Reviewer's report

Title: Clinical instability of breast cancer markers is reflected in long-term in vitro estrogen deprivation studies

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Reviewer: Chin-Yo Lin

Reviewer's report:

This report provides interesting and important observations regarding the expression of molecular markers and key regulators of hormone signaling and growth pathways and gene expression in general in response to LTED. This is of particular interest to those who rely on the cell line models described. There are a number of issues to be considered and addressed, however, before the paper is ready for publication.

Major Compulsory Revisions

1. The protein expression data were obtained exclusively by IHC. Although it might not be practical to repeat the entire study, repeating the earlier time points and showing changes by western analysis can provide confidence in the approach, reagents, and the results obtained.

2. There has been a number of microarray studies describing estrogen-responsive changes in gene expression in breast cancer cells. A key question of the observed changes in gene expression following LTED is which of the changes are direct effects of inactivating estrogen receptor, including those that are in common with the AI treatment study. This analysis should be performed and the results included in the section describing the microarray data from the current study.

3. The authors cite Aguilar et al. who performed a similar microarray study in MCF-7 cells, but the comparison of studies and results is lacking. This should be provided (analysis and detailed discussion) so the readers can interpret and evaluate this study in the context of the previous study.

Minor Essential Revisions

1. In the Methods section, it states that "Each culture medium was supplemented further with 10% heat-inactivated FCS...” This should be omitted since the addition of serum was described in the previous sentence, unless the authors added an additional aliquot of serum.

2. The text within each of the circles in Fig. 5 can be enlarged for ease of reading.

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