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Comments addressed by author for 1st reviewer(00227487)

ESPS Peer-review Report
Name of Journal: World Journal of Gastroenterology
ESPS Manuscript NO: 9683
Title: Hepatitis B virus, HBx mutants and their role in hepatocellular carcinoma
Reviewer code: 00227487
Science editor: Qi, Yuan
Date sent for review: 2014-02-24 13:20
Date reviewed: 2014-02-25 10:00
COMMENTS TO AUTHORS
This review article by Ali et al. summarizes the ro9le of HBx in HBV-associated hepatocarcinogenesis. The manuscript is well written and well-organized. I would like to suggest minor points listed below. 1) Some important papers can be cited: World J Gastroenterol 13(1): 74-81, 2007; and Acta Med Indones 38(3):154-9, 2006. 2) Graphical summary can be added for better understanding the description.

Answer-

*World J Gastroentero l 13(1): 74-81, 2007 is cited in Reference no 2-Lupberger et al 2007.

*Acta Med Indones 38(3):154-9, 2006 is cited in reference no 6.

*Besides them we have also added more recent articles including its relationship with micro RNA .

*Figure and table have been provided to support the argument in favor of HBx role in pathogenesis

*We have cited some recent updates in references no 83,84,92,95 and 99.

*We have complied with the recommendations of the other reviewer and have deleted some references and also added a separate paragraph showing relationship with micro

RNA (As shown below)

4.Effect of HBx on micro RNA expression associated with HCC-

Micro RNAs are noncoding RNAs which are involved in the regulation of gene expression. Additionally, they play crucial roles in numerous pathobiological processes including tumor formation. Therefore, their possible role in the causation of hepatocellular carcinoma is not unexpected. However, the question as to how HBx actually regulates miRNA expression during HCC has no easy answer. However, Wu *et al* in 2014 have shown that HBx down regulates microRNA-15b via fucosyltransferase 2 induced Globo H(a cancer-associated carbohydrate antigen) expression ultimately influencing HCC proliferation [75]. In a recently published article Zhang *et al* 2013 have found that miR-205 is downregulated in 33 samples of HCC tissues in contrast to adjacent healthy areas of the liver. They suggested that miR-205 could be a potential tumor-suppressive gene in case of HCC. According to them, HBx inhibit tumor suppressor miR-205 and increases hepatocarcinogenesis by hypermethylation of miR-205 promoter [76]. Wei *et al* have demonstrated HBx induces epigenetic repression of miR-132 by methylation of DNA and suggested that it could be a promising biochemical marker for HCC. They have also shown that miR-101 is down regulated by HBx which was in turn induced by HBX targeting the methyltransferase 3A(DNMT3A) gene [77]. In a mouse model of liver cancer, it was observed that miR-148a is repressed by HBx and this leads to cancer growth and eventual metastasis. Expression of miR-148a in hepatoma cells reduces hematopoietic pre-B cell leukemia transcription factor-interacting protein (HPIP). This caused the suppression of AKT and ERK induced mTOR inhibition involving AKT/ERK/FOXO4/ATF5 pathways [78]. HBx also downregulates PDCD4 by influencing another micro RNA called miR-21 [79]. Recently Qui *et al* have

highlighted that HBx downregulates PDCD4 by upregulation of miR-21. HBx suppresses EGFR by miR-7 which confirms the role of HBX-miR-7-EGFR as a critical signaling pathway in controlling cell growth in HCC[80]. HBx perturbs the in vitro expression of miRNA in cancerous hepatocytes of the host liver, especially by downregulating the mi-16 family. Its suppression was c-Myc mediated and is a necessary requirement for the HBx-induced transformation of HepG2 cells in vitro [81]. Another micro RNA, miR-152 is frequently down-regulated during HBx expression and it is also known to regulate DNMT1 in HBV-related HCC. Additionally, tumor-suppressive role of miR-152 in the epigenetic irregularity of HBV-related HCC has been observed [82]. Wild-type HBx and the high proliferation-inducing mutant HBx can influence the expression profile of miR-338-3p and miR-551b by its downregulation in L02 cells. Here, the cell growth inhibition occurs by direct modulation of cyclinD1, cyclinG1, and E2F[83, 84]. In another study showing linkage of mi RNA with HBx, it was found that HBx could inhibit apoptosis of HepG2 cells through down-regulation of miR-192 which induces apoptosis of HepG2 cells [85].

