Author's response to reviews

Title: Prognostic significance of proline, glutamic acid, leucine rich protein 1 in triple-negative breast cancer: a retrospective study on 129 cases

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Version: 3 Date: 15 June 2015

Author's response to reviews: see over
Reviewer#1

Reviewer's report

Title: The prognostic significance of Proline, glutamic acid, leucine rich protein 1 in triple-negative breast cancer: a retrospective study on 129 cases

Version: 2 Date: 5 May 2015

Reviewer: Pellegrino Michele

Reviewer's report:

In the present manuscript the Authors aim to address the role of Proline, glutamic acid, leucine rich protein 1 (PELP1) as prognostic markers for TNBC patients. PELP1 overexpression was reported to induce the malignant transformation of normal cells, accelerate cell cycle progress, promote tumor cell proliferation, and enhance the migration and invasion of tumor cells. Here, the Authors assessed the PELP1 expression in 129 patients with TNBC and correlated the status of PELP1 independently or in combination with other clinic-pathological variables to the outcomes of the patient. In particular, they demonstrated that the high PELP1 protein expression is correlated with positive lymph node status in TNBC.

Moreover, the Authors showed that for the TNBC patients with characteristics of smaller tumor size or high Ki-67, high PELP1 protein expression in tumor may predict a poorer outcome and double high expression of PELP1 and Ki-67 is associated with poorer outcome of patients.

The reported observations are interesting, the paper is well written. Thus, the resulting data are convincing and the conclusions are strongly sustained by data obtained.

There is just one change to do: in the figure 1 the morphological pictures should have a
size bar indicated and should be displayed also at a higher magnification.

Response: The size bar had been added, high magnification image had been added in the upper right corner of each panel in Figure 1.

Level of interest: An article of importance 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

Reviewer#2

Reviewer's report

Title: The prognostic significance of Proline, glutamic acid, leucine rich protein 1 in triple-negative breast cancer: a retrospective study on 129 cases

Version: 2 Date: 14 April 2015

Reviewer: Julie Ostrander

Reviewer's report:

This study describes the analysis of 129 triple-negative breast cancers for PELP1 expression and clinicopathological variables. The authors found that PELP1 was expressed at moderate and high levels in all tissues, and that high PELP1 expression correlated with lymph node stage. Combining PELP1 expression with Ki-67 LI, the authors found that the double high population had reduced disease free survival and overall survival. This study adds to the body of work on PELP1 expression in breast cancer, specifically in the TNBC population and is suggestive that PELP1 expression in
combination with Ki-67 could be used as a biomarker of aggressive TNBC.

Minor essential revisions.

1. The explanation for a lack of cytoplasmic PELP1 staining in the current study and the study by Habashy et al should be more comprehensive. The authors suggest that the studies of cytoplasmic functions of PELP1 are flawed because a cell line model expressing an NLS mutant of PELP1 has been utilized to study cytoplasmic functions. This explanation does not accurately represent the published data. There are a number of papers that show PELP1 IHC staining in the cytoplasm. It was the IHC results that lead to the in vitro studies. The PELP1 NLS mutant is a tool to study the signaling, not evidence that cytoplasmic PELP1 occurs in vivo. Alternatively, both the Bethyl laboratory and Novus Biologicals PELP1 IHC antibodies were raised to PELP1 amino acids 1000-1050, thus an alternate explanation could be that this epitope is masked when the protein is in the cytoplasm. It is recommended that the authors modify this section of the discussion to include alternate explanations for the discrepancy in the literature. Of note, most of the PELP1 interacting proteins that have a role in metastasis are cytoplasmic proteins (page 12, line 19).

Response: Modification had been made accordingly; alternate explanations had been included in this paragraph (page11-12, line 216-233).

2. The authors note that the limitation of the study is the small sample size. But is this study powered to observe differences in DFS and OS comparing PELP1 low vs. high groups?

Response: In our study, in the PELP1 low group, 13/59 (22.0%) experienced recurrence, and 10/59 (16.9%) dead; while in the PELP1 high group, 25/70 (35.7%) experienced
recurrence, and 22/70 (31.4%) dead. According to the sample size estimating formula for log-rank test designed by Schoenfeld (reference to: Wittes J. Epidemiol Rev. 2002;24(1):39-53), the minimal sample size for comparing DFS between PELP1 low and high group should be 165.5 at $\alpha=0.05$ and 80 percent power, as well as 132.4 at $\alpha=0.05$ and 70 percent power; while the minimal sample size for comparing OS between PELP1 low and high group should be 129.4 at $\alpha=0.05$ and 80 percent power, as well as 103.5 at $\alpha=0.05$ and 70 percent power. Thus, we had to admit the relative low power for comparing DFS and OS between PELP1 low and high groups in this study due to the smaller sample size. However, the main finding of this study is “Considering PELP1 and Ki-67 expression systemically in TNBC will enhance the prognostic sensitivity of the two biomarkers”, the sample size of this study is powerful enough to comparing the DFS and OS between PELP1/Ki-67 double high group and others according to Schoenfeld’s formula ($\alpha=0.01$, power>95 percent for DFS; $\alpha=0.001$, power>95 percent for OS).

Whether PELP1 could be used independently as a prognostic biomarker for TNBC still need more studies with big sample size to be verified.

3. There should be more about the similarities/differences between the Habashy et al study in the discussion.

Response: More similarities/differences comparing with the Habashy et al study had been included in the discussion section (page 12, line 226-231; page 12, line 236-245; page 12, line 247-249).

4. Page 4, line 6. "lacks" should be "lack"

Response: Modification had been made accordingly (page 4, line 41)

5. Page 6, line 5-7. Alternative version... "Twelve patients were excluded from the study
cohort due to gender (male), or acceptance of neo-adjuvant chemotherapy. The pathological slides of the remaining 147 patients were reviewed... 18 were excluded for discordance between the reviewers, leaving 129 patients in the study.

**Response:** Modification had been made accordingly (page 6, line 90-94).

6. sliders should be slides.

**Response:** The word had been corrected accordingly (page 6, line 92)

7. Instead of writing "Habashy's paper", in the manuscript by Habashy et al.

**Response:** Modification had been made accordingly (page 7, line 122)

8. Page 8, line 10. 38 of 129 is 29.4%.

**Response:** The number had been corrected (page 8, line 142)

9. Define Ki-67 LI.

**Response:** The definition of Ki-67 LI had been added (page 6, line 87-88)

**Level of interest:** An article of importance in its field

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

I declare that I have no competing interests'

**Additional Editorial Request:**

1). Copyediting: We recommend that you copyedit the paper to improve the style of written English. If this is not possible, you may need to use a professional language editing service. For authors who wish to have the language in their manuscript edited by a native-English speaker with scientific expertise, BioMed Central recommends Edanz (www.edanzediting.com/bmc1). BioMed Central has negotiated a 10% discount to the
fee charged to BioMed Central authors by Edanz. Use of an editing service is neither a requirement nor a guarantee of acceptance for publication. For more information, see our FAQ on language editing services at

http://www.biomedcentral.com/authors/authorfaq/editing.

Response: Copyediting had been performed by Edanz.

2). Line numbering: kindly be informed that it should be continuously numbered and not per page.

Response: Line number had been reformulated as continuously.

3). Title page: kindly provide email addresses of all authors.

Response: E-mail addresses of all authors had been added in the title page.

4). kindly rename Introduction to Background.

Response: “Introduction” had been renamed to “Background” (page 3, line 2; page 4, line 36).