Dear Science Editor Ze-Mao Gong:

Thank you for your letter and for the reviewers' comments regarding our manuscript entitled "Zinc-α2-glycoprotein 1 attenuates nonalcoholic fatty liver by negatively regulating tumour necrosis factor-α" (Ref. No.: 49795). The comments are all valuable and were very helpful for improving our paper. We have made corrections as suggested by the reviewers and here by submit a revised manuscript. Corrections in the paper and responses to the reviewers' comments are listed below. We hope that the manuscript will now be accepted for publication in World Journal of Gastroenterology.

Corresponding author: Mingyi Xu, E-mail: xumingyi2014@163.com.

Once again, thank you very much for your comments and suggestions.

With all my best regards!

Sincerely yours,

Mingyi Xu

Revision Notes

The following is a point-by-point response to the reviewers' comments.

Reviewer 1:

(1) The importance of the research and the significance of the research contents; The authors of this article have been evaluate molecular mechanisms and therapeutic effects of ZINC-A2-GLYCOPROTEIN 1 (AZGP1) in non-alcoholic fatty liver disease (NAFLD). The importance and significant of the research is high. A chronic inflammatory state is a major condition for developing of various aspects of non-alcoholic steatohepatitis (NASH). Neutralization of TNF-alpha activity is an important therapeutic target in management of fatty liver disease.

(2) The novelty and innovation of the research; The novelty of the research represents the evidence that AZGP1 loss aggravates liver inflammation, promotes intracellular lipid accumulation, suppresses lipid degradation metabolism, reduces cell proliferation and promotes cell apoptosis. AZGP1 reverses these effects and attenuates NAFLD by blocking the TNF-α. The

authors propose AZGP1 as a promising therapeutic candidate for NAFLD. (3) Presentation and readability of the manuscript; Original article is well organized.

R1-1 (4) Ethics of the research. All patients provided written informed consent, and the study was approved by the ethics committee of hospital. (Important to write name of the of institution).

Answer: Thank you for kindly reminding us this point. We changed it in the revised paper in page 7 line 19: "the study was approved by the ethics of Shanghai General Hospital".

R1-2 Necessary to write how were carried animal experiments. (Like - Animal experiments were carried out according to the guidelines of the local university Institutional Committee for the Care and Use of Laboratory Animals and with the committee's approval)

Answer: Thank you for kindly reminding us this point. We changed it in the revised paper in page 8 line 13-15: "Animal experiments were carried out according to the guidelines of Shanghai Jiao Tong University School of Medicine and approved by the ethics committee of Shanghai General hospital".

SPECIFIC COMMENTS Title: accurately reflects the major topic and contents of the study. Abstract: it is gives a delineation of the research background. The methods are presented clear. Results contain clear, most important information, all abbreviations have explanation. Conclusion reflected the result. Introduction: present relevant information about zinc-α2-glycoprotein and aims of the study.

R1-3 Methods: Better to have delineation between human sample and all method which were use and animal sample.

Answer: Thanks for the careful review opinion. In the article, only human samples and all methods which were used are described. We supplemented all the methods used in the animal samples in the revised paper in page 8 line 9-13: "All samples of the four groups were subjected to Haematoxylin and eosin (HE) staining, Sirius Red staining, qPCR, Western blotting, Immunofluorescence (IF) staining, Measurements of serum alanine transaminase (ALT) and Oil Red O (ORO) staining".

R1-4: Statistical analysis need to be with more informative.

Answer: Thank you for carefully review. We completed the information of statistical analysis in the revised paper.

Results: authors present interesting and original result, the main point of this

study is directed to biological importance of exposed results. Figures and table: very good explain the result of study, relevant and understandable Conclusions: Conclusion reflect the result. References: references are appropriate, relevant, and updated.

Reviewer 2:
I believe the manuscript can be accepted after minor revisions:

R2-1 the authors must be more detailed in the references: Any method, protocol or score must be correctly cited

Answer: Thank you for kindly reminding us these points. The methods and protocols have been improved in the revised paper. And we supplemented NAFLD activity scoring, NASH Clinical Research Network scoring system and Scheuer's classification with corresponding references in the revised paper.

R2-2 the authors should broaden the discussion by hypothesizing further AZGP1 effects, even not directly related to NAFLD

Answer: Thank you for this constructive opinion which also provided us with an idea of further study of AZGP1. We supplemented the relevant content in the revised paper. In Page 20, line 4-21: "Regarding the effect of AZGP1, our previous study also found that the deletion of AZGP1 induced epithelial-mesenchymal transition (EMT), accompanied by confusion of energy metabolism, reduction of cell proliferation and apoptosis, and increased invasion, finally AZGP1 suppressed hepatic carcinogenesis by blocking TGFβ1-extracellular regulated protein kinases (ERK2) signaling pathway [31]. A study by Hyun Sik et al. found that AZGP1 aggravated the development of inflammatory diseases by forming a vicious circle with IL-17, such as rheumatoid arthritis with HFD [43]. It had also been found that AZGP1 inhibited the inflammatory response associated with obesity by blocking the NF-κB signaling pathway [44]. Moreover, AZGP1 reduced insulin resistance by promoting Insulin receptor substrate-1 (IRS-1)/protein kinase B (AKT) signaling pathway [44]. Research on AZGP1 and fatty liver found that AZGP1 reduced intracellular lipid deposition by up-regulating lipolysis genes and down-regulating the expressions of adipogenic genes [44]; Xiao et al. also found that AZGP1 can significantly inhibit lipogenesis, promote lipolysis and fatty acid β oxidation, and reduce PA-induced intracellular lipid deposition [8], and our results were similar to these studies."

