Author's response to reviews

Title: Prostate tumor overexpressed 1 overexpression correlates with tumor progression and predicts poor prognosis in breast cancer

Authors:

Fangyong Lei (leify@sysucc.org.cn)
Longjuan Zhang (zhlongj@mail.sysu.edu.cn)
Xinghua Li (xinghuali22@gmail.com)
Xi Lin (linxi@sysucc.org.cn)
Shu Wu (wushu@sysucc.org.cn)
Fengyan Li (lify@sysyucc.org.cn)
Junling Liu (liujl@sysucc.org.cn)

Version: 4
Date: 16 May 2014

Author's response to reviews: see over
May, 16th, 2014

Dear editor,

Thank you for your decision letter of April 14th, 2014 regarding our manuscript MS: 1179119073123351, entitled “Prostate tumor overexpressed 1 overexpression correlates with tumor progression and predicts poor prognosis in breast cancer” by Fangyong Lei et al., and for the opportunity of resubmitting a revised manuscript to BMC Cancer. We have very carefully studied all the points raised by editor and reviewers and have added necessary data to address the reviewers’ concerns. We have changed the title to “Overexpression of prostate tumor overexpressed 1 correlates with tumor progression and predicts poor prognosis in breast cancer” and are now submitting a revised manuscript with point-to-point response to the critiques to BMC Cancer.

We also would like to thank you for important suggestion, to which we have made modifications as follows:

1. We summarized ER status, PR status, HER2 status and P53 status of the tested cell lines in supplemental Table 1 and analyzed the correlation between PTOV1 expression and these statuses of the cell lines as the reviewers suggested.

2. We have made modifications to clarify the Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was as reference gene that acts as an internal reference to normalize the mRNA expression.

3. We included the analysis correlation of PTOV1 with PR and HER-2 in Table 3.

4. In Figure 4, the survival curves were modified to start from 100% as the reviewer 2 suggested.

The details of point-by-point description of the changes made are as follows:

**Reviewer #1:**

A. Major Compulsory Revisions:

1. Fig 1. MCF10A is an immortalized "normal" breast epithelial cell line. The authors stated that this is breast cancer cell line. This needs to be corrected. Since the tested cell lines vary in their ER status, mutant p53 status, and they belong to either
luminal or basal-like type, it would be helpful if the authors can comment on whether the PTOV1 expression levels is correlated with these differences.

Response: We apologize for the mistake about MCF10A and thank the reviewer for the comment. Appropriate correction has been made in the revised manuscript.

As regard to the differences in ER status, PR status, HER2 status and P53 status between the tested cell lines, we summarized these statuses in the table below (supplemental Table 1). We define the high or low PTOV1 expression by the median of the density scan of Western blotting detection of the protein expression of the cell lines. The results showed that there were no significant differences between PTOV1 expression and these features of the breast cancer cell lines. As the backgrounds of these cell lines we used in the study are not well understood and some of them are lack of reports about these statuses, such as ER, PR, HER2, P53 status or their subtype, we could not investigate that correlation between these statuses and PTOV1 expression. Moreover, the breast cancer cell lines cultured in vitro might perform different ER, PR or HER2 status, compared with the breast cancer cells grown inside the patient’s body, since tumor microenvironment plays an important role in tumor microenvironment during breast cancer progression [8-10]. Therefore,

Supplemental Table 1 Subtype classification and ER, PR, HER2 and P53 status in breast cancer cell lines

<table>
<thead>
<tr>
<th>Breast cancer cell line</th>
<th>Subtype</th>
<th>ER</th>
<th>PR</th>
<th>HER2</th>
<th>P53</th>
<th>PTOV1 expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCF-7</td>
<td>Luminal</td>
<td>+</td>
<td>+</td>
<td>+/−</td>
<td>WT</td>
<td>Low</td>
</tr>
<tr>
<td>MDA-MB-231</td>
<td>BasalA</td>
<td>+</td>
<td>+</td>
<td>+/−</td>
<td>WT</td>
<td>High</td>
</tr>
<tr>
<td>MDA-MB-435</td>
<td>BasalA</td>
<td>+</td>
<td>+</td>
<td>+/−</td>
<td>WT</td>
<td>Low</td>
</tr>
<tr>
<td>MDA-MB-453</td>
<td>BasalB</td>
<td>+</td>
<td>+</td>
<td>+/−</td>
<td>WT</td>
<td>Low</td>
</tr>
<tr>
<td>BT474</td>
<td>Luminal</td>
<td>+</td>
<td>+</td>
<td>+/−</td>
<td>WT</td>
<td>High</td>
</tr>
<tr>
<td>BT549</td>
<td>BasalA</td>
<td>+</td>
<td>+</td>
<td>+/−</td>
<td>WT</td>
<td>High</td>
</tr>
<tr>
<td>T47D</td>
<td>Luminal</td>
<td>+</td>
<td>+</td>
<td>+/−</td>
<td>WT</td>
<td>High</td>
</tr>
<tr>
<td>ZR-75-1</td>
<td>Luminal</td>
<td>+</td>
<td>+</td>
<td>+/−</td>
<td>WT</td>
<td>Low</td>
</tr>
<tr>
<td>ZR-75-30</td>
<td>Luminal</td>
<td>+</td>
<td>+</td>
<td>+/−</td>
<td>WT</td>
<td>High</td>
</tr>
<tr>
<td>----------------</td>
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</tr>
<tr>
<td>SKBR3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>High</td>
</tr>
<tr>
<td>Bcape37</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Low</td>
</tr>
</tbody>
</table>

