Author's response to reviews

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

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Version: 2 Date: 27 February 2009

Author's response to reviews:

27th February 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 was returned on the 9th February 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 editors, for their helpful 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 additional data and clarification they have requested. As requested, all changes have been outlined in the online section in a point-by-point response. For clarity, we have numbered the Reviewer’s reports 1
to 4 in the response. We have also underlined all changes to the manuscript in the updated document.

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 – 1.
Title: Expression profiling identifies genes involved in neoplastic transformation of serous ovarian cancer
Version: 1 Date: 5 November 2008
Reviewer: Teri Longacre

Reviewer’s report:

Major Revisions:
1. The percent epithelial content needs to be correlated with the array data. The authors suggest that similar expression profiles in benign tumors and normal tissue may reflect stromal content as opposed to epithelial expression, but they should be able to correlate to stromal content in these cases.

We included in Additional file 1 data showing epithelial (tumor) percentage of each sample, as reviewed by an experienced histopathologist associated with the study (D.J.P.). The number of samples (7 benign tumors and 4 normal ovaries) was too small to further subdivide this group for additional statistical analyses of factors like stromal content. We can however state that there was no obvious correlation of epithelial (tumor) content in the benign tissues and clustering shown in Figure 1 of the manuscript. The benign tissues used in the study varied between 1 and 10% epithelial content (Additional file 1), and cluster tightly together in no particular order. Importantly, tissues containing 1 and 10% epithelial tissue clustered together, whereas both of the samples with 10% epithelial content were further apart. We feel that this suggests the epithelial content did not have a major impact on the clustering.

The only data subset with a sufficient number of samples to correlate gene expression with stromal content was within the invasive tumor groups. Within the subset of invasive tumors (n=28), we carried out separate analyses to determine whether a lower percentage of tumor cell content influenced the gene expression profile. Specifically, seven of 28 invasive tumors that were analyzed by microarray had a percentage tumor cell content less than 50%, but no lower than 20%. When these seven tumors were removed and analyses were repeated, we observed a high degree of overlap with the gene lists reported in the current manuscript and hierarchical clustering patterns were also similar. We also carried out a comparison of 7 invasive tumors (epithelial content <50%) vs 21 tumors (epithelial content # 50%) but the number of genes identified was less than that
expected by chance.

To clarify this point, we have included a reference in the “Materials and Methods” section to the additional data describing the epithelial or tumor content (page 4), and a statement in the “Results” section, which reads “Further, hierarchical clustering was not affected by epithelial or tumor percentage content (data not shown)”. Please see page 9 for the additional text.

We appreciate the Reviewer’s suggestions and plan to evaluate this issue in future expression profiling studies that utilize microdissected tissues.

2. The Venn diagrams in Figure 1c needs more explanation. For example, does the 44 entry reflect co-clustering of SLP1 and GLTSCR2? If so, what does 43 represent? WNT7A is differentially expressed in invasive vs normal as well as vs benign, but it appears to be represented only in the benign vs invasive group in the Venn diagram.

Thank you for pointing out the need for further clarification in this figure. The list of 44 genes represents those that are unique to the ‘Normal vs Invasive’ comparison and were not in the other two comparisons (Benign vs Invasive and LMP vs Invasive). The middle number of genes (e.g. ‘43’ gene list) refers to genes that overlapped in two separate analyses (specifically Normal vs Invasive (89) and Benign vs Invasive (311)). We have added further explanation of the Venn diagram when it is first presented in the “Results” section. Please see page 10 for the revised text.

We state in the text and in the legend to Figure 1C that WNT7A was identified in the “benign vs invasive” (n=311) microarray comparison. The Reviewer is correct that WNT7A is also differentially expressed in the invasive vs normal comparison (and this was confirmed further later on by real time PCR), however with the analyses restrictions presented in Venn Diagram (p < 0.01, Student’s t-test with Benjamini and Hochberg FDR), WNT7A did not make the cutoff for this particular pair wise comparison.

3. The discovery that a small gene set distinguishes LMP and invasive cancer is not new. This was reported in the Gilks et al study. The authors are not citing the latter study correctly.

Reference to the Gilks et al. study has been corrected as they also found that only a small set of genes distinguished serous LMP and invasive tumors. We apologize for this oversight. Please see page 13, where the Gilks et al. study has been included in the references to the statement “Our results are in agreement, however, with previous findings in serous ovarian tumors”.

4. Representative images of the frozen tissue sections of benign, cancer and LMP tumors should be presented.

We have added representative H&E images of normal ovarian tissue, 2 benign tumors, 2 LMP tumors and 2 invasive tumors in a new Additional file, and alerted the reader to the new file (page 11). Please see Additional file 6 for details.
Minor Revisions:

1. Abstract, Results, 47 should be 46.

We apologize for the oversight in the manuscript. All references to “47” tumors have now been changed to “46”.

