Author's response to reviews

Title: Identification of gene fusion transcripts by transcriptome sequencing in BRCA1-mutated breast cancers and cell lines

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Author's response to reviews: see over
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Elaine Cruz
Journal Editorial Office
BioMed Central

**RE: Revisions for manuscript submission #2929062295802141**

Dear Ms. Cruz,

Thank you for responding to our initial submission of manuscript 2929062295802141 entitled “Identification of gene fusion transcripts by transcriptome sequencing in BRCA1-related breast cancers and cell lines” with the editors’ and reviewers’ reports. We valued the opinions of the reviewers and appreciated their constructive comments. Our responses to each of the reviewers’ concerns are addressed in the subsequent pages.

The sequence data of the breast cancer cell lines will be uploaded to the NCBI Sequence Read Archive (SRA) under the accession number SRA046769 (currently in progress). We will be able to release this data to the public as soon as our revised manuscript receives final approval from the editors. This accession number has been noted in the Methods section of the revised manuscript (page 12).

Unfortunately, due to privacy concerns of patient-specific data, we do not have consent to release the sequence data for any of the tumor samples at this time.

To conform to manuscript structure guidelines for a research article, we have moved the Methods section to follow the Background section and precede the Results section. We have also revised some of the text in the Results section to reduce redundancy with the Methods. To address the editorial comments regarding the table format, we have re-formatted the tables by removing merged cells and vertical lines. The legends have also been moved below the table.

As a result of one of the comments raised by one of the reviewers, we have made a minor revision of the manuscript title to: “Identification of gene fusion transcripts by transcriptome sequencing in BRCA1-mutated breast cancers and cell lines” (‘BRCA1-mutated’ has replaced ‘BRCA1-related’). We feel that the revised manuscript has sufficiently addressed all concerns and hope that will be acceptable for consideration as a research article in BMC Medical Genomics.

Sincerely, on behalf of all the authors,

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RESPONSE TO REVIEWERS’ COMMENTS

REFEREE #1

1) Availability and deposition of the RNA-seq data should be discussed.

We will upload the sequence data of the breast cancer cell lines (HCC1937, HCC2337, SUM149PT, SUM1315O2, HCC3153, and MCF10A) to the NCBI Sequence Read Archive (SRA) under the accession number SRA046769. This has been noted in a new Methods sub-section titled “Data access” of the revised manuscript (page 12, paragraph 2). Unfortunately, due to privacy concerns of patient-specific data, we do not have consent to release the sequence data for any of the tumour samples at this time.

2) Authors talk about 57 breast cancer samples in 3rd paragraph of “Discovery of gene fusions” and 3rd paragraph of “Discussion”, but there is no reference for this dataset.

We have added a statement in the Methods section to introduce this dataset (page 6, paragraph 3). In particular, we note that it was obtained from our collaborators at the Institute of Cancer Research (UK) and its purpose was to experimentally screen our candidate gene fusions for recurrence.

3) In 3rd paragraph of “Discovery of gene fusions” talks about HCC3153 being re-sequenced by Jorge Reis-Filho (who is an author). Is this experiment included in 4 lanes of Illumina sequencing summarized in additional file 1? If so, why authors mentioned this experiment separately? If not, authors should explain this experiment as well.

The re-sequencing of HCC3153 was indeed from a separate experiment and was not one of the primary datasets used in this study. We have revised this statement in the manuscript in order to make this clearer and indicate that the results for this dataset are not shown (page 16, paragraph 1).

4) In 5th paragraph of “Discovery of gene fusions” visual inspection of mutations with IGV does not sound good.

We have removed the mention of IGV in this statement, and instead made a note of its use under “RNA-Seq post-processing” of the Methods section (formerly titled “Sequence alignment to genome and splice junctions”) [page 8, paragraph 1].
RESPONSE TO REVIEWERS’ COMMENTS

REFEREE #2

1) What dose “BRCA1-related breast cancers” refer? Does it mean BRCA1 mutated, deleted…?
   Based on information listed in Table-1, I would like suggest to use “BRCA1-mutated breast cancers”
   The term “BRCA1-related breast cancers” is often used in literature to refer to the subset of breast cancers that can be attributed to BRCA1, but not necessarily due to mutation. Indeed, the breast cancers studied in this manuscript are due to BRCA1 mutations. We have revised our manuscript to use term “BRCA1-mutated” in place of “BRCA1-related” where appropriate, as suggested by the reviewer. This change is also reflected in the title of the manuscript.

2) Authors stated that they using “one normal breast epithelial cell line” as normal control. This cell line is MCF10A which actually is not considered as “normal”. Most of researchers in the field agree called it “non-tumorigenic breast epithelial cell line”, as authors correctly labeled in Table-1. Please keep consistency by using this term in the manuscript.
   We acknowledge the inconsistency in the manuscript and have replaced all instances of “normal breast epithelial cell line” with “non-tumorigenic breast epithelial cell line”.

3) The conclusion stated “…those fusions that are identified may still serve as potential “individualized” biomarkers to aid in patient monitoring”. Based on the results presented in paper, there is no recurrence for any genetic fusions reported in this study. Therefore the exaggerated conclusion should be corrected appropriately.
   The point that we wished to convey in this statement was that non-recurrent fusions may still be of value for treating the patient in which the fusion was identified. For example, the fusion that we have reported in a primary tumour may be useful for monitoring the health of that specific patient. Indeed, we are actively planning to explore this possibility in future studies. We agree that our statement is misleading and appears exaggerated. We have rephrased the statement and hope it will be clearer for the reviewer and readers (page 4, paragraph 1).

4) Some typos in manuscript:
   1) Background, page 5, last line “…estrogen growth factor receptor..” should be “…epithelial…”
   2) page 13, last line “…promoter or enhancer in MTAP…” should be “…WWC1…”
   We thank the reviewer for catching these typos. We have corrected the above typos in the revised manuscript (page 5, paragraph 1 and page 17, paragraph 1, respectively).