Dear Dr. Ma,

We thank you for your encouragement and advice. We would like to resubmit the manuscript entitled “Intracellular alpha-fetoprotein mitigates hepatocyte apoptosis and necroptosis by inhibiting endoplasmic reticulum stress” (Manuscript 74989) for your further consideration as an original research article for publication in Would Journal of Gastroenterology. We also thank the reviewers for their constructive comments and suggestions. We have revised the manuscript accordingly, and please see our point-by-point responses below. All amendments are indicated by red font in the revised manuscript. In addition, we have carefully checked every sentence in the revision to eliminate/reduce any potential syntax and this revision has been proofread by two native English biologists from *Medjaden*, a professional publication service company. We think that this manuscript is easily understood in terms of a scientific story and its language writing.

If I can be of any assistance regarding the process of this manuscript please contact me. I look forward to hearing from you soon.
Sincerely,

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Responses to the Reviewer #1:

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Minor revision

Specific Comments to Authors: I would like to suggest that the figures legend need to be more descriptive and better label pointing out the findings in the immunohistochemistry sections/pictures. How “hepatocyte apoptosis and necroptosis” were identified?

Response: We appreciate his/her advice. Accordingly, we have modified the legends by describing the findings of the Figure.

Hepatocyte apoptosis and necroptosis are two different forms of cell death, both of which are strictly regulated by intracellular signals. In this study, we assessed the effect and mechanisms of AFP on the liver injury, including hepatocyte apoptosis and necroptosis. Apoptosis is characterized by activated caspase and DNA fragmentation. ER stress mediates apoptosis by activating the CHOP pathway. In this study, we assessed hepatocyte apoptosis by detecting the relative levels of CHOP and cleaved caspase-3, and the TUNEL assay in vitro and in vivo following AFP silencing (Figure 6F). Necroptosis is a caspase-independent programmed cell death. It is characterized by activation of MLKL phosphorylation with morphological changes, similar to necrosis. In this study, the area of necrosis and the phosphorylation levels of MLKL were used to evaluate hepatocyte necroptosis.

Reviewer #2:

Scientific Quality: Grade A (Excellent)

Language Quality: Grade A (Priority publishing)

Conclusion: Minor revision

Specific Comments to Authors: The authors have investigated the intracellular AFP expression in hepatocyte under the condition of liver injury in vivo and in vitro experiments. Their results indicated that intracellular AFP expression induced by ER stress-mediated hepatocyte injury. The AFP expression was attenuated by ATF6 silencing meanwhile AFP silencing deteriorated the ER stress and hepatocyte apoptosis and necroptosis. Although correlation of AFP expression and liver injury/fibrosis has been reported, the findings regarding regulatory relationships of AFP and ER stress are interesting and distinct. In addition, the study is well conducted, experiments are appropriately performed, and the manuscript is well-written. I consider this paper has enough potential for publication. I have following comments.

1. In Figure 3, why AFP which induced by ER stress do not secrete to serum or supernatant? How about fraction of AFP (especially L3 fraction), and the fraction of AFP is the same or different between HepG2 cells and injured hepatocytes?

Response: We understood his/her comment. APF can be divided into the L1, L2 and L3 fractions, based on their binding to lens culinaris agglutinin (LCA), whichever form mainly refers to AFP in serum. The L1 fraction is associated with liver injury, and multiple previous studies have confirmed that the serum AFP in most patients with liver injury is not elevated. A recent study has shown that ER stress can increase the AFP expression in hepatocytes, but does not elevate the serum AFP levels in animals and the levels of AFP in the supernatants of cultured hepatocytes. Similarly, our previous study has achieved similar findings in HepG2 cells (PMID: 34374292). Actually, ER stress can inhibit the secretion of certain proteins, such as apolipoprotein A-I (PMID: 23154241), hepatitis B virus (PMID: 33983562; 33614671) in hepatocytes. Apparently, ER stress can down-regulate the secretory process of cells. Therefore, we speculate that AFP induced by ER stress may be a new type of AFP that

mainly accumulates in injured hepatocytes. However, it is unclear why AFP induced by ER stress is not secreted by hepatocytes. Given that the induced AFP is not secreted, it is unknown whether it can bind to LCA.

2. In Figure 1, the images of immunohistochemistry of AFP, positive staining of AFP seems to be found at cytoplasm and nuclear. How assess the positivity of AFP stain, cytoplasm only or including nuclear expression?

Response: We understood his/her comments. Our immunohistochemical staining indicated that AFP was mainly distributed in the cytoplasm and nucleus of hepatocytes, which was different from the previous report that AFP was mainly distributed in the cytoplasm of hepatocytes. Previous studies have shown that AFP exists in the cytoplasm as a cell signaling molecule involved in many important functions of cells (PMID: 25846475; 22521346). In this study, we found that AFP was present in the cytoplasm and nucleus of hepatocytes. We counted positive AFP staining regardless of its cytoplasm and nucleus staining. Functionally, we found that the intracellular AFP might act as a signaling event to negatively regulate ER stress, and reduce hepatocyte apoptosis and necroptosis (Figure 5 and 6).

3. In Figure 3G, is it “TG” (is it TM?)? Please confirm.

Response: It was right that the LO2 cells were treated with TG, which is commonly used in vitro experiment by inhibiting intracellular calcium balance to induce ER stress.

