Author’s response to reviews

Title: Vav3 oncogene activates estrogen receptor and its overexpression may be involved in human breast cancer.

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Author’s response to reviews:

Dr. Melissa Norton
Editor-in-Chief
BMC Cancer

Dear Dr. Norton,

We are submitting the revised version of our manuscript, entitled “Vav3 oncogene activates estrogen receptor and its overexpression may be involved in human breast cancer” (MS: 1236477421179147), for publication in BMC Cancer. In last a couple of months, I have had a few communications with your team and appreciate very much for the professional handling of our manuscript by Ashleigh Manning, Assistant Editor. We have carefully studied the comments from the reviews and our responses to address the comments are indicated below:

Reviewer #1:

1. Page 10: MCF10 are non tumoral breast epithelial cells, but these cells can not be considered as normal. Change its definition to “non tumoral”.

Reply: I appreciate this comment. Indeed, MCF10A cells are not normal cells, but non tumoral breast epithelial cells. This issue has been addressed in Result section, pages 6 and 10, lines 6 and 19.

2. Page 11 lane 20 (and throughout the rest of the manuscript): please clarify well which are the controls used in each experiment.

Reply: We have further emphasized the empty vector controls for reporter assays and control GST protein for GST pull down experiment. This issue was addressed at page 12, lines 13 and 17; page 13, lines 4, 19, and 22; page 15, lines 3 and 5, as well as figure legends 3, 4, 5, and 7.

3. Fig 2B does not show a clear increase of proliferation of vav3 cells respect the
control since the starting point (0 EGF) is also different. Normalization of the graph to equal starting point is essential to compare both curves.

Reply: We respectfully disagree with this comment. We found that Vav3 overexpression stimulated MCF7 cell growth in the absence of EGF and also significantly potentiated the cell growth in response to EGF treatment in a dose-dependent manner. An increased proliferation of Vav3 cells relative to the control cell at the starting point (0 EGF) suggests a significant growth stimulatory effect of Vav3 oncogene. Furthermore, with stimulation by an increased concentration of EGF, Vav3 cell proliferation is always better than that of the control cells.

4. ...... repeat the expression of vav3 in MCF10a and see whether ER increases.

Reply: We have repeated the expression analysis of ERa in MCF10A cells and found that a very low level of ERa protein can be detected with an increased exposure time of western blot analysis, as shown in Figure 2A. This issue was addressed in Result section, page 10, line 20.

Reviewer #2:

1. Vav3 does not appear from Figure 1 to decrease the proliferation rate of breast cancer cells, yet the authors maintain that it is involved in their growth; please explain.

2. If Vav3 is involved in both estrogen-dependent and estrogen-independent growth of breast cancer cells, a clonogenic assay to assess long-term proliferation rates would be important to determine.

3. In Figure 3, tamoxifen appears to increase ERα activity, please explain.

4. The GST pull-down data is not convincing, as there appears to be no change in the presence of Mcf-7 cells.

Reply: We respectfully disagree with Referee 2’s comments in that both Figure 1 and 7 are clearly explained. For Figure 3, it has been well known that Tamoxifen has a weak ER agonist activity in the absence of estrogen, although it is an ER antagonist.

I appreciate your consideration of our manuscript for publication in BMC Cancer.

Sincerely

Shan Lu, Ph.D.