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May 24, 2013

Dear Editor; Jin-Lei Wang,
Director, Editorial Office, World Journal of Hepatology
Baishideng Publishing Group Co., Limited

Please find enclosed the edited manuscript in Word format (file name: 3104 Igarashi revised.doc).

Title: Participation of peribiliary glands in biliary tract pathophysiologies

Author: Saya Igarashi, Yasunori Sato, Xiang Shan Ren, Kenichi Harada, Motoko Sasaki, and Yasuni Nakanuma

Name of Journal: *World Journal of Hepatology*

ESPS Manuscript NO: 3104

Thank you very much for your kind e-mail letter dated on May 6, 2013, regarding our above manuscript entitled "Participation of peribiliary glands in biliary tract pathophysiologies by expressing fetal pancreatic and endodermal stem/progenitor cell markers".

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

1 Format has been updated. The language of our manuscript has reached Grade A. The revised manuscript has included detailed contents, comparisons and evaluations and in-depth discussion.

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

(1) According to the suggestion of Reviewer No. 02444977, we checked the paper again and corrected typographical errors.

(2) According to the suggestion of Reviewer No. 00227487, we corrected as follows.

i) Abstract, line 11: Abstract, line 11: “hepatolithiati” must be “hepatolithiatic”.

We corrected “hepatolithiati” to “hepatolithiatic”.

ii) M & M section, page 5, line 9: More than 20 consecutive sections were made. I guess one section was stained by hematoxylin and eosin and the remaining sections for Immunohistochemistry. This should clarify.

We corrected: More than 20 consecutive 4- μ m-thick sections were cut from each paraffin block, and one section was stained with hematoxylin and eosin (H&E) for histologic observations. The remaining sections were used for immunohistochemistry.

iii) M & M section, page 5, line 8 from the bottom: Give word(s) after “at 4”.

We added °C after 4.

iv) M & M section, page 5, line 4 from the bottom: In addition to “negative controls”, we need the short description regarding “positive controls”, as listed in Table 1.

We added the following sentence in the revised manuscript: As positive controls, islet cells in normal pancreatic tissue (one case) for PDX1, fibroblasts in cirrhotic liver tissue (one case) for HES1, neural cells in a normal human brain (one case) for CXCR4, fibroblasts in cirrhotic liver tissue (one case) for CD44s, normal human embryonic tissue (one case) for SOX9, and bile ducts in a normal liver (one case) for EpCAM, were used as shown in Table 1.

v) M & M section, page 5, the last paragraph: The methods of immunohistochemical scoring is unclear. This should be described in detail.

We described this sentence in details as follows: The immunoreactivity of epithelial cells in the lining epithelia and peribiliary glands were semi-quantitatively graded based on the percentage of positive cells, as follows: 0, no expression of each marker in the lining epithelia or peribiliary glands; 1+ (focal), the expression of each marker in less than one third of the lining epithelia and peribiliary glands, respectively; 3+ (extensive), the expression of each marker in more than two thirds of the lining epithelia and peribiliary

glands, respectively; and 2+ (moderate), the expression of each marker in the lining epithelia and peribiliary glands between 1+ and 3+, respectively. The staining intensity was rather stronger in the cases of extensive expression, and was rather weaker in the cases of focal expression.

vi) Results section: The authors mention “hepatolithiatic liver characterized by proliferation of the peribiliary glands” in the Abstract and “All stone-containing bile ducts showed marked proliferation of peribiliary glands” in the 1st paragraph in the M & M section. Relationship between the proliferative activity of the peribiliary glands and the expression of markers examined is interesting. If the authors possess data, provide them and discuss.

As the reviewer pointed out, the proliferative activities of peribiliary glands and expression of S/P cell markers are interesting. In this study, we did not examine the proliferative activities of peribiliary glands. So, we could not add such data in the result section. However, in our previous study PDX1 expression in biliary cells was related to their proliferative activities. Thus, the following sentence was added in the discussion section of the revised manuscript.

Our previous study (4) showed that PDX1 expressed in preneoplastic and neoplastic biliary epithelia was related to their proliferative activities; therefore, so it also seems likely that PDX1 expression in the peribiliary glands of the hepatolithiasis may be related to their proliferation and neoplastic process [6,7,12].

3 References and typesetting were corrected.

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

Sincerely yours

Yasuni Nakanuma, MD