OPEN PEER REVIEW

Open Peer Review (OPR)

As a new feature for progressing towards transparency, we decided to open a new window for all of our editors as well as authors titled "**Open Peer Review**". We hope by this new facility, our reviewers will be more motivated and authors will be more satisfied with the review process. We believe that publishing our peer review reports could make a transparent and clear environment for all our efforts within a journal, but not all reviewers tend to publish their comments.

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An "Open Peer Review" process is making the details of all review process (including reviewers, associate editors, and EICs comments) as "Public" as it is agreed by EIC, Authors, and reviewers.

Advantages of "Open Peer Review" process

- More transparency, constructiveness and tactful comments of the peer review process: leads to an increase in the quality of reviews
- More motivations for all involved roles in the review process
- Authors' satisfactions from the review process: Increases honesty between authors and reviewers
- Education of both authors and new students
- Prevents reviewers from following their individual agendas and leading to the detection of reviewers' conflicts of interests

You can find out more at:
https://brieflands.com/briefland/knowledgebase/category/tree.html#opr.html

Hepatitis Monthly
Article DOI: 10.5812/hepatmon-128150
Published in: Hepatitis Monthly: 22(1); e128150

Peer Review Report for "Inhibitory Effects of Gabapentin on the Proliferation and Cell Motility of Hepatocellular Carcinoma Cells"

Author(s): Ye-Fan Mao, Wei-Jia Zhao, Satvinder Mudan, Jia-Cheng Lai, Li-Ming Xu, Qi-Yu Zhang, Yi-Hu Zheng, Chao Ye

Review Timeline:

Submit Date: 21 May 2022
Revised Date: 10 Aug 2022
Accepted Date: 15 Aug 2022

Revision (0)

Here, you can see the **Reviewers**, **Associate Editors** and **EICs'** comments from the beginning to the end of the revision process.

REFEREE: Reviewer | Revision (0)

21 Jun 2022

The manuscript "The studies on inhibitory effects of Gabapentin on the proliferation and cell motility of hepatocellular carcinoma cells" aimed to analyze the anti-cancer effects of Gabapentin against HCC cells in vitro and in vivo. The paper is generally well written and, with some changes, may be published in the Journal.

I recommend deleting "studies" from the title and including only the role of Gabapentin.

The abstract is well written, structured, and balanced and covers the paper's main aspects.

The Introduction correctly covers the field and the objectives of the present study. The methods are well presented regarding the materials, research steps and statistical analysis.

Regarding the presentation of the results, I consider that the part about the RNA-sequencing profile in Huh7 cells must be improved to be easy to understand what was done and how the role of Gabapentin treatment was evaluated. The rest of the results are well presented, including images to make the presentation easy to follow. There is no repetition of the data presented.

The Discussion chapter is appropriate and states correctly that these data support a preliminary explanation of the inhibitory effect of Gabapentin on HCC cells and the possible

role in the clinical environment. It is clear that further clinical studies are needed in this field. The data presented here support the conclusions of this paper.
The study was performed according to ethical approval from the academic institution.
The list of references includes 24 titles, only 11 being more recent than 5 years. Probably some more recent studies may be included.
The use of the English language is generally correct, but still, some minor errors should be corrected.

REFEREE: Reviewer | **Revision** (0) 23 Jun 2022

1. Please mention the number of ethical approval
2. Please describe the flow chart of this study
3. Please explain the conclusion of this study
4. Please compare Gabapentin and other cytotoxic drug for hepatoma (or liver tumor)
5. Please add more references in discussion

REFEREE: Associate Editor | **Revision** (0) 23 Jun 2022

The manuscript needs "major revision" based on
raised comments of the reviewers. To this end, authors may exactly indicate the number of ethical approval, describe the flow chart and conclusion of the study and compare Gabapentin and other cytotoxic drug reported as therapy against HCC by using more and updated references in the discussion section. The "scientific writing style might also be improved by considering the reviewers comment.

Seyed Moayed Alavian: EIC | **Revision** (0) 23 Jun 2022

Dear Authors,

Thank you for your submission. Please follow the peer-review comments and submit a revision. I have additional comments:

1. Please add the study design and type in the beginning of methods section of abstract. A subheading describing the study design can be added in the beginning of methods section of the main manuscript body.

