Author's response to reviews

Title: Genetic and epigenetic characteristics of human multiple hepatocellular carcinoma

Authors:

Kazuya Taniguchi (taniguti-ka@mc.pref.osaka.jp)
Terumasa Yamada (yamada-te@mc.pref.osaka.jp)
Yo Sasaki (sasaki-yo@city.yao.osaka.jp)
Kikuya Kato (katou-ki@mc.pref.osaka.jp)

Version: 2 Date: 14 December 2009

Dear Editor,

The followings are response to reviewers. Changed parts of the manuscript are marked by red characters.

Reviewer 1453659993320498

One of the clinical distinction of hepatocellular carcinoma (HCC) is multiple carcinogenesis, and discrimination of intrahepatic metastasis (IM) and multicentric carcinogenesis (MC) is critical for decision making of therapy. The authors investigated genetic aberrations of 15 pairs of multiple HCCs consisting of 11 MCs and 4 IMs using mutation analysis, array CGH, and methylation analysis. However, they could not demonstrate dominant conclusion, for example, difference of genetic alteration between MC and IM. And that, several conclusions the authors came to would not have the readers acceptable.

1) First, the authors’ assertion in ‘Somatic mutation analysis’ in Results is not clear.
It seems just a negative data.

The additional aim of this manuscript is evaluation of factors for differentiation of IM and MC. Because there have been papers reporting positive results [1], it is worthwhile to report negative results, which deny the general application of positive results. To clarify the objective, we revised the corresponding part (page 5, last paragraph, last lines) (page 12, first paragraph).

2) Second, the authors concluded the multiple tumors were derived from common precancerous or cancerous ancestors due to common chromosomal aberrations.
However, the number of common chromosomal aberrations in pair samples is quite few (Table 2), and I don’t think they could form such a conclusion. (‘Chromosomal aberration’ in Results).

The authors agree with this comment. We changed the corresponding parts of the manuscript (precancerous or cancerous ancestors -> the common lineage) (last line of page 9, first line of page 10).

3) Third, as with ‘Somatic mutation analysis’, the aim of ‘Methylation analysis’ is quite obscure. Why did the authors perform methylation analysis for multiple HCC?

As responded in the reply to the first comment, the additional aim of this manuscript is evaluation of factors for differentiation of IM and MC. Because hypermethylation of the selected genes was once tried to differentiation of IM and MC [2], we performed the methylation analysis. This literature is cited in the second paragraph of page 5.

4) Through genome research using IM and MC samples, the readers would like to know what is the difference of genetic change between two groups, I guess. That is, is it possible to make a molecular diagnosis about multiple HCC as IM or MC?

Occurrence of the common aberrations depended on the total number of chromosome aberrations, and was not likely to correlate with the history of multiple tumors. Thus, it is not possible to apply them to differential diagnosis of IM and MC. To clarify this point, we added sentences (from page 13 bottom to page 14 top).

5) Responding comment for language.

The original manuscript was edited by a language service recommended by OUP. The revised manuscript was edited by another service.

Reviewer 1376125669319190

Major Comments

(1) Is there any speculation on the higher similarity in the pattern of methylation levels from the same patients, e.g., there may be individual difference in susceptibility for specific promoter methylation?

There were no rules common to similar pairs of multiple HCC. If there were some environmental factors, they would induce changes to specific genes. Similar methylation patterns were likely to be trace of the common lineage. Short discussion was presented in the second paragraph of page 13.

(2) It would be better to clarify that the very high similarity (i.e., r>0.8), not the
relatively higher r associated with the individual difference, is a potential indicator of IM to avoid confusion.

Edited as the suggestion (from page 11 bottom to page 12 top).

(3) It would be informative to include clinical variables (e.g., tumor size, histological differentiation, encapsulation, presence of nodule-in-nodule structure, presence of cirrhosis,...) in Table 1. It will provide clue to have a sense about clinical likelihood of IM or MC and its correlation to r for each case. Instead, the results for mutation and chromosomal aberration in Table 1 can be moved to Table 2.

Clinical data except nodule-to-nodule structure were added to Table 1. In our hospital, nodule-to-nodule structure is not regarded as informative. However, from the status of operation, all tumors were not likely to have the nodule-to-nodule structure. We keep results of mutation and chromosome aberrations in Table 1, because we want to make it as a comprehensive result table.

(4) The negative results for mutation and chromosomal aberration analysis are likely due to small sample size, rather than technical limitation of the assays as mentioned in the last paragraph of Discussion, given the huge clinical and molecular heterogeneity in HCC.

Edited as suggested (page 14, second paragraph).

Minor comments
(1) It would be better to rephrase "adjacent normal liver" to "adjacent non-tumor liver" to avoid confusion.
(2) Results, Chromosomal aberration, "nucleotide resolution": More precisely, "probe-level resolution" would be better because the data were generated on CGH array.

Edited as suggested (With only these changes we did not changed the color).

(3) It would be ideal to deposit the datasets to public database like NCBI’s Gene Expression Omnibus.

We will submit the data to the corresponding database in DDBJ (CIBEX or related database).

References