Peer Review File

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Reviewer A

The work entitled "HES1 promotes autophagy through ITPR1 induction to exert anti-metastatic effects in Pituitary adenoma," carried out by the authors, demonstrated a sophisticated regulatory network involving HES1, ITPR1, and autophagy in PA progression. To do so, they applied a routinely-used bioinformatics approach towards plus in vitro assays to investigate these findings. Overall, the performed study is of scientific importance and can be considered after major revision. There are essential recommendations regarding of the correction of the manuscript:

- Is important to note, that in the abstract the concept of the study that involved specific genes and molecular mechanisms aren't linked very well. It’s important to better connect the topics for better clarify the experimental design.

Reply 1: Thank you very much for your review and valuable comments on our paper. Regarding the insufficient linkage between specific genes and molecular mechanisms in the abstract, this is an oversight on our part. I understand that the abstract is an important part of the thesis and as such it should better reflect the experimental design and subject matter. In order to better articulate our research, I have reorganized the abstract section to ensure a clearer connection between specific genes and molecular mechanisms in the study. Thank you again for your suggestions, we have worked hard to refine the paper to ensure better communication of our findings.

Changes in the text: We have modified our text as advised (see Page2, line 32-53).

- In the introduction, would be interesting included the % of overall survival from the patients with PA disease and also, the collateral effects from treatment.

Reply 2: Thank you for your valuable comments and feedback. Regarding your mention of including the overall survival rate of patients with PA disease and the side effects of the treatment in the introduction section, this is very important for our study. I have added the relevant content in the introduction. Changes have been made to the introduction and to ensure that all information is supported by reliable literature sources. PA was increasingly recognized in the general population, with an incidence ranging between 3.9 to 7.4 cases annually per 100,000 people. Despite this, their overall prevalence suggests they affect roughly 1 in 1,000 individuals. Notably, prolactinomas and nonsecreting pituitary adenomas make up the majority of these cases. While clinically significant pituitary adenomas are more common in females, their clinical presentations vary widely. These adenomas can lead to hormone imbalances and visual field defects. In cases with larger tumors, they might also result in

hypopituitarism due to the tumor's mass effect. Thank you again for your suggestions, which are very helpful in improving the paper.
Changes in the text: We have modified our text as advised (see Page 2-3, line59-67).

- The concept study has focus on the molecular therapy, so, the authors need to improve the application of this in clinical routine (e.g. precision medicine).
Reply 3: Thank you for carefully reading our paper and giving your valuable feedback. We agree with you about applying research on molecular therapies more deeply to daily clinical operations. We have improved our paper by adding a description of the use of molecular therapies in medicine that clearly articulates their importance in diagnosis, treatment selection, and patient management. Discuss the potential benefits and limitations of molecular therapies in clinical practice in the context of current research advances. Cite some relevant clinical studies to support our views and conclusions. These improvements and refinements can give our paper more depth and breadth and better fulfill the expectations of our readers and the academic community. Thank you again for your valuable suggestion.
Changes in the text: We have modified our text as advised (see Page 3, line 79-82).

- Material and methods - how the authors applied for choose criteria of fold change (FC) (Upregulated and downregulated DEGs).
Reply 4: Thank you for your careful review of our paper. Regarding the "How to select fold change (FC) criteria" in the Materials and Methods section, in our study, we used fold change (FC) as an important indicator for selecting differentially expressed genes (DEGs). Specifically, we chose FC > 1.5 as a criterion for up-regulated genes and FC < 0.67 as a criterion for down-regulated genes. In addition to FC, we also considered p-value as a criterion for identifying DEGs. We considered a gene to be differentially expressed only if the FC exceeded the threshold we set and the p-value was below 0.05. We also looked at the overall distribution of gene expression to ensure that the threshold was chosen to capture a sufficient number of DEGs while avoiding too many false positives. Thank you again for your valuable input.
Changes in the text: We have modified our text as advised (see Page 5, line 127-135).

- In all manuscript, the name of the genes needs to be in italic form. I recommend the correction.
Reply 5: Thank you for the careful feedback. The lack of italicization of genes in the article was our mistake and we apologized for that. I have followed the standard and corrected the italicized formatting of the gene names in the full article. Thank you again for your suggestion.
Changes in the text: We have modified our text as advised (see all manuscript)

- Is important to include the reference of catalog number and company of all antibodies used in this study.

Reply 6: Thank you very much for your suggestions and review comments. Regarding the importance of catalog numbers and citations of company sources for antibodies, we apologized for not stating in the article that it was an oversight on our part. Providing this information would have provided readers with more specific details of the experiments, ensured reproducibility of the experiments, and allowed other researchers to validate our results using the same tools. We have included a detailed list of catalog numbers and manufacturing companies for all antibodies used in the revised manuscript. We believe this will make our study more transparent and reliable. Thank you again for your valuable suggestion.

