Reviewer’s report

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

Version: 1 Date: 05 Nov 2016

Reviewer: Chris Slape

Reviewer's report:

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Again, this point is of particular concern because Santa Cruz have withdrawn the product from sale, promoting instead their "much superior" antibody which despite it's superiority is not recommended for IHC, and also because the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.

All other changes are acceptable.

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

No

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

No

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I recommend additional statistical review

Quality of written English
Please indicate the quality of language in the manuscript:

Needs some language corrections before being published

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