Author's response to reviews

Title: Histotype-specific copy-number alterations in ovarian cancer

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Version: 3 Date: 9 October 2012

Author's response to reviews: see over
Dear Dr Morin,

Thank you for the review. We like to take this opportunity to thank the reviewers again for their constructive comments.

We have made revision to the manuscript according to the minor essential revisions indicated by the reviewers. Response to the list of revisions is attached at the end of this letter. We hope the revised manuscript has addressed the concerns raised.

Yours sincerely,

Liang Goh, PhD, Assistant Professor,
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Duke-NUS Graduate Medical School
Reviewer 1: Terry Furey

Minor Essential Revisions:

The authors have addressed my previously stated concerns in this revised manuscript. I only have a few additional comments on this revision:

Minor Essential Revisions:

1) In the Results when discussing “significant” amplifications and deletions (page 6-7), either a definition of what constitutes significant should be stated, or an explicit reference to the Methods section where this is defined should be made. In the description of significance in the Methods (Data Analysis section), I assume the q-values refer to the false discovery rate – this should be explicitly stated to avoid confusion.

As suggested, we have included an explicit reference to Methods and indicated in “Data Analysis” section that q value refers to false discovery rate.

2) In the Methods, when describing the association between expression and copy number, it would be better to be as explicit as in the reviewer’s comments about how the correlations were done. That is, state that a Spearman correlation was calculated independently in each dataset for each gene, followed by the Fisher’s combined probability test. I believe this will make this description clearer and will then correspond to Figure S1 more closely.

Thank you for this suggestion. We have revised the paragraph on the association between expression and copy number in Methods accordingly.

3) I appreciate the removal of the serous samples in the overlap analysis. But, I think it would be interesting to include as supplementary material or in the text how many of the amplifications and deletions seen in the non-serous histotypes were also seen in serous cancers. This would emphasize the uniqueness of alterations in these non-serous histotypes. My original intent was to point out that it would be premature to claim that the changes in the serous cancers were unique, but the opposite comparison is still valid.

We have included the Venn diagram comparing between the 4 histotypes as a supplementary figure and added summary statements on the comparison between the 3 lower prevalent histotypes with serous tumors in the same section.
Reviewer: Charles Warden

Minor Essential Revisions:

1) “We performed ANOVA test on ~200 of these genes and observed no significance (FDR<0.05), thus supporting the PCA plot that batch effects was minimal.” I think this is a reasonable justification, and I noticed that this comment was added in the methods section. However, I think it would be nice to present this information in a supplemental table (for all of the probes, not just the housekeeping genes). That way, other users who analyze the data presented in this study can confirm that their gene(s) of interest don’t show any bias in the combed dataset. It would also be nice to provide these values for ERBB2 in the text, since that gene is emphasized so much in this paper.

*We agree with the reviewer and included Supplementary Table S5 containing the ANOVA results of all genes plus indication of housekeeping genes used in this analysis. In the section describing the ANOVA analysis, we have also included a statement that ERBB2 did not show any significance as well.*

2) “We have also included comparison with TCGA in revised manuscript.” I think the TCGA comparison provides the strongest data for the overlap analysis. However, the methods need to include a description of what defines an overlapping region. For example, do the regions show at least 50% overlap in genomic coordinates? A list of overlapping regions would also be helpful. If possible, it would be nice to add a column to Table 1. If that is not feasible for reasons of space, a supplemental table would also be acceptable.

*The comparison was done based on overlap of genes (if available) or genomic regions reported by TCGA in their Figure 1. A new comparison has been done with respect to their list of significant gain and loss reported in their supplementary table S5.2, where 46% (29/63) of gain and 54% (27/50) loss were also observed in our study. As suggested, we have included results of the comparison as Supplementary Table S3.*

3) “Similarly, deletion in 9p21 of mucinous tumors was also reported (Campbell, Gareth Beynon et al. 1995; Devlin, PA Elder et al. 1996)” These papers aren’t cited anywhere in the main text and should be added to the discussion.

*We have included these references in the manuscript.*

4) The table presenting the t-test statistics for the 3 groups (response to my previous major revision #2) needs to be included in the paper. A supplemental table is OK, but it needs to be clear that the qualitative trend is the same for all 3 studies but the difference is only significant with p-value < 0.05 in one study.

*We have included the table as Supplementary Table S4 and added a statement on this in the section on ‘Copy number alterations in known cancer genes’.*

5) Review again for grammatical errors
   a. This was similarly observed in other study [25]. Tense is not consistent (“study” needs to be “studies” or a modifier is need, e.g. “in another study”). Plus, there are actually 3 studies that can be cited to justify this claim.

*We have reviewed the manuscript for grammatical error.*