Reviewer's report

Title: An integrated analysis of genes and pathways participating in adaptive survival in estrogen receptor positive breast cancer cells

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Reviewer: Chin-Yo Lin

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General
The authors utilized publically available data from Serial Analysis of Gene Expression (SAGE) studies to identify genes differentially expressed in estrogen receptor-positive (ER+) breast tumor cells under estrogen starvation conditions as compared to normal breast tissue. Differentially expressed genes were further categorized based on gene ontology annotations. The aim of the study was to identify genes and pathways that may potentially be involved in the "adaptive survival" of ER+ tumor cells.

Overall, the scientific rationale was unclear and the results and conclusions are unconvincing.

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Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

1. The title and introduction refer to the term "adaptive survival" in describing the biology that the authors aimed to investigate. This term was coined to describe the ability of cells to survive large doses of radiation or cytotoxic drugs when previously exposed to lower doses of radiation or drugs. How this is related to the aim of the study is unclear. The authors also stated in the introduction that "differences in adaptive mechanisms favoring survival of ER+ breast cancer cells are largely unexplored." The simplest explanation for the survival and proliferation of ER+ cancer cells, which is noted by the authors but quickly dismissed perhaps due to a number of publications already addressing this, is that estrogens function as mitogens in both normal and cancerous ER+ breast epithelial cells. Whether there is "adaptive survival" to be seen in ER+ cells is questionable.

2. If the rationale seemed unclear, the confusion is further compounded by the experimental design of comparing two ER+ cell lines under estrogen starvation or in their "basal state," as the authors put it, with a normal breast tissue control. First, there is no explanation of what is considered "normal" tissue. Was it tissue adjacent to the excised breast tumors or from an immortalized but non-transformed cell line? Second, this comparison appears to be examining estrogen responsive genes after all, albeit in reverse. If normal breast tissues were examined, these tissues would have contained ER+ epithelial cells which would have been exposed to estrogens present in the tissues. If this is the case, then the authors would have presented a round-about way of identifying estrogen responsive genes rather than delineating genes involved in "adaptive survival."

Finally, it is still not clear how the comparison between the three datasets could yield an understanding of "adaptive survival."

3. The results from the analysis suggest differences between the two cell lines. In addition to biological differences between the cell lines, and there are potentially many due to differences in genetic background of the patients and different somatic mutations giving rise to the cancer cells, could there be technical differences in how the cells were treated, the RNA samples were prepared, and the SAGE libraries were constructed which may also have contributed to the observed differences? For example, ZR75-1 cells used to generated the published results were estrogen deprived for 7 days whereas the MCF-7 cells were starved for 48 hours. These and other technical disparities would likely confound the major conclusions of this analysis.

4. Since none of the differentially expressed genes or pathways were confirmed experimentally (Northern or real-time PCR), it is difficult to assess the validity or the potential functional impact of the results from a purely computational analysis.

5. The authors stated in the conclusion section that ZR75-1 cells mimic ER- breast tumors. Can they provide experimental data (SAGE results in an ER- cell line?) or appropriate references to demonstrate these similarities? It also seems overly ambitious to draw conclusions on clinical parameters and disease
outcome based on an analysis using two cell lines and one control sample.

6. A large volume of microarray results examining both estrogen responsive gene expression in cell lines and gene expression profiling of breast tumor samples have been published, and the data are publicly available. The authors should reference results obtained using other technologies, and the study may benefit from a comparison of an analysis of the microarray results vs. the SAGE results reported here.

7. Computational tools were used for gene ontology and pathway analysis but the authors did not describe the statistical methods and selection parameters for determining significance.

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article of limited interest

Quality of written English: Needs some language corrections before being published

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.