**Format for ANSWERING REVIEWERS**

December 13, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 7074-Edited_Dec13.doc).

**Title:** Role of HBV DNA integration in human hepatocarcinogenesis

**Author:** Hoang Hai, Akihiro Tamori, Norifumi Kawada

**Name of Journal:** *World Journal of Gastroenterology*

**ESPS Manuscript NO:** 7074

We appreciate your constructive comments regarding our manuscript.

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

(1) Reviewer No. 02449596
Minor comments:
*"#1. Page 7 line 5 from bottom: " ...including: hTERT, the PDGF receptor, MLL, calcium signaling-related genes, and ribosomal protein genes...." signaling should be corrected by signalling."*

World J Gastroenterol accepts American English spelling. So we use "signaling" as a previous published article "**Park KS** et al. Switching-on of serotonergic calcium signaling in activated hepatic stellate cells. *World J Gastroenterol.* 2011;17:164-73. doi: 10.3748/wjg.v17.i2.164."

*"#2. In Page 9 Line 10 to 13 from bottom, authors report that the locus of HBV integration was localized in... : " ... within or upstream of the TERT gene in 4 of the 11 HBV-related HCC. These findings are consistent with previous reports of recurrent HBV integration at the TERT gene locus[55]..." However, in page 10 line 4 from top, and in the same sentence, authors claim: "Currently, the most common integration site has not been identified." Both sentences seem contradictory. It should be clarified."*

We deleted the sentence "Currently, the most common integration site has not been identified."

*"#3. Authors discuss about accumulation of genetic and epigenetic changes and instability caused by HBV integration. However, authors do not cite the work by Fernández-Fernandez A., et al The DNA Methylomes of Double-Stranded DNA Viruses Associated with Human Cancer. GENOME RESEARCH 2009; 19(3):438-451. In this paper, authors performed a study of methylation of viruses*

*associated with human cancer. They studied de metilation of HBV genome in different stages of cacinogenesis and found that the HBV genome was almost completely unmethylated in the early stages of carcinogenesis, such as hepatitis and cirrhosis, while it became more methylated in the established liver tumors, both in patients and in cultured cancer cell lines. Most importantly, the presence of DNA methylation at the HBVgp4 and HBVgp2 genes, which, respectively, code for the C and S viral proteins, was associated with their lack of expression, while HBx gene was in an unmethylated state in the malignant lesions. It is recommended to add the contribution of this paper to the manuscript."*

We added a highlighted paragraph in page 12:
Beside genomic alterations, epigenetic factors like methylation-associated gene silencing have been shown to be involved in the deregulation of cellular function in HCC. HBV genome was almost completely unmethylated in the early stages of carcinogenesis, from chronic active hepatitis to hepatic cirrhosis, while it became more methylated in the established liver tumors, both in patients and in cultured cancer cell lines[74].

(2) Reviewer No. 00504885
Major concerns:
*"#1. The main issue with the manuscript is that it is poorly written, the authors stated that they had some native English persons to edit the MS. However, it contains many typos, grammar problems and many redundant sentences. For examples, the sentences from the abstract are repeated later in the text without changing a word. Even in the text, there are many redundant sentences. The authors need to work on this carefully."*

English language editing certificate is provided when we resubmit the edited manuscript. We revised carefully by deleting several unnecessary sentences (page 8 lines 1-3, page 10 lines 12-17) as well as adding highlighted sentences.

*"#2. I suggest the authors to make a table to list the genes affected by HBV DNA integration. By doing so, it will make reader to easily understand the possible mechanism that HBV uses to cause HCC."*

According to your suggestion, we created **"Table 1. The main integration sites in human genome and in HBV DNA"** in the last page.

**Table 1**
**The main integration sites in human genome and in HBV DNA**

| Integration sites in | |
|---|---|
| **Host genome** | **HBV DNA** |
| hTERT | 3' end of HBx |
| MLL | Pre-S2/S |
| RAR-b | |
| CCNE1 | |
| Cyclin A2 | |
| FN1 | |
| ROCK1 | |

SENP5
ANGPT1
PDGF receptor
Calcium signaling-related genes
Ribosomal protein genes
Epidermal growth factor receptor
Mevalonate kinase
Carboxypeptidase
Platelet growth factor receptor

(3) Reviewer No. 02538457

*"HBV infection is one of the major causes of human hepatocarcinogenesis, however, the mechanisms are still to be clarified. Recently, there are many progresses published with vary technologies in clinic, especially with NGS technology and GWAS. The authors review in detail the role of HBV DNA integration in human hepatocarcinogenesis mainly from the two potential consequences of integration: 'cis' effect and 'trans' effect. This review is very useful for basic and clinical researchers to understand the mechanisms of hepatocarcinogenesis and to prevent patients from developing HCC in the future, though the role of integrated HBV DNA in hepatocarcinogenesis remains controversial. The text is also present very well."*

No revision is necessary.

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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