

Manuscript NO.: 84648

Title: Suberoylanilide hydroxamic acid upregulates reticulophagy receptor expression and promotes cell death in hepatocellular carcinoma cells

Dear Editor:

We would like to take this opportunity to thank the editors and reviewers for their constructive suggestions. Accordingly, we have made the necessary changes to the manuscript to acknowledge the concerns of the editors and reviewers, as well as to fully meet the requirements of the publishing policies of World Journal of Gastroenterology. As a result, we believe that the quality of the manuscript is greatly strengthened. All amendments are highlighted in red in the revised manuscript. In addition, point-by-point responses to the comments are listed after this letter. The comments are copied in black and italics, while our responses are in red font.

We hope that it is now acceptable for publication in your journal, and we look forward to hearing from you soon.

Yours sincerely,

Dr. Rujia Xie

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First of all, we would like to express our sincere gratitude to the reviewers for their helpful and valuable comments.

Replies to Reviewer 1

Specific Comments

Comments

1. *It is recommended to deepen the introduction section, especially in the context of epigenetic regulation and hepatocellular carcinoma. What's already known?*

Response: Thank you for raising this concern. In the revised manuscript, we have incorporated the two articles recommended by the reviewers to emphasize the background of the study in the introduction section, as suggested. Moreover, we have added recent advances in the treatment of liver cancer to make the background of the study more coherent.

The revised texts : Epigenetic regulation occurs primarily through DNA methylation, post-translational histone modification, chromatin remodeling, and non-coding RNA-mediated gene silencing. Current therapies targeting epigenetic modifications to cancer mainly include DNA methyltransferases and histone deacetylases, as well as microRNAs. Due to the widespread existence of DNA methylation variation in HCC, a variety of corresponding regulators of DNA methyltransferase have been developed. At the same time, microRNAs such as miR-148a are used in anti-HCC therapy by combining with oncolytic viruses.

Replies to Reviewer 2

Specific Comments

Comments

1. *Introduction section: We believe the evolving systemic treatment scenario for HCC*

should be further discussed and some recently published papers added within the introduction (PMID: 33820447; PMID: 34798793; PMID: 36633661; PMID: 35403533), only for a matter of consistency. We think it might be useful to introduce the topic of this interesting study.

Response: Thank you for the valuable suggestion. We have incorporated these four articles into the introduction section as recommended; moreover, we have added relevant information based on a deep exploration of the systematic treatment options for hepatocellular carcinoma (HCC), which has improved the coherence of the revised introduction section of manuscript. The text added to the revised manuscript is highlighted in red.

2. The authors should expand the Discussion section, including a more personal perspective to reflect on. For example, they could answer the following questions – in order to facilitate the understanding of this complex topic to readers:

① what potential does this study hold?

Response: Thank you for the suggestion. SAHA, a classic HDACi drug, has been approved for the treatment of hematological malignancies and is currently under clinical trials for its therapeutic effect on other solid tumors. In this study, we explored the anti-tumor mechanism of SAHA inhibiting hepatocellular carcinoma and identified a new target of its action, which is associated with the reticulophagy pathway. We explored epigenetics to elucidate the upregulation of histone acetylation and the transcription of related genes. Histone acetylation, whether induced by HDACi alone or in combination with drugs, has been revealed to be a promising target for this anti-cancer drug.

②What are the knowledge gaps and how do researchers tackle them?

Response: Thank you for raising this concern. Firstly, Suberoylanilide hydroxamic acid (SAHA) is the most common histone deacetylase inhibitor that triggers multiple forms of cell death in HCC. It is not clear whether

SAHA utilizes a novel mechanism to induce Huh7 and MHCC97L cells death through a number of different therapeutic targets. The authors found that SAHA can induce cell death through the reticulophagy pathway. When SAHA-induced HCC death forms other than apoptosis were still identified, which could be autophagic death triggered by excessive autophagy, the authors further verified this using BafA1, a specific inhibitor of the late phase of autophagy that restrains autophagosomal fusion with lysosomes. Secondly, the mechanism by which SAHA upregulates reticulophagy receptors is unclear. SAHA is a classic broad-spectrum histone deacetylase inhibitor, and the acetylation of histone H4 lysine-16 (H4K16ac) is closely related to the induction and regulation of autophagy. Therefore, the authors used ChIP to verify whether SAHA can induce reticulophagy by enriching H4K16ac in the *FAM134B* promoter region of HCC cells. Finally, we verified that SAHA treatment augmented *FAM134B* expression and facilitated Huh7 and MHCC97L HCC cell apoptosis in the present study. However, the regulatory mechanisms underlying this effect remain unknown. Recent findings have also identified that *FAM134B*-mediated reticulophagy may regulate ER-mitochondrion interaction and Ca^{2+} deregulation between ER and mitochondria by MERCS led to mitochondrial calcium overload, thereby activating the mitochondria-associated apoptotic pathway. We evaluated the protein level of AMFR and IP3R-GRP75-VDAC1 in Huh7 and MHCC97L cells and found that SAHA could significantly increase its protein levels.

③How do you see this area unfolding in the next 5 years?

Response: Thank you for raising this concern. Our extensive research suggests that epigenetic research will primarily focus on antitumor therapy in the next 5 to 10 years. Moreover, research on the mechanism and the role of epigenetics, which includes studies on the establishment and maintenance of epigenetic information, epigenetic modification, and non-coding RNAs related to epigenetic regulation, will be continued in the next 5 years.

Integrated cell signaling networks that include regulatory networks at different stages, such as epigenetic modification, chromatin remodeling, and gene expression, need to be further elucidated. A deep understanding of the molecular mechanisms, including cell signaling and epigenetic regulation, regulating individual growth, development, and adaptation to the environment is crucial for further research and development. The comprehensive knowledge of these cellular mechanisms would be an important milestone in anti-tumor therapy.

3. One additional little flaw: the authors could better explain the limitations of their work, in the last part of the Discussion.

Response: Thank you for highlighting this deficiency. This section has been revised and modified as suggested by the reviewer. This study has a few limitations: (1) Despite the presence of numerous reticulophagy receptors, we focused on only FAM134B in this manuscript. For comprehensive knowledge, our team will verify multiple receptors in subsequent laboratory studies. (2) Knocking down of the gene encoding FAM134B could verify if it is a key gene in the pathway leading to HCC death. (3) In this experiment, H4K16ac, a highly relevant acetylation site, was selected for the study. However, SAHA acts as a broad-spectrum deacetylase inhibitor influencing numerous acetylation sites, which need to be further verified in subsequent experiments. Results of this study indicate that SAHA can inhibit the proliferation of liver cancer cells *in vitro*; however, *in vivo* analyses could confirm the consistency of its effectiveness. Basic medical research serves the clinic, and the clinical verification of various aspects is more persuasive. Our study provides basic information that aids ongoing and prospective *in vivo* experiment.

Replies to 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 1 Pathological 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. 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) 2023.

Response: Thank you for your valuable suggestion. We have provided the decomposable Figures and organized them into a single PowerPoint file.