Author's response to reviews

Title: Elevated C1orf63 expression is correlated with CDK10 and predicts better outcome for advanced breast cancers: a retrospective study

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Answers to the Reviews

We thank you very much for your comments, we have changed the manuscript following our suggestions, as explained “point by point” below (the revision was marked red in the manuscript),

Reviewer1 (1670316615163190_comment)

Major Compulsory Revisions 1): Seek editorial revision by a native English language speaker or comparable expert.

Answers:

The paper was submitted to a native English language speaker, and the language of our paper has been polished.

Major Compulsory Revisions 2): Controls: 8 tumors served as controls with adjacent normal tissue. Only 4 of these showed C1orf63 staining. The authors should obtain at least two (2) more control breast cancer tumor tissue samples with normal adjacent tissue to demonstrate that C1orf63 is not expressed in non-tumor tissue.

Answers:

C1orf63 staining and scoring were performed in 4 more breast cancer tissues paired with matched adjacent normal tissues, as shown in the sections of Methods (Page 5, line 16) and Results (Page 8, line 22-25; Page 9, line 1-2).

Minor Essential Revision 1): Confirm adherence to journal format requirements.

Answer:

We have checked the journal format again and confirm that current manuscript
meet the journal format requirements.

Minor Essential Revision 2): Verify that references properly correlate with in-text citation.

Answer:
The references are verified to correlate with in-text citation properly.

Minor Essential Revision 3) Verify that figure references properly correlate with the in-text citations.

Answer:
All 5 figures are verified to correlate with in-text citation properly.

Minor Essential Revision 4): In the introduction note that C1orf63 is also known as Arginine/serine-rich protein 1 (RSRP1, NCBI gene ID: 57035)

Answer:
Thank you very much for this suggestion, we have thus put this in the Introduction section of current manuscript (Page 4, line 8-9).

Minor Essential Revision 5): “Comparable to adjacent non-cancerous tissues, there was a higher positive rate of C1orf63 expression in tumors, which demonstrated the early stage of breast cancer, C1orf63 might serve as a tumor promoter….Thus, C1orf63 expression in breast might not involve ER, PR, HER-2 expression. In the advanced stage of breast cancer however, C1orf63 might act as a tumor suppressor.” p.12

a. This is confusing. What were the criteria used to designate the “early stage of breast cancer”? Clarify in methods the histopathological definitions of “early cancer” and revise discussion.

Answer:
Thank you very much for your comment, we apologized for the inconvenience, In our study, Clinical tumor stage (TNM stage) of breast cancer were was grouped in accordance with the American Joint Committee on Cancer (AJCC) 6th Ed Cancer Staging Manual (2002). Stage III and IV was designated as advanced stage, and relatively, stage I and II was early stage. We have clarified this in methods, and one reference was provided (Page 5, line 10-13), following your suggestion.

b. How may C1orf63 function as a tumor promoter and subsequently a tumor suppressor? Provide proposal of a mechanism supported by the literature of similar protein arginine/serine rich proteins functions.

Answer:
Thank you very much for this very important comment. The dual functions of C1orf63 were observed in our study, however, the mechanism is still unclear. C1orf63 encodes a protein of 290 amino acids enriched in arginine and serine
residues. Following your suggestion, we searched several websites for scientific literatures, and found that SRSF1, a member of the arginine/serine-rich protein, and discussed in the section of Discussion. ----SRSF1 is a proto-oncogene that is overexpressed in many different cancers. However, recently, it has been demonstrated SRSF1 might involve in stabilization of p53, and increased SRSF1 expression in primary human fibroblasts could ultimately triggers oncogene-induced senescence. The known mechanism of SRSF1 will enlighten the future study of C1orf63. Revision was done in the section of Discussion (Page 14, line 19-25).

Discretionary Revisions:
1) Examination of C1orf63 expression in other tumors (colorectal and lung adenocarcinoma) available microarray data would strengthen the manuscript and should be added.

Answer:
We appreciate very much for this very important comment, which enabled us to analyze eight more microarray datasets, including breast cancer (GSE15852, GSE42568), lung cancer (E-MEXP-231, GSE19804), prostate cancer (GSE6956, GSE6919), and hepatocellular carcinoma (GSE14323, GSE6764) achieved from public databases. The difference of C1orf63 expression between cases and controls of each dataset was tested by student t-test. Revision was done both in the sections of Methods (Page 7, line 10-15) and Results (Page 9, line 2-6).

2) Consider these as sources for C1orf63 functions and discussion:

Answer:
Thanks the reviewer very much. Following this suggestion, we read these very important literatures as well as the information of C1orf63 in Gene database of NCBI (http://www.ncbi.nlm.nih.gov/gene/). Your suggestion contributed to more discussion about C1orf63 functions shown in the section of Discussion of current manuscript (Page 13, line 1-12).

Reviewer 2 (2119578842169420_comment)
Major Compulsory Revisions

1. In the section "Impact of C1orf63 expression on OS in all breast cancer patients" the authors compare C1orf63 expression levels with OS of all breast cancer patients included in their project. However, since breast cancer is a heterogeneous disease with defined subtypes, I suggest to perform this analysis separately for luminal A, luminal B and basal like tumors.

Answer:
We appreciate very much for this very constructive suggestion. Following what
you suggested, We have checked the original Pathology Report to try to find the Ki67 IHC score of these patients involved in current study. According to 2005 St. Gallen International Expert Consensus, ER+ and/or PR+ breast cancers with Ki67 positivity > 14% is classified as Luminal B subtype regardless of Her-2 status. Unfortunately, these breast cancers were not stained for Ki67 before. Therefore we could not completely follow the reviewer’s suggestion since we can not further divide these luminal breast cancers into A or B. However, we tried our best to perform survival analyses to correlated C1orf63 expression to the survival rate of individual breast subgroups, namely luminal, HER-2-enrich and triple-negative breast cancer, and no significant result was observed. Revision was done both in the Sections of Results (Page 10, line 11-15).

2. The authors describe a trend towards better outcome of C1orf63 positive patients, however, no statistical significance was reached. I suggest to use the kmplot tool (http://kmplot.com/analysis/) and search for associations between C1orf63 expression and PFS / OS of breast cancer patients. This database includes expression data of more than 3000 breast cancer patients which can be classified according to PAM50 and other scores.

Answer:
Following the reviewer suggested, we used the KM Plotter tool to further assess the relationship between C1orf63 mRNA expression and RFS (relapse free survival)/OS of breast patients, and the results are significant. Revision was done in Sections of Methods (Page 7, line 21-25; Page 8, line 1-2) and Results (Page 10, line 5-9).

3. The authors describe a positive correlation between C1orf63 expression and CDK10 expression based on Affymetrix microarray data. Although p-values were statistically significant, R values indicate only weak correlations. To strengthen their findings, I suggest to repeat this analysis using RNA-seq data from TCGA using the cBio portal (http://www.cbioportal.org).

Answer:
Following this very important suggestion, we downloaded one normalized RNA-seq dataset of breast cancer from GEO (GSE60788) to further assess the relationship between C1orf63 and CDK10 expression (Page 7, line 17-19; Page 12, line 11-13).

If anything has to be adjusted further, please don't hesitate to contact us. Thank you very much for your time and considerations.