Ruan et al.:
"Construction of replication-competent hepatitis B virus vector carrying secreted luciferase transgene and new hepatitis B virus replication and expression cell lines"

Dear Editor,

First of all we would like to thank the reviewers and the Academic Editor for their general appreciation of the novelty of this work and their helpful and insightful comments. Below we provide a point-by-point response to all issues raised. We go through them in the same order as they appeared in the reviewer reports we received.

We hope that the revised ms is now acceptable for publication in *World Journal of Gastroenterology.*

With kind regards,

Dianxing Sun

**Reviewer #1: The authors describe the construction of a replication competent HBV vector tagged with a luciferase transgene to obtain modified HepG2 cells in which further mechanistic and therapeutic assays can be done in an easy way. There are several publications describing the construction of HBV vectors to be used in similar ways. Here the authors demonstrate that this construction is able to replicate and to show some of the characteristics of the parental HBV. However, the novelty is not clear in the field, unless the authors provide convincing evidence in support of the originality of this construct vs other that have been prepared in different forms, including nucleocapsids, adeno-associated constructs, etc. This needs to be a little be more elaborated or assayed (effect on cell differentiation; changes in gene expression, proliferation as cell xenotransplants in mice, etc.). I am ready to recognize the value of a new tool for the study of HBV physiopathology in the HepG2 model. Authors need to put in value their construction vs other prepared by other groups. Overall, the manuscript needs an in depth revision of the English structure since in some parts, in addition to grammatical errors, the wording is complex and difficult to understand de idea behind the mind of the authors.**

A: We thank the reviewer for the careful reading our ms and the comment, suggestions for improvement.

1. There are several publications describing the construction of HBV vectors. **To illustrate the originality of this construct, we suppled some discussion in the ms as following:**
   Various Replication-defective HBV vectors were constructed by substitution of the S gene or the Core gene with the gene of interest. We reported the construction of double shRNA expression on

HBV Core and S region. But these HBV vectors require complementation in Trans by a helper virus genome, which provide the essential functional proteins. Cotransfection of the chimeric genome of HBV vector and helper genome into permissive cultured hepatoma cells result in the release of enveloped infectious chimeric virions.

Several published studies report attempts to generate replication-competent HBV vectors. Chaisomchit demonstrated that a functional human immunodeficiency virus (HIV) *tat* was expressed before the preS1. Except for that the 276 bp small insertion almost completely abolished generation of recombinant HBV, infectivity of its particles remained uncertain and most probably lost because the expression of preS1 might missing. Hanafusa showed that the HBV could carry 63 bp of extra DNA by destroying the DR2 region, which should be a critical cis element of HBV replication. The only defined data is that a few of HBV DNA was observed by southern blot in the cell line HuH-7 but not HepG2. Bai constructed a new kinds of HBV vector by in insert transgene at the spliced HBV polymerase spacer region and proved it could replicate in hepatocytes. But as the PreS1 region was replace by the transgene, it should have lost the infection ability. We reported the successful construction of replication competent HBV vector by inserting two 22 nt Rbm3 IRES sequence and transgene in between the overlap region of Core and Polymerase gene. In this study, the transgene was replaced by Nanoluc Luciferase.

2. **About the needs to be a little more elaborated or assayed (effect on cell differentiation; changes in gene expression, proliferation as cell xenotransplants in mice, etc.):**

As this is a replication-competent HBV vector, it only used for experimental infection of human hepatocytes or HBV-infectable cell lines. The replication of HBV vector in the cell may really change the gene expression or other function of the cell. But as the background of HBV or the luciferase is clear, so we did not test the changes of cell.

3. **Put in value their construction vs other prepared by other groups:**

Yes. The cell line and the secreted recombinant viral particle could trace of HBV replication or infection. This was add at the abstract and discussion section.

4. **About the language problem:**

Yes. The language was further mordified by professionals as you can see the attached certificate.

**Reviewer #2: This is an interesting manuscript on the construction of replication-competent hepatitis B virus vector carrying secrete luciferase transgene to improve the efficiency of development of new anti-HBV drugs and contribute to clarify many issues regarding HBV research. Methods are fine and the results confirm this interesting study.**

A: Thank you.