

Response Letter

Manuscript ID: 89519, Basic Study

Manuscript Title: Effects of Rifaximin on Epigenetic and Autophagy Markers in an Experimental Model of Hepatocellular Carcinoma Secondary to Non-alcoholic Fatty Liver Disease

We received the reviewers' comments on the manuscript entitled "Effects of Rifaximin on Epigenetic and Autophagy Markers in an Experimental Model of Hepatocellular Carcinoma Secondary to Non-alcoholic Fatty Liver Disease" (89519, Basic Study). We are grateful for their valuable time and useful contributions. We have addressed each of their concerns as outlined below:

Reviewer's Comments - 1: Significance of Dietary Flavonoids: The authors have provided a comprehensive analysis of the impact of RIF on NAFLD-HCC. However, there is an expanding body of literature suggesting that dietary components, particularly flavonoids, have significant effects on fatty liver disease through modulation of epigenetic and autophagic pathways. For instance, a recent study (DOI: 10.1016/j.numecd.2023.03.005) elaborates on the relationship between dietary flavonoids and fatty liver disease, which may offer additional insights into the epigenetic mechanisms discussed. The authors are encouraged to incorporate these findings into the discussion to provide a more holistic view of the potential dietary influences on NAFLD and HCC.

Answer: As per the reviewer's suggestion, the information has been incorporated into the manuscript's discussion.

Reviewer's Comments - 2: Inflammatory Diet and Biomarkers: The manuscript would benefit from a discussion on the role of inflammatory diets and related biomarkers in the progression of fatty liver disease. The incorporation of studies highlighted by DOIs 10.3389/fimmu.2022.925690 and 10.1007/s40520-023-02410-1 would substantiate the current understanding of how inflammation and dietary patterns contribute to NAFLD-HCC pathogenesis and might interact with the epigenetic mechanisms and autophagic processes described in the study.

Answer: Information from both manuscripts mentioned by the reviewer has been added to the manuscript's discussion with the aim of contributing to the understanding of the disease's pathogenic mechanisms.

Reviewer's Comments - 3: Other Nutritional Metabolic Factors: The authors should consider broadening the scope of their discussion to include other nutritional and metabolic factors that impact fatty liver disease. References with DOIs 10.3389/fendo.2023 would provide additional context to the study, especially concerning how various nutritional components may synergize or interfere with the epigenetic and autophagy markers that are the focus of this study.

Answer: We thank the reviewer for the contribution. The corresponding reference to the role of flavonoids on fatty liver disease through modulation of epigenetic and autophagic pathways was included in the text in response to reviewer comments #1. The specific article suggested with DOI 10.3389/fendo.2023 was not found in Pubmed.

Reviewer's Comments - 4: Specific Recommendations: The authors should revise the Introduction to mention the potential role of dietary components, such as flavonoids, and their known interactions with epigenetic mechanisms in the context of NAFLD and HCC.

Answer: Information added to the manuscript's introduction.

Reviewer's Comments - 5: In the Results section, while the focus on RIF is pertinent, the discussion would be enriched by considering the broader implications of diet, as evidenced by the listed studies. This would not only help contextualize the findings but also potentially open avenues for combinatorial therapeutic approaches involving diet and pharmacological agents like RIF.

Answer: We thank the reviewer for the comment. However, due to the study design, our results do not allow analysis of dietary factors. References to the inflammatory role of different diets was included in the discussion in the response to reviewer comments #2.

Reviewer's Comments - 6: The Discussion should be expanded to speculate on the potential for dietary interventions to modulate the epigenetic and

autophagy markers that were affected by RIF in this study. How might dietary components such as flavonoids alter these markers, and what implications does this have for the prevention or management of NAFLD-HCC?

Answer: It is beyond the scope of this study to discuss additional dietary intervention, however it can be speculated that the model could be reproduced considering dietary intervention with the addition of other elements, such as fiber and flavonoids. They could be beneficial. This was included in the discussion section.

Reviewer's Comments - 7: It would also be beneficial if the authors could address the limitations of their study regarding the exclusion of dietary factors and propose future research directions that include these variables.

Answer: Information inserted into the manuscript's discussion.

Reviewer's Comments - 8: Concluding Remark: The study presents important findings that contribute to the field of hepatology, especially in the context of NAFLD-HCC. However, by integrating the suggested literature into the manuscript, the authors would significantly strengthen the comprehensiveness and relevance of their work to the current scientific dialogue surrounding the impact of diet on liver disease.

Answer: We thank the reviewer for the comments. Dietary aspects were included in the discussion. A recent Brazilian guideline draws attention to the causal aspects related to diet and its control in the management of MASLD, reference DOI 10.20945/2359-4292-2023-0123.