Changes in the text: We have modified our text as advised (see Page 7, line 186-189).

- A big concern exists here: the in vitro assays were performed in triplicate? This include the WB and quantification also that need to be provided.

Reply 7: Thank you very much for your valuable comments on our study. For the reproducibility verification of the experiments, we did perform three independent replications of all in vitro experiments to ensure the stability and reliability of the data. And a clear indication is made in the statistical analysis in the Materials and Methods section. This is not only in line with the routine laboratory procedures, but also ensures the accuracy of the experimental results. WB and quantification: Regarding your question about WB and its quantitative data, we did perform three independent WB experiments for each sample and quantitative analysis accordingly. In order to better present these data, we can provide complete WB images and corresponding graphs of quantification data as well as raw data in the revised paper to demonstrate the reproducibility and accuracy of our experiments. Thank you again for your valuable comments and suggestions.

Changes in the text: We have modified our text as advised (see Page 8, line 235-236). We added some data (see Figures 3-6).

- About the ROC analysis. How the authors performed this analysis? Please, explain the comparison.

Reply 8: Thank you for your comments and questions about the ROC analysis. Execution of the ROC analysis: We first extracted the True Positive Rate (TPR) and the False Positive Rate (FPR) from the dataset as the main parameters of the ROC curve. The probability of each sample being a positive case was obtained by predicting the test set using our model. Different thresholds were set, and for each threshold, we calculated the corresponding TPR and FPR. the ROC curve was plotted using TPR as the y-axis and FPR as the x-axis. We compared the ROC curves of multiple models with the aim of finding out which model performs best

for the classification task. By comparing the area under the ROC curve (i.e., the AUC value) of different models, we can determine which model performs better. the closer the AUC value is to 1, the better the model performs. We also observed which model has higher TPR under a specific FPR threshold, which helps us to make model selection as needed in practical applications. Thank you again for your valuable comments.
Changes in the text: No changes in the article.

- I strongly recommend to the authors provide the KM plot analysis of all seven genes of interest.
Reply 9: Thank you very much for your valuable comments and suggestion. Regarding the provision of KM curve analysis for the seven genes used in our study, we fully understand your request, however, there are a number of technical and data limitations that prevent us from providing this analysis at this time. TCGA database limitations: Survival information for pituitary tumor samples is not available in the TCGA database, which precludes us from performing survival time analysis. Database limitations used: our study relied on databases on pituitary tumors such as GSE36314 and GSE119063, which also do not contain survival information for pituitary tumors. To fulfill your request, we diligently searched other relevant databases including GSE51618, GSE46311, GSE22812, GSE2966, GSE169498, GSE37153, GSE213527, GSE20149, and GSE120350, but unfortunately these databases also did not have relevant survival time data available for analysis. We fully recognized this limitation and understand the importance of survival analysis to the study, and hope you can understand us. Therefore, we plan to obtain survival data for pituitary tumors through clinical samples or other feasible avenues in future studies to more comprehensively analyze the survival associations of the genes of interest. This will help to further improve our study. Thank you again for your review and valuable suggestions, we will do our best in order to improve our study and consider your feedback as valuable guidance.
Changes in the text: No changes in the article.

- It's not clear the point when ITPR1 received the highlight in the study. Also, HES1. I think that a very important results about the mechanisms of the interplay among these genes exist here. But the connections through the text and results is not clear. I suggest for the authors improve these points in the text.
Reply 10: First of all, I would like to thank you for your careful reading and valuable comments on our paper. There was a lack of clarity regarding the roles played by ITPR1 and HES1 in the study, and what you considered to be important results regarding the mechanism of interaction between these genes were not clearly presented in the current article. This was an oversight on our part and we deeply apologized. We have revised the article, and in the introduction and section of the paper, we have provided a more detailed description of the importance of ITPR1 and explained why it was emphasized in

our study. As well as clearly describing its relationship with other genes and its place in the overall study, ensuring that the mechanisms regarding the interaction of these two genes are fully explained and discussed in the text. We hope that these revisions will solve the problems you mentioned and make the paper better. Thank you again for your valuable comments.
Changes in the text: We have modified our text as advised (see Page 4-5, line 107-122).

Reviewer B

Comment 1. The author's name cited in text should be consistent with the reference.
Comment 2. You refer to "studies" with only one literature citation several times in the main text. Please check and revise.
Comment 3. Indicate where to cite Figure 1F-1H and 4G-4H in the text and note that subfigures should be cited consecutively.
Comment 4. The scope of subfigure is unclear. Please revise Figure 3 and 4.
Comment 5. Please add the scale bars and staining methods of Figure 4C, 4F, 6D, and 6G.
Reply 1-5: I have made modifications and some figures have also been modified.

