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.

What is "Open Peer Review" process?

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://sites.kowsarpub.com/kowsar/knowledgebase/category/tree.html#opr.html

Jentashapir Journal of Cellular and Molecular Biology
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Peer Review Report for "Effects of Toxicity Induced by Gentamicin on the Kidney of Killifish *Aphaniops hormuzensis* and the Role of Wt1 and MMP9 Genes in Response to This Toxicity"

Author(s): Mina Motamedi, Atefeh Iranmanesh, Azad Teimori, Sara Soltanian

Review Timeline:

Submit Date:	26 Aug 2020
Revised Date:	22 Sep 2020
Accepted Date:	23 Sep 2020

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) — 28 Aug 2020

Dear AE,
Comments on the article titled "The effects of toxicity induced by gentamicin on the kidney of killifish Aphaniops hormuzensis and the role of Wt1 and MMP9 genes in response to this toxicity
- Plagiarism detection in the manuscript: ok
- Analysis method of the manuscript: ok
- Results: ok
- Correct referencing and citations: ok
- Correct and proper English writing: The findings of this study on gentamicin-related kidney toxicity are interesting and beneficial, particularly in genetic evaluating. The study is well structured and only requires some lexical revising,(Native English editing)
- The decision about this manuscript (accept/revision/reject).: minor revision
Thanks.

REFEREE: Reviewer | **Revision** (0) — 31 Aug 2020

Dear AE,

The authors examined the effect of gentamicin toxicity in kidneys using small fish species belonging to the family Aphaniidae. It is a well-considered and unique study for the trait of the endemic fish in West Asia. I recommend this manuscript is published in the Jentashapir Journal of Cellular and Molecular Biology. However, some minor revisions and more detailed discussions are required to be done by the authors. The point-by-point suggestion is described below.

Major

Introduction

- You must explain a more detailed significance why you analyzed Aphaniops hormuzensis.
- Do they have a unique characteristic in kidney function because of living in salty water?
- Has anyone already analyzed that in related species? Or, are you the first one to analyze that using the family Aphaniidae?
- The author suggests that MMP9 plays a role to remove the damaged tissues. However, the substrates for MMP9 is known to be extracellular matrixes (ECMs) not cell component. Which type of cells cleans up the dead cell-derived component? Did you check macrophage aggregation or the expression of related genes? Does the MMP9-mediated ECM digestion assist a macrophage migration into the injured tissue? It might require a deeper discussion about renal regeneration.
- The author suggests that Wt-1 is downregulated on the time to nephrogenic bodies' maturation. If the hypothesis is true, the expression level for wt-1 should be near to the untreated sample (control). However, the result of the 7-dpi showed grossly lower expression than the control. How do the authors explain that?
- Related the above, the reference 16 & 17 showed the expression of wt-1 by whole-mount in situ hybridization (WISH). The method has an advantage over pPCR. That is information for gene expression location. Could you add WISH data and improved discussion including spatial information?

Minor

Figure 1

- You provided the data in separated three images. The band size looks like differences between the first and second images. Please add a molecular weight marker in each image. Or, all samples should be loaded in a single gel. The RNA in lane 4 looks completely degraded. What happened in this sample? Why did you use this image?

Figure2

- I did not sure of the band size from this image. As possible, please improve the quality of the image as Figure 1. Besides, higher density agarose is suitable in the case of such small nucleotides. Please retry this assay using approximately 2.0% agarose gel.

Figure3

- What does the "hematopoietic tissue" mean? To my knowledge, it is a complex structure of hematopoietic stem cells (including differentiated blood cells) and hematopoietic niches. Do the epithelial cells or nephron cells form a part of the hematopoietic niche or not? It must be a precise description. I think the "hematopoietic tissue" you indicated in the figure is the blood cell population including HSC.

Figure5

- The label on the vertical axis is "MMP6".

Thanks.

Seyyed Ali Mard: Associate Editor | **Revision** (0) 31 Aug 2020

Dear EIC,
A Major revision is recommended by reviewers.
Thanks.

Seyyed Ali Mard: EIC | **Revision** (0) 31 Aug 2020

Dear Authors
According to the comments forwarded by reviewers your manuscript needs to be revised before getting acceptance. I encourage you to submit it and correct your manuscript in accordance with their comments point-to-point to avoid extra revision. The main policy of this journal is to speed up the time of p Peer- review as soon as possible. Thus, I encourage you to submit your revision as soon as possible.
Thanks.