2. This study was conducted in Australia. Why use US cancer statistics? Australian statistics would be more relevant to their data.

As the manuscript was being sent to a US-based journal, we felt that including US cancer statistics would be more appropriate. The incidence rates of ovarian cancer in Australia and the US are very similar. To address the Reviewer’s comments, we have since edited the manuscript to present Australian in place of US statistics as this is the population being studied. Please see the top of page 3 of the revised manuscript for the changes.

3. "Positive staining" for SLPI should be described more fully: cytoplasmic, nuclear, both??? The figure depicting staining for SLPI suggests it is cytoplasmic.

Was there stromal staining in any of the tumors? Also, the figures that are depicted are a bit deceptive, since the data in Figure 1d indicates that LMP tumors were more frequently positive than invasive cancers.

We have modified the text in the Results section to state that SLPI protein expression was cytoplasmic and also that no staining was observed in stromal cells. Please see page 11 of the revised manuscript for the changes. The legend to both Figure 2 and Table 3 also state that SLPI staining is cytoplasmic, to clarify this to the reader.

The Reviewer is correct that Figure 1D illustrates that SLPI mRNA expression is higher in LMP than in invasive tumors in the microarray analysis. However, the further studies presented in this manuscript showed equal or higher levels of SLPI in invasive compared with LMP tumors by real time PCR and immunohistochemistry, respectively (Tables 3 and 4). We expect the real time PCR results to be the more reliable measure of mRNA expression as it is optimized on a gene-by-gene basis and we evaluated gene expression in a second, additional set of tumors. Based on the real time PCR and immunohistochemistry results, we believe that Figure 2 presents an accurate representation of the results and trends that were observed in the data.

Reviewer’s report – 2.

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

Version: 1 Date: 6 January 2009

Reviewer: Lin Zhang

Reviewer’s report:

The authors compared mRNA expression profiles during malignant
transformation of ovarian surface epithelium. And several potential targets were identified by microarray, further validated by RT-PCR. WNT7A was able to increase cell migration in vitro. This is well-designed study, and should contribute to both diagnosis and treatment for human ovarian carcinoma.

Minor Essential Revisions:
How do the authors over-express WNT7A in OVCAR3? Using full length cDNA? Is it transient or stable over-expression? The authors should provide more detailed information in the method section.

The reviewer is correct, over-expression of WNT7A was using a full length cDNA. The over-expression was by stable transfection of the cell line. These points were left out of the original manuscript to minimize space, but are now clarified in a new section in Materials and Methods section. Please see page 8 for the additional description.

The function of WNT7A was examined in only one cell line, OVCAR3, by over-expression. If authors can test it in other ovarian cancer cell lines, it will be more robust conclusion. E.g. using siRNA to knock-down WNT7A expression in high level expressing cell lines. If the authors are not going to perform further experiments, they might discuss it in the discussion section to point out the weakness of the current study and future direction.

We agree that further studies into the exact role of WNT7A in ovarian cancer progression are very desirable, but would represent a major addition to the current manuscript and are outside the scope of the stated major aim. We have therefore altered the “Discussion” section to read “The data presented here is limited by the use of a single cell line. Further analyses of additional ovarian cancer cell lines with either overexpression or ablation of WNT7A would be needed to precisely identify the role of WNT7A in ovarian cancer progression. These studies will be undertaken in the future”. Please see page 15 for the additional text.
Major Compulsory Revisions

1. Since the authors focus on Wnt7A for the verification studies, the paper should really be about the WNT pathway. The authors fail to give additional details about the other wnt factors expressed in the ovarian cancers and OVCAR-3 and the other proteins in the pathway. In addition, do the other serous cell lines that have WNT7A expression show increased migration and invasive characteristics compared to OVCAR-3? Are other Wnt proteins present in the OVCAR-3 cells? The authors should show that a knockdown of WNT7A in one of the other cell lines has the opposite effect on migration and invasion. Could the authors use a different Wnt family member and get the same effect? Lastly, since WNT7A has its effect extracellularly, this means that the pathway must be intact in those cells.

The major aim of the current study was to clarify the relationship of the different clinically observed types of serous ovarian tumor, given the uncertainty about the relationship between these different forms. We selected five genes for further analyses and carried out functional validation of WNT7A in ovarian cancer lines. The finding of increased WNT7A expression in ovarian cancer is a novel and interesting result and holds promise to advance understanding of the pathogenesis of this disease. We agree that the WNT7A studies comprise one of the most interesting aspects of this manuscript, however our overall aim was to apply an unbiased experimental approach (microarray analysis) to learn more about molecular pathways that may play a role in serous ovarian tumorigenesis. We feel that any further examination of the potential effectors of these differences (such as WNT7A) would be outside the scope of the current study. We agree that further studies into Wnt family members, and additional investigations into the exact role of WNT7A in ovarian cancer progression are very desirable, but would represent a major addition to the current manuscript and are outside the scope of the stated major aim.

