Reviewer's report

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

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Reviewer: Lauro Sumoy

Reviewer's report:

This is an article of interest because of the fact that it tries to apply microarray comparative genomic hybridization to build a classifier to aid in diagnosis of early and advanced gastric cancer and metastatic subtypes. However promising in its potential, as the same authors acknowledge toward the end of their discussion, a larger sample collection would be needed in order to validate such a tool for real application in clinical diagnostics. Nevertheless, this study provides a proof of principle of the feasibility of using a BAC array with a reduced number of probes to classify gastric cancer samples based on DNA copy number alterations. This is the first time this has been proposed and the authors achieve a relatively significant degree of success.

- Major Compulsory Revisions

The article in its current form has methodological information missing that makes it advisable that authors make their data available with sufficient detail to allow other researchers to reproduce their results.

1. Data should be made publicly available following standards for microarray data publication. These should have a) details describing platform probe details with accession numbers, genomic coordinates or specific BAC clone end sequence information, b) sample preparation, labelling and hybridization methods; c) data preprocessing, normalization and specific data filtering criteria applied. If this has already been published elsewhere a reference should suffice; if not it should be explained in larger detail in the manuscript or in the form of supplementary material, if applicable.

2. The authors only state that a log2ratio cutoff was set arbitrarily at ±0.25, but there is no measure of variance in signal among replicate spots or across probes arraywide. The fact that the threshold is set so low could be due to the mosaic nature of tumors, but the effect of tissue heterogeneity on DNA copy number detection is well addressed by testing several microdissected regions of the same tumor in Table 4. However there is no mention of possible dye bias or CNV false positive results. The small log2ratio values achieved using cancer samples raise the concern that there may be some of these two factors influencing the results. Authors should include at least one dye swap hybridization per platform to show negligible effects (or a reference to such an experiment if already published for the larger commercial platform). The array annotation should be in sufficient detail as to allow mapping of probes to the
genome to establish possible CNV content.

3. For clone selection method 1 authors use a decision-tree classifier. They don’t indicate the parameters used and proper citation is needed (Witten et al).

4. The difference in probe content between the large and small platforms raises issues on reproducibility of normalization when the number of clones is brought down to such a small number as 138. There is no quantitative data shown on replicability between the large and small platforms. The authors only show a picture of two profiles from the 1.4K arrays and the mini-array on a single sample in Figure 1b, but there is no specific data on clone agreement. A log2ratio correlation plot for common clones in both platforms in this sample should be shown. Ideally a large subset of samples used in the large platform should have been analyzed with the small platform and, if this has been performed, an average and standard deviation of correlation values should be provided.

5. The authors state that they used the two different clone selection methods in the small platform to assess classification power. From the statement on p.8 that says that ‘The criteria for the training set where also applied to data analysis of the mini-array’ it is unclear whether they perform the clone selection again or just used the clones previously selected by these two methods to perform sample classification on the independent set of 30 samples. Since the data needed to compute the Chi-squared test are not available, it was not possible to reproduce the p-value calculations for the large platform nor to test their variation in the small platform.

6. The authors end up using the sum of two methods but it is unclear, given the fact that they rely on a practically non-overlapping set of clones, if perhaps one of the two may be significantly more accurate than the other. Overall, the approach seems rather arbitrary and it raises questions on its universal applicability to other cancers, other sample sets or other array platforms. Since the authors chose as their decision criteria to give higher priority to the method whose diagnosis indicated a more advanced stage, it would be interesting to know the degree of agreement between both methods and whether one of the two may be more or less accurate or more or less prone to over or under-diagnose. There is only a vague statement in p.12 regarding the fact that ‘diagnostic accuracy was considerably high even in a single method’. Authors should compare the two.

- Minor Essential Revisions

The manuscript holds several imprecisions that need to be addressed:

1. There is a misleading statement in the number of clones detected as discriminant and used in the mini-array. Authors state in their abstract (p.2) that they included 50 clones selected by either of the two methods, but they say they chose six, four, four and seven clones on one hand and 26 on the other. This adds up to 51 -only in the text it becomes apparent that the reason they chose 50 is because there is a one clone overlap between the two selection methods. This reviewer interprets that the common clone is the one on 5q13.2. Is this correct? Please clarify.

