Author’s response to reviews

Title: Integrated Sequence And Expression Analysis Of Ovarian Cancer
Structural Variants Underscores The Importance Of Gene-Fusion Regulation

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Author’s response to reviews:

Dear Editor:

We thank the reviewers for their most helpful comments. We have made all of the requested changes (see below) and hope you will now find the MS suitable for publication in BMC Medical Genomics.

Yours,

John McDonald

REVIEWER 1

In general, Reviewer 1 found the MS to be well written and suitable for publication with minor suggested changes.

COMMENT 1: The authors should be careful for the limitation of the genomic data from a small set of tumors in this study to draw a conclusion, and SVs found in this study may be basically the scar of genomic instability and not be the “drivers” in tumor development and progression.

RESPONSE: Certainly, many of the SVs uncovered may not be of functional significance but there are several SVs (particularly gene fusions) that are of at least potential functional significance. We have now “toned down” the conclusions in both the abstract and in the text to more conservatively state what we think is supported by our results. The changes in the text are highlighted in yellow.

COMMENT 1: The author should be aware of at least two of the five cases in this study carrying BRCA2 mutations. It is unclear for BRCA status of P1 in the TCGA data. BRCA1 or BRCA2 mutations are the drivers of 30% of the high-grade serous ovarian cancer. These mutations result in deep deficiency of homologous recombination repair with high genetic instability. Large amount of SVs and unique patterns of SVs distribution in tumor genome may be associated with the deficiency. The authors should compare SVs in tumors carrying mutated BRCA to those with wild-type BRCA.

RESPONSE: We detected no BRCA germline mutations in any of the patients analyzed. We
detected BRCA 2 (somatic) mutations in the tumor samples of two patients, P2 and P5. However, we detected no significant elevation in overall frequency of SVs in these patients relative to the other patients in the study. We have now included these findings on page 10 of the manuscript (highlighted in yellow).

COMMENT 3: In page 14 last paragraph, is change zero to capital O correct when the gene region RP1-2705.3 became fusion gene RP1-27O5.3-ZNF643? Why did repeat 1p34.2-1p35.1 deletion?

RESPONSE:
We have corrected the gene symbol RP1-2705.3 to RP1-27O5.3. (see-http://www.proteinatlas.org/ENSG00000254553-RP1-27O5.3/gene). Deletion typo has also been corrected. The changes in the text are highlighted in yellow.

COMMENT 4: In page 18 first line of conclusion, the first sentence “more important factors.....than previously envisioned” was probably over-stated. This study didn’t show any recurrent fusion gene(s) with potential functional impact in multiple tumor cases.

RESPONSE: We have revised this section (changes highlighted in yellow) so as not to overstate the conclusions.

REVIEWER 2

Reviewer 2 also considered the MS to be “an article of importance in its field.” The reviewer suggested 2 major compulsory revisions, 3 minor compulsory revisions and 2 discretionary revisions. Each of these changes have been addressed as follows:

Major Compulsory Revision 1: ....the conclusions have been overstated both in the abstract section as well as at the end of the discussion. These conclusions do not follow from the work presented. Therefore, they need to be changed to those that follow from the results.

Response: We have now revised the wording of both the abstract and conclusions in the text (highlighted in yellow) to conservatively convey what we believe is supported by the results.

Major Compulsory Revision 2. Page 14: "All 8 coding gene fusions detected by RNA-seq were somatically derived". This line seems to be out of context as these 8 gene fusions have not been mentioned previously. Could you add in a few lines to describe how your analyses resulted in 8 gene fusions?

Response: The wording of this section has now been revised to explain that of all of the gene fusions detected by the DNA-Seq analysis, only 8 were expressed (i.e., detected by RNA-Seq) and that all of these were of somatic origin (changes in text highlighted in yellow).

Minor Essential Revision 1: Remove hyphen from the following: gene-fusion, inter-genic, intra-genic.
RESPONSE: Done

Minor Essential Revision 2: Page 9: Please replace "2X" with "twice".
RESPONSE: Done

Minor Essential Revision 3: Page 17: un-nnotated should read as "unannotated".
RESPONSE: Done

Discretionary Revision 1: Was there any criterion in choosing these six patients or are there any similarities in the genetic background of these six patients such as BRCA1 mutations. If so, this information would be important to include.

RESPONSE:

These patients were selected because the results of DNA sequence analysis, RNA sequence analysis and microarray gene expression analyses were available for each of the patients. A sentence to this effect has been added to the M&M section and highlighted in yellow.

Discretionary Revision 2: Did you discover any hotspots for SVs in these six ovarian cancer patient samples. If so, this would be important information to include.

RESPONSE:

No hotspots were detected.

No coding gene-fusion hotspots were detected. We detected recurrence in SVs (predominantly germline-see paragraph 3 on page 10), but were cancer specific gene-fusions of potential functional significance.