Title: Localization of phosphorylated ErbB1-4 and heregulin in colorectal cancer

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Version: 7 Date: 2 October 2014

Author's response to reviews: see over
Dear Dr. Milo Frattini,

Enclosed is a re-submission of MS: 1771760274130307, titled “Localization of phosphorylated ErbB1-4 and heregulin in colorectal cancer” by Mitsui, Yonezawa, Tatsuguchi, Shinji, Gudis, Tanaka, Fujimori, and Sakamoto.

We would like to thank the reviewers for their thoughtful and helpful comments. We have addressed each of the reviewers’ comments in the revised manuscript and trust that the manuscript is improved from the original submission. We highlighted all changes with red coloured text. A point-by-point explanation of how the comments were addressed follows.

We believe that the BMC Cancer readership will recognize the importance of this work and receive the paper with significant interest.

Sincerely,
Atsushi Tatsuguchi, M.D., Ph.D.
Reviewer's report:
Minor essential revisions
1) While the authors have now clarified the methodology used in Figure 1, it remains unclear why the authors cannot validate the purity of their cytoplasmic and nuclear fractions by performing a western blot (using an aliquot of the total lysates prior to immunoprecipitation) for a protein(s) known to be restricted to the respective compartments. In addition, given that the immunoprecipitations of the cyto and nuclear fractions used the same amount of total protein rather than the same number of cell equivalents of each fraction, it is not possible to assess the relative proportions of the proteins in each compartment. At a minimum, the authors should state in the text that a conclusion regarding the relative amounts of p-ErbB2 and p-ErbB3 in the cyto and nuclear fractions is not warranted given the data presented.

Authors’ response:

We agree with the reviewer’s comment. We added the following text in the Discussion section on page 22, lines 16-23:

“The limitation of this study revolves around the fact that calculation of the relative amounts of pErbB2 and pErbB3 in the cytoplasm and nuclear fractions in cancer cell lines is not warranted given the data. The nuclear and cytoplasmic fractions of cancer cell lines were normalized by total protein amount (1 mg) prior to immunoprecipitation. However, following immunoprecipitation, it was not determined during western blot analysis whether both pellets contained the same number of cells or the same amount of protein, since the pellets contained only the phosphorylated form of proteins in the nuclei and cytoplasm.”
Reviewer's report
Title: Localization of phosphorylated ErbB1-4 and heregulin in colorectal cancer
Version: 6 Date: 4 September 2014
Reviewer: Cornelis Sier

Reviewer's report:
The authors have adequately answered the points raised by the reviewers. They should consider to mention the additional IHC for EGFR/ErbB1 into the abstract and aim of introduction.

Authors' response:
We agree with the reviewer's comment. We added the following text in the Abstract section on page 3, lines 20-22:

“Phosphorylated EGFR (pEGFR) immunoreactivity was observed in the cytoplasm and nuclei of cancer cells, whereas the pattern of EGFR staining was membranous and cytoplasmic.”

We also added “EGFR,” in the Background section on page 6, line 24.
Reviewer's report
Title: Localization of phosphorylated ErbB1-4 and heregulin in colorectal cancer
Version: 6 Date: 4 September 2014
Reviewer: Myung-Geun Shin

Reviewer's report:

Authors revised manuscript according to reviewer's comment. They didn't do some additional experiments because of sample shortage (limitation of retrospective study) and technical failure and difficulties.

Authors' response:

We appreciate the reviewer for showing consideration of our problem and evaluating the revised data presented.