Author's response to reviews

Title: Fibroblast growth factor 19 expression correlates with tumor progression and poorer prognosis of hepatocellular carcinoma

Authors:

Seiki Miura (smiura@msd.biglobe.ne.jp)
Noboru Mitsuhashi (noborumitsuhashi@gmail.com)
Hiroaki Shimizu (h-shimizu@faculty.chiba-u.jp)
Fumio Kimura (kimura-f@faculty.chiba-u.jp)
Hiroyuki Yoshidome (h-yoshidome@faculty.chiba-u.jp)
Masayuki Otsuka (otsuka-m@faculty.chiba-u.jp)
Atsushi Kato (akato@faculty.chiba-u.jp)
Takashi Shida (shidax812@yahoo.co.jp)
Daiki Okamura (okamura.chiba@gmail.com)
Masaru Miyazaki (masaru@faculty.chiba-u.jp)

Version: 2 Date: 12 December 2011

Author's response to reviews:

Miss Marinette Lacson
Journal Editorial Office
BMC medicine journals

Dear Miss Lacson,

Thank you very much for your letter of October 31, 2011, with regard to our manuscript together with the comments from the two reviewers. We apologize for the delayed response. I am sending herewith a copy of our revised manuscript. Our incorporation of the reviewers' suggestion is as follows:

Reviewer: Dr. Allan Tsung

Major comments

1. The patients consisting of the group of 40 HCC tissues are different from the 29 patients whose serum was examined. We understand that serum FGF19 in those 40 patients should be examined. However, these patients were operated before 2001, and we did not collect the serum samples of them. Therefore we are unable to do that experiment. We added serum FGF19 level of health controls at Figure 7. Serum FGF19 was significantly higher in the HCC patients than normal controls. We also added the sentence about the serum FGF19 level of normal control in Method #p. 10, line17#, Result#p. 15, line10-14#, and Figure legend#p. 25, line8-9#. 
2. We did not notice about that. Thank you very much for pointing out the mistake. We have changed the statement accurately (p. 12, line17-18, p.16, line22-23).

3. We thank the reviewer for this comment. As reviewer’s comment, it may not be appropriate to assess the amount of the protein by immunohistochemistry. We agreed with additional information on western blot to quantify the amount of FGF19 and FGFR4 protein would be valuable. FGF19 is a protein of the secretion type, we believe the concentration of the FGF19 in the medium is the most important molecule. Actually, the level of FGF19 secreted in the medium is high in JHH7 and HuH7 (Fig 3B). We also agreed with the level of the FGF19 secreted in the medium from primary normal hepatocytes are very important information, so we examined and added the data of the FGF19 concentration of the primary hepatocyte culture medium (Fig 3B).

4. We apologize about that we did not show the data of the time course. As we had showed in Fig4, all 5 cell lines demonstrate the highest Proliferation Index at the FGF19 concentration of 1ng/ml. We had changed the original Fig4. to Fig4A. and had added the Fig4B. along the time axis in the FGF19 concentration of 1ng/ml as the reviewer suggested. We are also sorry about that our statement is hard to understand about the numbers. “n=12” for in Fig.4, Fig5A, Fig. 6E, Fig 6F means the number of the chambers we examined at the same time in the same condition. We added this comment on the Methods section(p10 ,l12-14 ).

Minor points

1. We thank the reviewer for this comment. It is our mistake to forget showing what rectangles indicate. The closed rectangles indicate the same cells stained immunohistochemically using control antibody. We added this information on the Methods section (p6, l25).

2. We agreed with the reviewers comment. We deleted the sentence “RNA interference (RNAi) is a new technique”.

Reviewer: Dr. John Chiang

1. We appreciate the reviewer’s concerns on this point. We agreed with the reviewer about the number of human HCC samples should be increased for accuracy of the results. Unfortunately, we didn’t have the samples of old cases. Our data showed significant difference between two groups statistically. Therefore, we would like to retain the original text.

2. We agreed with the relevance of this reference, and have added it to the Discussion (p.16, line5-9) and References (29). Han Kiat Ho et al., reported that one third of HCC patients exhibited increased FGFR4 mRNA expression in the matched tumor/normal tissue. In our study, mRNA expression of the FGFR4 tended to increase (Fig1B, P=0.055) in cancer tissue compared with adjacent tissue. We believe there is no confliction of the data between both groups.
3. We did not have the evidence that FGFR4 protein expressed on the membrane in our study. According to reviewers comment, we removed this sentence (p.14, line25).

4. We wish to thank the reviewer for this comment. Accordingly, we have added label of “survival rates to Fig 2.

5. This error has been corrected in accordance with the reviewer’s comment. (Fig6D., Fig6C, and p.13, line22-23)

6. We acknowledge the reviewer’s comment on this point. We performed and added the data of the cell migration assay.

We thank reviewers again because the manuscript has been improved drastically by their comments. We hope it will be accepted for publication in Biomedical Cancer.

Sincerely yours,

Department of General Surgery,
Chiba University Graduate School of Medicine,
Chiba, Japan.

Noboru Mitsuhashi, MD., PhD