Author's response to reviews

Title: Global Gene Expression Analysis in Time Series Following N-Acetyl L-Cysteine Induced Epithelial Differentiation of Human Normal and Cancer Cells In Vitro

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Author's response to reviews: see over
RE: Re-submission of manuscript to BMC Cancer

Dear Editor,

Please find enclosed the revised manuscript "Global gene expression analysis in time series following N-Acetyl L-Cysteine induced epithelial differentiation of human normal and cancer cells" by Anna Gustafsson et al. that we wish to re-submit for publication in BMC Cancer. We are sorry for the delay in our response but this is due to the two first authors having new research and working positions, which has hindered a coordinated effort to improve the paper, as well as family reasons for the corresponding author.

We are happy to find that the reviewers found some merit in our work and we also acknowledge the valuable comments provided by the referees to improve and clarify the paper. Below is our response to the reviewers' comments in a point-by-point fashion and we hope that the reviewers find the improvements sufficient for a publication in BMC Cancer.

Best Regards

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Reviewer 1: John Mariadason

1. We have as the reviewer suggested performed a gene ontology analysis of the results to further support our claims and two new tables have been included in the manuscript together with comments in the results and discussion section. The new additions demonstrate the abundance of differentially expressed genes grouped according to their functional category and show a general trend for the cells to change their transcriptome from a proliferation to a differentiation state using two separate pathways.

2. The phenotypic effects are, as the reviewer points out, included in another paper by a collaborating group. That paper is rather extensive in terms of describing the morphological, biochemical and molecular outcome of N-acetyl-L-cysteine treatment and gives firm evidence of the claims stated in our paper. That paper is currently being revised for another journal and is accessible to the reviewers by our homepage ([http://biobase.biotech.kth.se/NACmanuscript](http://biobase.biotech.kth.se/NACmanuscript)) using the password Parasassi. We ask the reviewers to keep the pending paper confidential. We have tried to improve the paper to further stress on our results in the context of biological context giving additional references to this previous work.

3. We agree to a certain extent that the graphs contain limited information but we still believe that the time response is important especially when comparing to different cell systems that could have different kinetics. Furthermore the combination of the new gene ontology information and the apparent differences in observed differentially expressed genes for the two cell types (although having similar phenotype) are unexpected and the graphs give a further overview of the transient and the more constant gene expression patterns. The supplementary material has been included as tables in the revised manuscript, as requested by the reviewer.

4. We agree with the reviewer that the differences in gene expression was expected to be more convergent considering the similar morphological and biochemical data provided in the paper by the collaborating group. However the new gene ontology information gives support to the switch of proliferation to differentiation. The differences in the actual transcriptome could then indicate that different pathways are employed. Obviously this is very interesting and needs further experimental work.

5. The weakness of the study is obviously that we could not include more replicates due to our limited research funds, which is a common problem with Affymetrix analysis. The Affymetrix experiments are good but very expensive. We have tried to approach the issue by having the time series design (that in part confirms gene expression patterns over time), RT-PCRs that are included in the paper and give support to the array results and we have also recently switched to more affordable arrays by using in house produced spotted arrays. We believe that future studies on these cell systems will be more extensive to better define true biological changes from technological noise. The p-value cutoff is still within reason (we had to simply consider for the lowered power due to replicates), but also, a vast majority of the genes (>90%) are far below the 0.05 p-value cutoff. We have nevertheless been able to confirm many genes in the current manuscript.
Reviewer 2: Pierfrancesco Marconi

1. The phenotypic effects are, as pointed out to reviewer 1, included in another paper by a collaborating group. This paper is now accessible to the referees as pointed out to reviewer no 1. Please keep the information confidential. We have, as indicated earlier, tried to improve the paper to further describe our results in the biological context from the previous paper.

2. We agree with the reviewer and we have included the gene ontology comparison as two new tables in the paper to better compare the different functional groups in terms of cell types and their regulation and we have also slightly expanded the Background to give some more information of the previous results.

3. The rationale of using two different concentrations is that this is required for the two cell types to demonstrate similar morphological and biochemical properties as described in the submitted paper that now is accessible at our web site. We have included an explanation in the Methods section.

4. This study did not have a focus on the apoptosis machinery although this is one of the important issues in the other submitted paper. They demonstrate that apoptosis is not a key event explaining or contributing to the conversion from proliferation to differentiation. Our data support these previous findings and we have now added a comment on the subject in the revised manuscript (in the discussion of GO analysis).

Minor essential revisions

1. The sentence and reference have been removed.
2. Volumes and pages have been included.