[31] Xu MY, Chen R, Yu JX, Liu T, Qu Y, Lu LG. AZGP1 suppresses epithelial-to-mesenchymal transition and hepatic carcinogenesis by blocking TGFβ1-ERK2 pathways. Cancer Lett, 2016; 374: 241-9. [PMID: 26902423 DOI:

10.1016/j.canlet.2016.02.025]
[43] Na HS, Kwon JE, Lee SH, Jhun J, Kim SM, Kim SY, Kim EK, Jung K, Park SH, Cho ML. Th17 and IL-17 Cause Acceleration of Inflammation and Fat Loss by Inducing α2-Glycoprotein 1 (AZGP1) in Rheumatoid Arthritis with High-Fat Diet. Am J Pathol, 2017; 187: 1049-58. [PMID: 28284716 DOI: 10.1016/j.ajpath.2016.12.023]
[44] Xiao XH, Wang YD, Qi XY, Wang YY, Li JY, Li H, Zhang PY, Liao HL, Li MH, Liao ZZ, Yang J, Xu CX, Wen GB, Liu JH. Zinc alpha2 glycoprotein protects against obesity-induced hepatic steatosis. Int J Obes (Lond). 2018; 42: 1418-30. [PMID: 30006580 DOI: 10.1038/s41366-018-0151-9]

R2-3 the authors should point out that the research focuses on hepatocytes in NAFLD patients and there are no associated hepatitis or toxic substances use

Answer: Thank you for carefully review. We supplemented the diagnostic criteria for NAFLD disease in our revised article, and pointed out in page 7 line 7-9: “Our research focused on hepatocytes in NAFLD patients, so there were no associated hepatitis or toxic substances use”.

Reviewer 3:

In this paper the expression of AZGP1 and its effects on hepatocytes were examined in NAFLD patients, CCl4-treated mice fed a high fat diet (HFD) and human LO2 cells. The expressions of AZGP1 were significantly down-regulated in liver tissues of NAFLD patients and mice. In LO2 cells, AZGP1 had the effects of alleviating NAFLD by means of blocking tumour necrosis factor-α (TNF-α) signalling mediated inflammation, intracellular lipid deposition, proliferation and apoptosis.

R3-1 In the section “Materials and methods Human liver tissues” the authors should report the appropriate reference each time that they discuss “a score” or a “Research network”.

Answer: Thanks for pointing out our negligence. We added the corresponding references of NAFLD activity scoring, the NASH Clinical Research Network scoring system and Scheuer’s classification in the revised paper, and we reported the corresponding references each time when mentioned the scoring systems.

R3-2 Focusing on patients, the authors should report how the diagnosis of NAFLD was formulated (and how alcohol intake was considered).

Answer: Thank you for carefully review. We supplemented the diagnostic criteria for NAFLD disease in our revised article.

R3-3 When the authors describe "Mice were injected with CCl4 (Sigma-Aldrich Corp, St. Louis, MO) three times a week during the final 6 weeks to establish a liver injury and cirrhosis model in HFD+CCl4 and HFD+CCl4+AZGP1 groups; while oil (2ul/g, 3 times a week) for LFD and HFD groups.", was this protocol reported in previous works?

Answer: Thanks for the careful review opinion. Our method of model construction was similar as the previous literature [De Minicis S, Rychlicki C, Agostinelli L, et al. Hepatology, 2014, 59(5): 1738-49; Donthamsetty S, Bhave VS, Mitra MS, et al. Hepatology, 2007, 45(2): 391-403; Kubota N, Kado S, Kano M, et al. Clin Exp Pharmacol Physiol, 2013, 40(7): 422-30; Chheda TK, Shivakumar P, Sadasivan SK, et al. BMC Gastroenterol, 2014, 14:89; Wehr A, Baeck C, Ulmer F, et al. PLoS One, 2014, 9(11): e112327].

R3-4 When the authors report "Adiponectin is an important adipokine that suppresses lipid accumulation in the liver." To support their sentence, they should cite the review by Prof. Abenavoli, entitled Adiponectin in hepatology. Minerva Biotcenologica 2018; 30:36-40.

Answer: Thanks for the reviewer's valuable suggestion. We cited this review by Professor Abenavoli in the revised paper [L Abenavoli, L Boccutoto, M Masarone, R Pellicano, M Persico. Adiponectin in hepatology, 2018; 30: 36-40].