1. The phrase in p.12 stating ‘There were no BAC clones common to these
parameters except for one clone in 5q13.2 harboring FCHO2' is ambiguous in its context because it may be wrongly interpreted as indicating that this clone was able to distinguish among all types of clinical 2 class comparisons. Authors should clarify this by referring to method 1 versus method 2 instead of using the word 'parameters'. In the discussion, in p.15, further reference to this clone is made by stating that 'The copy number loss of the clone in 5q13.2 was frequent in gastric cancers with neither node nor liver metastasis, but the converse was not true'. This statement is not supported by Table 2. Authors should resolve if there is a mistake either in the table or in the statements in the text. In fact, this raises the question of why there is such a small overlap if the clones are selected to distinguish tumors based on the same phenotypes. Authors should address this in their discussion.

2. There is confusion in the way the clinical summary (Table 1) and diagnostic accuracy (Table 3) address the early versus advanced cancers. In Table 1, while in the screening sample subset the authors provide an early/advanced ratio (6/35 for intestinal-type and 4/38 for diffuse-type) in the validation set they provide only a number of 3 (which one can only assume refers to early cases). Therefore it should state 3/27. Likewise, in Table 3 values are provided for 'Early or Advanced' while they apparently refer to advanced or deep invading tumors and therefore should be labelled as 'Advanced'.

3. Figure 1b has slightly poor graphical resolution (chromosome numbers on the x axis are difficult to read) and could be plotted to expand the y axis by setting the maximum and minimum log2ratio coordinates to 2. Shared differential clones may be highlighted or colored identically in the two plots.

4. Table 2 requires further clarification to describe what Presence(%) and Absence(%) mean. There are two figures (N=59 and N=24 in the table header); it is unclear what they refer to. It would appear that the numbers of tumors belonging to each of two classes in every characteristic should be different and these are not indicated.

5. Table 3 appears to have errors in a few numbers since these don't add up and this is also reflected in the text in p12 and p17. Node metastasis diagnostic sensitivity should be 20/21 (0.90) -it says 19/21- and specificity 0/9 (0.00) -it says 0.11-. Liver metastasis diagnostic specificity should be 24/27 (0.89) -it says 23/27 (0.85). Peritoneal dissemination diagnostic false positives should be 0/25 (0.00) -it says 0/24-. As said before, the 'Early or Advanced' category should be changed to 'Advanced' for the sake of clarity.

6. The statement in p.13 comparing accuracy of histological classification by two methods may be out of context and could be part of the discussion comparing both methods (see point 6 under major compulsory revisions).

7. On p.13 there is an awkward sentence: 'The slight variation.... was observed... from a tumor'. This may be changed to 'A slight... from a single tumor'.

8. Typographical and/or grammatical errors:

8.a) In p.4: 'type of cancers' should be 'types of cancers'.
8.b) In p.5 'diseases states' should be 'disease states'
8.c) In p.6 some numbers followed by words are not separated by a space ('induced10', '41tumors')
8.d) On top of p.8 colon (:) signs should be replaced by semi colon (;) signs
8.e) In p.11 'it is possible to divided gastric cancers into two groups' should be '... divide...'
8.f) In p.15 '...the examination of only a few genomic markers insufficient for the precise estimation...' should be '...is insufficient...'.
8.g) In p.16 'The mini-array developed in this study allows the estimation ... is unique' should be '...allowing...'.
8.h) In p.17 'the false positive and false negative rate was...' should be '...rates were...'.
8.i) In p.19 'coomented' should be 'commented'.
8.j) In p.28 in the Figure 1 legend '138 clones was spotted....' should be '...were spotted...'
8.k) Table 4 shows two asterisks which should be a single one referring to histologic type (misspelled as 'hitologic', please correct)

- Discretionary Revisions
1. This reviewer's quick survey of the literature shows very little agreement on a common association to recurrent gains and losses in specific cytogenetic regions and this work brings up yet new possible targets reflecting the heterogeneity of the stomach cancer disease. While there is no operational requirement for an efficient diagnostic tools to necessarily provide markers directly related to causality or biological mechanism of the disease, the authors attempt to propose markers that may be related to metastatic and invasive behavior or histotype by proposing a list of candidate genes. However there is no attempt to go into more depth. they are invited to discuss this further if space allows.

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being published

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:
'I declare that I have no competing interests'