Peer Review File

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Reviewer A

The authors provide a good basic science experiment evaluating the pathogenesis of lenvatinib resistance (LR). It is an area of good need. I would recommend the authors address following, prior to consideration for publication

1) The first 3 sentences in introduction and discussion need references
Reply: This paragraph shares the reference with the following sentences. See line 59-63，line 418-421.

2) The authors mention the results in the end of introduction section. These should be referred to only in the results, and discussion section
Reply: It has already been mentioned in the text. See line 247，line429-482

3) In the study, the authors show an association between AKR1C1 levels and LR. However, this is far from causation. The authors do not run experiments to confirm causation of LR through AKR1C1. If they have, these should be mentioned in limitations discussion of the study
Reply: In future experiments, we will further investigate.

4) The discussion section needs a limitations section as well.
Reply: We mentioned the shortcomings of existing research.

5) The author's conclusion is not supported by data. Would say they show association,
Reply: Our experimental data confirms our hypothesis.

6) How should this association be assessed in clinical studies? Few sentences about future direction in confirming, next steps about LR should be discussed.
Reply: Thank you for your comment. We will further investigate.

7) Small grammatical mistakes through the manuscript. Would recommend another revision of English language.
Reply: Thank you for your comment. We will make further language modifications.

This is a good basic science experiment, that addresses a new area of need. Lenvatinib resistance has been studied by them. However, the authors suggest causation based on 1 experiment and linking findings to online databases, KEGG and cibersort, to speculate on involved biochemical pathways.

In the experiment, the authors need further testing to assess the causative association. This has not been provided, and would likely need to mentioned in limitations, or recommended as next steps.

Reviewer B

This article demonstrates that overexpression of AKR1C1 induces Lenvatinib-resistance in HCC. The authors identified AKR1C1 through RNA-seq and in silico analysis. Although the authors present a substantial amount of data, there is a significant gap between their conclusion and the data they provide, which renders the manuscript unsuitable for publication.

1. The concentration of Lenvatinib used in this study is relatively high, as suggested by previous literature (e.g., PMID: 34884875). Although the authors mention 20uM Lenvatinib as a low dose, it would be helpful if they presented the IC50 value and provided further justification for their chosen concentration.
Reply: This depends on the cell state.

2. The authors identified AKR1C1 and SERPINE1 using MHCC-97H cells, which their data suggests are relatively resistant to Lenvatinib. This approach may not be suitable for identifying genes related to Lenvatinib resistance at different concentrations, as these cells have already acquired resistance. It would be more appropriate to compare the results from MHCC-97H cells with those from other cell lines to validate the findings.
Reply: We use tissue chips for validation.

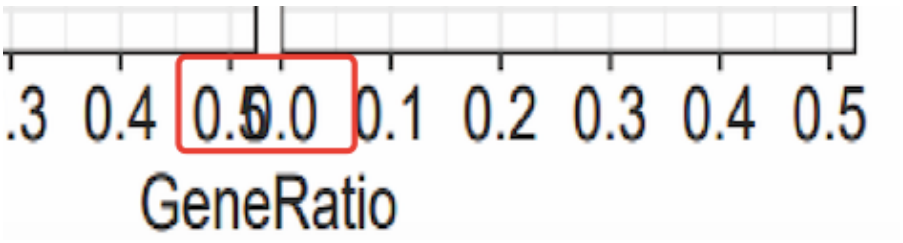
3. The authors do not show any scientific evidence of AKR1C1 inducing Lenvatinib resistance though the title states "AKR1C1 overexpression leads to Lenvatinib Resistance". They should conduct experiments to demonstrate AKR1C1 overexpression and knockdown induce Lenvatinib resistance and Lenvatinib sensitivity in HCC respectively.
Reply: We validated this hypothesis through experiments. See line 159-255.

4. In line of 178, OS means "overall survival", not over survival.
Reply: Thank you for your comment.

Reviewer C

1. **Figure 2C**

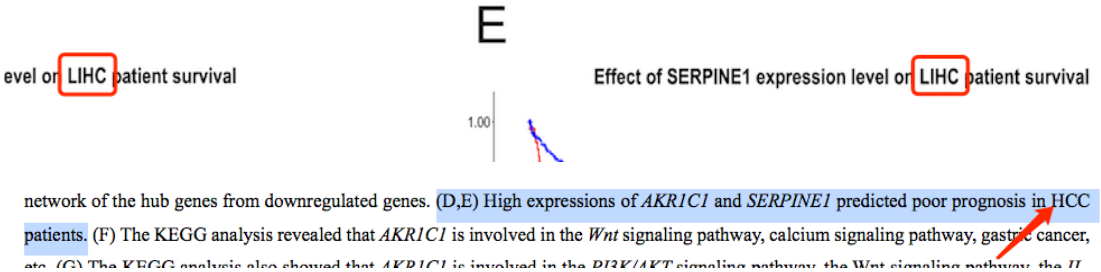
Please check the numbers below, as they overlapped.



Reply: We have made modifications.

2. **Figure 3**

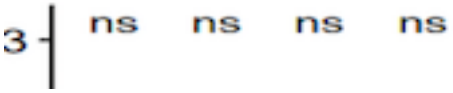
a. Please check if "LIHC" is correct here, as it is "HCC" in figure legend.



network of the hub genes from downregulated genes. (D,E) High expressions of *AKR1C1* and *SERPINE1* predicted poor prognosis in HCC patients. (F) The KEGG analysis revealed that *AKR1C1* is involved in the *Wnt* signaling pathway, calcium signaling pathway, gastric cancer, etc. (G) The KEGG analysis also showed that *AKR1C1* is involved in the *PI3K/AKT* signaling pathway, the Wnt signaling pathway, the *IL*

Reply: We have made modifications.

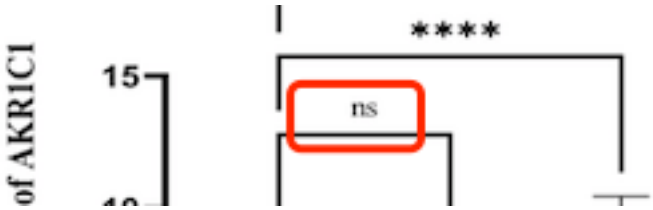
b. Please indicate the meaning of "ns" in figure legend.



Reply: We have made modifications.

3. **Figure 4**

Please indicate the meaning of "ns" in figure legend.



4. **Please indicate the source of animals (bough from where) in the Methods section.**

##Tumor xenografts

Females BALB/c nude mice (5 weeks old, weight: 18–21 g) were used for the xenograft models. A

Reply: We supplement the purchasing sources of nude mice here.