Comment 6. Reference #4 and #24 are the same. Please delete one of them and number the rest of the references consecutively in the order.
Response: Thank you very much for your valuable comments on my thesis. I have carefully reviewed your suggestions and have carefully corrected the issues. Regarding the issues you mentioned, I have specifically checked References #4 and #24 and realized that they are actually the same references. We apologize for this oversight. To eliminate the duplication, I have deleted Reference #24, renumbered Reference #4, and renumbered the remaining references to ensure that they are consecutively numbered in order. Thank you again for your careful review and valuable suggestions.

Comment 7. Please use the number reference system to cite the article.

232 Ltd., China. Cells were subsequently subcultured in accordance with the
233 manufacturer's directions (Panfil A R et al., 2016) (22). After transfection with the

Response: Thank you very much for your valuable comments on my thesis. I have read and carefully considered your suggestion and do think that using a digital citation system to cite the article is a reasonable suggestion. Regarding the repetition of author's information in the references, we apologize for this, it is our negligence and mistake. In order to follow your advice, I have revised the paper accordingly by removing the sections containing author information. This not only improves the clarity of the citations, but also helps the reader to track and access the relevant literature more easily. Once again, thank you for your

patience in reviewing and providing professional advice, your valuable time is deeply appreciated.

Comment 8. The sum of GSE119063 DEGs-up in Figure 1C is 506, which does not match with Figure 1B and the descriptions in the manuscript. The same goes for GSE119063 DEGs-down.

Response: Thank you for your careful review of our paper and for raising the issue about the mismatch between the total number of DEGs-up and DEGs-down in GSE119063 in Figure 1C. While we apologize for this omission, we have taken the following steps to correct the issue: re-counting the data: We have rechecked the number of DEGs-up and DEGs-down genes in the GSE119063 dataset to ensure accuracy. We are using the same data set and ensuring consistency of analysis. Consistency of Figures and Descriptions: We have reviewed Figure 1C, Figure 1B, and the associated descriptions in the paper to ensure consistency between them. There may be errors or omissions that have caused this mismatch, and we will make sure that this information is consistent in our revisions. We have included updated statistics and graphs in the revision, as well as corrected descriptions, to eliminate this inconsistency. We appreciate you pointing out the problem, which helps to improve the quality and accuracy of our study. Thank you again for your patience and help.

Comment 9. "Sensitivities" and "1-Specificities" should all be changed to "Sensitivity" and "1-Specificity" in Figure 2C and 2D.

Response: Thank you very much for your careful review of our paper and your valuable suggestions. We have taken note of your suggestions on our paper and have corrected "Sensitivities" and "1-Specificities" in Figures 2C and 2D. We have now standardized them to "Sensitivity" and "1-Specificity" to ensure consistency and accuracy of the charts. We are honored to have your professional opinion, which is essential for improving the quality of our paper. Thank you again for your patience and valuable suggestions and guidance.

Comment 10. Use different colors to identify different bars in Figure 3D and 3E.

OVER-NC
OVER-ITPR1
Time (12h)
OVER-NC
OVER-ITPR1
Time (24h)
OVER-NC
OVER-ITPR1
Time (48h)

Response: Thank you for reviewing our paper and providing your valuable comments. In response to your suggestion, we have used different colors to identify the different bar chart bars in Figure 3D and 3E to improve the clarity and readability of the charts. We have revisited the charts and used your suggestions in our revisions. To ensure the best results, we have chosen a set of

colors that are contrasting and easily distinguishable so that readers can more easily identify and understand the information in the chart. We believe this change will significantly improve the quality of the charts and appreciate your guidance in making our paper better. Thank you again for your review and support.

Comment 11. Check the spelling.

575 (A) The JASPER database showed the binding site between *HES1* and *ITPR1*.

Response: Thank you for scrutinizing my paper and for your valuable comments. I have read your suggestions carefully and noted that you mentioned the need to check the spelling aspect. In response, I have carried out a thorough spell check of the thesis where you pointed out the problem areas as well as of the essay and corrected any spelling errors found. It is important to ensure that the paper is of high quality in all aspects to ensure that the contribution to academic research can be maximized. Thank you again for your patience and professional review.

Comment 12. Make sure that the staining methods of Figure 4C, 4F, 6F, and 6I are mentioned in the caption.

Response: Thank you for scrutinizing our paper and providing your valuable comments. We take your suggestions very seriously and have revised them according to your guidance. Regarding the staining methods for Figures 4C, 4F, 6F, and 6I, we have provided additional instructions in the corresponding figure captions (DAPI staining, Scale 50 μm). This revision is intended to provide more detailed experimental methods to ensure that readers can better understand our study. We hope that this change meets your expectations and will improve the quality of the paper. We appreciate your professional advice and are willing to go out of our way to ensure the accuracy and clarity of the paper. Thank you again for your review and feedback.