Peer Review File

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Reviewer A

Study of pheochromocytoma biology; a neuroendocrine tumor; results ln large amounts of catecholamines into the blood – which drives up blood pressure. PLAT protein converts plasminogen to plasmin (also known as TPA); it is elevated with low blood supply (see overexpression in PGGL when low blood supply). This study looked at overexpression of PLAT in PC-12 cells and looked for differentially expressed genes when compared with control PC-12 cells. Study team finds enriched functional pathways related to mitochondrial respiration chain and energy metabolism.
Although this is an important line of investigation, the methods and results fall short of being informative.

Minor edits
Spell out first incidence of an acronym: MOI
Reply: The full name of MOI is Multiplicity of Infection, which we have now labeled at its first occurrence in the manuscript.
Changes in the text: We added annotations where MOI first appeared. (see line 96)

Use proper and single convention and nomenclature for genes, proteins. (T-PA, TPA, tPA). All caps, italic for human genes; all caps not italic for human protein (lower case for mouse)…….
Reply: We decided to standardize the above descriptions throughout the entire manuscript as PLAT and TPA.
Changes in the text: We have standardized the terminology throughout the full text.

Major issues
1. Methods: Manuscript does not adhere to MDAR checklist. Additional detail needed so that experiments can be replicated by another team. Need exact reagents and concentrations, methods and appropriate references (analysis computational tools). Examples follow but many additional instances in the manuscript.
a. Need more detail on Lentiviral transfection. Product number for lentiviral vector. What user manual? Concentration of lentiviral vector added to cells. Define and describe NC (control). Define “complete culture medium”. Are you cloning in rat Plat gene or human PLAT gene? What secondary antibodies were used; what chemiluminsecent substrate is used?
Reply: Thank you. We have made the necessary revisions. We have added more experimental details to the manuscript so that the experiment can be replicated by another team, making it more in line with the MDAR checklist.
The content of the user manual is described in lines 97 to 98, which states that the complete culture medium should be replaced after 12 hours, and the cells should be further cultured for 72 hours before they can be used for subsequent experiments.
The definition of NC is given in line 92, which refers to a set of empty vectors constructed.

Lines 86-87 describe the complete culture medium.
The remaining information is supplemented in the manuscript.
Changes in the text: The relevant text has been added in lines 92, 93, 94, 118, 119, and 120.

b. Line 96 duplicate of line 75.
Reply: Thank you for the reminder. Yes, we have noticed the issue and have deleted the statement on line 96.
Changes in the text: The duplicate statement in line 96 (new document line 124) has been deleted.

2. Results:
a. Figure 2A: state that these are the result of qPCR (and not from RNA sequencing).
Reply: The relevant instruction has been provided in lines 207 to 209 of the manuscript.
Changes in the text: Nothing changes in the text about this comment.

b. What is Figure 3A showing? What genes are on 3B (supplemental data?); 3C is not necessary because it is replicated in legend of 3E; Figure 3D is not necessary: If you're looking at differential expression of OE vs NC; you shouldn't be testing NC.
Reply: Figure 3A shows the correlation between samples. The correlation of gene expression levels between samples is an important indicator for assessing the reliability of experiments and the rationality of sample selection. The closer the correlation coefficient is to 1, the higher the similarity of expression patterns between samples. Figure 3B depicts a clustered heatmap of differentially expressed genes. It is presented here to provide a more intuitive visualization of clustering relationships between samples or genes. Due to the large volume of data, we have not displayed all gene names. The most significant differentially expressed genes are presented in Table 2. We deleted Figure 3C and retained Figure 3E, and renumbered them. The retention of Figure 3D is meaningful as it visually reflects the number of different genes detected between the two sample groups, as well as the number of commonly expressed genes. We will retain this figure accordingly.
Changes in the text: Figure 3C has been deleted and all images have been renumbered.

c. Line 189: How were differentially expressed genes selected in Table 2? Need indication if genes shown over expressed or under relative to NC.
Reply: We arranged the top 10 genes with the most significant changes in the OE group compared to the NC group in descending order of Padj values. This includes genes that are either over-expressed or under-expressed relative to the NC group, distinguished by the positive and negative values of Log_2FC.
Changes in the text: We have added an instruction on line 232 of the manuscript.

d. What is the biologic overlap of GO and KEGG that you observe? What specific genes are driving the significance of these pathways?
Reply: Thank you for your question. We have added the specific genes enriched in each pathway to the supplementary file for readers to review.

Changes in the text: We have added Supplementary appendices S1 and S2 and added wording in lines 242-243 and 248-249 of the manuscript.

3. Discussion:

a. Discuss strengths and weaknesses of paper.

Reply: Thank you for your suggestion. We added the strengths and weaknesses of this study at the end of the article DISCUSSION section.

Changes in the text: We have added a discussion in lines 399 to 433.

b. Discuss how PLAT gene expression relates to energy metabolism.

