Reviewer's report

Title: Hepatitis B virus X protein suppresses caveolin-1 expression in hepatocellular carcinoma through regulating DNA methylation

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“Hepatitis B virus X protein suppresses caveolin-1 expression in hepatocellular carcinoma through regulating DNA methylation” by Yan et al.

The authors examined the DNA-methylation status of the caveolin-1 promoter by n-MSP in 33 HBV-infected HCC. Methylation of the caveolin-1 promoter was detected in 84.8% (28/33) of HBV-infected HCC samples. Expression of caveolin-1 was significantly suppressed. Transfection HBx significantly suppressed caveolin-1 promoter activity and induce methylation in HCC cell line. They concluded that HBx protein induces methylation of the caveolin-1 promoter region and suppresses its expression.

1) The authors examined the methylation status of HCC tissues using nested-MSP. However, quantitative analysis of methylation level should be better for this type of evaluation. More specifically, is there a correlation between methylation level and expression of corresponding mRNA or protein? In addition to the in vitro data of HCC cell line, analyses using MethyLight, COBRA, RT-real-time PCR and IHC are required to make the result more confident.

2) Fig 2 D: For representation of bisulfite sequence analysis, which part of the targeted gene did the authors analyzed? The location of bisulfite sequencing should be shown in Fig 2 A. In addition, putative core promoter region of the caveolin-1 showed be indicated.

3) The authors showed that methylation event of the caveolin-1 gene was unique in HBV-infected HCC and the role attributed to the action of HBx protein. Is there an association between HBx protein expression and methylation level and downregulation of the caveolin-1 gene? How is the methylation status in HCV-infected or virus negative HCC?

4) Previously, the authors reported the association between caveolin-1 downregulation and HCC progression using human HCC tissues. Is the methylation level also increasing according to the tumor size, vascular invasion etc.?

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable
Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

Nothing to declare.