2. Add the limitations of your study to discussion.

Thanks
EIC

OPEN PEER REVIEW

Revision (1)

Reply to Reviewers

Ideally, the reviewing process can significantly improve the submitted manuscripts by allowing the authors to take into account the advice of reviewers. Author(s) must reply to all reviewers' comments in a separate Word file, point by point. A "**Reply to Reviewers**" document is submitted along with revised manuscript during submission of revised files, summarizing the changes that the authors made in response to the reviewers' comments. The responses to reviewers' comments specifies how the authors addressed each comment the reviewers made.

You can read the authors' responses to the reviewers' comments in the next page.

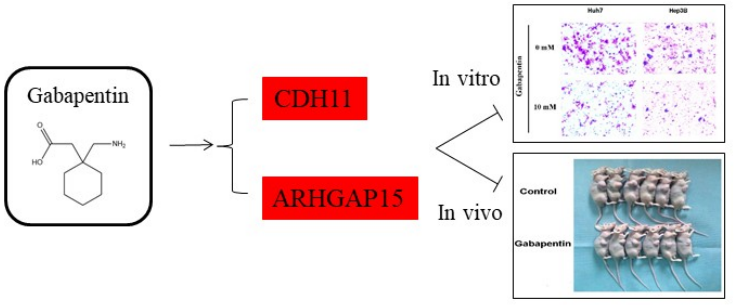
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Reply to Reviewer1:

Thank you very much for your valuable advice. For your questions, I would like to give the following answers :

1. This study did not refer to human biology materials and did not need ethical approval involving human participants. All animal procedures were carried out in compliance with the National Institute of Standards and Practices for the Care and Use of Laboratory Animals. The first affiliated hospital of wenzhou medical university started to assigned every animal experiment a series number in 2020. But we completed animal experiments before 2020. So our animal experiments did not have a number of ethical approval.
2. The flow chart has been shown in the article (fig.7).



HCC Cell Proliferation

3. In this study, MTT assay, Transwell cell migration assay, RNA-seq analysis and xenotransplantation tumor model in mice were used to demonstrate that Gabapentin has inhibitory effects on the growth, motility and tumorigenesis of hepatocellular carcinoma cells.
4. Previous our studies have shown that BCAT1 is a key enzyme in BCAA catabolism, and its expression is significantly correlated with HCC cell proliferation and chemoresistance to cisplatin [1-2]. This article our research focus on gabapentin as an inhibitor of BCAT1 to explore its effect on HCC treatment. Moreover, other cytotoxic drugs have been reported in previous studies, and their mechanism of action on HCC is comparatively definite. So we did not pay more attention to other cytotoxic drug for HCC treatment in this study.
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[1] Luo L, Sun W, Zhu W, Li S, Zhang W, Xu X, Fang D, Grahn THM, Jiang L, Zheng Y. BCAT1

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Reply to Reviewer2:

Thank you very much for your valuable advice. For your questions, I would like to give the following answers :

1. It has been deleted "studies" from the title according to your suggestions, Title changed to ” The inhibitory effects of Gabapentin on the proliferation and cell motility of hepatocellular carcinoma cells”.
2. The experimental and analysis processes for the RNA-sequencing profile in Huh7 cells are complicated. We tried our best to illustrate it concisely. But most of the experimental procedures are confirmed, which described by other studies. Moreover, the role of Gabapentin treatment was evaluated by DEGs and KEGG analysis. Some genes such as CDH11 and ARHGAP5 are supposed to account for the possible mechanism of Gabapentin. We have tried our best to modify them.
3. We have added more discussion and literature. However, there are a few in-depth studies on

the effect of Gabapentin on cancer research recently. Hence, there is not enough references available for adding. We are sincerely sorry.

4. We have tried our best to polish the language of the article. And the minor errors regarding english grammar have been corrected.

The manuscript needs "major revision" based on raised comments of the reviewers. To this end, authors may exactly indicate the number of ethical approval, describe the flow chart and conclusion of the study and compare Gabapentin and other cytotoxic drug reported as therapy against HCC by using more and updated references in the discussion section. The "scientific writing style might also be improved by considering the reviewers comment.

