March 27, 2014

Dear Editor,

Please find the edited manuscript in Word format (file name: 8051-Review.doc).

Title: Significance of downregulation of liver fatty acid-binding protein in hepatocellular carcinoma

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Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 8051

The manuscript has been improved according to the suggestions of reviewers:
1 Format has been updated

2 Revision has been made according to the suggestions of the reviewers

Reviewer No. 00069630

(1) This reviewer pointed out that convincing conclusion may not be drawn from the present study because the number of liver fatty acid-binding protein (L-FABP)-negative hepatocellular carcinoma (HCC) cases was only 16. In the revised manuscript, we described this problem as the limitation of the present study (page 22, line 20-24) and described only the facts accurately throughout the paper.

(2) This reviewer required us to detect expression of hepatocyte nuclear factor 1 (HNF1)α protein or mutation of *HNF1A*. Accordingly, we performed mutation analysis of *HNF1A* (page 12, line 16 – page 13, line 9; page 16, line 16 – page 17, line 3; page 21, line 5-22).

Reviewer No. 00159425

We appreciate this reviewer's kind comments. This reviewer required no revision.

Reviewer No. 00182114

(1) This reviewer asked us the reason why the downregulation pattern of L-FABP expression was different between cases of small and large HCC. We speculate that most small HCCs with downregulation of L-FABP expression represent focal phenotypic changes in tumor progression. In large HCCs, although the regions with such phenotypic changes may grow to demonstrate diffuse distribution, several cases may represent malignant transformation of HNF1α-inactivated HCA (H-HCA) (page 19, line 6-9, 11-13).

(2) This reviewer asked us the reason why we decided that small HCC was ≤2cm and large HCC was >2cm. In this study, we classified HCCs into small and large tumors using the standard, because tumor stage of HCC is classified using the same standard in the General Rules for the Clinical and Pathological Study of Primary Liver Cancer in Japan. Actually, staging systems using the same standard have been reported to better reflect patients' prognosis (page 11, line 3-8).

Reviewer No. 00503516

(1) Ten cases exhibiting focal downregulation and 6 cases exhibiting diffuse downregulation belong to the 16 L-FABP-negative cases on tissue microarrays. This was clarified in the revised manuscript (page 14, line 11).

(2) The cases with focal or diffuse downregulation belong to the 16 L-FABP-negative cases on tissue microarrays. This was clarified in the revised manuscript (page 14, line 16-17).

(3) This reviewer described that some *in vitro* studies might be performed and/or some considerations should be introduced. According to the request of this reviewer, we performed *in vitro* experiments using cell lines (page 11, line 10 - page 12, line 15; page 16, line 7-15; page 20, line 22 - page 21, line 4).

(4)This reviewer required an explanation on the reason why we considered the cut-off of 2cm to discriminate small and large HCC. In the present study, we used the standard because tumor stage of HCC is classified using the same standard in the General Rules for the Clinical and Pathological Study of Primary Liver Cancer in Japan. Actually, staging systems using the same standard have been reported to better reflect patients' prognosis (page 11, line 3-8).

(5) We revised Core tip according to the suggestion of this reviewer.

(6) We thank this reviewer for pointing out our careless mistake. We corrected L-LABP to L-FABP in the revised manuscript (page 4, line 18).

Reviewer No. 00680628

Major comments:

This reviewer pointed out the necessity to establish strict criteria in evaluating the results of immunohistochemical staining for L-FABP. According to this reviewer's suggestion, in the revised manuscript, we evaluated the results as positive if more than 5% of tumor cells stained positive (page 10, line 6-7, 10-16). We believe that these strict criteria increased the objectivity of the data. This reviewer also pointed out the problem of sampling bias that could occur from usage of tissue microarrays. In the present study, we used tissue microarrays to examine the rate of L-FABP-positive or L-FABP-negative cases because it was difficult to perform immunohistochemical staining using whole-tissue sections for as many as 146 HCC cases. As this reviewer pointed out, the possibility of sampling bias may not be negligible, and we described this problem in Discussion in the revised manuscript (page 22, line 6-16).

Minor comments:

(1a) We categorized age and tumor size and presented all data as % in the revised Table.

(1b) In viral infection, "negative" means nonB nonC. This was clarified in the revised Table.

(1c) According to the suggestion of this reviewer, we separated non-tumor part and tumor part in the revised Table.

(1d) According to the suggestion of this reviewer, the prevalence of liver cirrhosis and Child-Pugh classification were presented in the revised Table.

(1e) According to the suggestion of this reviewer, we categorized tumor size in the revised Table.

(2) According to the suggestion of this reviewer, we omitted Table 1 and described the procedure of immunohistochemical staining in detail (including dilution of antibodies and the method of pretreatment) in the revised manuscript (page 9, line 7 - page 10, line 5).

(3a) According to the suggestion of this reviewer, we changed "Statistics" to "Statistical analysis" in the revised manuscript (page 13, line 10).

(3b) We used Mann-Whitney U test only for tumor differentiation, tumor stage, background liver tissue, and Child-Pugh classification (page 13, line 13-15). These data were not presented as mean ± standard deviation. The statistical methods were clarified in the revised manuscript (page 13, line 10-17), and number of patients analyzed were shown in each Table.

(3c) As this reviewer pointed out, it is incorrect to use Fisher's exact test for tumor differentiation, tumor stage, background liver tissue, and Child-Pugh classification. We used Mann-Whitney U test for these items (page 13, line 13-15).

This reviewer's comment is truncated at (3d).

Reviewer No. 02528139

(1) According to the suggestion of this reviewer, we described the results following the several main topics in the revised manuscript.

(2) This reviewer pointed out that the sample size was very small to conclude the correlation between L-FABP downregulation pattern and tumor size. In the revised manuscript, we described this problem

as the limitation of the present study (page 22, line 20-24) and described only the facts accurately throughout the paper.

(3) In the present study, among those with large HCC, L-FABP-negative patients were significantly younger than L-FABP-positive patients. We speculate that this distribution is probably because several L-FABP-negative cases of large HCC represent malignant transformation of H-HCA (page 19, line 16-20). The reason behind the age-related differences, where L-FABP-negative patients were significantly older than L-FABP-positive patients for small HCCs, is difficult to be answered, and further studies are needed to address this issue (page 19, line 21-24).

(4) This reviewer required us to do some *in vitro* experiments. Therefore, we performed *in vitro* experiments using cell lines (page 11, line 10 - page 12, line 15; page 16, line 7-15; page 20, line 22 - page 21, line 4).

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

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