Author's response to reviews

Title: Comparing gene expression data from formalin-fixed, paraffin embedded tissues and qPCR with that from snap-frozen tissue and microarrays for modeling outcomes of patients with ovarian carcinoma

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Author's response to reviews: see over
To the editors of BMC Pathology,

Please accept our thanks for the consideration of our manuscript and kind comments from the reviewers. We appreciate the opportunity to share our work and believe it has been improved by the comments made and replies provided. See below our point by point reply and we have uploaded the revised manuscript. We are open to other suggestions should they be needed, and thank you again for the consideration. Sincerely,

William H. Bradley, M.D.

Reviewer's report:
Minor Essential Revisions
1. The authors do not address the differences in "warm ischemia" time between obtaining a fresh specimen and placing in formalin, which could be hours, versus the typically brief time between obtaining a specimen and snap freezing the tumor tissue. Delay in fixation can variably alter expression patterns and mRNA levels of housekeeping versus target genes.

Reply: We agree that warm ischemia time is clearly an important issue in the acquisition and processing of tissue samples. In our institution the pathologic specimen is brought immediately to the pathology laboratory, and any specimens acquired by the tissue bank were removed from the pathology specimen at that time. The sample was then snap frozen, temporally at the same point the tissue was fixed in formalin. We have adjusted the methods and discussion section to reflect this.

2. Although FFPE RNA integrity is measured by RQI, this does not appear to be integrated into expression outcome other than by delta Ct considerations. All cases used the same 40 ng of RNA regardless of the RNA quality. Are the individual RT-PCR assays for each of the 91 genes designed to be similar in robustness to the housekeeping genes for one specimen type?

Reply: We used ready made “best coverage” wells in our TaqMan plates, which are designed to be equivalent in robustness. We did examine changes in RNA input without significant changes in expression readings. Some of the gene levels (e.g. WNT3) were noted to be among the lowest in the TCGA (bottom 5%). Alternative technology may be required (e.g. IonTorrent), or alterations in modeling.

3. Can the authors address the reproducibility of their findings. Were similar results found across the 18 specimens when run several days after the initial experiment?

Reply: This is a critical question and we have provided data in the results and discussion on a second set of qPCR assays run on the snap frozen blocks. The correlation was consistent and strong.

4. The authors state that an assay testing only "highly differentially expressed genes may
more easily translate from microarray to qPCR" (line 94) but is there evidence that ovarian carcinoma response to chemotherapy is best predicted by highly differentially expressed genes?

Reply: Our work seems to point to the conclusion that lower expressing genes may very be relevant. We have emphasized this in the text and discussed our lower expressing genes.

5. The work was done to demonstrate that expression results obtained from a technique using frozen specimens are similar to expression results obtained form a technique using FFPE specimens. This particular study did not prove the PSRP 91 gene TaqMan assay can predict ovarian tumor response to chemotherapy, as proclaimed in the first sentence of the Discussion, and that statement should be reconsidered.

Reply: We appreciate this measured comment and have moved to temper the discussion appropriately.

Major Compulsory Revisions
1. Although there is good correlation between the two test platforms, the sample size (10) is too small to draw a definitive conclusion.

Reply: We accept this fair comment and have tempered the discussion.

2. Fourteen out of 91 genes (15%) were unexpressed in at least one TaqMan assay including two (AKT2 and DNTT) of the top 3 genes in the MAPK pathway (see reference 1) and other genes involved in multiple pathways. Figure 3 showed normal distribution of gene expression in all the pathways examined. It was also mentioned in the manuscript that these 14 genes were those that had lowest expression in TCGA microarray but it raises the concern that the qPCR assay may miss some of the low expressing yet important genes.

Reply: We agree on the importance of this, and have expanded the discussion to address drop outs. Of note, our original modeling used a “Lasso” technique for gene selection, and from the MAPK pathway, DNTT was not selected as a gene of interest in the measurement (supplemental data table 2).

3. In Figure 2, could the author show the degree of correlation for the rest of the 10 samples?

Reply: The range of correlation for all 18 samples is provided in the text.

Minor Essential Revisions
1. The running title is unclear: correlation of ovarian carcinoma tissue with ?-need to specify.
Reply: This is expanded to add more clarity.

2. Several typos.

Reply: Reviewed and corrected.