Author's response to reviews

Title: Establishment of monoclonal HCC cell lines with organ site-specific tropisms

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Author's response to reviews: see over
Dear editors and reviewers:

Thank you very much for your constructive suggestions on our manuscript “Establishment of monoclonal HCC cell lines with organ site-specific tropisms”. Your comments are helpful for us to improve our paper. We have revised the manuscript according to your comments one by one. The followings are our responds on the reviewer’s comments. The revised has been marked in red in the manuscript.

We appreciate the editors and reviewers once more and hope that our revision will meet to be published in BMC Cancer.

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To reviewer, Dr. Siu Tim Cheung:

1. The origin of the cell line HCCLM3 should be clearly described. Cite the relevant paper. Have noted the below paper which described the cell line: Li Y, Tang Y, Ye L, Liu B, Liu K, Chen J, Xue Q. Establishment of a hepatocellular carcinoma cell line with unique metastatic characteristics through in vivo selection and screening for metastasis-related genes through cDNA microarray. J Cancer Res Clin Oncol 2003;129(1):43-51. However, this paper has not been cited in the manuscript. Authors should clarify. Cite appropriate paper and acknowledge the cell line source.

Responds:

HCCLM3 was established in our institute (Liver Cancer Institute, Fudan University). And this work is based on HCCLM3 cell transfected with RFP-expressed vector, so
we have cited the original paper in our reference according to your suggestion.

2. Provide information on the cell line source, acknowledge the gift source or cell bank. Present the standard cell line authentication on the original cell line and the derived clones.

Responds:
Thank you for your kindly suggestion. The cell line can be used freely in our institute for scientific research.

3. The authors are describing the work on lung metastasis and lymph node metastasis. Provide suitable literature review on HCC metastasis to lung and lymph node in terms of frequency etc. This will allow the readers to understand the clinical relevance. Check entire manuscript and cite appropriate references.

Responds:
Thank you for your suggestion. We have cited appropriate references in the manuscript for reference.

4. Justify in detail the use of formula: Total metastatic foci number of each monoclonal HCC cell line = grade I ×1 + grade II ×2 + grade III ×3 + grade IV ×4.

Responds:
The formula was used by Dr. Gao (see reference 17). It is a semi-quantitative scoring system to evaluate tumor metastatic potentials, especially when the metastatic foci in lung are in cluster. The results from the formula are the indirect parameter of tumor metastatic potentials.

5. The figures are in poor quality. For example in Figure 1, photos are in low resolution, and charts too. In the charts, the two lines cannot be read for their respective data, which one is “Lymph node” or “Lung” cannot be recognized.
Similarly improve the other figures.

Responds:
Thank you for your suggestions. We have redone our Figure 1. Different color lines were used, in detail, green line is for HCC metastasis in lung and Red line for HCC metastasis in lymph node.

6. The cell line subclones would need comprehensive examination to differentiate the metastasis key drivers.

Responds:
Thanks you for your suggestions. The results in this study is our preliminary ones, more comprehensive examinations are going on by exosome DNA and mRNA sequencing. The differential expression levels of IGTA1, VCAM1, SPARAC and ANGPTL4 in these subclonal cells have been further confirmed in our mRNA sequencing data (GEO accession: GSE38945). Thus, more key metastasis drivers will believe to be found lately.

To reviewer, Dr. Sandra Muñoz-Galván

a) Major compulsory revisions:
- The authors must provide a list of the 30 genes whose expression levels were analyzed, references of the works where these genes were involved in the regulation of tumor metastatic tropism in other cancers, as well as their expression levels measured by RT-qPCR. This could be done, for example, as a supplemental figure or table. Moreover, why do the authors focus only in up-regulated genes? Is there any down-regulated gene that could be involved in metastasis?

Responds:
Thanks you very much. We pick out 30 tumor tropism-related genes from published paper (Supplementary Table 1) and 21 genes of them were detected in our subclonal cells by RT-PCR. The name and primary sequences have been provided in Supplementary Table 2. And their relative expression levels were analyzed in
Supplementary Figure 1. In our study, we could not find any down-regulated gene involved in HCC metastatic tropisms. Actually, these subclonal cells have been further confirmed in our mRNA sequencing data (GEO accession: GSE38945) and more comprehensive examinations are going on by exosome DNA sequencing and the results will be published in the future.

- The authors have to discuss deeper the functions of the selected 4 up-regulated genes and the possible implications of these findings.

Responds:
Thanks, we have discussed more biological functions of these 4 up-regulated genes in the manuscript, as show in line 378-383.

b) Minor essential revisions:
- The specific statistical tests used in each figure and why the authors chose those tests must be indicated in the corresponding figure legend.

Responds:
Thanks, proper revisions were made according to your suggestions. The specific statistical tests were indicated in the corresponding figure legend.

- Fig 1B and D: which line corresponds to lung or lymph node in the graphs should be defined more clearly, for example by using different colors. It is hard to distinguish the black line from the grey one in such small graphs.

Responds:
Thank you for your suggestions. We have updated our Figure 1. Different color lines were used, in detail, green line is for HCC metastasis in lung and Red line for HCC metastasis in lymph node.

- What do error bars represent in Fig. 2C, 3B-C, 4 and 5B? Are they the standard deviation as indicated in the Materials and Methods section, or are they the standard error of the mean? Because it is hard to believe that some differences observed
throughout these graphs are not statistically significant, given the small standard deviations they have.

Responds:
Thanks, the statistical analyses have been redone according to your suggestions.
Error bars in Fig. 2C, 3B-C, 4 and 5B were the standard error of the mean.

- The migration and invasion experiments (page 11, lines 296-300) should be better explained.

Responds:
Thanks, the migration and invasion experiments has been explained in the text, as show in line 297-306.