Author's response to reviews

Title: Elevated levels of circulating microRNA-200 family members correlate with serous epithelial ovarian cancer

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Author's response to reviews: see over
Dear Sir,

Ms. Ref. No: MS: 2122805585811564

Thank you for the assessments of our original manuscript titled “Elevated levels of circulating microRNA-200 family members correlate with serous epithelial ovarian cancer” submitted to BMC Cancer. Below are the reviewers’ comments (in italics) and a point-by-point description of how these comments have been addressed. Please find attached the revised manuscript.

Reviewer 1

...The aim of this study is very interesting and method of using patient’s serum is interesting. However, the authors did not showed why they selected only 4 cell lines, they selected microRNA from array data of these cell, so this point is very important...

As our focus was serous epithelial ovarian cancer (SEOC) we performed miRNA profiling on four SEOC cell lines available in our laboratory at the time, and compared these to a model of normal human ovarian surface epithelial cells (OSE(tsT)). As described in the text in Methods, analyses were conducted relative to a commercial RNA pool (Ambion FirstChoice Human Total RNA Survey Panel, Applied Biosystems). Triplicate and duplicate arrays were performed on the OSE(tsT) and SEOC cell lines respectively, and included a dye-swapped array for each cell line.

This approach was validated by our serum miRNA studies, given that putative marker miRNA that were selected from the cell line discovery platform were differentially expressed in serum from women with SEOC and age-matched healthy volunteers. No changes have been made to the manuscript.

...almost all 28 patients were advanced stage, I think that early diagnosis is required in ovarian cancer, the authors should mention this point.

The authors agree with Reviewer 1 that early diagnosis is required in ovarian cancer. This is stated in Background, paragraph 1, line 5 of the manuscript: “This disparity in survival
between early and late stage diagnosis emphasizes the need to improve early detection of EOC.”

To emphasize the importance of this fact, further studies including patients with early state disease is suggested in the last sentence of the Conclusion of the manuscript: “Testing of a larger cohort that includes patients with early stage disease is warranted to determine whether the addition of miR-200a, b or c, to a panel of serum biomarkers may improve diagnostic sensitivity.”

Unfortunately, the vast majority of women diagnosed with ovarian cancer are diagnosed at a late stage. This sample bias towards late stage ovarian cancer is reflected in our own, and in the majority of, collaborative gynaecological tumour banks. We are addressing the issue of sampling earlier stage ovarian cancer in our studies of mouse models of this malignancy; however, this work is beyond the scope of the current manuscript.

For these reasons, no changes have been made to the manuscript.

Page 6, line15 /acc.cgi?acc is type error?

The complete URL as stated in the text is: http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE35951
This is the correct URL for NCBI Gene Expression Omnibus (GEO) where the microarray data presented in this manuscript are stored according to internationally accepted GEO and MIAME (Minimum Information About a Microarray Experiment) guidelines. This URL provides ready access of these data to readers of this manuscript. Therefore, no changes have been made to the manuscript.

Reviewer 2
1A. miRNA expression profiling for the cell lines were performed with Exiqon MiRCURY Locked Nucleic Acid arrays. They should describe the data manipulation as well as analysis in detail….

The following has been added to the text in Methods, miRNA Expression Profiling, and three new references have been included as indicated.

“For each array, background correction was performed by fitting a mixture model of a normal and exponential distribution where normal distribution captures the non-expressed probes and exponential distribution the expressed probes [ref;20]. Loess print-tip normalization was performed within arrays. Scale normalization was performed between arrays. As each probe was printed as adjacent duplicate spots, these were expected to be positively correlated. Therefore, technical replicates were corrected for using the duplicateCorrelation function to
assess differential expression via Linear Models for Microarray data (LIMMA), using the value for the average correlation to merge data from duplicate spots [ref:21,22]

New References


1B... it is obscure why they selected miR-103, -92a and -638 for the candidate endogenous normalizers. Is there any statistical analysis for the selection? They should add explanation on this matter.

Yes, we did conduct statistical analyses. The following has been added to the text in Results, miRNA investigated for uniform expression between SEOC and OSE(tsT) cell lines:

“In order to select miRNAs with potential suitability as endogenous controls for normalization, we generated a list of 451 miRNAs that were expressed at a constant level across all SEOC cell lines and OSE(tsT) (test based on one-way ANOVA with asymptotic P values > 0.05 using Benjamini and Hochberg False Discovery Rate for multiple testing correction). From this list, miRNA previously reported to be detected in serum or plasma were selected for further analysis, specifically, miR-92a, miR-103 [12, 14, 27] and miR-638 [28] (Figure 1B). miR-103 had also been reported to have similar expression across ovarian cancer and normal tissues [29].”

The above text replaced the following original text:

“We assessed the SEOC and OSE(tsT) cell line miRNA microarray expression data for miRNA that were expressed with minimal variation across all cell lines as candidate endogenous normalizers for SEOC-associated serum miRNA analysis. Three miRNA, miR-103, miR-92a and miR-638 were chosen as their expression was relatively invariant across the SEOC and normal cell lines (Figure 1B); they were known to be expressed in serum or plasma [12, 14, 24], and at least for miR-103, also reported to have similar expression across ovarian cancer and normal tissues [25].”
2. Although they performed the leave one out cross validation for the predictive model, to be appropriate for this journal, independent validation analysis should be performed to have a robust conclusion that combing miR-200b + c normalized to serum volume and miR-103 is a positive classifier of SEOC.

Leave one out cross validation is standard methodology for data analysis and uses a single data point as validation data, and the remaining data points as training data. The analysis is repeated for the entire data set so that each data point is used once as validation data.

Examples of high impact publications that have used this methodology include:

- Jingjing Ye et al. On the analysis of glycomics mass spectrometry data via the regularized area under the ROC curve *BMC Bioinformatics* (2007) 8:477

We believe this is appropriate methodology for our dataset and therefore have not made changes to the manuscript.

Thank you for considering our resubmission of this manuscript for publication in *BMC Cancer*.

Yours sincerely,

Viive Howell PhD