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 reviewer:

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 your comments. The revised has been marked in red in the paper.

We appreciate you 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. Standard cell line authentication, for example by short tandem repeats, on the original cell line and the derived clones, are essential for cell line reporting. The authors have not addressed these.

Responds:

Thanks for your opinions that use short tandem repeats to check the linage of original cell and the derived clones. Though "Short Tandem Repeats" (STRs) have proved to be versatile molecular markers, particularly for DNA analysis in forensics cases and paternity testing, but they are not without limitations. (1) Microsatellites developed for particular species can often be applied to closely related species, but the percentage of loci that successfully amplify may decrease with increasing genetic

(2) Stochastic effects of sampling that occurs during mating may change allele frequencies in a way that is very similar to the effect of null alleles; (3) In tumour cells, where controls on replication may be damaged, STRs may be gained or lost at an especially high frequency during each round of mitosis. Hence a tumour cell line might show a different genetic fingerprint from that of original ones.

Now the next generation sequencing technology for exon sequencing reveals more advantages compared with traditional STRs in determining the paternity of original cells and the derived clones. Thus, we have done the exon sequencings of HCCLM3-R, LM-1 and LnM-1 cells. The related data are going on analysis and not released in the paper. To answer your concern, we provide some LOH data of these 3 cell lines to show their original. In the supplementary-2 (only for review), as you can see, similar changes existed in chromosome 1, 9 and 21, representing the characteristic chromosome deletion and amplification in these 3 cell lines. In addition, HCCLM3-R, LM-1 and LnM-1 cells are all expressed red fluorescence. All these data confirm their paternity between original cells and the derived clones. Please see line 244-247.

2. The cover letter indicated the authors agreed on the revision to describe “the Literature review on HCC metastasis to lung and lymph node”. But has not listed where they have incorporated the change. Cannot find the content in the revised submission, including text or reference list highlighted in red.

Responds:

Please forgive our negligence last time. The literatures on lung and lymph node metastasis have been summarized in Supplementary Table 1, and see the changes of line 316-318, 385-389, and line 485-509 in our text.
3. **Justification on the formula for total metastatic foci number.** The authors cited the reference, but did not explain the reason for using this formula.

**Responds:**

Theoretically, metastasis potential should be evaluated directly by the number of metastatic foci in a second organ. However, if metastasis foci are overlapped into a cluster, it is difficult in practice to be counted exactly. Therefore, we used a semi-quantitative scoring system introduced by Dr. Gao (refer 16) to evaluate the tumor metastatic potentials (please see line 147-151).