R3-5 Pag 18, line 1 please report Liu et al. I suggest to the authors to verify all times this aspect.

Answer: Thank you for pointing out the error. We changed it in the revised paper. This aspect was modified throughout the manuscript.

R3-6 Since the role of AZGP1 remains unclear, in the discussion the authors should discuss the possibility that AZGP1 loss could be a consequence of an unknown cause and not a cause of damage.

Answer: Thanks for the careful review opinion. We included the discussion of this issue in the revised paper in Page 20 line 22-29 and Page 21 line 1-13 : "Since the role of AZGP1 remains unclear, so there is the possibility that AZGP1 loss could be a consequence of an unknown cause and not a cause of damage. Our study found that the expressions of AZGP1 were reduced in both NAFLD patients and CCl4-treated mice fed a high fat diet (HFD), which were similar to previous studies [8, 45-46]. We found that the disease state of NAFLD was aggravated after knocking down AZGP1 by artificially applying plasmid-based shRNA to LO2 cells. However, there was no relevant literature on the exact cause of AZGP1 loss. Although the specific mechanism was still unclear, the expression of AZGP1 could be partially attributed to

the acetylation of histone, which regulated gene viability by altering the structure of chromatin [47]. Tian et al. reported that deacetylation of histone H4 decreased AZGP1 expression by inhibiting the transcription factor Ikaros bind to the promoter of AZGP1 in HCC cells [48]. Daniel and his colleagues found that AZGP1 is upregulated in lung adenocarcinoma due to acetylation [49]. Kong et al. had also reported that AZGP1 is absent from pancreatic ductal adenocarcinoma due to histone deacetylation [50]. However, whether histone deacetylation contributes to the decreased expression of AZGP1 found in our study requires further investigation. If we want to study whether the loss of AZGP1 alone will directly induce the occurrence of injury, we may need AZGP1 knockout mice, molecular biology studies and so on to verify whether the loss of AZGP1 can directly lead to injury."

[8] Xiao X, Li H, Qi X, Wang Y, Xu C, Liu G, Wen G, Liu J. Zinc alpha2 glycoprotein alleviates palmitic acid-induced intracellular lipid accumulation in hepatocytes. Mol Cell Endocrinol, 2017; 439: 155-64. [PMID: 27264075 DOI: 10.1016/j.mce.2016.06.003]
[45] Mracek T, Gao D, Tzanavari T, Bao Y, Xiao X, Stocker C, Trayhurn P, Bing C. Downregulation of zinc-{alpha}2-glycoprotein in adipose tissue and liver of obese ob/ob mice and by tumour necrosis factor-alpha in adipocytes. J Endocrinol, 2010; 204: 165-72. [PMID: 19934249 DOI: 10.1677/JOE-09-0299]
[46] Yilmaz Y, Yonal O, Eren F, Kurt R, Celikel CA, Ozdogan O, Imeryuz N, Kalayci C, Avsar E. Serum zinc-α2-glycoprotein concentrations in patients with non-alcoholic fatty liver disease. Clin Chem Lab Med, 2011; 49: 93-7. [PMID: 21077793 DOI: 10.1515/CCLM.2011.022]
[47] Huang Y, Li LZ, Zhang CZ, Yi C, Liu LL, Zhou X, Xie GB, Cai MY, Li Y, Yun JP. Decreased expression of zinc-alpha2-glycoprotein in hepatocellular carcinoma associates with poor prognosis. J Transl Med, 2012; 10: 106. [PMID：22625427 DOI：10.1186/1479-5876-10-106]
[48] Tian H, Ge C, Zhao F, Zhu M, Zhang L, Huo Q, Li H, Chen T, Xie H, Cui Y, Yao M, Li J. Downregulation of AZGP1 by Ikaros and histone deacetylase promotes tumor progression through the PTEN/Akt and CD44s pathways in hepatocellular carcinoma. Carcinogenesis, 2017; 38: 207-17. [PMID: 27993894 DOI: 10.1093/carcin/bgw125]
[49] Albertus DL, Seder CW, Chen G, Wang X, Hartojo W, Lin L, Silvers A, Thomas DG, Giordano TJ, Chang AC, Orringer MB, Bigbee WL, Chinnaiyan AM, Beer DG. AZGP1 autoantibody predicts survival and histone deacetylase inhibitors increase expression in lung adenocarcinoma. J Thorac Oncol, 2008; 3:12366-44. [PMID: 18978557 DOI: 10.1097/JTO.0b013e318189f5ec]
[50] Kong B, Michalski CW, Hong X, Valkovskaya N, Rieder S, Abiatari I, Streit S, Erkan M, Esposito I, Friess H, Kleeff J. AZGP1 is a tumor suppressor in pancreatic cancer inducing mesenchymal-to-epithelial transdifferentiation by inhibiting TGF-β-mediated ERK signaling. Oncogene, 2010; 29: 5146-58. [PMID: 20581862 DOI: 10.1038/onc.2010.258]