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Comments addressed by authors for 2nd reviewer (02444752)

ESPS Peer-review Report
Name of Journal: World Journal of Gastroenterology
ESPS Manuscript NO: 9683
Title: Hepatitis B virus, HBx mutants and their role in hepatocellular carcinoma
Reviewer code: 02444752
Science editor: Qi, Yuan
Date sent for review: 2014-02-24 13:20
Date reviewed: 2014-03-04 09:16
COMMENTS TO AUTHORS
The content of this review article is overlap with previously published review (Kew MC. Hepatitis B virus x protein in the pathogenesis of hepatitis B virus-induced hepatocellular carcinoma. Gastroenterol Hepatol. 2011 Jan;26 Suppl 1:144-52). It should be focused on the relationship between HBx mutants and their role in hepatocellular carcinoma. It is best to condense something, especially those Kew MC has reviewed

Answer - Thank you for bringing this matter to the attention. Yes we agree some sections on HBx were previously covered by Kew et al., 2011 but this review is now 3 years old and new references are added to make relevance to the updated information on the HBx role in induction of pathogenesis. The grammar and spelling have corrected. Some more recent article related to HBX mutants and HCC have been added. We have also included a separate paragraph showing relationship with micro RNA to make it more relevant.

*We have cited some recent updates in references no 83,84,92,95.

*We have complied with the recommendations of the other reviewer and have deleted some references and also added a separate paragraph showing relationship with micro RNA (as shown below) .

4.Effect of HBx on micro RNA expression associated with HCC-

Micro RNAs are noncoding RNAs which are involved in the regulation of gene expression. Additionally, they play crucial roles in numerous pathobiological processes including tumor formation. Therefore, their possible role in the causation of hepatocellular carcinoma is not unexpected. However, the question as to how HBx actually regulates miRNA expression during HCC has no easy answer. However, Wu *et al* in 2014 have shown that HBx down regulates microRNA-15b via fucosyltransferase 2 induced Globo H(a cancer-associated carbohydrate antigen) expression ultimately influencing HCC proliferation [75]. In a recently published article Zhang *et al* 2013 have found that miR-205 is downregulated in 33 samples of HCC tissues in contrast to adjacent healthy areas of the liver. They suggested that miR-205 could be a potential tumor-suppressive gene in case of HCC. According to them, HBx inhibit tumor suppressor miR-205 and increases hepatocarcinogenesis by hypermethylation of miR-205 promoter [76]. Wei *et al* have demonstrated HBx induces epigenetic repression of miR-132 by methylation of DNA and suggested that it could be a promising biochemical marker for HCC. They have also shown that miR-101 is down regulated by HBx which was in turn induced by HBX targeting the methyltransferase 3A(DNMT3A) gene [77]. In a mouse model of liver cancer, it was observed that miR-148a is repressed by HBx and this leads to cancer growth and eventual metastasis. Expression of miR-148a in hepatoma cells reduces hematopoietic pre-B cell leukemia transcription factor-interacting protein (HPIP). This caused the suppression of AKT and ERK induced mTOR inhibition involving AKT/ERK/FOXO4/ATF5 pathways [78]. HBx also downregulates PDCD4 by influencing another micro RNA called miR-21 [79]. Recently Qui *et al* have highlighted that HBx downregulates PDCD4 by upregulation of miR-21. HBx suppresses EGFR by miR-7 which confirms the role of HBX-miR-7-EGFR as a critical signaling pathway in controlling cell growth in HCC[80]. HBx perturbs the in vitro

expression of miRNA in cancerous hepatocytes of the host liver, especially by downregulating the mi-16 family. Its suppression was c-Myc mediated and is a necessary requirement for the HBx-induced transformation of HepG2 cells in vitro [81]. Another micro RNA, miR-152 is frequently down-regulated during HBx expression and it is also known to regulate DNMT1 in HBV-related HCC. Additionally, tumor-suppressive role of miR-152 in the epigenetic irregularity of HBV-related HCC has been observed [82]. Wild-type HBx and the high proliferation-inducing mutant HBx can influence the expression profile of miR-338-3p and miR-551b by its downregulation in L02 cells. Here, the cell growth inhibition occurs by direct modulation of cyclinD1, cyclinG1, and E2F[83, 84]. In another study showing linkage of mi RNA with HBx, it was found that HBx could inhibit apoptosis of HepG2 cells through down-regulation of miR-192 which induces apoptosis of HepG2 cells [85].

