Author's response to reviews

Title: An integrated analysis of genes and pathways exhibiting metabolic differences between estrogen receptor positive breast cancer cells

Authors:

Soma SM Mandal (mandals0@cc.umanitoba.ca)
James JRD Davie (davie@ms.umanitoba.ca)

Version: 5 Date: 24 August 2007

Author's response to reviews: see over
Respected Dr. Edmunds:

Thank you very much for your decision on our manuscript. As the first and second reviewers’ have suggested acceptance without further revision, we have addressed the concerns raised by the third reviewer before and now (please refer to the point-by-point response below). We have incorporated these comments by this reviewer in the appropriate sections. These revisions have improved the manuscript significantly, and we would like to extend our thanks to the reviewers. You have indicated some revisions for the manuscript, including formatting. We have done the required formatting in the manuscript, such as adding the web links as references from the text as suggested by you.

Incorporating these final revisions, we are now submitting the final draft of the manuscript. We hope to hear back from you soon.

Sincerely yours,

James Davie, Ph.D.
Professor, Department of Biochemistry and Medical Genetics
Canada Research Chair in Chromatin Dynamics
Director and Margaret A. Sellers Chair, MICB
Provincial Director, Research, CancerCare Manitoba
675 McDermot Avenue
Winnipeg, Manitoba R3E 0V9 Canada
Tel: (204)787-2391
Fax: (204)787-2190
E-mail: davie@ms.umanitoba.ca
Addressing the comments of Reviewer 3

The authors’ efforts to address previous comments are appreciated but still do not address previous concerns regarding the validity of the SAGE results and correlation with clinical parameters. First, there was no attempt to experimentally verify that the genes involved in the key pathways or ontology groups that the authors used extensively to define and explain the differences between the two cell lines are indeed differentially expressed between the two cell lines. Confirmation by Northern analysis or quantitative PCR is two possible approaches for accomplishing this, even if it is for only one of the ontology groups.

As pointed out by the Editor and the panel of medical and biology editors of this journal, this is an in silico study to show how a plethora of stat-of-the-art bioinformatics tools can be utilized to elucidate a biology in a study and identify pathways of the expressed genes to take advantage of pathway based drug design besides other downstream applications. Besides, we have given sufficient validation by comparing our results with the real tumor tissue sample (suggested by second reviewer in the previous revision) as well as ER negative cell lines to validate our findings in the two cell lines. We agree with the Editor that this is a preliminary work and we did not make any prognostic claims in this study nor is this a pharmacologically oriented study. The importance of this study is pointed out as above.

Second, when the authors introduced GSEA data from clinical samples representing luminal A and B and basal epithelial cell tumors to demonstrate similarities or differences in gene set enrichment, there is no clear indication in the figure or the results section how these similarities or differences are measured and statistically supported. It is also not clear whether the tumor data represent three tumor samples or three groups of tumors.

To support our cell line data, reviewer 2 had suggested that we compare our results with tumor samples by means of GSEA analysis. So in our previous revision, we incorporated these results to address the second reviewer’s concerns. The data we have presented is representative of the tumors of specific groups, which were only used to compare and validate our data. As pointed by this reviewer, we will be more explicit about his comments. This was reviewed by reviewer 2 in the third round of revisions and he found it befitting to the contents of the present manuscript. The relevance of tumor data is to compare trends in the light of this manuscript as suggested by the reviewer (#2) and we have complied with that.

Wouldn’t a more direct comparison be using gene expression profiles to hierarchically cluster the cell lines with the tumor samples to show similarities and differences, as it has been done previously (Perou et al, Nature 2000 and many other subsequent papers) rather than “eyeing” gene ontology results and trying to discern trends? As it is, the results comparing cell lines with tumors are not convincing and do not support the authors’ contention that there is prognostic value in the results.

We agree with the editor that we do not have any intention of giving any prognostic value to our results. The results are based on in silico findings. Moreover, comparison by using gene expression profiles to hierarchically cluster the cell lines with the tumor samples
only provides changes in gene expression, but gives no information about how the group of genes fall into any pathway(s), Gene Ontology, and protein domains; therefore, this is the significance of this group(s) of genes in the light of their expression differences. By showing the pathway of the groups of genes here, we have correlated them in the context of our study, which is to discern similarities and differences between cell lines which are representative of a subset of ER(+)ve tumors.

Finally, if it is the authors’ aim to show that their approach represent a significant advance in identifying prognostic genes or gene sets, then they should complete the study by taking expression data from patient samples with corresponding clinical and follow-up data (a number of studies have been published and the data should be publicly available) and show that their gene sets can distinguish the different tumor types and are associated with disease outcome (Kaplan-Meier survival analysis?).

In response to this, we would like to emphasize that we do not wish to make any prognostic claims, rather show possibilities that such genes are of importance in this subset of breast tumors. We have supported our findings at all stages with literature evidence and also pointed out that further studies will be required to use these findings for downstream applications such as drug design or prediction of patient outcome. Reviewer 2 had suggested that we should clearly mention this in the manuscript and we have complied with it. All our results are thus supported by available data and the possibilities of further investigation under certain appropriate conditions including disease outcome have been mentioned as suggested.