Author’s response to reviews

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

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Author’s response to reviews: see over
BMC Cancer

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 reviewer for his thoughtful and helpful comments. We have addressed the reviewer’s comment 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:
The statement that the authors have added on page 22, lines 16-23 is helpful. I do think it makes more sense to add this statement to the results section (for example, page 12 after line 17) rather than to the discussion.

Also, to make the issue clearer to readers, I recommend a slight modification of the second sentence: "Prior to immunoprecipitation, the nuclear and cytoplasmic fractions of cancer cell lines were normalized by total protein amount (1 mg) rather than by the number of cell equivalents of each fraction."

I also think that the third sentence of the statement can be removed, as it is not entirely clear.

Authors' response:

We agree with the reviewer’s comment. We removed the following sentences “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.” from the Discussion section. Instead we added the following text in the Result section on page 13, lines 17-22:

“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. Prior to immunoprecipitation, the nuclear and cytoplasmic fractions of cancer cell lines were normalized by total protein amount (1 mg) rather than by the number of cell equivalents of each fraction.”