Author's response to reviews

Title: The development of a mini-array for estimating the disease states of gastric adenocarcinoma by array CGH

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Author's response to reviews: see over
Dr. Robin Cassady-Cain  
Assistant Editor  
BMC-series journals

Dear Dr. Cassady-Cain,

Thank you for your kind letter together with the comments from the three reviewers. The reviewers' comments and suggestions were very helpful. We should like to express my gratitude to you and reviewers for suggesting the best way to improve our article. We have edited carefully to fulfill reviewers' requests and response to their comments. In addition, all figures have been replaced by images with higher resolution than those originally submitted, and according to a reviewer’s suggestion, Figure 3 that shows profiles of the dye-swap experiment has been added to the manuscript. The manuscript has been revised so as to meet the format. The first version of this paper submitted was checked by an English speaking person, but according to your recommendation, the manuscript has been checked again by a native speaker of English. The manuscript has been revised at many points as stated in accompanying sheets. I am sending a file of the revised manuscript entitled "The development of a mini-array for estimating the disease states of gastric adenocarcinoma by array CGH" (Ms. 1915766214173097) by Furuya, et al. together with an array-CGH raw data file of 83 gastric cancers. We hope that the revised manuscript will meet with your approval.

Sincerely yours,

Kohsuke Sasaki, MD
Responses to comments and suggestions from reviewers

Reviewer 1

1. The reviewer questions whether there is a difference in number between clones identified in screening chips and clones spotted on mini-arrays. This point was mentioned in the first version of the manuscript (page 12, 1st paragraph in the first version). To avoid confusion, however, a statement `the method 1 identified 24 BAC clones of which one was shared between node metastasis and liver metastasis, and the method 2 identified 26 clones.' have been added to the section of `Customization of BAC mini-array specific for gastric cancer' (Page 8, 2nd paragraph). Instead, the relevant part of page 12 has been deleted.

2. The reviewer indicates to provide the detailed CGH data of 83 cases analyzed in this study. Since the data size was very large, they have been provided as supplemental information by another file, which has been sent together with the manuscript to the editor.

3. The reviewer requests to confirm the mini-array data with other methods. In this study, DCNAs from the mini-arrays were confirmed by neither FISH nor quantitative RT-PCR. However, we compared DCNAs detected by the mini-array with DCNAs detected by screening arrays using the same DNAs. We confirmed that the data from the mini-array were equivalent to those from the screening chip. We have added following statements to the section of `Validation of the mini-array (page 12)` in the text: Although the density of the dots was less in the mini-array than in the screening 1.4K arrays, data by the mini-array analysis were virtually equivalent to those by the screening array (Fig. 1c). The copy number of each clone in the mini-array correlated well with that of the corresponding clone in the screening array (average correlation coefficient r=0.747, ranging from 0.664 to 0.920).

4. The reviewer claims the incorporation of BAC ID of selected clones into the Table 2. According to the instruction, we have added BAC ID allotted by Macrogen to the Table 2.
Reviewer 2

1. The reviewer indicates to show the algorithms used in `Method 1’. To avoid long description of the algorithms, we showed original website of the algorithms in the section of `aCGH data analysis, and in addition, a reference cited (reference # 17) has been added to the text (page 8).

2. The reviewer requires a statement about DNA amplification sites. Before describing DNA amplification sites, we have defined DNA amplification. A statement `the log\(^2\) ratio >1.0 was regarded as amplification.' has been added to the last part of `Array CGH screening` in `Materials and methods` (page 7). Then, a sentence `DNA amplification was detected at chromosomal regions 17q21.1 (harboring HER-2), 11q13.3 (CCND1), and 11q13 (FGF4) in four, three, and three tumors, respectively, though the amplification was not correlated with any clinicopathological feature of the gastric cancers.' has been added to the text (page 12, 3\(^{rd}\) paragraph).
Reviewer 3

1. The reviewer requires providing information on data including BAC clone sequence and array CGH technologies used in this study. The technologies used in this study have been published as scientific papers and Macrogen website. According to the reviewer’s instruction, we have cited three published paper on array CGH experiments that used the same platform as this study (Oncology 2007:72:132-138, Journal of Clinical Pathology 2006;59:978-83 and Gynecologic Oncology 2005;99:545-551). Two of them were published by us. In addition, we have shown the website of Macrogen (http://www.macrogen.co.kr/eng/biochip/kin which aryo_summary.jsp) where detailed information on end-sequenced BAC clones and data processing procedures is available (page 7; the heading of ‘Array CGH for screening’).

