BAISHIDENG PUBLISHING GROUP INC
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242 Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com http://www.wjgnet.com

Reviewer 1:

Dr. Hong and colleagues have presented an interesting study in which they aimed to investigate the expression and clinical significance in terms of HCC-development of B7-H4 in dependency of HBx in cell culture and HBV-positive patient samples. The colleagues found that B7-H4 up-regulation in HBx-positive samples were correlated with hepato-carcinogenesis in cell culture and patients. The authors conclude that B7-H4 may be involved in facilitating HBV-related HCC-development. Overall, the manuscript is well written and concise in its content and the methodical design is well performed. However, there are some comments which should be addressed.

1) There are some minor spelling and typing errors which should be polished, e.g., in the Abstract, Conclusion first sentence it should read "was" correlated instead of "were".

Response: We have corrected those errors. And the revision was proofread by a native English professional with science background at Elixigen Corporation.

2) Abstract section, Results; the correct abbreviation for the hepatoma cell line is HepG2.2.15

Response: We have corrected the name of hepatoma cell line, using HepG2.2.15 instead of HepG2215.

3) Material and Methods, Cell lines; the HepG2.2.15 cells are stably HBV-expressing hepatoma cells derived from HepG2 cells and were firstly described by Sells et a 1987 and 1988 (PNAS 84:1005-1009 and J Virol 62:2836-2844).

Response: We have increased this description of HepG2.2.15 in Material and Methods, Cell lines.

4) The Introduction section is informative but much too long and reads like a review article. Please shorten it and focus on the essentials.

Response: We have shortened the introduction and focus on the essentials.

5) Table 1 and 2. There are some cryptic signs for Tumor grade and TNM stage. This might be due

to the word file; however, I can't read it.

Response: We have added the description in Page 10. Tumor grade Ⅰ is well-differentiated and in a low-grade; tumor grade Ⅱ is medium-differentiated and in a moderate malignant; tumor grade Ⅲ is poor-differentiated and in a highly malignant. TNM stage refers to the Tumor Node Metastasis stage.

6) Fig. 1a; In my opinion and as a suggestion the density data should be presented as a graph rather than a table.

Response: We have presented the density data as a graph rather instead of a table.

7) Fig. 1 legend; the last sentence of the figure legend is incomplete **, vs HepG2 cells should read HepG2.2.15 vs HepG2

Response: We have completed the figure legend with HepG2.2.15 vs HepG2.

8) Fig.2; it is hard to identify the intracellular localisation of B7-H4 due to the minor quality of the figures. Please enhance the quality of the figures by, e.g., enhancing the magnification.

Response: We have presented figures with higher magnification showing clearly B7-H4 and HBx expression and staining.

9) Fig. 3. The staining of HBx and also B7-H4 is not really convincing. Please present figures with higher magnification showing clearly B7-H4 and HBx expression and staining.

Response: We have presented figures with higher magnification showing clearly B7-H4 and HBx expression and staining.

10) There are two recent reports on B7-H1 expression showing that HBeAg supresses the cellular immunity by Han et al (Cell Immunol 2013 283:25-30) and especially of Xu et al (Clin Immunol 2010

136:30-41) showing that B7-H4 expression attenuates con-A induced hepatic injury. These reports could be discussed in the context of the findings of the authors.

Response: We have discussed those reports on Page 14.

Substantial research has been performed regarding the role of B7-H4 in tumor immunity. However, the pathophysiologic function of B7-H4 has yet to be fully elucidated. In the present study, we suggested a detrimental role for B7-H4 in HBV-HCC patients. Costimulatory molecules including VCAM-1 [37], CD40 [38], CD28, and PD-1 [39], as well as several members of the B7 superfamily, including B7-1, B7-2 [40], B7-H1 [12,33], and B7-H3 [41] have been reportedly expressed in HCC tissues. These costimulatory molecules could provide positive (VCAM-1, CD28, B7-1, B7-2, and CD40) or negative (PD-1 and B7-H1) signals to local T cells that determine an optimal T cell response and regulate the pathogenesis of HCC. It is reported that HBeAg suppresses the specific cellular immunity which was to clear the virus through up-regulating B7-H1 expression, and eventually lead to immune tolerance to HBV infection [42]. B7-H4 was originally described to be a membrane costimulatory ligand of the B7 superfamily, which is involved in the down-regulation of T cell activation under certain circumstances. Ectopic B7-H4-Ig may protect animals from liver injury induced by ConA, which could be associated with reduced serum levels for IL-2, IFN-gamma and IL-4 as well as enhanced IL-10 production [43]. Thus, B7-H4 may lead to immune tolerance to HBV infection, and play an important role in immune suppression in chronic HBV-HCC patients.

Reviewer 2:

The authors reported that the elevated expression of B7-H2, known as a co-stimulatory molecule, was detected not only HepG2.215 cell lines (transfected with HBV DNA) but also HBV-related hepatocellular carcinoma clinical tissues in this manuscript. Although they showed a positive correlation between HBx and B7-H4 expression in hepatocellular carcinoma of HBV positive patients,

they provide a mere phenomenon. They did not show a role of B7-H4 in this manuscript. Even though they showed a role of B7-H4 in pancreatic carcinoma cells through their previous study, we would like to urge roles of B7-H4 in hepatic cancel cell. For instance, During B7-H4 suppression with its siRNA in HepG2.215 cells, roles of B7-H4 including cell to cell interaction, cell growth, and apoptosis are examined.

Response: We suspected that intracellular B7-H4 might possess an anti-apoptotic effect in HCC cells. We will next determine if this is the function of B7-H4 in this context with future research. We will detect the roles of B7-H4 on cell to cell interaction, cell growth, and apoptosis in HBV-HCC cells by B7-H4 specific shRNA. And we will identify if there is an epitope change of B7-H4 which might be involved in immune-mediated tumor escape, and thereby contributing to tumor development.