Author's response to reviews

Title: Vascular measurements correlate with estrogen receptor status

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Author's response to reviews: see over
March 5, 2014

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: 3 Date: 25 January 2014
Reviewer: Rachael Natrajan

Reviewer’s report:
1+ 8) The authors say that the ER IHC DAB stain and hematoxylin counterstain are not sufficient to identify vasculature with confidence - could they do this by staining with co-IHC of ER and CD31, or CD31 in serial sections?
In order to demonstrate enhanced confidence in the effect of ER positivity near vasculature, the authors did compare CD34 and ER in serial sections and quantified the ER stain intensity of each cell and measured the distance of each cell to the nearest co-registered blood vessel (see figure 5 and additions to the methods on pg 8 and 9 and results on pg 11).

This suggestion encouraged the authors to add data to this project which significantly strengthen the manuscript.

3) Given that VEGF expression can be used as a surrogate for endogenous estrogen, could the authors use this to assess estrogen in the microenvironment?
This was a good suggestion. VEGF staining was performed. VEGF expression was found to be strong in regions of high vascularity. However, it should also be noted that VEGF expression was observed to be high (often higher) in regions of low vascularity (ie near necrosis (see below)). Furthermore, near many vessels, VEGF expression appears low near vessels. The authors hypothesize that this is due to the dynamics of the cells adapting to a low or high vascular environment. The difference between VEGF and estrogen is fundamentally significant. Namely, VEGF is secreted by cells in regions of poor blood flow in an effort to stimulate vascular genesis, whereas conversely, estrogen diffuses mainly from the blood vessels. Therefore, the authors believe the new data regarding VEGF expression would only add confusion to the existing manuscript. For this reason, additional information regarding this comment was not included in this manuscript but can be found here:
**Figure X.** VEGF positivity in a single sample relative to vasculature location where A) illustrates relative VEGF negative staining near vasculature (black arrows); B) VEGF production is upregulated near a vessel and; C) VEGF stain intensity is observed to be high near necrosis (red arrows). Scale = 100μm

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