

Peer Review File

Article information: <https://dx.doi.org/10.21037/tcr-24-1523>

Reviewer A

Comment:

Nice article. Congratulations on the work.

Reply:

Thank you very much for your kind words and positive feedback on our article. We are delighted to hear that you found our work commendable. Your encouragement is greatly appreciated and motivates us to continue our research with the same dedication and rigor.

Reviewer B

The study employed a two-sample Mendelian randomization approach to examine the causal relationships between 2,821 protein level ratios and non-small cell lung cancer (NSCLC), identifying 142 protein level ratios with significant associations. Notably, the AKR1B1/SUGT1 and PLPBP/STIP1 ratios showed the most significant negative correlations with NSCLC risk, while the ARHGEF12/IRAK4 and BANK1/LBR ratios exhibited the most significant positive correlations. Despite these findings, the study's conclusions necessitate further validation through extensive clinical research due to the limitations inherent in Mendelian randomization studies, such as potential violations of the core assumptions and the reliance on observational data. Clinically, these results introduce new insights that could inform personalized treatment strategies and diagnostic approaches for NSCLC, although their direct clinical applicability is still subject to confirmation in future studies.

Comment 1:

In the abstract, please describe the controversy regarding the causal links between protein level ratios and NSCLC in the background, describe the datasets used and instrumental variables selection in the methods, and have more detailed comments for the clinical implications of the findings.

Reply 1:

In accordance with your suggestions, we have incorporated a contentious description of the causal relationship between protein ratios and non-small cell lung cancer (NSCLC) in the background section of the abstract. Additionally, we have provided a description of the dataset utilized and the selection of instrumental variables in the methods section. Furthermore, we have expanded on the clinical significance of the study results. A detailed review has been conducted. To enhance the precision of the abstract, we have removed the introductory content regarding NSCLC and omitted the

sections pertaining to the sensitivity analysis and odds ratio (OR) value.

In the subsequent revised manuscript, the revised contents were marked one by one using the annotation method.

Changes in the text:

See Page 1; line 5-10, line 13-18. line 20-25, line 28-30. Additions are found in the revised manuscript below.

Comment 2:

In the introduction, the authors need to review controversy regarding the causal links between protein level ratios and NSCLC, analyze the potential reasons, and explain why MR is needed to address this.

Reply 2:

Based on review comments, we have added a review of the controversy regarding the causal relationship between protein level ratios and NSCLC, analyzing the underlying causes, and explaining why MR is needed to address this issue.

In order to refine the content, I have deleted some content.

We have deleted the content about the study of protein level ratio in other tumors and added the relevant content about the study of NSCLC. For the specific modification position, please see: deleted line 59-69.

In the subsequent revised manuscript, the revised contents were marked one by one using the annotation method.

Changes in the text:

Deleted line 39-40, line 59-69, line 71-74. Additions are found in the revised manuscript below.

Comment 3:

In the methodology, please describe the two sample and data sources, how the NSCLCL was diagnosed, how the protein levels were measured, how the healthy controls were recruited, and the clinical characteristics of the clinical samples. This information would help assess the external validity of the findings such as population differences between the two cohorts. In statistics, please describe P value for statistical significance.

Reply 3:

Based on review comments, we added to Methods a description of the two samples and data sources, how to diagnose NSCLCL, how to measure protein levels, how to recruit healthy controls, and the clinical characteristics of clinical samples, and in Statistics, describe Statistical significance of P value.

In the subsequent revised manuscript, the revised contents were marked one by one using the annotation method.

Changes in the text:

Deleted line 91-96, line 101-102. Additions are found in the revised manuscript below.

Comment 4:

Finally, please consider to cite several related papers: 1. Wei Y, Xu J, Huang X, Xie S, Lin P, Wang C, Guo Y, Zou S, Zhao Z, Wen W, Song Y, Bao Z, Zhang L, Liu W, Kong W, Wang W, He B, Zhang S, Zhou C, Chen Y, Yu Z. C-reactive protein and lactate dehydrogenase serum levels potentially predict the response to checkpoint inhibitors in patients with advanced non-small cell lung cancer. *J Thorac Dis* 2023;15(4):1892-1900. doi: 10.21037/jtd-23-240. 2. Onodera R, Chiba S, Nihei S, Fujimura I, Akiyama M, Utsumi Y, Nagashima H, Kudo K, Maemondo M. High level of C-reactive protein as a predictive factor for immune-related adverse events of immune checkpoint inhibitors in non-small cell lung cancer: a retrospective study. *J Thorac Dis* 2023;15(8):4237-4247. doi: 10.21037/jtd-23-85. 3. Sung M, Jang WS, Kim HR, Park JA, Lim SM, Kim HR, Cho BC, Park YR, Hong MH. Prognostic value of baseline and early treatment response of neutrophil-lymphocyte ratio, C-reactive protein, and lactate dehydrogenase in non-small cell lung cancer patients undergoing immunotherapy. *Transl Lung Cancer Res* 2023;12(7):1506-1516. doi: 10.21037/tlcr-23-7. 4. Lu D, Ma Z, Huang D, Zhang J, Li J, Zhi P, Zhang L, Feng Y, Ge X, Zhai J, Jiang M, Zhou X, Simone CB 2nd, Neal JW, Patel SR, Yan X, Hu Y, Wang J. Clinicopathological characteristics and prognostic significance of HDAC11 protein expression in non-small cell lung cancer: a retrospective study. *Transl Lung Cancer Res* 2022;11(6):1119-1131. doi: 10.21037/tlcr-22-403

Reply 4:

We cited these four articles in the introduction when reviewing the research and controversies between protein level ratio and NSCLC. References:13-16.

Changes in the text:

See revised manuscript for details. References:13-16.