Author’s response to reviews

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

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Author’s response to reviews:

On Sunday, November 13, 2016 8:52 PM, BMC Clinical Pathology - Editorial Office

Date: To: "baongoc nasri" pbngoc2001@yahoo.com

Subject: Your submission to BMC Clinical Pathology - CPAT-D-16-00023

Reply-To: "BMC Clinical Pathology - Editorial Office" bmcclinpathol@biomedcentral.com

CC: elaine.zhang@biomedcentral.com

CPAT-D-16-00023

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

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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.

“The positive and negative controls for EphA3 immunohistochemistry are still inadequate. The concern I had was whether your antibody was specific for EphA3. Your negative control addresses only the specificity of your secondary antibody (which is important, but not what I asked for). Your positive control is confusing - you say in your comments to me (although not in the manuscript, where it is listed simply as "Normal positive control") that it is "normal gastric tissue", although 1) it doesn't look like normal gastric tissue, and 2) you show elsewhere that normal gastric tissue is negative for EphA3 (except for mesenchyme). I think what you have done is stained gastric tissue for another marker that you knew would be positive, to show that your technique works. Again, this is useful but not what I asked for and doesn't address the specificity of the EphA3 antibody. What you need for the positive control is some tissue that is known to express EphA3 - this is difficult because EphA3 is not widely expressed, but the use of cell lines (such as LK63) could overcome this. What you need for the negative control is something known to not express EphA3.

Again, this point is of particular concern because Santa Cruz have withdrawn the product from sale, promoting instead their "much superior" antibody which despite it's superiority is not recommended for IHC, and also because the positive control on the Santa Cruz page for this antibody demonstrates staining of human kidney, which is regarded as being negative for EphA3 expression, although there is some dispute.”

I have adjusted figure 1A, 1B to “non cancerous gastric tissue” which was stained without 1st antibody, did not show immunostaining for EphA3 or showed staining in the mesenchyme not in the mucosal layer.

We have two cell lines: KATO III EphA3 (undifferentiated type) and MKN 74 EphA3 (differentiated type) which showed weak to strong staining EphA3, however these staining are not considered as specific for EphA3.

The antibody used in this paper is eligible for immunohistochemical staining as highlighted in yellow in the “EphA3 instruction”. EphA3 immunohistochemical staining is also utilized in other publications such as Xi HQ et.al in J Gastroenterol 2012; 47:785-794, Lu CY et.al in Oncol
Rep 2013; 30:2179-2186. These articles also use Santa Cruz antibody. Even though the brand code is not listed, these articles also use polyclonal rabbit antibody as we do.

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

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