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Thank you very much for your valuable advice. For your questions, I would like to give the following answers :
Relevant changes have been shown in the manuscript and attachments.

1. Please add the study design and type in the beginning of methods section of abstract. A subheading describing the study design can be added in the beginning of methods section of the main manuscript body.
2. Add the limitations of your study to discussion.

Reply to EIC:

Thank you very much for your valuable advice. For your questions, I would like to give the following answers :

1. Study design and type have been added at the beginning of the abstract and subheadings have been added at the beginning of the text of the manuscript.
2. The limitations of additional studies have been addressed in the discussion.

OPEN PEER REVIEW

Revision (1)

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REFEREE: Reviewer | **Revision** (1) 1 Aug 2022

The authors answered to my comments as follows:
"1. It has been deleted "studies" from the title according to your suggestions, Title changed to ”The inhibitory effects of Gabapentin on the proliferation and cell motility of hepatocellular carcinoma cells”.
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Still, there are some minor improvements that I considered needed:
- I would not mention in the Abstract that this is a Research study. That is clear!
- I would not use abbreviated words in the list of keywords
- all abbreviated words must be explained at their first use in the Abstract and in the Text of the manuscript

REFEREE: Associate Editor | **Revision** (1) 9 Aug 2022

The manuscript requires minor revision based on the referee's comments to reach to the acceptance state.

Seyed Moayed Alavian: EIC | **Revision** (1) 9 Aug 2022

Dear Authors,

There are additional comments on your revised manuscript. Please follow them and submit a revision.

Regards,

EIC

OPEN PEER REVIEW

Revision (2)

Reply to Reviewers

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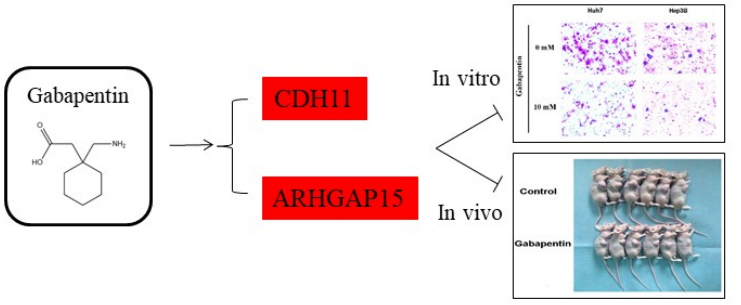
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OPEN PEER REVIEW

Revision (2)

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REFEREE: Reviewer | **Revision** (2) 13 Aug 2022

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The paper is generally well written and may be published in the Journal in the present form after changes recommended by the reviewers and addressed by the authors.

The Abstract is well written, structured, balanced, and covers the paper's main aspects.
The abbreviate swords were explained at the first use. The keywords were corrected.
The Introduction covers the field and the objectives of the present study as gabapentin may have the potential to inhibit BCAT1 and inhibit the growth, migration, and tumorigenesis of hepatoma cells.
The methods are well presented regarding the reagents, cell cultures, cell proliferation, transwell cell migration assays, RNA-seq and analyses, mouse xenograft tumor models, quantitative real-time PCR, and statistical analysis.
The results are well presented, including figures. There is no repetition of the data presented.
The Discussion chapter is appropriate and states correctly that these data support a preliminary explanation of the inhibitory effect of Gabapentin on HCC cells and the possible role in the clinical environment. It is clear that further clinical studies are needed in this field. The authors included some changes asked for by the reviewers.
The conclusions of the study are supported by the data presented.
The study was performed according to ethical approval from the academic institution.
The authors updated the list of references from 24 titles to 31 titles, including four more titles more recent than five years.
The English language is generally correct, but still, there are some minor editing errors (space).

REFEREE: Associate Editor | **Revision** (2) 15 Aug 2022

Authors have successfully addressed the last comments of the esteemed referee in their second revision of the manuscript. In this condition I suggest acceptance of the manuscript for publication.

Seyed Moayed Alavian: EIC | **Revision** (2) 15 Aug 2022

Dear Authors,

Thank you for submitting the revised manuscript. I am delighted to inform that your manuscript is accepted for publication in Hepatitis Monthly journal.

Regards,
EIC