Author's response to reviews

Title: Vascular measurements correlate with estrogen receptor status

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Author's response to reviews: see over
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Editor
BioMed Central

Dear Sir/Madam,

My colleagues and I would like to resubmit an article titled “Vascular measurements correlate with estrogen receptor status” for consideration by the BioMed Central.

Revisions to this manuscript have directly responded to reviewer’s comments following our initial submission. We believe that the comments provided by the reviewers have given us the opportunity to significantly strengthen this manuscript. For that, we thank the reviewers and look forward to publishing these works.

Specific responses to the reviews are available here:

Reviewer’s report
Title: Vascular measurements correlate with estrogen receptor status
Version: 2 Date: 18 December 2013
Reviewer: Rachael Natrajan
Reviewer’s report:
The authors have addressed some comments, however have failed to address others, and simply state that this is for future research. It would be advisable, if the authors could attempt to perform some of the analyses suggested.

Major Compulsory Revisions

1. Why did they only look at cases >90% ER+ cells if one of their aims was to assess the regional distribution of ER+ and ER- cells. This is a very important point and the authors thank this reviewer for ensuring that additional analysis was performed. The experiment now described significantly strengthens this manuscript. The authors address this point on pages 6 with the statement ‘an additional five cases were selected with <60% ER positivity and used to specifically evaluate the spatial distribution of ER positivity with respect to vasculature’ and again on page 10 and 11 ‘The spatial distribution of ER positivity with respect to vasculature has been of great interest. Five IDC cases were stained with ER (<60% positivity) were used to specifically evaluate the spatial distribution of ER positivity with respect to identifiable blood vessels. Here the study pathologist (MMB) identified visible vasculature directly from the ER stained slides. Larger vessels were clearly identifiable and demonstrated proximal (<30µm) ER positivity in 76.5% (26 of 34) visible vessels (Figure XX). However, due to the fact that the ER IHC DAB stain and hematoxylin counterstain are not sufficient to identify vasculature with confidence (in particular, small vessels), a more rigorous quantitative study has not been performed.”

3. The authors hypothesize that ER will be expressed only if there is estrogen in the microenvironment. Is there a way to test this? After the first review the following statement was added to the manuscript: It is plausible that ER expression only occurs in the microenvironment when estrogen is present may be a testable hypothesis. This was addressed on page 14 with the statement “Regardless, this unavailability of estrogen is one reasonable explanation for estrogen-independent tissue selection. This may be a testable hypothesis in vitro or using techniques including laser capture microdissection to isolate specific regions of high vascularity within patient tumors and evaluating the estrogen concentrations. This is a key future direction for this research.”

This, understandably, did not satisfy this reviewer. Unfortunately, using the pathology slide resources available it was not possible to test for the presence of estrogen itself in the environment. Techniques do exist for the quantification of estrogen levels including E-SCREEN (Soto, A. M., Sonnenschein, C., Chung, K. L., Fernandez, M. F., Olea, N., & Serrano, F. O. (1995). The E-SCREEN assay as a tool to identify estrogens: an update on estrogenic environmental pollutants. Environmental health perspectives, 103(Suppl 7), 113.) and others. In vivo measurements are also becoming increasingly available and interestingly these same measurements have been found to correlate with VEGF production (Garvin, S., & Dabrosin, C. (2008). In vivo measurement of tumor estradiol and vascular endothelial growth factor in breast cancer patients. BMC cancer, 8(1), 73.).

7. The authors conclude that ‘This suggests that as ductal carcinoma in situ progresses towards invasion, if necrosis does not increase with the cancer progression, then ER+ cells are more likely to dominate the population.’ Isn’t it more plausible here that the blood vessel size means the larger they are the less necrosis you get? and is actually determined quite early on? Is there a difference between vasculature in the adjacent normal breast and whether the tumor/DCIS lesion is ER+ or ER-? Were the authors able to look at this
association with DCIS lesions?

These are very good questions and the authors address these questions on page 1 with the statement “Our second hypothesis, that ER status would be inversely correlated with necrosis, was even more strongly supported. This suggests that as ductal carcinoma in situ progresses towards invasion, 1) the larger the vasculature is early in disease progression, the lower the volume of necrosis and 2) if necrosis does not increase with the cancer progression, then ER+ cells are more likely to dominate the population.” And again on page 12 “Finally, adjacent normal breast tissues were also investigated to understand whether or not the vasculature of adjacent normal tissues correlated with the ER positivity in the nearest lesions. The vascularization studies were performed on ten samples which we either 0% ER+ (n=5) or >90% ER+ (n=5). However, there were no observable differences between the vessel features in the adjacent normal tissues.”

8. On page 15 the authors state “Furthermore, it may be possible that ER+ cells cluster around vasculature and effectively act as a barrier’. Did the authors see this?
The authors did not observe vessels large and prominent enough to confidently identify them as vessels in areas of dense and heterogeneous (<60% ER+) ER expression. The authors did include this statement and data “Of great interest has been the spatial distribution of ER positivity with respect to vasculature. Five cases were stained with ER (<60% positivity) were used to specifically evaluate the spatial distribution of ER positivity with respect to vasculature. Here the study pathologist (MMB) identified visible vasculature directly from the ER stained slides. Larger vessels were clearly identifiable and demonstrated proximal (<30µm) ER positivity in 76.5% (26 of 34) visible vessels. However, due to the fact that the ER IHC stain and hematoxylin counterstain are not sufficient to identify vasculature with confidence, a more rigorous quantitative study have not yet been performed.”

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
I declare that I have no competing interests

My colleagues and I thank you for your consideration.

Best wishes.

Mark Lloyd