Author's response to reviews

Title: Expression profiling identifies genes involved in neoplastic transformation of serous ovarian cancer

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Author's response to reviews:

11th August 2009

Prof. M. Norton,
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Dear Professor Norton,

We wish to resubmit for publication in BMC Cancer a manuscript entitled “Expression profiling identifies candidate genes involved in neoplastic transformation of serous ovarian cancer” by Merritt et al. This manuscript (version 2) was returned on the 15th July 2009 with a decision of “revise and resubmit”. The material in this manuscript has not been published nor is it under active consideration by another journal. All authors have reviewed the manuscript.

We thank the reviewers, as well as the Editorial Board Member, for their considered comments and willingness to review this manuscript. We believe that many aspects of the manuscript have been improved from the comments and suggestions made by the reviewer’s and Editorial Board Member, as well as the additional data and clarification they have requested. As requested, all changes
have been outlined in the online section in a point-by-point response. We have also underlined changes to the most recent version of the manuscript in the updated document. We have also included the GEO Series Accession Number, as requested.

We hope that the changes to the manuscript will now make it acceptable for publication. We thank-you for reconsidering this manuscript for publication.

Yours Sincerely,
Glen M. Boyle PhD

Reviewer’s report.
Title: Expression profiling identifies genes involved in neoplastic transformation of serous ovarian cancer
Version: 2 Date: 15 July 2009
Reviewer: Barbara Vanderhyden
Reviewer’s report:

Major Compulsory Revision:
The stated goal of this work was “to identify genes associated with neoplastic progression in the serous type of ovarian tumors”. The value of this type of work (i.e. microarrays) is not simply to show that there are differentially expressed genes (that has been done before), but to demonstrate that the candidate genes identified have some relevance. The authors do not propose to be identifying biomarkers of clinical outcome; i.e. there is no association made between expression of these genes and clinical outcome. The value of these candidate genes then is assumed to be their functional role in tumor progression. As it is presented here, the authors have identified and validated five candidate genes, and performed an analysis with only one of those genes in one cell line to investigate its function. While very promising, it is a superficial analysis and any subset of the experiments proposed by Reviewer 3 would have been helpful. Even without additional experimentation, this section could be strengthened by a re-evaluation of the microarray data for the expression of other wnt factors and members of the WNT pathway to differentiate between the potentially unique role of WNT7A vs. a more global alteration in WNT pathway activity. While I agree with the authors that a full investigation of WNT7A is beyond the scope of this manuscript, the investigation of the one gene whose function they did chose to validate should be done more convincingly.

As suggested by the original Reviewer 3, and the Editorial Board Member, we now include the data showing expression of WNT7A in a panel of 11 ovarian cancer cell lines, and make the comparison against immortalized human ovarian surface epithelium (HOSE cells). Importantly, 8 of the 11 ovarian cancer cell lines show higher expression levels of WNT7A compared to HOSE cells. These results are presented as a new part of Figure 3 (A), and are described in the Results section. Please see page 13 of the revised manuscript for the additional
We have also performed bioinformatic analyses of all of the gene lists obtained in this study using Gene Set Enrichment Analysis (GSEA) and Expression Analysis Systematic Explorer (EASE) pathway analysis as originally suggested by Reviewer 3. This analysis attempted to identify if the defect was possibly a global alteration of the WNT pathway, and other members of the WNT pathway were differentially regulated. No specific signature relating to the WNT pathway was identified from any gene list presented in the manuscript in either analysis; in fact no significant signature was observed for any pathway. We have added a description of the additional analyses in the “Results” section. Please see pages 10 and 11 of the revised manuscript for the additional text.

Additionally, as suggested by the original Reviewer 3 and the Editorial Board Member, we now include data showing the expression level of all of the WNT family members present on the microarray as new Table 5, and of all WNT pathway members (taken from GSEA and EASE lists) present on the microarray as Additional file 7. Expression of WNT3 and WNT8A were found to be increased in LMP/invasive tumors when compared to normal/benign tissues, possibly indicating a more widespread activation of the WNT pathway in these tumors. These results are presented on pages 12 and 13 of the revised manuscript, and discussed on page 16, with a statement indicating that this was not detected with initial pathway analysis.

Minor Compulsory Revision:
The authors have stated in the Abstract that the expression profiling was done on the surface epithelium of four normal ovaries, but the Methods indicate that the whole ovaries were used. The Abstract needs to be revised if the Methods are correct.

The Abstract has been changed to reflect the tissue used. The Abstract now reads “Expression profiling was carried out on samples of 7 benign, 7 LMP and 28 invasive (moderate and poorly differentiated) serous tumors and four whole normal ovaries using oligonucleotide microarrays representing over 21,000 genes.”