Author's response to reviews

Title: Genomic profiling of rectal adenoma and carcinoma by array-based comparative genomic hybridization

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Author's response to reviews:

Subject:
Revised BMC medical genomics-1537320196646526 Version 1 “Genomic profiling of rectal adenoma and carcinoma by array-based comparative genomic hybridization” by Zhi-Zhou Shi et al.

Dear Editor-in-Chief,

Thank you for your letter dated June 14, 2012. We appreciate your careful evaluation of our work and are grateful to the Reviewers for their valuable suggestions. We are very sorry to reply you so late, because we have been doing complementary real-time PCR and immunohistochemistry of rectal adenoma and rectal carcinoma tissues for determining expression of candidate genes (following the Reviewers’ advice). Combined with the results of the complementary experiments, we have made major revisions to our manuscript with taking into account all your and the Reviewers’ comments point by point.

According to the reviewer’s suggestion, we have deleted the section of integrative analysis on the seven colorectal cancer cell lines. Further we selected 14 genes by literature analyses to compare their expression between rectal cancer tissues and paracancerous tissues as well as from rectal adenoma to carcinoma. We also analyzed the expression of two genes from rectal adenoma to carcinoma by immunohistochemistry.

Importantly, we found that copy number and mRNA expression of EFNA1 were increased from rectal adenoma to carcinoma. And C13orf27 and PMEPA1 with gains in both adenoma and carcinoma were overexpressed in rectal cancer.
tissues. Interestingly, mRNA and protein expression of GPNMB was both higher in cancer tissues than rectal adenoma tissues.

All the amendments have been highlighted by underline in the revised manuscript. Here is how we have addressed each revision request or comment:

Reviewer: Manny Bacolod

A. Major Compulsory Revisions

1. The study would have been much better if the authors analyzed the genome-wide expression of the 16 samples themselves (instead of using the expression data from 7 CRC lines), when trying to figure out which among the resident genes are most relevant in the rectal cancer progression. If tissue samples remained (either fresh froze, or even FFPE), expression analyses can certainly be done. Additional expression data will make up for the low sample size in this study.

Because all specimens are small biopsy samples, all tissues have been used to extract DNA for array CGH assay. There are no tissue samples of the 8 adenomas and 8 carcinomas in the array-CGH assay to analyze the genome-wide expression. To further identify copy number related genes, we analyzed expression of 14 genes by real-time PCR and two genes by immunohistochemistry in other specimens of rectal adenomas and carcinomas, see the “Results” in page 9-10 and Figures 3-5.

2. The authors listed the aberrant (gain, loss) chromosomal regions that are common to the tumors and adenomas. That is fine. In my opinion, I think it is also important to identify the difference between the adenomas and carcinomas (in particular, the gains/losses present in carcinomas, but not in adenomas). Then the authors may ask the next question: which genes had significant expression level change when going from adenoma to carcinoma? The authors may want to pay particular attention to the copy number-dependent, upregulated genes (in carcinoma relative to adenoma), verify their expression at the protein level (or histochemistry), and discuss the possibility of such genes/proteins being therapeutic targets.

According to the reviewer’s suggestion, we have analyzed and listed the aberrant chromosomal regions that were present in carcinomas, but not in adenomas, see The “Results” in page 9. Then we selected 14 genes of chromosomal regions that are common (7 genes) or distinct (7 genes) between rectal adenoma and carcinoma to analyze their expression status by real-time PCR. Copy number and mRNA expression of EFNA1 were increased from rectal adenoma to carcinoma. And C13orf27 and PMEPA1 which gained in both adenoma and carcinoma were overexpressed in rectal cancer tissues see Figures 3 and 4.

3. The authors can strengthen this paper by: sequencing SMAD4 (18q) (and examine if the gene gets mutated at the adenoma stage), and by checking the expression of MET (7q), if it has significantly increased from adenoma to carcinoma. It wouldn’t hurt to sequence p53, and APC as well.

In our real-time PCR assay, SMAD4 and BCL2 were underexpressed in rectal
cancer tissues compared with paracancerous normal tissues, but not significantly decreased from adenoma to carcinoma, see Figure 3. We didn’t detect the mutation of SMAD4 and expression of MET.

