Author's response to reviews

Title: Loss of heterozygosity at the ATBF1-A locus located in the 16q22 minimal region in breast cancer

Authors:

Kazuharu Kai (kai-k@sc.itc.keio.ac.jp)
Zhenhuan Zhang (zhangz@fc.kuh.kumamoto-u.ac.jp)
Hiroko Yamashita (hirokoy@med.nagoya-cu.ac.jp)
Yutaka Yamamoto (ys-yama@triton.ocn.ne.jp)
Yutaka Miura (member@sunprom.med.nagoya-cu.ac.jp)
Hirotaka Iwase (hiwase@kumamoto-u.ac.jp)

Version: 3 Date: 28 July 2008

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

Thank you very much for your reviews and helpful comments. We would like to address these comments point by point in each reviewer as follows:

To all reviewers

We made a big change especially in the section of Discussion. We would be grateful if you review our paper, again.

To reviewer (1)

1. Although microdissection is obviously necessary for allelic loss and mutational analysis as you pointed out, we have been performed macrodissection, whose fidelity was verified as added in “Methods” in the main manuscript, “Breast cancer specimens were verified not to contaminate normal cells at 10% or more of each sample on the hematoxylin and eosin stained slides as previously described (19)”. The macrodissection technique have been empirically validated in our previous paper (1,19), though it is not described in main manuscript.

2. As you described in your comments, there is no direct evidence that a mutation account for reduced expression of ATBF1. However, somatic mutations with frameshift were considered to confer instability of their transcripts, and maybe in somatic mutations without frameshift, but with the substitutions of amino acids. Therefore, we prospectively reached for the mutations which may confer the instability of their transcripts, and we assumed these mutations as “mutations succeeding to the instabilities of their transcripts” described in Result and finally assessed the gene alterations in the view from their functions. Functional assessment of gene alterations in this paper was done citing previous your and others’ papers and added in Discussion.

Although you recommended to search for the mutation in all the cases, we could not performed it due to the limited manpower and grant in our laboratory. Therefore, we carefully moderated the statement about the results of gene alterations not to overestimate our results. For example, “These findings support the view that the ATBF1-A ” were changed to “These findings could support the view that the ATBF1-A “ described in Background.

3. The total number of the cohort is definitely 43, consisted of 22 (LOH) and 21 (ROH). We have not counted for the patients who have not determined the clinicopathological factors, listed in Table 3. Therefore, we added the numbers as in the category “Not measurable” or “unknown” in Table 3. As the undetermined factors in each patient was at random, we chose these patients
with the profile of 16q22 LOH status as study cohort to strengthen the statistical power.

4. Although it might not be so clear statement according to your comment, we added the statement in the section “Relationship between LOH status of the ATBF1-A locus and clinicopathological factors” in Result as follow; “As the reduced ATBF1-A mRNA expression levels correlated with unfavorable characteristics of tumors, ER-α- negative and lymph node metastasis, in our previous study, we wondered whether LOH status at ATBF1-A locus were related with clinicopathological factors as well”.

5. We added the explanation for the meaning of “not determined (ND)” as described in “Loss of heterozygosity at chromosome band 16q22” in Result as follow; “not determined (ND), when there was discrepancy in LOH status between D16S3106 and D16S3018, because ATBF1-A is located between these markers, LOH status at ATBF1-A is not rigorously determined in this case”.

To reviewer (2)

1. We cited your interesting paper and added the consideration in Discussion based on the newly cited papers.

2. As you described in your comments, there is no direct evidence that transcriptional down regulation instead of LOH is the mechanism by which ATBF1 expression is decreased. Although we wanted to prove experimentally this hypothesis, we could not performed additional experiments due to the limited manpower and grant in our laboratory. Therefore, we carefully moderated the statement about the speculation not to be misunderstood by the readers. For example, “These findings support the view that the ATBF1-A” were changed to “These findings could support the view that the ATBF1-A” described in Background.

3. Although microdissection might be fundamentally necessary for allelic loss and mutational analysis, we have been performed macrodissection, whose fidelity was verified as described in “Methods” in the main manuscript, “Breast cancer specimens were verified not to contaminate normal cells at 10% or more of each sample on the hematoxylin and eosin stained slides as previously described (19)”. The macrodissection technique have been empirically validated in our previous paper (1,19), though it is not described in main manuscript.

4. According to your comment, we changed the statement “chromosome arm 16q22” to “chromosome band 16q22” or “chromosome 16q22”.

5. It is just because manufacture’s instruction recommend as the protocol described in Method.

6. According to your comments, we revised the description in Page 9 as described below, “Allelic loss at each microsatellite locus was considered to be present in tumor samples’ DNA
when there was at least a 65% peak reduction at one of a pair peak compared with the corresponding peak of normal DNA”.

7. As you reported in BMC Cancer, in breast cancer, there were no somatic mutations. However, we used the primers reported in prostate cancer, in which frequent somatic mutations were observed. In addition, we intended to research the somatic mutations at the beginning of research as described in revised Method, “With intent to analyze ATBF1-A mutations”. Therefore, we used the description in Methods as follow, “all the mutational spots in exon 9 and the most frequent deletion spot in exon 10 reported in prostate cancer”.

8. According to your recommendation, we deleted the majority of the description in sequencing procedures.

9. According to your recommendation, we indicated the position of ATBF1 in Fig 2.

To reviewer (3)

As the study subjects for LOH and mutational analysis were so small as you described in your comments, we wanted to add the study subjects to get more accurate results. However, we could not performed it due to the limited manpower and grant in our laboratory. Therefore, we carefully moderated the statement about the results of gene alterations not to overestimate our results. For example, “These findings support the view that the ATBF1-A “ were changed to “These findings could support the view that the ATBF1-A “ described in Background.