Author’s response to reviews

Title: High expression of EphA3 (erythropoietin-producing hepatocellular A3) in gastric cancer is associated with metastasis and poor survival.

Authors:

Baongoc Nasri (pbngoc2001@yahoo.com)

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High expression of EphA3 in gastric cancer is associated with metastasis and poor survival.

Baongoc Nasri, MD

BMC Clinical Pathology

IRui Henrique, M.D., Ph.D.

BMC Clinical Pathology

Attention to Editor of BMC Clinical Pathology

Dear Dr Irui Henrique

First of all, on this opportunity I am very happy to express my sincere gratitude to two reviewers contributed to reviewing my article and giving me instructive and productive comments on it.

Please kindly find my responses to each of them as itemized below.

To Reviewer #1

Thank you so much for sharing your time to review my article.

I am very impressed with your kind and instructive comments on my article. Please kindly find my responses to your questions as itemized below.
<Major points>

I fully agreed with your opinion.

I resubmitted the positive and negative control for EphA3.

I also included image which showed no immunohistochemical staining in normal gastric tissue. Scale bars are included in all images.

Negative control of normal gastric tissue which was prepared without 1st antibody, did not show immunohistochemical staining for EphA3. Normal gastric tissue showed staining in the mesenchyme not in the mucosal layer. Positive control of normal gastric tissue showed strong immunostaining. Representative primary gastric carcinomas with intensity score of 0 (negative), 1 +, 2 + were included. The images were captured under magnification 400 x. Scale bar in the left lower corner is 50µm.

I am very sorry for these confusing images.

<Minor essential revisions>

I totally agreed with your opinion.

Multiple passages are directly lifted from other research articles - while every instance of this that we identified was correctly referenced, it is inappropriate to copy whole sentences word-for-word.

I already edited all of the paragraphs which could be confusing for their similarity.

Although I am not qualified to review the application of statistics in this article, the statistical analysis techniques appear to be consistent with standards for this type of study. A little more clarity in the Methods section regarding this might be helpful, and the sentence "Prognosis was evaluated based on the length of Overall Survival and Relapse Free Survival" should be edited for clarity.

The Method part also edited with more details regarding statistical analysis. I also edited all of incorrect English wording.

Table 1 has categories for "distant metastasis or recurrence", etc,
Table 1 included “distant metastasis and recurrence” which was changed to “distant recurrence”. Patients with Stage IV at time of surgery were excluded from this study. Stage IV is defined as distant metastasis M1 and/or positive peritoneal lavage cytology. Hence any metastasis occurs after the surgery is considered as recurrence.

Authors state that staining extensity was classified into four categories, but then go on to list only three categories.

I deeply apologized for the wrong information in the categorization. We categorized the staining extensity to THREE categories not four as initially written. They are 0 (0-9%), 1 (10-49%), 2 (50-100%).

"separately counted stained tumor cells in at least three field per section, including the deepest site invaded by tumour cells, the surface of the lesion and the intermediate zone" does this mean three fields per section, or one field per section for three sections?

We counted stained tumor cells three field PER SECTION.

<Discretionary revisions/comments>

I am uncertain of the value of combining "staining intensity" and "staining extensity" into a single composite score, given that they reflect very different biological situations. Why not analyse each component separately?

Thank you for your comments. I have read some articles whose composite scores were utilized to analyze the effect of molecule of interest on specific tissue, such as Xi et al (J Gastroenterol. 2012 Jul; 47(7):785-94), Nakagawa et al (Ann Surg Oncol. 2015 Jul; 22(7): 2329-35). Moreover, gastric cancer is known as one of the heterogeneous diseases which require comprised scores of both staining intensity and extensity for accurate analysis (BMC Clin Pathol. 2013 Jun 5; 13(1): 19). Hence, I utilized composite scores for this study.

It's not clear from the introduction why EphA3 was chosen to study.

Other subclasses of EphA such as EphA1, EphA4 have been studied for their effects on gastric cancer. As one of the largest subclass EphA3 has been reported certain effects on solid tumor. However, there is just one study regarding EphA3 effect on gastric cancer. Hence I planned to elucidate its effect on gastric cancer.
The Discussion is largely irrelevant to the Results. There is substantial discussion of mechanism which is wholly unaddressed by the analyses performed in this study and this section could be greatly reduced in length. Discussion should also mention the limitations of the study, and discuss in more depth the differences between their study and the recently published Xi et al study.

I totally agreed with you.

I focused more on limitations of this study such as lack of cell lines study or Western-blotting. I already omitted most of the paragraphs which were not mentioned in this study. I also discussed in more depth the differences between my study and the recently published Xi et al study. For examples, even though we shared certain similar outcomes, my study showed that high expression of EphA3 associated with worse relapse-free survival and higher rate of liver recurrence. My study along with Xi et al is the only two articles regarding the effect of EphA3 on gastric cancer.

To reviewer #2

Thank you for reviewing my article with many instructive comments based on your profound experiences.

I would like to answer all of the questions as itemized below.

Do you have cell culture experiment results? Such as the cell inhibition expression of gastric adenocarcinoma cell lines..

The paper only studied the protein expression level by IHC staining, did you try by Western-blotting? Have you checked the mRNA expression level?

Unfortunately we did not have any cell line experiment results, and we only studied the protein expression level by IHC staining. These are limitations I mentioned in the revised version. As a newly discovered receptor EphA3, very limited resources are available. I included Xi et al findings regarding cell line study, western-blotting in the discussion.

Needs some language corrections before being published

I totally agreed with you. I have corrected all of the English in the revised version
All that I have revised and added sentences that were related to your questions were shown in blue for your quick reviewing and confirmation.

Thank you very much again for sharing your time in reviewing my article.

Best regards

Baongoc Nasri M.D.
1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8519, JAPAN
Tel: + 81-3-3813-6111
Fax: + 81-3-5803-0139
E-mail: pbngoc2001@yahoo.com