   The reviewer and editor recommend publicizing microarray data together with the paper. Accordingly, we have sent an array CGH data file of 83 gastric adenocarcinomas examined in this study as suppliment information together with the manuscript.

2. The reviewer points out that since array CGH analysis is influenced by several factors, it may be difficult to determine the threshold level. I agree with the reviewer. In this study, we determined the cutoff value based on repeated exploratory experiemnts and previously published data. The present study followed the same cutoff value as previous experiments. Almost all spots were within the area between cutoff lines (±0.25) for male/female DNA samples. The coefficient of variation for normal male/female DNA samples was less than 10% (usually approximately 6.5%). Previous experiments (references: 14-16) were cited in the revised version as well as in the first version of the paper. In addition, according to the reviewer’s indication, we have added a figure of dye swap hybridization for the mini-array (Fig. 3).

3. The reviewer claims to add a reference on a decision-tree classifier together with an address of a website to the section of ‘aCGH data analysis’ (page 8). We have added them to the manuscript.


4. The reviewer requests to show the replicability between the mini-array and screening chip. According to the reviewer’s instruction, we have referred to the correlation efficiency (r=0.745) to clear the equivalence of data between mini-arrays and screening chips on the selected clones. There was the close similarity of CGH profiles between two platforms. Statistical analysis data have been added to the section of ‘Validation of performance for mini-array’ in ‘Results’ (page 12). In addition, the relevant part of ‘Materials and Methods’ has been revised.
5. The reviewer points out that a sentence `The criteria for the training set were also applied to data analysis of the mini-array.' was an equivocal description. We have edited this part so as to avoid ambiguity of the description as follows: Since CGH profiles of the mini-array are equivalent to those of screening arrays, ratios greater than log$_2$ 0.25 or less than log$_2$ 0.25 were considered as abnormal in the mini-array (page 9).

6. In this study, we used two analytical methods, though we showed only overall accuracy of these methods in the first version of the manuscript. The reviewer claims to show the accuracy for each method. According to reviewer’s instruction, we have added respective accuracy of methods 1 and 2 to Table 3. We have also added explanatory statements to the relevant part of the text (page 13).

Others
1. To clarify the number of BAC clones used for the mini-array, the following sentence has been added to the section of ‘aCGH data analysis’ (Page 8, 2nd paragraph); `the method 1 identified 24 BAC clones but one of them was shared between node metastasis and liver metastasis. The method 2 identified 26 clones.' In addition, a sentence `in the method 2, 26 clones were determined.' has been added to the last part of the paragraph. Instead, the relevant part of page 12 has been deleted.

1. (Page 12) As indicated by the reviewer, the statement `There were no BAC clones common to these parameters except for one clone in 5q13.2 harboring FCHO2` might lead to misunderstanding. To avoid misunderstanding, the sentence has been replaced by a simple statement `There were no BAC clones shared between methods 1 and 2.'

2. The reviewer points out the imbalance in the number between early and advanced cancers. This is true. We examined consecutive cases, resulting in such an imbalance. According to the reviewer’s suggestion, a label `Early and advanced' in Table 3 has been replaced by a label `Advanced cancers'.

3. According to the reviewer’s instruction, we have improved the resolution of Fig. 1b in the first version. In the revised version, it corresponds to Fig. 1c.

4. According to the reviewer’s instruction, Table 2 has been revised.

5. The reviewer points out a few incorrect figures in Table 3. The table has been checked and revised. Then, the relevant parts of the text have been corrected (pages 13 and 17).

6. The statement in page 13 pointed out by the reviewer has been deleted.

7. The sentence has been changed following the reviewer’s instruction (page 13).
8. We have corrected all typographical and grammatical errors indicated by the reviewer. A native speaker of English has checked the manuscript, again.