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.

Dear respected editor,

Many thanks for sending our manuscript for revision. We also thanks to the respected reviewers for their valuable comments. They were really constructive and we did almost all of them and explains a few of them. Our point by point answers to the comments of the respected reviews is included below. Our answers are highlighted in yellow.

Accordingly, we submitted two versions of the revised manuscript. One is a “clean” version, and one is “with track changes” version so that all the changes requested by the reviewers could be followed.

We hope the manuscript is now acceptable.

Best wishes

Mina Motamedi

Azad Teimori

POINT BY POINT ANSWERS TO THE COMMENTS

Reviewers' Comments:

Reviewer 1:

Comments on the article titled "The effects of toxicity induced by gentamicin on the kidney of killifish Aphaniops hormuzensis and the role of Wt1 and MMP9 genes in response to this toxicity

Plagiarism detection in the manuscript: ok

Analysis method of the manuscript: ok

Results: ok

Correct referencing and citations: ok

Correct and proper English writing: The findings of this study on the gentamicin-related kidney toxicity are interesting and beneficial, particularly in genetic evaluating. The study is well structured and only requires some lexical revising,(Native English editing).

ANSWER: many thanks for your positive view on this manuscript. The English is checked once again and improved.

Your decision about this manuscript (accept/revision/reject).:minor revision

Reviewer 2:

The authors examined effect of gentamicin toxicity in kidney using small fish species belonging to the family Aphaniidae. It is well considered and unique study for the trait of the endemic fish in the West Asia. I recommend this manuscript is published in Jentashapir Journal of Cellular and Molecular Biology. However, some minor revision and more detailed discussion are required. The point-by-point suggestion is described below.

Major
Introduction
You must explain a more detailed significance why you analyzed Aphaniops hormuzensis.

ANSWER:Thanks for the comment, this comment is applied in the introduction part.

Do they have unique character in the kidney function because of living in salty water?

ANSWER: We added a paragraph to the introduction to apply this comment of the respected reviewer

Has anyone already analyzed that in related species? Or, are you the first one to analyze that using the family Aphaniidae?

ANSWER: We are the only group that started an investigation on the material toxicity and regeneration aspects of this Iranian endemic species and the family Aphaniidae. We notify this in the manuscript.

The author suggests that MMP9 plays a role to remove the damaged tissues. However, the substrates for MMP9 is known to be extracellular matrixes (ECMs) not cell component. Which type cells clean up the dead cell-derived component? Did you check macrophage aggregation or the expression of related genes? Does the MMP9-mediated ECM digestion assist a macrophage migration into the injured tissue? It might require deeper discussion about the renal regeneration.

ANSWER: Thanks for the comment, that's right, that MMPs play role in the infammatory phase of regeneration, by activation of chemokines and then mediating leukocyte recruitment. Moreover, the macrophage most probably involves in the renal regeneration after toxicity in our fish model. We have not checked the macrophage related genes, but we aware of this good comment of the respected reviewer and will certainly consider this comment (and the other one in below) for our new grant and next project. However, to consider the comment of the respected reviewer comment, we added more detail regarding MMPs role in ECM digestion and there after, its contribution to a macrophage migration into the injured tissue in the discussion part.

The author suggests that Wt-1 is down regulated on the time to nephrogenic bodies' maturation. If the hypothesis is truth, the expression level for wt-1 should be near to the untreated sample (control). However, the result of the 7-dpi showed grossly lower expression than the control. How do the authors explain that?

ANSWER: Thanks for the comment. Regarding Wt1 expression, this gene has shown various expression patterns during kidney development or regeneration. These expression alternation have been studied in medeka fish by ISH and we added this into the discussion part (lines 189- 193). Moreover, in other

studies on the mice nephrogenesis, there is a down regulation of Wt1 expression in the adult mice in compare to the neonatal and embryonic stages (Metsuyanim et al., 2020).

As respected reviewer correctly mentioned, in our result the expression of Wt1 down regulated than the control. We have rechecked our qRT-PCr and there was no mistakes on result. Howover, the possible explanation for such a expression pattern of Wt1 in our study is that it might be upregulated on several days after 7dpi and it come close to the control. Therefore, investigation on days after our last time point (i.e., 7 dpi) should be applied to show the regulatin of this gene. We have included this explanation and recommend this in our manuscript text.

