COMMENTS FOR THE AUTHOR:  
  
**Reviewer #1**: The study by Dr. Chen et al. examined the integration sites of host genomic DNA and HBV-viral DNAs comprehensively from tumor tissues, their background tissues, and plasma.  
They successfully found the integrated DNA fragments from plasma in HCC patients.  
In addition, they found that some viral integration pattern contained the entire ORF of HBs, which theoretically retained the ability of viral protein expression. Moreover, RNA sequencing confirmed the possibility of their protein expression.  
  
The study revealed the status of HBV integration in HCC tissues and the possibility of their detection from plasma. Also, they found the possibilities of the viral protein expression (some of which may be fused with the host transcripts) from the integration sites.  
  
These results may provide with the comprehensive information of the HBV DNA integration in the host genome, although several reports have already reported similar reports.  
  
I have a few concerns on this manuscript as below.  
  
1) The hypotheses the authors provided were that they may be able to detect the viral integrant DNA from plasma, which may be useful to obtain the HBV integration information in a non-invasive way. These are novel approaches in these kinds of studies.  
Indeed, in the manuscript, the authors could detect such integrants mainly derived from HCC-tissues. Please explain in more detail about the patients' information and describe if these information can be sued for the early detection of HBV-related HCC.  
  
2) The authors concluded in the abstract and discussion that the integrate sequences which express viral proteins may be targeted immunotherapies. This concept is interesting, but the evidence is lacking. The detectability of the fusion proteins or neoantigens possibly produced from the integrants as the authors stated should be shown.  
  
3) The concentration of HBV integrants by probe-hybridization-method is not entirely new. Please explain in more detail the new aspects of the methodology.  
  
  
  
**Reviewer #2**: HEPI-D-19-00487  
 This is a manuscript entitled "Noninvasive chimeric DNA profiling identifies tumor-originated HBV integrants contributing to viral antigen expression in liver cancer "  By Wei Chen1, et al.  
  
In this study, the authors developed a probe-based capture strategy to enrich integrated HBV DNA for deep sequencing analysis of viral and host genome integration sites in paired patient samples derived from tumor, liver tissue adjacent to tumor and plasma. By using this, the authors reveal that plasma samples from HCC patients, viral-host chimeric DNA fragments were successfully detected and those were from tumor rather than in adjacent liver tissues. Additionally, the authors showed that among four resolved viral patterns, the majority of Pattern I events retained the complete  
opening reading frame for HBV surface proteins.  
  
 This study is well constructed and the results include virological and clinical important findings.  
This reviewer has some concerns before publication in hepatology international  
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1.      In patients with multiple HCC, the integration site might be different in each HCC. The prevalence of detected integration site in cfDNA were different according to tumor size or vascular invasion.  
  
2   Please show whether the host-viral chimeric proteins could be detected in HCC, not only RNA.  
  
3   Were the prevalence of HBV integration and serum HBs antigen titer correlated, because most integrants has ORF of HBs protein.  
  
4. The number of integration events were different among the investigated patients. Please discuss the reason why the differences are observed, viral titer, HBV genotype, HBe antigen positive are related?  
  
5. Please show the data of HBV genotype.  
  
  
  
  
  
**Reviewer #3**: With recent advances in molecular and genomic investigations, several biomarkers associated with surveillance, diagnosis, prognosis prediction and monitoring treatment response of HCC has been explored. In this article, Chen et al. investigated the association of host genome integration of HBV sequence and development of HCC. They found that plasma cell-free chimeric DNA was associated with HCC development. Although viral-host chimeric DNA may serve as a new biomarker of HCC, several issues are worthy of attention.  
  
1.      Previous studies have been reported that integration of HBV DNA into liver cell DNA were not uncommon in HCC patients with occult HBV infection. In this study, HBsAg negative patients were used as negative controls. The authors should provided the status of anti-HBc and anti-HBs of these HBsAg negative patients.  
2.      The sizes of the HBV integrations should be analyzed.  
3.      The association between the plasma cell-free chimeric DNA and the serum levels of HBV DNA should be anlyzed. The authors should describe whether the HBsAg-positive HCC patients received antiviral therapy or not.  
4.      Please clarify whether the presence of cirrhosis influence the detection of plasma cell-free chimeric DNA.  
5.      The authors should summarize the distribution of breakpoints in the human genome and HBV genome. In addition, the pathway of breakpoint should be analyzed to identify the target gene of integration in tumorigenesis.  
6.      The authors should analyze the correlation between number of HBV integrations and overall survival in HCC patients.  
  
  
  
Reviewer #4: General  
In the manuscript entitled "Noninvasive chimeric DNA profiling identifies tumor-originated HBV integrations contributing to viral antigen expression in the liver cancer", the author developed a sensitive low-pass sequencing assay to enrich integrated HBV DNA for deep sequencing analysis. They clarified that most plasma chimeric fragments were derived from integrations in tumor tissues and also identified that there were 4 distinctive viral sequence patterns.  
This manuscript is well written and interesting. However, there are some concerns to be clarified. Some areas of particular concern are outlined below.  
  
Major pints  
1) The author should investigate the detection rate of HBV integrations in the plasma cfDNAs according to tumor size.  
  
Minor points  
1) Page 8, lines 21-23;The author should describe the reasons why the detection rate of HBV integration rate in saliva is lower than that in plasma in the discussion .  
2) Page 10, lines 12-13; References are required why the author assumed that independent integrations in host genome should be far away from each other.  
3) Page 14, line 30; including liver occurrence→liver tumor occurrence.