Author's response to reviews

Title: DNA methylation profiling reveals novel diagnostic biomarkers in renal cell carcinoma

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Dear editors:

Thank you for the considerate reviews and comments on manuscript 1273016012136123 titled DNA methylation profiling reveals novel diagnostic biomarkers in renal cell carcinoma. Please find below our responses to each reviewer:

Reviewer #1:

1. The information presented about current treatment of RCC is accurate. To make this clearer, we have added additional, recently published references (Bhatt, 2014; Bielecka, 2014) to the first background paragraph (page 3).

2. We now provide additional information concerning immune and GPCR signaling pathway deregulation reported by previous groups (page 10, paragraph 1). We also discuss previously-implicated RCC genes (page 1p). Additionally, as stated in the manuscript (page 11), all but one of our CpGs identified in the PAM diagnostic panels were significant and we discuss several of those genes, including their relationship to other studies, in the Discussion (page 15 and 16).

3. In addition to the literature review of SAA1 and other genes already presented in the Discussion (top of page 15), we now include additional information about epigenetic regulation of the potentially associated genes (bottom of page 15 and top of page 16).

4. We think SEPT9 and PCA3 are relevant because they demonstrate non-invasive diagnostic clinical tests that detect DNA methylation marks, particularly as there are no diagnostic tests available in RCC. These examples demonstrate the clinical utility and feasibility of implementing diagnostic DNA methylation-based tests in the clinic. We identified the usefulness for this diagnostic panel, as well as diagnostic approaches for application, in the Discussion as a whole, and specifically on page 16 and 17.

5. We are performing survival analysis, but it is a separate study and will be included in another manuscript later. The markers in the current paper are for diagnostic and not prognostic testing.

6. We have amended the Figure Legend for 4C to make it clear that it is TCGA data that is presented.

7. We updated Suppl. Fig 2 legend to include both cg and gene identifiers.

8. We have added the abbreviation explanation for AUC on page 12. We now include the additional list of all abbreviations on page 17.
9. Figures were presented for review as a PDF, but are uploaded as high quality TIFFs for publication. We have reviewed the manuscript for any linguistic deficiencies.

Reviewer #2:
Additional information regarding tissue resection is now included in the manuscript in the first paragraph of the Methods section (page 5).

Reviewer #3:
We agree that Illumina arrays yield high-quality data and that our data was very comparable to TCGA DNA methylation data. We validated 127 CpGs from this study in 19 benign adjacent tissues by Bis-seq (a capture sequencing method) that will be submitted in a separate manuscript. Additionally, our group has validated this same platform previously in prostate cancer via PyroMark. We have information regarding this on page 9.

Editorial:
1. We provided additional information about the institutional review boards (page 5).
2. We provided an abbreviation section as suggested (page 19).
3. Adjusted formatting of author’s contributions to match suggested (page 19)

We hope that these comments and changes answer the reviewers' and editors' concerns and hope that you will find our manuscript acceptable. We look forward to hearing from you.

Yours sincerely,

Richard M. Myers, Ph.D.