Dear Reviewers,

Thank you for your thoughtful suggestions and insights, which have benefited from the manuscript. I am looking forward to working with you to move this manuscript closer to publication in "World Journal of Gastroenterology ".

The manuscript has been rechecked and the necessary changes have been made in accordance with your suggestions. The responses to all comments have been prepared and attached below. We have tried our best to solve the problems you proposed, and we hope that the revised manuscript is now suitable for publication in the journal "World Journal of Gastroenterology". If you have any questions remained about this paper, please feel free to contact us.

Reviewer #1:

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Major revision

Specific Comments to Authors: Dear Editor, To maintain WJG's rapid peer review process, I just focus on individual concerns and make decision finally. According to your esteemed guidelines for publishing by WJG, this paper is interesting and applicable to clinical practice in hepatic ischemia-reperfusion injury. However, lack of important detail of vito model explained and bias affected results description to provide information for readers.

RE: Thank you for pointing out this issue. In order to facilitate the reader's understanding, we have added the principles of model construction in the section "Establishment of the AP cell model and mouse model" . In addition, we explained the reasons for detecting the expression of these factors (amylase, lipase, TNF-α, IL-6, and IL-1β) in the section "tRF-36 is upregulated in AP and contributes to AP progression" .

It's my pleasure to have an opportunity to review this manuscript. I hope I will have more invitation from WJG to review manuscript submitted to your esteemed journal in the future. Sincerely yours, I-Shiang Tzeng, PhD Associate Professor This is a basic study discussing about the regulatory mechanisms of tRNA-derived fragments (tRFs) in AP based on small RNA sequencing and experiments. This study results confirms through in vitro model. I think the topic is important and contributive to the clinical practice with an empirical approach quite valuable for acute pancreatitis professionals.

1 Title: Appropriate.

2 Abstract: Well described in the manuscript.

3 Key words: Appropriate.

4 Background: Please add references about review article related to similar issue.

RE: Thank you for pointing out this issue. We have added the corresponding references in the background section, as detailed in the manuscript.

5 Methods: Appropriate.

6 Results: Appropriate.

7 Discussion: I am not familiar the hypothesis proposed in this study. The authors should clarify this concern for discussion.

RE: Thanks for the comments. The tRNA derived fragments (tRFs) are non-coding RNAs (ncRNAs) that have recently been found to be produced by processing of mature tRNA or tRNA precursors. In this study, we first obtained the differentially expressed tRFs (DE-tRFs) between AP and normal samples by bioinformatic methods. Subsequently, RT-qPCR was used to mine our primary target tRF-36, which was the most significantly differentially

expressed tRF. After exploring the regulatory mechanisms of tRF-36 in the progression of AP, we found that tRF-36 was mainly associated with ferroptosis-associated P53 and mTOR signaling pathways. Finally, by constructing animal and cellular models, we found that tRF-36 may recruit IGF2BP3 to the p53 mRNA m6A modification site by binding to IGF2BP3 to enhance the stability of p53 mRNA and promote ferroptosis in pancreatic follicular cells.

8 Illustrations and tables: I am not familiar in vitro model. Please introduce commonly used illustrations and tables for vitro model. Does any vivo model involve the similar topic?

RE: Thanks for the comments. The in vitro model in this study, namely the cell model of AP, was obtained by administration of 10 nM cerulein in to the mouse pancreatic acinar carcinoma cell line (MPC-83). The cell model was constructed mainly to facilitate the purpose of tRF-36 low expression by adding inhibitor. In vitro model has also been constructed in the published literature PMID: 36611865 for your reference. The published literature (https://doi.org/10.3389/fphys.2020.614591) explained in detail the in vivo model of AP.

9 Biostatistics: Does the manuscript examined by experienced biostatistics?

RE:Thanks for the comment. There was no problem with biometrics.

10 Units: Does the manuscript meet the requirements of use of international system of units?

RE:Thanks for the comment. Based on your suggestion we have further verified the use of international system of units.

11 References: Please cite appropriately the latest, important and authoritative references in the introduction and discussion sections.

RE: Thanks for the comment. We have updated the references based on your suggestions.

12 Quality of manuscript organization and presentation: Please provide English editing certificate.

RE: Thanks for the comment. Based on your suggestion we have polished the revised manuscript and provided a certificate.

