

## Peer Review File

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### Reviewer A

“This study investigated the role of IRAK3 in prognosis, immune cell infiltration, and therapeutic response in lung adenocarcinoma (LUAD) using bioinformatics analysis of public datasets and a local LUAD cohort. The data showed that the level of IRAK3 was reduced in LUAD, which was supported by immunohistochemistry. Decreased IRAK3 was associated with poor clinical outcomes. IRAK3 expression was correlated with immune cell infiltration and related to several immune-related pathways, as well as therapeutic responses to various drugs.

Overall, this manuscript provides new insights into the role of IRAK3 in LUAD. The analyses were comprehensive with detailed method descriptions. The results were effectively presented, and the study limitations are discussed. However, there are some areas where the manuscript can be improved:”

**Comment 1:** Because this study demonstrated a correlation between IRAK3 expression and immunotherapy response using bioinformatics, the potential role of IRAK3 in immune-related pathways (such as the B cell receptor signaling pathway, chemokine signaling pathway, and leukocyte transendothelial migration) was suggested through gene set enrichment analysis. However, experimental validation on these issues was not conducted. Therefore, the authors should interpret the data cautiously and avoid overclaiming their conclusions. The statements regarding these findings should be reframed.

**Reply 1:** Thank you for your insightful comments on our manuscript. We appreciate your feedback and have made the following revisions accordingly. About interpretation and reframing of findings, we totally agree with your precise comment. We have reframed our statements with extra caution just to clarify the potential role of IRAK3 involved in immune-related and inflammation-related pathways. Additionally, we have clearly stated that these conclusions are based on bioinformatic analysis and have not been experimentally validated.

**Changes in the text:** We have modified the result and discussion sections to reflect this cautious interpretation (see Page 18, Line 354, Page 19, Line 368, Page 24, Line 488-489, Page 25, Line 493-494) and have ensured that our conclusions do not overstate the findings (see Page 28, Line 562). Specifically, we have rephrased our statements to highlight the need for further experimental validation (see Page 27, Line 554).

**Comment 2:** Additionally, the words “promotes immunotherapy resistance through inflammation-related pathways” in the title should be revised.

**Reply 2:** In line with your suggestion about title revision, we have revised the title to avoid the implication that IRAK3 directly promotes immunotherapy resistance through inflammation-related pathways without experimental evidence.

**Changes in the text:** The Title has been revised to “IL-1 receptor-associated kinase 3 (IRAK3)

in lung adenocarcinoma predicts prognosis and immunotherapy resistance: Involvement of multiple inflammation-related pathways”

**Comment 3:** In Figure 2B, a scale bar should be included for all IHC images. The label "50  $\mu$ M" for the scale bar should be changed to "50  $\mu$ m".

**Reply 3:** Thank you for your constructive feedback on our figures. We appreciate your attention to detail and have made the following revisions accordingly. We have added a scale bar to all IHC images in Figure 2B to provide a clear reference for measurement. The label for the scale bar has been corrected from "50  $\mu$ M" to "50  $\mu$ m" to accurately represent the unit of measurement.

**Changes in the text:** The updated Figure 2B now includes the appropriate scale bars and the corrected label (see Page 39, Line 844).

**Comment 4:** The manuscript needs to be more concise. The language could be further simplified for better readability, and the flow of the article could be improved by connecting sections more cohesively.

**Reply 4:** We appreciate your suggestions for improving its readability and cohesiveness. We have revised the manuscript to make it more concise by eliminating redundant phrases and unnecessary details. This has helped in conveying our findings more clearly and succinctly. We have simplified the language throughout the manuscript to enhance readability. Technical jargon has been minimized, and complex sentences have been restructured for clarity. To improve the flow of the article, we have restructured certain sections to ensure a more logical progression of ideas. Transitional phrases have been added to better connect sections and maintain a cohesive narrative.

**Changes in the text:** The changes are reflected throughout the document. For instance, the introduction and discussion sections have been streamlined, and transitions have been added to improve the overall flow (see Page 6, Line 78-80, 94, Page 26, Line 524 for specific examples).

## **Reviewer B**

“The presented manuscript is concerned around IRAK3 and its connection to LUAD progression and survival.

Overall the manuscript is well written, although some minor issues need to be addressed before publication.

.....Despite the fact that manuscript presented for review is mainly retrospective with bioinformatics databases used, it is valuable and provides new insights for IRAK3 in lung cancer.”

**Comment 1:** Firstly, according to HUGO the gene symbols should be italicised, please make necessary changes through all manuscript. It will also help to distinguish when authors are writing about protein and when about gene expression.

**Reply 1:** Thank you for your constructive feedback on our manuscript. We appreciate your attention to detail and have made the following revisions accordingly. In accordance with

HUGO guidelines, we have italicized all gene symbols throughout the manuscript. This change helps to clearly distinguish when we are referring to genes versus proteins.

**Changes in the text:** All gene symbols have been italicized to comply with HUGO standards (see Page 3, Line 33 for examples).

**Comment 2:** Next, there is some chaos in bioinformatics databases: in some of them the necessary citing is missing, in other the accession data is not provided. Please provide missing information.

**Reply 2:** We appreciate your careful review and have ensured that all bioinformatics databases used in our study are properly cited. Any missing citations have been added to the manuscript. In addition, we have included the necessary accession data for all relevant databases to ensure transparency and reproducibility.

**Changes in the text:** The updated manuscript now includes proper citations and accession data for all bioinformatics databases (see Page 9, Lin148, Page 11, Line 235-236, Page 14, Line 262, for specific additions).

**Comment 3:** Please provide better resolution of figure 4 part B and C as the font is too small to be readable.

**Reply 3:** We appreciate your attention to the quality of the figures and have improved the resolution of Figure 4B and 4C to ensure clarity and readability.

**Changes in the text:** The updated figure with better resolution is now included (see Page 41, Line 861). And the pdf version of Figure 4 is provided.