4. The authors may want to discuss further the difference between rectal and colon tumors (in terms of chromosomal aberrations, etc.), if there are any.
We discussed the difference between rectal and colon tumors in page 11.

B. Minor Essential Revision
1. The manuscript needs a few minor grammatical corrections. For example, in abstract (methods part): use “interesting” instead of interested.
We have done our best to correct the English errors in wording, spelling, grammar and punctuation in the revised manuscript.

C. Discretionary Revision
1. Do the authors have information on who among the patients sampled for adenomas eventually developed tumors (and who did not)? If these information are available, the authors may want to examine the difference in chromosomal aberrations between these two groups (subgroup which remained adenoma, subgroup which progressed to cancer).
The information on who among the patients sampled for adenomas developed tumors and who did not is unavailable.

Reviewer: Jordi Camps

Major compulsory revisions
1. The number of samples is by far very limited. In order to make any statement correlating the chromosome imbalances that are involved in the adenoma-carcinoma progression, ideally one would need to approach the samples from the same patient and consider performing FISH to assess heterogeneity and confirm the findings.
Because all specimens are small biopsy samples, all tissues have been used to extract DNA for array CGH assay. There are no tissue samples of 8 adenomas and 8 carcinomas in array CGH assay to confirm the findings by FISH.
2. The meta-analysis using the NCI-60 panel of cancer cell lines is confusing. To my knowledge, none of the cell lines in that panel represent the tumor entity that the authors are assessing (i.e., rectal cancer), therefore the analysis of gene expression at this level can arise some concerns.
We deleted this section in the revised manuscript.
3. Besides the bioinformatic assessment of gene expression, it would very interesting to know whether the genes that the authors identified as potential candidates based on the NCI-60 panel of cell lines or the Oncomine database are also up-regulated or underexpressed in the tumors that the authors analyzed by array CGH.
There were no more tissues of patients in array CGH study to analyze the expressions of candidate genes. Therefore we compared the expression of 14 selected genes between rectal cancer and paracancerous tissues, or between rectal adenoma and carcinoma tissues by real-time PCR. We also analyzed the expression of GPNMB and DIS3 in rectal adenoma and carcinoma by immunohistochemistry, see the “Results” in page 9-10 and Figures 3-5.

4. The finding of driver genes is important to nail down the pathways involved in carcinogenesis. More work could be presented towards the understanding of how those genes that the authors suggest to be involved in rectal carcinogenesis might play a role in cancer in the context of molecular and cellular pathways. The validated genes by real-time PCR or IHC were little, and not suitable for analyzing the pathways changed in rectal carcinogenesis. More work on this maybe be conducted in the further study.

5. It has been very well established by several groups the correlation between copy number changes and levels of gene expression. Therefore the authors would need to refer to this evidence in greater detail and frame their results based on previous findings.

We have added discussion on the differentially expressed genes with copy number changes in colorectal carcinogenesis in page 11.

Minor essential revisions:

1. Although the manuscript is generally well written, it might need some editing and more accuracy when wording some sentences and statements.

We have done our best to correct the English errors in wording, spelling, grammar and punctuation in the revised manuscript.

2. The authors could have supported their background and discussion with a much more extensive list of literature references.

We have enriched the introduction and discussion sections with much more literature references.

3. Study design is unnecessary as it summarizes the abstract.

We have deleted the study design in Methods.

4. Percentage of contamination with normal cells in the tumor specimens should be indicated in the section Patients and Samples. There is the need to indicate how this determination was performed.

We added the explanation “An experienced pathologist confirmed that normal cell content of all the samples was less than 40% by HE staining” in Patients and Samples.

5. Analysis of array CGH data requires much more details, including what algorithm was used, and how the minimal regions of gains and losses were defined.
We added the explanation about the algorithms and details used in CGH data analysis in Microarray Data Analysis.

6. It has been known for a while that loss-of-function of SMAD4 is associated with deletions of 18q in colorectal cancer.
We accept the reviewer’s suggestion about SMAD4.

Many thanks again for your interest in our work. We hope that in its present form the article will be suitable for publication in BMC Medical Genomics.

Sincerely Yours,

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