Author's response to reviews

Title: Loss of heterozygosity: what is it good for?

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Version: 2 Date: 24 May 2015

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Response to reviewers

Anna Sablina:

Discretionary Revisions:

As a proof of principle the authors should apply a suggested combined approach to identify novel tumor suppressor genes in ovarian cancer.

We agree this would be valuable information, however, it is well beyond the scope of the current paper.

Xiaoyang Ruan:

Major Issues

The paper tries to provide insight into a topic (the mechanism and consequence of LOH) with somewhat inappropriate sample set. Comparing to other types of cancer, ovarian cancer (OC) has a relatively more complex genetic origin, confounding environmental factor, and histological subtypes with very different prognosis. Genetically, there are familial breast and ovarian cancer that is mostly attributable to autosomal dominant BRCA1/2 mutation, HNPCC (Hereditary Non-Polyposis Colorectal Cancer) related OC that is caused by mismatch repair deficiency, and sporadic cases. Environmentally, virus infection plays an important role in OC. The paper did not aware of this and used samples with mixed histological types without clearly defined the genetic background. This is disturbing in that the observations described in the paper might be for different diseases.

We strongly disagree with the reviewers comments. While we fully acknowledge the diversity of ovarian cancer subtypes, our detailed understanding of the copy number profiles of these subtypes (see Gorringe et al., 2007) leads us to believe that LOH is a mechanism common to all cancer types. Indeed, the type of analysis we have carried out in this paper could equally have been done in a pan-cancer fashion, since the objective was to understand the impact of LOH on cancer development, rather than identifying cancer specific genes per se. While some cancer types may have greater or fewer LOH events than ovarian cancer, there is no reason to believe that the underlying biology driving these events will not be the same regardless of cancer type or absolute event frequency. We chose to analyse ovarian cancer because of the wealth of data we had available, both from our own analyses and also from the TCGA.

In addition, while our initial mutation screen included a number of different subtypes, these data led us to propose our hypotheses, which we then primarily addressed using TCGA data. The TCGA set comprises almost entirely high-grade serous carcinomas. While we could have further stratified this data set (BRCA vs non-BRCA, by Tothill subtypes etc), the inter-tumour genetic heterogeneity in ovarian cancer is so extreme that each case could be viewed as a distinct entity, making it impossible to do anything at all.
Nonetheless, to address the reviewers concerns, we have added the following to the introduction:

“...we previously used SNP mapping arrays to analyse LOH in ovarian carcinomas of diverse histological subtypes,...”

“We evaluated a number of different histological subtypes, since these have different etiologies and causative genes.”

We also point out that in Table 1, we listed the subtypes affected by significantly mutated genes. There were no recurrently mutated genes exclusive to the low-grade serous, mucinous or clear cell carcinomas, and only PTEN mutations were significantly associated with a subtype (endometrioid), which is well known.

Minor:

Page 7: “the variant was not called in the matched normal sample or identified as a germline alteration in another tumour/normal pair”. Not clear.

Changed to:

“the variant was not identified as a germline alteration in any normal sample”.

Page 7: “A selection of variants which met the above criteria ...”. Please give a proportion and explain briefly the selection criteria.

The criteria were as listed in the above paragraph (i – iii). To clarify:

A selection of variants that met the above criteria for a somatic mutation (n=202/621)...

Page 7: “Affymetrix SNP Mapping array data was obtained for the sequenced cases”. Give a total number here.

“86” has been added to the sentence.

Page 7: “The Cancer Genome Atlas Affymetrix SNP6 data were downloaded from the Data Portal”. Problematic sentence. Also, where the Affymetrix 500k data from? The author needs to describe the source of sample more clearly. Are all the wet lab samples from TCGA?

As stated, the 500K arrays were previously published in our landmark ovarian SNP array paper (Gorringe et al, Clin Ca Res, 2007), however we have clarified this sentence (see below). The ovarian tumour cohort for these SNP arrays and the sequenced samples is clearly described in the first part of the methods as being distinct from TCGA. Further information about our samples and the arrays used is provided in Supp Table 1.
Affymetrix SNP Mapping array data was obtained for the 86 sequenced cases, 54 by SNP6 arrays (GSE19539, {Ramakrishna, 2010 #61}), 26 by 500K arrays (previously published in [6]), and six previously unreported low-grade endometrioid cases. Affymetrix SNP6 CEL files, HM27 methylation array data (level 3), Agilent expression array data (level 3) and somatic mutation data from 266 tumors generated by The Cancer Genome Atlas (TCGA) were downloaded from the TCGA Data Portal.

Page 8: “thus excluding regions of allelic imbalance where at least one copy of both alleles was present”. -- where at least one copy of both alleles was present – can be removed.

Because some of these concepts may be unfamiliar to many, even those with cancer genomics experience, we prefer to retain this explanation as it may be helpful for some readers.

Page 8: “A candidate TSG screen in ovarian cancer”. This section is very long. So sub-titles will be helpful for readers. The content is not organized efficiently.

We have divided this section into three subheadings:

A candidate TSG screen in ovarian cancer – selection of genes from LOH regions

A candidate TSG screen in ovarian cancer – correlation of mutations with LOH

Significance analysis of recurrently mutated gene candidates

We have also broken up the last section into three paragraphs.

Page 9: “A targeted mutation screen was conducted on 86 ovarian cancer cases including high-grade serous and endometrioid, low-grade endometrioid, clear cell and mucinous subtypes.”. Should be “on the 86 ovarian cancer cases...”.

Done

Also, add a total number to the “Ovarian tumour cohort” section on page 6.

Done

Page 9: “The classic two-hit hypothesis would suggest that driver genes should have”. “would suggest ... should” sounds very redundant.

We have changed this to:
“...would predict that driver genes have homozygous..”

Supp table 1: the table can be more efficient

We do not understand how this table can be more “efficient” as it provides a fairly minimal summary of each case. We feel that if readers wish to analyse our data in depth, they would need case-by-case information rather than a summary.