*NA: Unknown. There’s no research report about subtype classification or ER, PR, HER2, P53 status about the breast cancer cell line.

Further investigations are being required to elucidate reviewer’s comment. We do appreciate reviewer’s suggestion and apologize that we could not make a clear conclusion at present.

**References:**


2. Fig 2B. The authors must clarify how they normalized PTOV1 mRNA amounts against GAPDH. They stated that this was done by "normalized to the geometric mean of housekeeping gene". Here only one housekeeping gene was investigated. What does "geometric mean" mean? Does this mean "geometric mean" of expression levels of several housekeeping genes including GAPDH?

Response: We do appreciate the reviewer’s comments and we are sorry we did not write this point clearly in the originally submitted manuscript. The mRNA expressions of the genes, measured by qRT-PCR, were defined based on the threshold
cycle (Ct), and Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as reference gene that acts as an internal reference to normalize the mRNA expression, and calculated as $2^{-[(Ct \text{ of genes}) – (Ct \text{ of GAPDH})]}$. The geometric mean is a type of mean or average, which indicates the central tendency or typical value of a set of numbers by using the product of their values as opposed to the arithmetic mean which uses their sum. It is a calculate method in certain equations to properly analyze the relative changes in gene expression by real-time PCR (1). For a clear description, appropriate modifications have been made in the revised manuscript.

Reference:


B. Minor Essential Revisions

Although the manuscript is generally clearly written, the authors need to carefully check their grammar and correct errors which were found throughout the manuscript. A few examples:

1. Abstract/Results: line 2, "human breast epithelial" ; line 3, "protein level(s)"

Response: We thank the reviewer for the suggestion. The correction has been made in the revised manuscript.

2. Page 4, line 12, "further enhanced..."

Response: We thank the reviewer for the suggestion. The correction has been made in the revised manuscript.

3. Authors' contributions: line 2 "and editing of..."

Response: We thank the reviewer for the suggestion. The correction has been made in the revised manuscript.

4. Discussion: page 2, line 4 and line line 7, "PTOV1 plays an important role in cell proliferation status", "could contribute to the proliferative status"."Proliferation status" does not seem to be a right phrase.

Response: We thank the reviewer for the suggestion. The correction has been made in
the revised manuscript.

The title may also need to be modified "prostate tumor overexpressed 1
overexpression"; it would be better to change to "overexpression of the prostate tumor
overexpressed 1 gene"

Response: We thank the reviewer for the suggestion. The correction has been made in
the revised manuscript.

Reviewer #2:

1. Some of the figure legends have abbreviations which are not clearly shown the
full meaning, such as Figure 2, HCC

Response: We apologize for the mistake and thank the reviewer for the comment.
Appropriate correction has been made in the revised manuscript.

2. Table 3, authors can also include the analysis with PR and Her-2 correlation, even
it is not significant.

Response: We appreciate the reviewer’s comment. Appropriate modifications have
been made in Table 3 in the revised manuscript.

3. Since all of the breast cancer cell lines showed high levels of PTOV1, is there any
correlation also can be identified with the status of ER, PR, or HER2 in cells? At
least authors can put this in a table.

Response: We do appreciate the reviewer’s comment and we have provide more
information about the ER, PR, HER2 status and P53 status of the breast cancer cell
lines and the correlation between these statuses and PTOV1 expression in
supplemental Table 1.

4. In Figure 4, the survival curves also start from 120%, it will be better to do it with
100%.

Response: We thank the reviewer for the suggestion. Appropriate modification has
been made in the revised manuscript.
We hope that you will find our revised manuscript suitable for publication in *BMC Cancer*. Your supportive consideration is greatly appreciated.

Best wishes,

Junling Liu, MD  
State Key Laboratory of Oncology in South China,  
Department of Medical Oncology,  
Sun Yat-sen University Cancer Center,  
Guangzhou 510060, P.R.China.  
E-mail: liujl@sysucc.org.cn