Related the above, the reference 16 & 17 showed the expression of wt-1 by whole-mount in situ hybridization (WISH). The method has an advantage than pPCR. That is information for gene expression location. Could you add WISH data and improved discussion including spatial information?

ANSWER: That's correct and the WISH experiments will clear up more exactly the expression parts of these studied genes. However, we have not included the WISH experiment because of the low amount of grant for this project. But we keep this valuable comment of a respected reviewer for our new grant and further detailed analysis regarding the renal regeneration aspect on this species.

Minor

Figure1

You provided the data in separated three images. The band size looks like different between the first and second images. Please add molecular weight marker in each image. Or, all sample should be loaded in single gel. The RNA in lane 4 looks like completely degraded. What happened in this sample? Why did you use this image?

ANSWER: Many thanks for notifying this point; we have changed the figure based on the respected reviewer comment. The lane 3 and 4 belong to the RNA extraction of 7dpi. We omitted the lane 4 in this figure because of its low quality. It should be noted that this sample has not been used in q-PCR. We also correct the caption.

Figure2

I did not sure the band size from this image. As possible, please improve the quality of image as Figure 1. Besides, higher density agarose is suitable in case of such small nucleotides. Please retry this assay using approximately 2.0% agarose gel.

ANSWER: Thanks for the comment, we have tried our best to improve the image quality in the revised version and also the ladder band size included to the image. With respected to the second part of the comment of the respected reviewer, since the student could not be in the lab because of the coronavirous condition, it is not possible to retry the aasy by 2.0% agarose gel. I hope this excuse would be acceptable.

Figure3

What does the "hematopoietic tissue" mean? As my knowledge, it is complexed structure of hematopoietic stem cells (including differentiated blood cells) and hematopoitetic niche. Do the epithelial cells or nephron cells form a part of hematopoietic niche or not? It must be precise description. I think the "hematopoietic tissue" you indicated in the figure is blood cell population including HSC.

ANSWER: In the bone of these fishes, there is no bone marrow and the kidney involves in the blood cell production besides its excretion and osmoregulation functions. the interstitial stroma of the kidney includes hematopoietic tissue which contains the hematopoietic stem cells (HSC) blood cells, renal progenitors and other mesenchymal cell types. This information is included in the introduction part to avoid ambiguity.

Figure5

The label in the vertical axis is "MMP6".

ANSWER: The MMP6 has already been indicated in the vertical axis.

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Revision (1)

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

REFEREE: Reviewer | **Revision** (1) 7 Sep 2020

Dear AE,
I am basically agreed to the publication of the manuscript.
However, I still have one question.
The authors described that Figure5 indicates the expression level of the MMP9 gene.
The figure title is "The transcription levels of MMP9..."; however, the label in the vertical axis is "MMP6".
Is it correct? Please check it again before publication.
Thanks.

Seyyed Ali Mard: Associate Editor | **Revision** (1) 7 Sep 2020

Dear EIC,
It is provisionally accepted.
Thanks.

Seyyed Ali Mard: EIC | **Revision** (1) 7 Sep 2020

Dear Author
Your manuscript is provisionally accepted now but a correction needs to be done in accordance with the reviewer comment. Please correct mentioned figure and add Ethical approval in the last part of your manuscript as well as in Method. Doing these corrections mean acceptance of your manuscript. I encourage you to submit your revision as soon as possible.
Best regards

OPEN PEER REVIEW

Revision (2)

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.

Dear respected editor,

Many thanks for sending our manuscript for revision. We also thanks to the respected reviewer for his/her careful check and sorry for our previous misunderstanding of the comment about Figure 5. Now it is corrected. We hope the manuscript is now acceptable. The Ethical approval is added in the Method section and also in the last part of the manuscript.

Best wishes

Mina Motamedi

Azad Teimori

OPEN PEER REVIEW

Revision (2)

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

Seyyed Ali Mard: Associate Editor | **Revision** (2) 23 Sep 2020

Dear EIC,
The authors added ethical approval and corrected figure 5. Therefore, their manuscript is now accepted for publication.
Best

Seyyed Ali Mard: EIC | **Revision** (2) 23 Sep 2020

Dear Authors,
You added ethical approval and corrected figure 5. Therefore, the manuscript is now accepted for publication.
Best