13 Research methods and reporting: Please provide appropriate research methods and reporting. Authors should have prepared their manuscripts according to manuscript type and the appropriate categories, as follows:

(1) CARE Checklist (2013) - Case report;

(2) CONSORT 2010 Statement - Clinical Trials study, Prospective study, Randomized Controlled trial, Randomized Clinical trial;

(3) PRISMA 2009 Checklist - Evidence-Based Medicine, Systematic review, Meta-Analysis;

(4) STROBE Statement - Case Control study, Observational study, Retrospective Cohort study; and

(5) The ARRIVE Guidelines - Basic study. 14 Ethics statements: Please provide appropriate ethics approval.

RE:Thanks for the comment. Based on your suggestion we have provided appropriate ethics approval.

Reviewer #2:

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Major revision

Specific Comments to Authors: Confidential The manuscript entitled " Exploring the regulatory mechanism of tRF36 in acute pancreatitis based on small RNA sequencing and experiments" has been reviewed. A lot of careful

work has gone into this project. However, a drawback of this manuscript is that, as the authors describe, the analysis was carried out on a small number of samples. On the other hand, the authors even describe the weakness of the paper in the discussion section. Apart from that, several issues need improvement. Remarks to the author The manuscript entitled " Exploring the regulatory mechanism of tRF36 in acute pancreatitis based on small RNA sequencing and experiments" has been reviewed. A lot of careful work has gone into this project. However, a drawback of this manuscript is that, as the authors describe, the analysis was carried out on a small number of samples. On the other hand, the authors even describe the weakness of the paper in the discussion section. Apart from that, several issues need improvement.

Comments. What are the causes of acute pancreatitis?

RE: Thanks for the comment.Acute pancreatitis (AP) is a local inflammation of the pancreas and even organ dysfunction due to self-digestion of the pancreas and surrounding organs after abnormal activation of pancreatic enzymes. We have explained about this issue in the introduction.

How many days after the onset of pancreatitis was the patient's blood drawn?

RE: Thanks for your comment. All AP patients were included in accordance with the "2013 Chinese Guidelines for the Diagnosis and Management of Acute Pancreatitis" developed by the Chinese Society of Gastroenterology. The diagnostic criteria for acute pancreatitis include the following 3 items: (1) acute sudden onset, persistent, severe upper abdominal pain, often radiating to the back, possibly accompanied by nausea, vomiting and abdominal distension; (2) serum amylase and/or lipase concentration at least 3 times higher than the upper limit of normal; (3) abdominal imaging findings showing blurring, edema, exudation and necrosis of the pancreas and/or peripancreatic tissue. Acute pancreatitis can be diagnosed when two of the above three criteria are met. Once the diagnosis is clear according to the diagnostic criteria of appeal, blood is drawn (usually within 1-2 hours).

How severe was the pancreatitis when the blood was taken?

RE: Thanks for the comment. The patients whose blood samples were obtained were all people with mild and moderately severe pancreatitis.

Patients with pancreatitis would have had mild to severe disease. Did the authors check the serum levels of P53 in the serum of the 20 pooled cases of patients with pancreatitis?

RE: Thanks for the comment. The 20 AP samples and 20 normal samples were collected here mainly to validate the expression differences of DE-tRFs. Therefore, we did not perform assays for P53 expression levels. When tRF-36 was significantly enriched to the P53 signaling pathway, we again constructed AP cell models to study the expression levels of P53.

And did tRF36 and P53 correlate with the severity of the pancreatitis?

RE: Thanks for the comments. In this study we firstly obtained the differentially expressed tRFs （DE-rRFs） in AP samples and normal samples by Small RNA sequencing, and selected the most significantly different tRF36. Next, we investigated the mechanism of action of tRF36 by constructing a cellular model and an animal model of AP. And in this process, the severity of AP samples was not analyzed. Therefore, we cannot explain the relationship between tRF36 and P53 and the severity of AP based on the existing findings. However, based on your suggestion, we will further investigate the differences between tRF36 and P53 in samples with different severity of AP, and we will continue to focus on the role of tRF36 in AP.

In the results section. As shown in Figure 2a, tRF-36 was downregulated in the serum of AP patients and its differential expression was the most significant ($p < 0.05$, Figure 2a). →Is this statement correct?

RE: We apologize for our error. The statement in the manuscript described the result of Figure 1C. And Figure 2a explained the efficiency of inhibitor in reducing the expression of tRF-36. We have made changes in the manuscript.

The expression of ferritin in the pancreatic tissues of the AP model mice was significantly reduced (Figure 3h). →Is this result correct?

RE: Thanks for the comment. The result that ferritin levels are lower in the AP mouse model is correct. In Figure 3, the blue color is the hematoxylin-stained nucleus, while the brown color is the expression of ferritin. Furthermore, this result is consistent with that in the AP cell model.

Ferritin should be increased in pancreatitis. Longer discussion. Please be concise as there are many results.

