

Peer Review File

Article information: <https://dx.doi.org/10.21037/tcr-22-2793>

Reviewer #1:

1. Comment: (Significance: The significance of studying the ZHX family expression is not clear and strong. This main issue needs to be addressed in the Introduction and Discussion..)

Reply: We fully understand the reviewer's concern and completely agreed with the reviewer's suggestions. According to the reviewer's suggestions, we have especially explained the significance of studying the ZHX family expression to make the study's significance more clear and strong. This issue has been addressed in the Introduction and Discussion sections.

Changes in the text: Line 101-103.

2. Comment: (Method and Results: The characteristics (age, race, sex, smoking, etc) and clinical outcomes (stage, histology, survival, follow-up, metastases, recurrence, progression, etc) for the patients are lacking. This key component needs to be included in Method and results. One detailed Table is needed.)

Reply: We fully understand the review's concern. We completely agreed with the reviewer's suggestions. The characteristics (age, race, sex, smoking, etc) and clinical outcomes (stage, histology, survival, follow-up, metastases, recurrence, progression, etc) should be summarized and presented in a table. However, the clinical information mentioned above was not available in the online database, which caused that we couldn't present the clinical characteristics and outcomes. This is an unsolvable shortcoming in this study. We have acknowledged the limitation in the Discussion section.

Changes in the text: Page 17, Line 503-512.

3. Comment: (The outcome analysis needs to be further stratified by stage, at by separate early/advanced. The smoking effect also needs to be addressed..)

Reply: We fully understand the review's concern. We completely agreed with the reviewer's suggestions. The clinical outcome analysis should be stratified by smoking status, stage, and other parameters. However, the clinical information mentioned above was not available in the online database, which caused we couldn't conduct the stratified analysis of the outcome. This is an unsolvable shortcoming in this study. We have acknowledged the limitation in the Discussion section.

Changes in the text: Page 17, Line 503-512.

Reviewer #2:

1. Comment: (This manuscript reveals that the expression of ZHX family is closely related to the poor prognosis and immune infiltration of lung adenocarcinoma (LUAD). It provides a new basis for further study of the potential biological function of ZHX family in LUAD and lays a foundation for further development of therapeutic targets for LUAD patients)

In this study, the authors first analyzed the ZHX family expression levels in various types of human cancers by using public databases. ZHXs expression were significantly decreased in LUAD tissues compared with normal tissue samples. Survival analysis showed that low ZHXs expression was associated with poor prognosis in LUAD patients. Functional enrichment and pathway analysis were performed with STRING, KEGG and CancerSEA databases. Importantly, there was a positive correlation between ZHX family members and the immune infiltration of monocytes, TAM, M1, M2 macrophages in LUAD patients. The expression of ZHX family was also significantly correlated with various immune markers in LUAD. Furthermore, GEO analysis and RT-PCR confirmed that the expression level of ZHXs in LUAD was significantly decreased.

Overall, this is the first study to find the association of ZHXs with OS and immune infiltration in LUAD. Several concerns should be addressed to improve the manuscript.)

Reply: We sincerely thank you for your careful reading of our paper and helpful comments in improving the quality of our submission.

Changes in the text: Not applicable (NA).

2. Comment: (1. In Figure 1, the figure legend of (C) and (D) were missing.)

Reply: Thank you for these valuable comments. We are deeply sorry and sincerely apologize for our carelessness. The figure legends of (C) and (D) were added in the revised manuscript.

Changes in the text: Page 22, Line 665-667.

3. Comment: (2. The figure legend format should be unified. Figure 2 is A/B/C, but Figure 1 is (A)/(B)/(C).)

Reply: Thank you for the constructive comments. The figure legends format of all figures has been unified according to your suggestion.

Changes in the text: Page 22, Line 665-667, 675-681, 689-713.

4. Comment: (3. In Figure 3, which databases were used specifically, what were the

criteria for the selected differentially expressed genes, Fold change? And it may be helpful to include a heatmap of gene expression in ZHXs-High Expression and ZHXs-Low Expression.)

Reply: Thank you for these valuable comments. We are deeply sorry and sincerely apologize for our inaccurate description of Figure 3. STRING (<https://string-db.org/>) database was used to identify enriched functional categories of ZHXs and related genes, but rather to analyze the selected differentially expressed genes. The functional relationships for the top 100 genes that most relevant to ZHXs were investigated by using the STRING database. In addition, adding a heatmap of gene expression in ZHXs-High Expression and ZHXs-Low Expression is a helpful and constructive suggestion for improving the quality of our study. However, as this is a study based on online bioinformatics analysis, the individual genes expression information was not available for us to conduct further analysis and construct a heatmap of gene expression in ZHXs-High Expression and ZHXs-Low Expression, which should be done in future studies. Thanks again for your valuable suggestions.

Changes in the text: Page 6 Line 143-145.

5. Comment: (4. The figure legend of Figure 4 missed..)

Reply: Thank you for these valuable comments. We sincerely apologize for our carelessness. The figure legend of Figure 4 has been added.

Changes in the text: Page 22, Line 683-687.

6. Comment: (5. Why choose the immune infiltration for further analysis?.)

Reply: Thank you for the reviewer's comments. Immunotherapy has significantly improved the clinical outcome of cancer patients. Strategies to therapeutically target the tumor microenvironment (TME) have emerged as a promising approach for cancer treatment in recent years. Immune cell infiltration was the most important component of TME, which plays a potentially critical role in immunotherapy. The cause of choosing immune infiltration for further analysis was that we want to clarify whether there were certain associations of the expression of the ZHXs family with the immune cells infiltration which might impact the immunotherapy. Thus, in this study, we further analyzed the relationship between the expression of the ZHXs family and immune cell infiltration.

Changes in the text: NA.

7. Comment: (6. It seems this study mainly focused on the bioinformatics analysis. Whether to consider the relevant cell or animal experiments, further verification of specific regulatory mechanisms?)

Reply: Thanks very much for your valuable and constructive suggestions. As the reviewer mentioned, this study aimed to explore the relationship between ZHX family expression and clinical outcome and immune infiltration in LUAD patients by using bioinformatics analysis. The findings of this study will be validated in vitro and in vivo experiments, and the specific regulatory mechanisms of ZHX family members will also be deeply investigated in future studies. Thanks again for your suggestions.

Changes in the text: NA.