Dear editor,

We respond to the reviewers's comments and revise our manuscript as followed:

Reviewer #1:
Scientific Quality: Grade C (Good)
Language Quality: Grade B (Minor language polishing)
Conclusion: Rejection
Specific Comments to Authors: The author presented interesting findings in the letter entitled"Autophagy related protein 9A increase in hepatitis B virus-associated hepatocellular carcinoma and the role in apoptosis" that ATG9A expression is up-regulated in HBV-associated HCC tissue ,and silencing it could trigger cell apoptosis. However, the scientific importance or clinic significant of these findings have not been well address. Firstly, the author has reported that ATG9A was upregulated in HBV related-HCC and associated with apoptosis[ref1]. The only progress here is tested these results in HCC tissue. Secondly, author's data showed that neither HBx could affect ATG9A nor ATG9A could affect HBV replication. These results disproof that ATG9A were connected with HBV. Thirdly, ATG9A is the only transmembrane protein of the core autophagy machinery. The domain-swapped homotrimer ATG9A has membrane-bending properties and internal cavities for lipid transportation[ref2]. Why the author pursues its role in apoptosis other than autophagy? In conclusion, rejection for amending was suggested. Reference： 1. Kunanopparat A , Kimkong I , Palaga T , et al. Increased ATG5-ATG12 in hepatitis B virus-associated hepatocellular carcinoma and their role in apoptosis[J]. World Journal of Gastroenterology, 2016, 22(37):8361-8374. 2. Carlos M.G, Xiao-FengT, Tengfei L, et al. Structure of Human ATG9A, the Only Transmembrane Protein of the Core Autophagy Machinery[J]. Cell Reports, 2020,31(13):107837. DOI： 10.1016/j.celrep.2020.107837

1. Firstly, the author has reported that ATG9A was upregulated in HBV related-HCC and associated with apoptosis[ref1]. The only progress here is tested these results in HCC tissue.

Answer Clinical samples from HCC patients were added in this letter to support our previous finding (Kunanopparat A et al., 2016). We also analyzed the role of ATG9A in HBV replication and apoptosis to clarify the function of ATG9A. Although there was no significant difference between HBV copies between ATG9A-knockdowned and control cells, but we found the effect of ATG9A on apoptosis. This result is beneficial information, which can be applied for HCC treatment or the development of new therapeutic approaches in the future.

2. Secondly, author's data showed that neither HBx could affect ATG9A nor ATG9A could affect HBV replication. These results disproof that ATG9A were connected with HBV.

Answer Our findings exhibited no change of HBV titer in HepG2.2.15 cells with silenced ATG9A compared with its mock control. The result suggested that ATG9A might not assist HBV replication in HCC. HBV may interact with other molecules to benefit its replication. Previous study showed that HBV replication related to ATG12 via IFN pathway in HCC [ref 1]. However, ATG9A might correlate with other functions in HCC. Therefore, we searched for the effect of ATG9A on apoptosis. Our data indicated that ATG9A might be an essential molecule for tumor survival in HCC.

Ref 1. Kunanopparat A, Hirankarn N, Kittigul C, Tangkijvanich P, Kimkong I. Autophagy machinery impaired interferon signaling pathways to benefit hepatitis B virus replication. Asian Pac J Allergy Immunology. 2016;34(1):77-85.

3. Thirdly, ATG9A is the only transmembrane protein of the core autophagy machinery. The domain-swapped homotrimer ATG9A has membrane-bending properties and internal cavities for lipid transportation[ref2]. Why the author pursues its role in apoptosis other than autophagy?

Answer Our previous studies provided evidence to support the role of autophagy in HCC as well as current study (ref 2-3). In addition, a most recent report in 2020 (ref.4) showed that ATG9A was validated as a target of miR-29b for HCC treatment. Apoptosis is a programmed cell death that occurs in multicellular organisms. The previous study showed that apoptosis is a tumor suppressor, and autophagy may promote the survival of cancer cells in the stress condition along with deficient apoptosis [ref 5]. In HCC, pro-apoptotic molecules are not only related to liver tumorigenesis but also the chemotherapeutic treatment in HCC [ref 6]. Hence, from the information about the crosstalk between autophagy and apoptosis in HCC, we aimed to study the effect of this autophagy component (ATG9A) on apoptosis.

Ref 2. Peantum J, Kunanopparat A, Hirankarn N, Tangkijvanich P, Kimkong I. Autophagy Related-Protein 16-1 Up-Regulated in Hepatitis B Virus-Related Hepatocellular Carcinoma and Impaired Apoptosis. Gastroenterology Res. 2018 Dec;11(6):404-410.

Ref 3. Kunanopparat A , Kimkong I , Palaga T , et al. Increased ATG5-ATG12 in hepatitis B virus-associated hepatocellular carcinoma and their role in apoptosis. World J Gastroenterol. 2016 Oct 7;22(37):8361-8374.

Ref 4. Shi Y, Yang X, Xue X, Sun D, Cai P, Song Q, Zhang B, Qin L. HANR Enhances Autophagy-Associated Sorafenib Resistance Through miR-29b/ATG9A Axis in Hepatocellular Carcinoma. Onco Targets Ther. 2020 Mar 9;13:2127-2137.

Ref 5. Su M, Mei Y, Sinha S. Role of the crosstalk between autophagy and apoptosis in cancer. J Oncol. 2013;2013:e102735.

Ref 6. Ma S, Chen GG, Lai PBS. Bcl-2 Family Members in Hepatocellular Carcinoma (HCC) – Mechanisms and Therapeutic Potentials Springer. Apoptosis in Carcinogenesis and Chemotherapy, 2009;219-235.

Reviewer #2:
Scientific Quality: Grade C (Good)
Language Quality: Grade A (Priority publishing)
Conclusion: Minor revision
Specific Comments to Authors: This letter reported that ATG9A is upregulated in HBV-associated hepatocellular carcinoma, and provides resistance to apoptosis. Their previous study (World Journal of Gastroenterology, 2016) Upregulation of ATG9A was already reported in hepatoma cell lines but clinical samples were added to support their issue in this letter. 1. It seems like that suppression of ATG9A decreases HBV DNA replication in Fig2.B. Therefore, statistical analysis should be added in Fig2.B although they described no difference HBV copies between ATG9A-knockdowned and control cells. 2. The author described the text as " In order to search for the effect of autophagy in HBV-associated hepatocellular carcinoma, we investigated apoptosis ". For performing Fig4 experiment, rationale is lack. This description seems to be inappropriate. For example, we recommend to describe " In order to search for the effect of ATG9A on apoptosis, we performed a flow cytometry.

1. It seems like that suppression of ATG9A decreases HBV DNA replication in Fig2.B. Therefore, statistical analysis should be added in Fig2.B although they described no difference HBV copies between ATG9A-knockdowned and control cells.

Answer

Thank you very much for the kind suggestion. We added statistical analysis ($p = 0.108/p>0.05$) in Fig2.B as reviewer's suggestion.

2. The author described the text as " In order to search for the effect of autophagy in HBV-associated hepatocellular carcinoma, we investigated apoptosis ". For performing Fig4 experiment, rationale is lack. This description seems to be inappropriate. For example, we recommend to describe " In order to search for the effect of ATG9A on apoptosis, we performed a flow cytometry.

Answer

Thank you very much for your valuable suggestion. We have revised the manuscript by adding the sentence "In order to search for the effect of ATG9A on apoptosis, we performed a flow cytometry" as reviewer's suggestion. (page4)