Reviewer's report

Title: Environmental conditions correlate with estrogen receptor status

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The authors used whole slide image acquisition and analysis algorithms to study the relationship between ER expression status, vascularization and extent of necrosis on a panel of primary breast cancer samples. The study revealed that vascularization positively correlates with expression of ER, whereas necrotic areas are more extensive in ER- tumors. Authors used these results to support a priori hypothesis that the correlation between expression of ER and vascularization arises due to selective pressures, where ER expression provides a selective advantage under conditions of adequate blood supply, but is disadvantageous under the conditions of poor vascularization and therefore undergoes negative Darwinian selection.

Contributions of selective pressures in tumor evolution appear to be underappreciated in the mainstream of cancer biology and studies on how tumor biology can be shaped by selective pressures can potentially alleviate this deficiency. However, current study makes an unjustifiable leap of faith between the data and the conclusions ignoring alternative explanations that appear to be much more likely based on current knowledge on the biology of ER.

Authors assume that ER expression is a fixed phenotypic trait that can serve as a substrate for Darwinian selection under positive selection pressure upon estrogen availability and negative selective pressure in estrogen absence. Whereas this assumption is formally possible, large body of evidence from many labs strongly indicates that ER expression is a dynamic trait under complex regulation (for review see PMID15143157).

Of relevance to the current study

1) Genetically homogeneous breast cancer derived cell lines display variability in ER expression even in presumably homogeneous in vitro culture conditions.

2) Similarly to cells from normal mammary epithelia, in breast cancer ER expression is absent in progenitor like cells and abundant in cells with luminal differentiation phenotype (PMID 17349583), i.e. heterogeneity in ER expression within tumor might reflect remnants of differentiation hierarchy.

3) Hypoxia has been reported to induce proteosomal degradation of ER (PMID 19952428, 12351689), therefore findings of the correlation between ER expression and vascularization can be explained on the basis of published knowledge.

Additional caveat, majorly overlooked by the study is that drawing the
conclusions on selective pressures based on comparisons between different tumors is hardly justifiable, especially in the case of comparisons between ER+ and ER- cancers which represent clinically and biologically distinct group of disease, with major differences in evolutionary trajectories (including commonly presumed difference in target cells for cancer initiation, different patterns of gene expression, different patterns of chromosomal instability and different putative drivers), different biological and clinical properties and are thought to originate from different target cells. The particular panel of samples with used in this study (with nearly uniform expression of ER) appears to be ill suited for addressing the roles of selective pressures; analysis of intra-tumor differences would be more revealing (while dealing with heritable differences, which is unlikely to include differences in ER expression).

I strongly disagree with the assumption “As with any common phenotypic trait, we assume that ER expression will be observed only if it provides an adaptive advantage. When estrogen is absent, ER expression represents a needless expenditure of resources and will be selected against”. Whereas these assumptions would be applicable to populations of unicellular organisms under competitive growth limiting conditions, it makes little sense in context of population of tumor cells. Overexpression or deletion of many genes has little to no effect on competitive fitness in tissue culture or xenograft studies, suggesting that energy considerations required for expression of a single gene are unlikely to play significant role selection pressure wise.

It should be at least noted in the discussion of the data that due to tumor-specific alterations in vasculature bed not all of the endothelial cells would necessarily display CD34 staining, therefore there is a possibility that some differences might be over or underestimated with the use of the antibody employed in the study.

I would like to advise more careful referencing to figures. For example, authors state “The average diameter of ER+ DCIS without central necrosis was 702 µM (Figure 4)”, whereas figure 4 contains no quantitative data instead showing some representative H&E pictures. Similar inaccuracies can be also found in references to other figures.

Throughout the manuscript some data is presented as mean numbers only without showing deviation, which hinders adequate evaluation of the results.

Likewise, I suggest greater care with references. Reference Alfarouk et al 2013 mentioned in the Introduction is missing in the list of references. To support statement “Similarly to molecular heterogeneity found in other cancers” authors reference 2 reviews, 2 primary papers and 1 mathematical modeling paper, which is a questionable choice, as the 2 primary papers do not exhaust massive body of experimental literature on the subject, referencing 1-2 comprehensive reviews would be sufficient, also see comment below regarding use of term “molecular heterogeneity”.

Authors appear to be using genetic heterogeneity, cellular heterogeneity and
molecular heterogeneity interchangeably, which not only lacks precision, but can also lead to unnecessary confusion, as terms cellular heterogeneity and molecular heterogeneity are commonly used to describe an entirely different phenomena of meaningful phenotypic differences within cell populations that arise due to stochastic fluctuations in gene expression networks (for example see high profile review PMID 20478246).

Statement “.. the source of estrogen in the breast is typically serum” is incorrect, serum is blood plasma depleted of coagulation factors, whereas cells in normal tissues and tumors are bathed in interstitial fluid, not serum.

In summary, the data presented in the paper might be useful for the cancer research community. However, sound support of author’s conclusions would require refuting large body of evidence on dynamic regulation of ER expression and its dependence on oxygenation levels. I suggest complete re-evaluation of conclusions and discussion of the