4 LANGUAGE POLISHING REQUIREMENTS FOR REVISED MANUSCRIPTS SUBMITTED BY AUTHORS WHO ARE NON-NATIVE SPEAKERS OF ENGLISH

As the revision process results in changes to the content of the manuscript, language problems may exist in the revised manuscript. Thus, it is necessary to perform further language polishing that will ensure all grammatical, syntactical, formatting and other

related errors be resolved, so that the revised manuscript will meet the publication requirement (Grade A).

Response: We have carefully checked every sentence in the revision to eliminate/reduce any potential syntax. In addition, this manuscript has been proofread by two native English biologists from *Madjaden*, a publication service company. We think that this manuscript is easily understood in terms of a scientific story and its language writing. Please see the language certification from the company.

Authors are requested to send their revised manuscript to a professional English language editing company or a native English-speaking expert to polish the manuscript further. When the authors submit the subsequent polished manuscript to us, they must provide a new language certificate along with the manuscript.

Once this step is completed, the manuscript will be quickly accepted and published online. Please visit the following website for the professional English language editing companies we recommend: https://www.wjgnet.com/bpg/gerinfo/240.

5 ABBREVIATIONS

In general, do not use non-standard abbreviations, unless they appear at least two times in the text preceding the first usage/definition. Certain commonly used abbreviations, such as DNA, RNA, HIV, LD50, PCR, HBV, ECG, WBC, RBC, CT, ESR, CSF, IgG, ELISA, PBS, ATP, EDTA, and mAb, do not need to be defined and can be used directly.

The basic rules on abbreviations are provided here:

(1) Title: Abbreviations are not permitted. Please spell out any abbreviation in the title.

(2) Running title: Abbreviations are permitted. Also, please shorten the running title to no more than 6 words.

(3) Abstract: Abbreviations must be defined upon first appearance in the Abstract. Example 1: Hepatocellular carcinoma (HCC). Example 2: *Helicobacter pylori* (*H. pylori*).

(4) Key Words: Abbreviations must be defined upon first appearance in the Key Words.

(5) Core Tip: Abbreviations must be defined upon first appearance in the Core Tip. Example 1: Hepatocellular carcinoma (HCC). Example 2: *Helicobacter pylori* (*H. pylori*)

(6) Main Text: Abbreviations must be defined upon first appearance in the Main Text. Example 1: Hepatocellular carcinoma (HCC). Example 2: *Helicobacter pylori* (*H. pylori*)

(7) **Article Highlights:** Abbreviations must be defined upon first appearance in the Article Highlights. Example 1: Hepatocellular carcinoma (HCC).

Example 2: *Helicobacter pylori* (*H. pylori*)

(8) Figures: Abbreviations are not allowed in the Figure title. For the Figure Legend text, abbreviations are allowed but must be defined upon first appearance in the text. Example 1: A: Hepatocellular carcinoma (HCC) biopsy sample; B: HCC-adjacent tissue sample. For any abbreviation that appears in the Figure itself but is not included in the Figure Legend textual description, it will be defined (separated by semicolons) at the end of the figure legend. Example 2: BMI: Body mass index; US: Ultrasound.

(9) Tables: Abbreviations are not allowed in the Table title. For the Table itself, please verify all abbreviations used in tables are defined (separated by semicolons) directly underneath the table. Example 1: BMI: Body mass index; US: Ultrasound.

6 EDITORIAL OFFICE'S COMMENTS

Authors must revise the manuscript according to the Editorial Office's comments and suggestions, which are listed below:

(1) Science editor:

This is a very interesting paper which aims to study whether and how AFP could regulate ER stress and hepatocyte injury. They analyze he distribution of AFP and the degrees of ER stress in liver tissues and liver injury were characterized by histology, immunohistochemistry, and western blot in biopsied human liver specimens, two mouse models of liver injury and a cellular model. The reviewers point a few things that need to be adressed, but overall they give positive feedback. I would suggest publication after review.

Language Quality: Grade B (Minor language polishing)

Scientific Quality: Grade B (Very good)

Response: Thanks for your comments.

(2) Company editor-in-chief:

I have reviewed the Peer-Review Report, the full text of the manuscript, and the relevant ethics documents, all of which have met the basic publishing requirements of the World Journal of Gastroenterology, and the manuscript is conditionally accepted. I have sent the manuscript to the author(s) for its revision according to the Peer-Review Report, Editorial Office's comments and the Criteria for Manuscript Revision by Authors. Before final acceptance, uniform presentation should be used for figures showing the same or similar contents; for example, "Figure 1Pathological changes of atrophic gastritis after treatment. A: ...; B: ...; C: ...; D: ...; E: ...; F: ...; G: ...". Please provide decomposable Figures (in which all components are movable

and editable), organize them into a single PowerPoint file. Please authors are required to provide standard three-line tables, that is, only the top line, bottom line, and column line are displayed, while other table lines are hidden. The contents of each cell in the table should conform to the editing specifications, and the lines of each row or column of the table should be aligned. Do not use carriage returns or spaces to replace lines or vertical lines and do not segment cell content. In order to respect and protect the author’s intellectual property rights and prevent others from misappropriating figures without the author's authorization or abusing figures without indicating the source, we will indicate the author's copyright for figures originally generated by the author, and if the author has used a figure published elsewhere or that is copyrighted, the author needs to be authorized by the previous publisher or the copyright holder and/or indicate the reference source and copyrights. Please check and confirm whether the figures are original (i.e. generated de novo by the author(s) for this paper). If the picture is ‘original’, the author needs to add the following copyright information to the bottom right-hand side of the picture in PowerPoint (PPT): Copyright ©The Author(s) 2022.

Response: Thank you for your insightful suggestion. We have prepared our manuscript accordingly.