Author's response to reviews

Title: Estrogen receptor alpha-coupled Bmi1 regulation pathway in breast cancer and its clinical implications

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Author's response to reviews: see over
Dear editor:

On behalf of my co-authors, we thank you very much for giving us an opportunity to revise our manuscript, we appreciate editor and reviewers very much for their positive and constructive comments and suggestions on our manuscript entitled “Estrogen receptor α-coupled Bmi1 regulation pathway in breast cancer and its clinical implications” (MS:2087415787104846)

We have studied reviewers’ comments carefully and tried our best to revise our manuscript according to the comments. And if you have any queries, please don’t hesitate to contact me.

Here a point-by-point response to the comments is listed as follows:

Reviewer 1:

1. Material and Methods: the tumor histological typing and more detailed clinical data should be presented.
   Response: All the 92 cases are invasive ductal carcinoma in histopathology, and their detailed clinical data have been summarized in Supplementary Table 1

2. The clinical potential of Bmi1 should be discussed. Since Bmi1 was shown to be regulated by ERα, the authors should explain why Bmi1 pathway was an ERα-coupled signal pathway, but not a part of ERα signal pathway as a downstream factor. In this context, the authors should discuss why the Bmi1 is more significant over other ERα regulated factors.
   Response: We have added some views in the last section in Discussion.
Reviewer 2:

Major Compulsory Revisions

1. Legend to Fig 1 could misled the non-specialist reader into thinking all ER-negative breast cancers are universally negative for PR, cyclin D1 and pRB, especially the last sentence in the fig legend, and contracts what is presented in Table 3. This needs addressing.
   Response: Based on Table 3, we have replaced the picture of pRB staining in ER-negative column, and have corrected the legend to Fig 1.

2. There is not “…marked down-regulation of ERα protein by these three siRNAs…” as described on pg 14 with reference to Fig 4. Only siRNA 3 shows this. The text and legend should be modified to reflect this.
   Response: It has been corrected as “The level of ERα protein was markedly reduced by siRNA3” in the text. And we have also added simple declaration in the legend to Fig 4.

3. The results section “ERα-coupled Bmi1 regulatory pathway in breast cancer” (pg15) is too long (4+ pages) and contains discussion points as well as the results. This section should contain just results with the discussion points being assimilated into the discussion section of the manuscript.
   Response: Discussion points in the results section “ERα-coupled Bmi1 regulatory pathway in breast cancer” have been deleted and assimilated into the Discussion section of the manuscript.

Minor Essential Revisions

1. Given the heterogeneity of breast cancer, the clinical cohort is relatively modest (n=92). The authors should acknowledge this in the context of the correlative results they describe in Table 1.
   Response: We have acknowledged this in Discussion section relating to the results described in Table 1.
2. Fig 2 is quite confusing in its presentation. I recommend the Western images be presented sequentially underneath each other (likewise for the quantification graphs) so that data for each cell line can be more easily compared.

   Response: The images in Fig 2 have been adjusted according to suggestion of the reviewer.

3. Fig 3 legend; best to say empty rather than blank vector.

   Response: It has been corrected as “empty vector” in Fig 3 legend.

4. The authors use some strong statements e.g. pg14 “…results conclusively demonstrated that ERα can specifically stimulate the functional expression of Bmi1”. While this may prove to be the case it needs validation by others so this statement should be toned down.

   Response: It has been corrected as “these results implied that ERα may specifically stimulate the functional expression of Bmi1”.

Discretionary Revisions

In the supplementary table please check SP1 antibody is supplied by Dako. Could you mean 1D5 rather than SP1?

Response: The anti-ERα antibody is supplied by Dako and the clone is 1D5. I am very sorry for this error.

Looking forward to hearing from you.

Thank you and best regards.

Sincerely yours,

Huali Wang, ph.D