure 2A it looks like for each Fzd receptor the empty vector control is set at zero. This makes sense when comparing relative fold inductions. In figure 2B the empty vector control varies. For ease or interpretation can this be presented in the same way or at least the differences explained. This is also the case for figure 3 where the empty vector control is less than 1 for the A549 cells.

Answer:
The empty vector control was minimal but not set at zero. Relative fold inductions were used for Figs 2A, 2B(now Figs1A and 1B), and 3A. The empty vector controls in 293 and 293T cell lines showed similar minimal background (<1, now Fig 1B). The empty vector control in A549 showed some activity (>2) is most likely due to the intrinsic Wnt signaling in this
cancer cell line. In Fig 3, dnhWnt2 inhibited the intrinsic Wnt (most likely Wnt2) signaling and result in the low background (less than 1, Fig 3A-A549). The sentences are added on p.11-line13 and p12-line7.

2) The second paragraph of the introduction is repetitive and confusing when describing canonical Wnt signaling. This should be shortened and simplified.

**Answer:**
Done as suggested. We have modified the second paragraph (p.4).

3) The paper is well written. There are a few issues with sentence structure on:
page 14-line 7 construct competes for binding or for the binding

**Answer:**
Done as suggested (p.14-line17).

line 17 Although the frizzled family of receptors

**Answer:**
Done as suggested (p.15-line1).

line 26 shown to play a role in Wnt signaling

**Answer:**
Done as suggested (p.15-line10).

Page 15 line 15 used the DNhWnt2

**Answer:**
Done as suggested (p.16-line3).

Line 16 the dnhwnt2 construct reduces...

**Answer:**
Done as suggested (p.16-line4).
Level of interest: An article of outstanding merit and interest in its field

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

'I declare that I have no competing interests'