We have therefore altered the “Discussion” section, as suggested by Reviewer 2, to outline the limitations of the current study, to read “The data presented here is limited by the use of a single cell line. Further analyses of additional ovarian cancer cell lines with either overexpression or ablation of WNT7A would be needed to precisely identify the role of WNT7A in ovarian cancer progression. These studies will be undertaken in the future”. Please see page 15 for the additional text.

2. The authors should calculate the chance number of genes that would be observed in their experiments. Could a number of the genes identified be observed by chance?

We applied very conservative restrictions to these analyses (using the False Discovery Rate multiple testing correction as well as a p-value of 0.01) so that only 1 in 100 genes (1%) would be expected to be identified by chance. A statement to this effect has been added in to “Materials and Methods” under the Microarray data analysis section. Please see page 6 for the additional text.

Minor Essential Revisions
3. Are the benign serous samples in fact serous cystadenoma samples? If so, this should be mentioned.

The benign samples were a mix of serous cystadenomas and cystadenofibromas and all tissues used in the study were independently evaluated by the study pathologist (D.J.P.). This detail has been added to the Methods/ Tissue collections section. Please see page

4. The authors overemphasize their point about what is a good control and that a Mullerian derived benign tumor is a better control. It is not clear what the best control is for any cancer study. Perhaps they need to tone down these statements.

We appreciate the Reviewer’s comments and agree that based on data from the current and previous studies, it is not clear what the best ‘normal’ reference/control would be. We therefore have removed all references and description of the Mullerian derived tumors, with the exception of one statement on this point in the Introduction.

Reviewer’s report – 4.
Title: Expression profiling identifies genes involved in neoplastic transformation of serous ovarian cancer
Version: 1 Date: 28 January 2009
Reviewer: Peter J Fuller

Reviewer’s report:
Apologies for the late response on this manuscript. In this microarray study, the authors examine 7 benign, 7 low malignant potential and 28 invasive epithelial ovarian tumours using an oligonucleotide microarray. The study follows a number of similar microarray studies in the ovarian cancer area. Numerically, it is somewhat dwarfed by some other studies. The number of tumours in the benign groups and for the normals is relatively small.

The advantage of our study was that all of the tissues were robustly verified histologically. Further, we performed validation on an equal number of tissues, a practice not usually performed in the field.

The array platform is to some extent superseded.

The array platform used has been widely used and is a robust one. Please see examples of references below, all using the array platform used in the current manuscript. We feel that the data is solid, as real time PCR confirmed the data not only in the tissues used in the microarray study, but also in an independent validation set of tissues.


Kojima S, Mulholland DJ, Ettinger S, Fazli L, Nelson CC, Gleave ME. Differential regulation of IGFBP-3 by the androgen receptor in the lineage-related androgen-dependent LNCaP and androgen-independent C4-2 prostate cancer models. Prostate. 2006 66(9):971-86.


The work has been conducted with appropriate rigour. It is perhaps less clear how much this adds to our knowledge of the field. The authors identify a number of transcripts that are differentially expressed and confirm this with RT-PCR. Although they are interesting, they are only partially characterised. As a consequence, the study is largely descriptive. One of the issues that is currently being discussed is the aetiology of epithelial ovarian cancers and the possibility that they arise from the Fallopian tube rather than from the ovary. This is not discussed. One ponders whether the appropriate control issue might have been the epithelium of the Fallopian tube? In some ways, given the clear morphological differences in the samples examined, it is surprising there aren’t more differences than were identified.

We appreciate the Reviewer’s comments in regard to “appropriate rigour”. We also agree that based on data from the current and previous studies, it is not clear what the best ‘normal’ reference/control would be, as also outlined by Reviewer 3. We also agree that given the morphological differences of the samples examined, it is surprising that there are not more differences in gene expression identified. We also feel that this is one of the key findings of the current manuscript, as we have outlined in the Abstract and Conclusion.

It is not clear that any of the differences identified represent pivotal changes that might be predicted to drive the neoplastic process. It also remains the matter of some contention that these lesions do in fact represent a continuum. This issue is not fully addressed in the Discussion nor considered in the context of the data.

We agree with the Reviewer on this point. The data presented in this manuscript may suggest that benign ovarian lesions are a distinct entity to LMP and invasive
tumors, and further may suggest that there is in fact not a continuum. To further strengthen this point in the manuscript, we include a statement in the "Discussion" section, which now reads “Further, our data may also suggest that benign ovarian lesions are a distinct entity to LMP and invasive tumors, given the differences or similarities in gene expression profile between the tissues”. Please see page 13 for the additional text.

Overall it is a sound study, but perhaps with only an incremental gain in knowledge.

We thank the reviewer for the assessment as a sound study. As serous ovarian cancer kills so many women, we feel that any incremental gain in knowledge is worthwhile.