Reply: Sure, we have supplemented the discussion on this.

Changes in the text: The sixth paragraph of the DISCUSSION section (lines 393-398) supplements the discussion on the relationship between PLAT gene expression and energy metabolism.

c. Lines 208-250 Remove, it's a biology lesson on blood clotting pathways, not discussion of the paper.

Reply: Thank you for your suggestion. We have made modifications to this section, and in accordance with the comments of Reviewer B, the text of these paragraphs has been deleted or supplemented.

Changes in the text: The third to fifth paragraphs of the DISCUSSION section have been specifically revised.

d. Lines 256-284 Remove most of this as it's a biology lesson on mitochondria.

Reply: We have followed your advice and deleted some of the content in these lines.

Changes in the text: Part of the text in the sixth paragraph of the DISCUSSION section has been deleted.

e. Discuss how this work relates to other molecular biology on pheochromocytomas? Possible look at results from: Fishbein, L., Leshchiner, I., Walter, V., Danilova, L., Robertson, A. G., Johnson, A. R., ... & Roach, J. (2017). Comprehensive molecular characterization of pheochromocytoma and paraganglioma. Cancer cell, 31(2), 181-193.

Reply: OK, we accept your suggestion and have discussed it.

Changes in the text: In the sixth paragraph of the DISCUSSION section (lines 356-359), we supplemented the discussion.

f. Discuss relationship of pheochromocytoma, PLAT and the KEGG and GO pathways identified.

Reply: Thank you for your suggestion. Yes, we have summarized the content of the discussion section and reorganized it to make it more logical, discussing the relationship between the identified GO and KEGG pathways and PLAT.

Changes in the text: The sixth paragraph (lines 360-398) of the DISCUSSION section has been revised.

Reviewer B

The paper titled “RNA sequencing reveals transcriptomic changes in PC-12 cells following PLAT overexpression” is interesting. This study provides some insights into the role of PLAT in pheochromocytoma and suggests directions for further research on its involvement in tumor development and angiogenesis. However, the specific regulatory mechanisms still require further validation. However, there are several minor issues that if addressed would significantly improve the manuscript.

1)The transcriptomics data is still premature without in-depth analysis and the authors' own perspective. Please supplement in the discussion.

Reply: Thank you for the reviewer's question. Yes, we are aware of this issue and have made some analysis of the transcriptome results at the beginning of the DISCUSSION section (lines 262-284). Meanwhile, in terms of discussing the limitations of the paper (lines 406-430), we also pointed out the shortcomings.

Changes in the text: We have added two paragraphs (lines 262-284) at the beginning of the DISCUSSION section of the paper.

2)There are reports indicating that PLAT was shown to be reduced with angiogenes in pheochromocytoma. It is suggested that the author attempt to analyze the functions related to angiogenesis through bioinformatics.

Reply: Thank you for your insightful suggestion. Indeed, there is a complex relationship between PLAT and angiogenesis, and we firmly believe that our research has clinical value. Our study provides some references for the involvement of PLAT in the development of pheochromocytoma and angiogenesis, which provides a direction for our future research. As for your suggestion of bioinformatics analysis, I think it's a great idea, and we plan to delve into it further in our future studies.

Nothing changes in the text about this comment.

3)The description of the results in the abstract is too simplistic, it is recommended to supplement it.

Reply: OK, we have expanded the description of the results in the abstract to make it more comprehensive.

Changes in the text: We have added some wording in the RESULTS section of the ABSTRACT (lines 42-44).

4)What are your future work plans? What is the guiding significance of this study?

Reply: Thank you for your suggestion. We included relevant content in the final part of the DISCUSSION to clarify our expectations for future work. Meanwhile, the guiding significance of this study has been added to the strengths section of the study.

Changes in the text: We have added some text at the end of the DISCUSSION section (lines 434-444) to describe future work plans. The guiding significance of this study is explained in lines 399-433.

5)What is the correlation between PLAT and the immune microenvironment? What are the possible goals of future drug development? It is recommended to add relevant content to the discussion.

Reply: We have made significant revisions to the third and fourth paragraphs of the DISCUSSION section (lines 285-295 and 303-327). We have summarized the relationship between PLAT and the immune microenvironment, and put forward expectations for potential targets in future drug development.

Changes in the text: The third and fourth paragraphs (lines 285-295 and 303-327) of the DISCUSSION section have been modified and supplemented.

6)It is recommended to add experiments to study the biological function of PLAT.

Reply: Thank you for your suggestion. What you said is very pertinent. It is indeed necessary to conduct basic experiments to study the biological function of PLAT. However, due to the constraints of article length and time urgency, we are unable to provide more experimental results in this paper in the short term. We also pointed out the research disadvantages in the DISCUSSION section of the paper. In subsequent studies, we will focus on exploring molecular biology-related experiments, and the relevant experimental plans are already in the pipeline.

Nothing changes in the text about this comment.