RE: Thanks for the comment. In this study, the expression level of ferritin was significantly lower in AP than in the normal group. The literature PMID: 27245739 also suggests that high expression of ferritin limits ferroptosis. Based on your comments we have added relevant content in Discussion.

What does Figure 2a mean? Please prepare a detailed explanation.

RE: Thanks for the comment. In the present study, tRF-36 inhibitor was transfected into MPC-83 cells to knock down the expression of tRF-36. The knockdown efficiency of tRF-36 was detected by qRT-PCR. Figure 2a demonstrated inhibitor significantly reduced the expression level of tRF-36.

Minor There is no comment on the standard ethical principles.

RE: We are sorry for our mistake. Based on your comments we have added standard ethical principles in "*Small RNA sequencing*" section and "*Establishment of the AP cell model and mouse model*" section.

Figures 3c and d are in reverse order.

RE: We apologize for our error. Based on your suggestion we have changed the order of Figure 3c and d in the manuscript.

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).

RE: The revised manuscript has been linguistically polished according to your suggestion.

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.

RE: The revised manuscript has been linguistically polished according to your suggestion and a new language certificate has been provided.

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.

RE : We apologize for our mistake. We have revised "tRF36" to "tRNA-derived fragments 36" in the title based on your suggestion.

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

RE: Thank you for your comment. We have added a running title of no more than six words based on your suggestion.

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

RE: Thank you for your comment. We have defined the abbreviations in the abstract.

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

RE: Thank you for your comment. We have defined the abbreviations in the Keywords.

(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)

RE: Thank you for your comment. We have defined the abbreviations in the Core Tip.

(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)

RE: Thank you for your comment. We have defined the abbreviations in the main text.

(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)

RE: Thank you for your comment. We have defined the abbreviations in the highlights.

(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.

RE: Thank you for your comments. Based on your suggestion we have defined the abbreviations in the title and legend of the figure.

(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.

RE: Thank you for your comments. Based on your suggestion we have defined the abbreviations in the tables and table title.

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:

The manuscript has been peer-reviewed, and it' s ready for the first decision.

(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. 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):

RE: Thank you for your comments. We have recombined the images in the PowerPoint according to your suggestion.

Revision reviewer

Specific comments to authors

Specific comments to the authors: Confidential The manuscript entitled "Exploring the regulatory mechanism of tRF36 in acute pancreatitis based on small RNA sequencing and experiments" has been re-reviewed. Comments. What are the causes of acute pancreatitis? RE: Thanks for the comment.Acute pancreatitis (AP) is a local inflammation of the pancreas and even organ dysfunction due to self-digestion of the pancreas and surrounding organs after abnormal activation of pancreatic enzymes. We have explained about this issue in the introduction. That is not my point. What causes acute pancreatitis? What is the underlying disease? For example, idiopathic, stone, alcohol? The reason for asking this is Does the primary disease make a difference in the expression of tRF36? How do the authors distinguish between mild and moderate pancreatitis?

RE：We apologize that we did not understand exactly what you meant. As you mentioned, conditions that can lead to acute pancreatitis include: gallbladder stones; alcoholism; certain medications; high triglyceride levels in the blood (hypertriglyceridemia); high calcium levels in the blood (hypercalcemia); parathyroid (hyperparathyroidism); pancreatic cancer; abdominal surgery; and obesity etc. In addition, endoscopic retrograde cholangiopancreatography (ERCP), a method used to treat gallstones, can also lead to pancreatitis. Sometimes, the cause of pancreatitis is never identified. This is referred to as idiopathic pancreatitis. We have also added the relevant description in the “Introduction” section, which reads as follows: There are many causes of acute pancreatitis, among which gallstones and alcohol are the main causes.

As for the effect of primary disease on the expression of tRF36 that you

mentioned, we cannot completely exclude it. In order to minimize the effect of extrinsic factors, we constructed both cellular and animal models to investigate the role of tRF36 in AP.

We differentiate mild and moderate AP according to RAC criteria, which are classified as follows: (1) mild acute pancreatitis (MAP): 80% to 85% of acute pancreatitis without organ dysfunction and local or systemic complications, usually recovering within 1 to 2 weeks, with a very low mortality rate. (2) moderately severe acute pancreatitis (MSAP): with transient (<48 h) organ dysfunction and/or local complications, with a low early mortality rate, but a higher mortality rate if necrotic tissue is combined with infection. (3) severeacute pancreatitis (SAP): accounts for 5%-10% of acute pancreatitis with persistent (>48 h) organ dysfunction and high mortality rate.