Author's response to reviews

Title: Simultaneous copy number gains of NUPR1 and ERBB2 predicting poor prognosis in early-stage breast cancer

Authors:

Seung-Hyun Jung (hyun@catholic.ac.kr)  
Ah-Won Lee (klee@catholic.ac.kr)  
Seon-Hee Yim (lyra@catholic.ac.kr)  
Hae-Jin Hu (haejin.hu@gmail.com)  
Chungyoul Choi (chungychoe@gmail.com)  
Yeun-Jun Chung (yejun@catholic.ac.kr)

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Author's response to reviews: see over
Dear Editor,

Regarding: MS 1968760341634343

Please consider the uploaded manuscript which has been second revised for publication in BMC Cancer.

We have addressed all the points raised by the reviewers. All the changes are marked in blue font in the revised manuscript. We also revised the supplementary materials.

Points by point replies to the reviewer’s comments are included in the ‘response to the reviewer’s comment’ below.

All authors listed in this manuscript have read and approved the content of the revised manuscript, new authorship and agreed to its submission.

Thank you very much for your helpful comments.

Yours Faithfully,

Yeun-Jun Chung M.D., Ph.D.
Integrated Research Center for Genome Polymorphism
The Catholic University of Korea, 505 Banpo-dong, Socho-gu
Seoul 137-701, Korea
Email: yejun@catholic.ac.kr
Phone: +82-2-2258-7343
FAX: +82-2-537-0572
Response to reviewer’s comments

Minor:
1. Please could there be more detail of the IHC scoring in the methods – what level of staining (e.g. Allred scale or 0-3) was considered to be positive for ER, PR and especially HER2?

: Our IHC scoring criteria is as follows; 0 or 1+ (Negative), ≥3+ (Positive).
In case of 2+ (equivocal), we performed SISH (Silver in situ Hybridization) for final confirmation.

2. Please could you add the description of “cancer genes” to the legend for Table2.

: Among all RefSeq genes located in the RARs, the genes which can be searched by the keyword “Cancer/Tumor” from NCBI Gene (http://www.ncbi.nlm.nih.gov/gene) are listed as “cancer-related genes” in the table. We added this comment in the table 2.

Major:
1. Was the FDR correction in Supp table 2 performed on a genome-wide scale? The values don’t seem to be very different from the original p-value, as I would have thought if correction was being made across hundreds of copy number segments.

: In the previous analysis, we used the NEXUS option which limits the statistical test to the CNAs showing a certain extent/amount of frequency gap between the categories to be compared. We set this threshold as 30%. Under this threshold, we identified 43 CNAs which were significantly more frequent in stage II group (P<0.05) and put them for the FDR correction.
In this revised manuscript, we dropped the rather arbitrary threshold of 30% and tested all 4,396 CNAs and put them all to the FDR correction. The number of CNAs differentially distributed between stages increased a bit based on unadjusted P values (P<0.05), which are presented in the Table S2, but, as expected, none of them passed the FDR threshold of 0.1. We revised the manuscript accordingly (page 9, line 3-6).

2. The definition of Luminal A and Luminal B is the same in the methods (ER+/PR+/HER2-). How were these distinguished (e.g. Ki67)? If ER/PR/HER2 is only available, these groups should be combined into a single Luminal group. As this point was originally discretionary in the first review, the authors could remove the subtype information from the paper if preferred.

: This confusion is due to our typographical errors. We are sorry about our mistake. Luminal type B should be ER + and/or PR +, HER2 + and HER2 overexpressed type should be ER - and PR -, HER2 +. We corrected the manuscript (page 5 line 4-7).

3. The numbers of HER2 cases do not add up. In Table 1 HER2 IHC positive cases are given as n=24 for the discovery set, but only 4 are listed in the HER Subtype group below. In addition, only 1 out of these 4 cases have HER2/ERBB2 copy number gain, which suggests
that the HER2 staining is not very accurate. Similarly, 29% of cases are stated to have high-level amplification of ERBB2 but 50% have positive staining. What is the correlation between HER2 staining and HER2 amplification? The copy number should be the gold standard for HER2 amplification. If the HER2 staining is determined to be unreliable, one suggestion could be to put the ERBB2 amplified samples (by array or QPCR CN) as a “HER2+” group (as in Thompson et al), and split the rest into Luminal and TNBC groups based on the ER/PR staining.

: According to the reviewer’s comments, we re-checked the HER2 staining. Most of the HER2 staining were performed more than 10 years ago (mostly done in 1988) and interpreted by several different pathologists. Also, some staining data (which was performed in Dankook University Hospital) is not available for re-checking because the pathologist who did scoring of HER2 staining passed away several years ago. Therefore, for this revision, we re-performed the HER2 staining/scoring for 30 samples out of 48. In other words, a total of 45 samples were re-validated (including staining for 15 samples without HER2 information for the previous revision) for this revision and all the staining was done by one breast pathologist (Dr. Ahwon Lee, second author of this paper).

Through the re-staining/scoring, some of previous HER2 positives were found to be overestimated. In our re-staining, HER2 was positive for 11 samples (22.9%) in the discovery set and 23 (23.7%) in the replication set. When we assessed the accordance of HER2 status between the IHC and array-CGH results, 43 out of the 48 cases were consistent (~89%). Based on the HER2 re-staining results, we grouped the molecular subtypes again and also re-calculate the distribution of the RARs by molecular subtypes.

We changed Table 1, manuscript (page 10, line 24 – page 11, line 2), Additional Figure 1 and Additional Table 5.