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Comments addressed by authors for 3rd reviewer(02715825)

ESPS Peer-review Report
Name of Journal: World Journal of Gastroenterology
ESPS Manuscript NO: 9683
Title: Hepatitis B virus, HBx mutants and their role in hepatocellular carcinoma
Reviewer code: 02715825
Science editor: Qi, Yuan
Date sent for review: 2014-02-24 13:20
Date reviewed: 2014-03-07 01:50

COMMENTS TO AUTHORS
This manuscript discusses the role of HBV-encoded X protein in the pathogenesis of hepatocellular carcinoma. In my opinion, although there is an excess of references for being a review, I recommend to include more recent original articles, specially describing its relationship with microRNAs, as miR-205.

Answer –

We are grateful for the reviewer on this comment on the lack of miRNA data in this review. We have complied with the recommendations of the reviewer and have deleted some references and also added a separate paragraph showing relationship with micro RNA . On the issue of miRNA, some recent articles were added and have mentioned micro RNA -205 in reference no 79 (Zhang et al 2013) as recommended by the reviewer.

*Figure and table have been provided to support the argument in favor of HBx role in pathogenesis

*We have cited some recent updates in references no 83,84,92,95, 99.

* As mentioned above we have added a separate paragraph showing relationship with microRNA(Shown below)

. **4.Effect of HBx on micro RNA expression associated with HCC-**

Micro RNAs are noncoding RNAs which are involved in the regulation of gene expression. Additionally, they play crucial roles in numerous pathobiological processes including tumor formation. Therefore, their possible role in the causation of hepatocellular carcinoma is not unexpected. However, the question as to how HBx actually regulates miRNA expression during HCC has no easy answer. However, Wu *et al* in 2014 have shown that HBx down regulates microRNA-15b via fucosyltransferase 2 induced Globo H(a cancer-associated carbohydrate antigen) expression ultimately influencing HCC proliferation [75]. In a recently published article Zhang *et al* 2013 have found that miR-205 is downregulated in 33 samples of HCC tissues in contrast to adjacent healthy areas of the liver. They suggested that miR-205 could be a potential tumor-suppressive gene in case of HCC. According to them, HBx inhibit tumor suppressor miR-205 and increases hepatocarcinogenesis by hypermethylation of miR-205 promoter [76]. Wei *et al* have demonstrated HBx induces epigenetic repression of miR-132 by methylation of DNA and suggested that it could be a promising biochemical marker for HCC. They have also shown that miR-101 is down regulated by HBx which was in turn induced by HBX targeting the methyltransferase 3A(DNMT3A) gene [77]. In a mouse model of liver cancer, it was observed that miR-148a is repressed by HBx and this leads to cancer growth and eventual metastasis. Expression of miR-148a in hepatoma cells reduces hematopoietic pre-B cell leukemia transcription factor-interacting protein (HPIP). This caused the suppression of AKT and ERK induced mTOR inhibition involving AKT/ERK/FOXO4/ATF5 pathways [78]. HBx also downregulates PDCD4 by influencing another micro RNA called miR-21 [79]. Recently Qui *et al* have highlighted that HBx downregulates PDCD4 by upregulation of miR-21. HBx suppresses EGFR by miR-7 which confirms the role of HBX-miR-7-EGFR as a critical signaling pathway in controlling cell growth in HCC[80]. HBx perturbs the in vitro expression of miRNA in cancerous hepatocytes of the host liver, especially by

downregulating the mi-16 family. Its suppression was c-Myc mediated and is a necessary requirement for the HBx-induced transformation of HepG2 cells in vitro [81]. Another micro RNA, miR-152 is frequently down-regulated during HBx expression and it is also known to regulate DNMT1 in HBV-related HCC. Additionally, tumor-suppressive role of miR-152 in the epigenetic irregularity of HBV-related HCC has been observed [82]. Wild-type HBx and the high proliferation-inducing mutant HBx can influence the expression profile of miR-338-3p and miR-551b by its downregulation in L02 cells. Here, the cell growth inhibition occurs by direct modulation of cyclinD1, cyclinG1, and E2F[83, 84]. In another study showing linkage of mi RNA with HBx, it was found that HBx could inhibit apoptosis of HepG2 cells through down-regulation of miR-192 which induces apoptosis of HepG2 cells [85].