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World Journal of Gastroenterology

Ms. No. 20133

Significance of hepatitis virus infection in the oncogenic initiation of hepatocellular carcinoma

Dear Prof. Ma,

We submit the revised version of our review article Ms WJG-20133 for publication in the **World Journal of Gastroenterology**. We appreciate the constructive comments of the reviewers and in response to the letter of the editorial office, we revised the previous version and prepared a point-by-point rebuttal (*As*) which addresses the comments (*Qs*) by the reviewers.

Reviewer #1

The manuscript is well written for hepatocarcinogenesis by chronic infection of HBV and HCV, and should be great impact to HCC researchers.

Thank you for your affirmative comment.

The concerns are following:

Q1: On page 3, line 17, aflatoxin B should be aflatoxin B1. On page 15, line 26, down regulated should be down-regulated.

A1: Thank you for noticing, both terms have been corrected in the revised version.

Q2: If there is the hypothesis/your idea of gender difference in HCC development, please introduce it.

A2: It has been widely known that the cumulative risk for HCC is higher in gender male compared to female (Ferlay et al., Int J Cancer 2015; Bosetti et al., Best Pract Res Clin Gastroenterol 2014; El-Serag HB, Gastroenterol 2012). It has been thought to occur because of life style, male is more prone to viral infection and alcoholic cirrhosis. However, it is important to note that hormones (testosterone, progesterone, and estrogens) also take part in viral infection and subsequent liver damages. This information had been introduced in Page 4 of revised Ms.

Reviewer #2:

This review focused on HCC and hepatitis virus (HBV and HCV). This review is well-organized and informative.

Q1: Are there any clinical significances of the integration of HBV DNA in bone marrow hematopoietic stem cells? Are there any diseases of the HBV infection of bone marrow hematopoietic stem cells?

A1: It is known that HBV DNA can integrate in PBMC (Michalak et al., J Clin Invest 1994) and in bone marrow hematopoietic stem cells (Shi et al., J Viral Hepat 2014) but little is known about the effect of this integration. In vitro exposure of human bone marrow to HBV results in a dose-dependent inhibition of erythroid, myeloid, and lymphoid hematopoietic stem cells. In general, HBV infection frequently causes the suppression of hematopoiesis and in some cases, this may lead to severe bone marrow failure, but the mechanism is still unknown (Zeldis et al., J Clin Invest 1986; Steinberg et al., J Virol 1990). Recently, Shy et al. demonstrated the integration of HBV into hematopoietic stem cells could lead to the generation of defective T cells (Shi et al., J Viral Hepat 2014). This information had been described in Page 12 of the Ms.

Q2: It is understandable that integration of HBV genome causes HCC. Still many HCC patients are accompanied with liver cirrhosis. How does chronic inflammation affect the carcinogenesis of HCC in HBV patients? Are the same mechanisms as HCV involved in the carcinogenesis of HCC?

A2: As described in our Ms (page 10), HBV infection causes immunological response and inflammation that may lead to oxidative stress and successive cellular damage. The accumulation of HBsAg in the endoplamic reticulum of hepatocytes causes histological appearance of ground glass hepatocytes (Hadziyannis et al., Arch pathol 1973). In transgenic mice of large surface antigens, the overproduction of large envelope protein can cause inflammation and regenerative hyperplasia to induce HCC development (Chisari et al., cell 1989). For the comparison between HCV and HBV, as reviewed by Higgs et al., J Gen Virol 2014 (Ref: 106), HBV and HCV viral proteins induce oxidative stress and this chronic infection by either virus triggers a non-specific immune mediated inflammation (hepatitis).

Q3: Regarding cancer stem cell markers. Are the stem cell markers different between HBV and HCV? If so, would the difference indicate the difference of carcinogenesis between HBV and HCV?

A3: The population of cancer stem cells is heterogeneous. Variability can be observed not only among patients but also within a single tumor. Moreover, these cells originate from either normal stem cells or dedifferentiation of adult hepatocytes (Anfuso et al. Clin Res Hepatol Gastroenterol 2015). All these factors render complication in the study of cancer stem cells in relation to HCV or HBV. In several studies cited in our Ms (Page 17-18), it was reported that the expression of EpCAM in HBV infected patients and animal model correlates with high expression of HBx (Wang et al., Hepatol 2012). The expression of an HCV subgenomic replicon in cultured cells resulted in the acquisition of CSC traits including CD133, AFP, and cytokeratin-19 (Ali et al., J Virol 2011). In human HCC tissues, histological analysis showed a positive correlation between HBV infection and CD90 and an inverse correlation with CD133 (Lu et al., Acta Histochem 2011; Yeh et al., BMC Cancer 2009). These observations seem to indicate a possible preferential expression of CSC markers between HCV and HBV, but its carcinogenetic mechanism must be further investigated.

Q4: Transgenic animal models are different species from human. In the future, hepatocytes differentiated from human induced pluripotent stem cells may be useful for experiments of carcinogenesis of HBV and HCV.

A4: We agree with the reviewer that animal model have many limitations due to different genetic backgrounds and species barrier. However, it is still an important tool because of its short life span, reproducibility, and flexibility in genetic manipulation and treatment. The use of human induced pluripotent stem cells (IPS) in animal model can give important aspects in the study of carcinogenesis, especially in the field of regenerative medicine. However, it is important to notice that the limitation in viral culture method could not be fully overcame with IPS cells technology. Improvement in the method of infection and maintenance of culture system will be the main way to increase our knowledge in carcinogenesis of HBV and HCV.

We hope to have comprehensively and successfully dealt with your critiques which helped improving the final quality of the Ms.

Sincerely yours,

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