Peer Review File

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Reviewer A:

In the present manuscript, Ke et al. investigated the potential of LPCAT3 as a prognostic biomarker in AML through pan-cancer analysis using various well-established bioinformatics analysis tools. The article has been read over and is worthy for publication in this Journal.

Reply: Thank you for your comments.

Reviewer B:

1.In general, legends should be expanded to allow the interpretation of the data in the figures.

Reply: Thank you for your suggestion and the changes have been made (see Page 23-24, line 598-619, 633-635).

2.Authors claim that they investigated new potential biomarkers for AML, however, the manuscript is LPCAT3 centered. The background introduction and study objective statement do not correspond to the data collection shown in the manuscript. Authors should give background in LPCAT3 and not AML

Reply: We would like to express our most sincere gratitude to your comment. We have reviewed and rewrote the background (see Page 4, line 73-101).

3.Figures 1A and 1B show the same dataset (TCGA). The legend is incorrect.

Reply: In our study, Figure 1A shows LPCAT3 chromosome localization in normal cells; Figure 1B shows the localization of LPCAT3 in the single-cell. These data are not from the TCGA database. So, the legend should be correct.

4.The AML HR in the forest plot (Figure 3A) indicates that LPCAT3 is a protector factor (HR<1), however, the Kaplan-Meier survival curve showed that upregulation of LPCAT3 expression was significantly correlated with poorer OS in AML. It is confusing why they performed the next in silico evaluation with AML focus

Reply: First, we would like to express our most sincere apologies to reviewer. We did not describe the statistical methods clearly, which led to some misunderstanding in our results. Using the high-LPCAT3 expression group as a reference, the hazard ratio for Low-LPCAT3 expression group was calculated through a Cox regression in our study. So, HR> 1 indicates the gene is a protective factor, and HR<1 indicates the gene is a risk factor. We are very sorry for our incorrect writing “HR> 1 indicates the gene is a risk factor, and HR<1 indicates the gene is a protective factor” in the legend of Figure 3, which has been corrected (see Page 23, line 608-609). In addition, we are hematologists, so we focused on AML.

5.Multivariate analyzes (subtype, age, mutation) of cancer types where LPCAT3 was observed to increase is necessary.

Reply: We are very sorry for our negligence of multivariate analysis data, which has been added to the supplemental table S2 (See Supplementary table S2; Page 9, line 247-249).

6.In Figure 4 there is a significant difference, however, the correlation is weak. It would be more illustrative if the authors reorganize the data ordering by the correlation or significance and also excluded the nonsignificant.

Reply: Thank you very much for your valuable advice. In our study, the result showed that the expression level of LPCAT3 was correlated with the infiltration level of various immune cells. Unfortunately, the correlations are relatively weak. We have shown the top four with the strongest correlation (positive and negative correlation).

7.In figure 5B it is necessary to expand the legend to interpret the data

Reply: We are very sorry for our negligence of the legend, which has been corrected in the new manuscript (see Page 23, line 616-619).

8.What is the biological relevance of investigating TMB and MSI in AML tumors?

Reply: TMB and MSI are considered essential factors impacting on the occurrence and progression of tumor. Previous research has shown that TMB can be used as a biomarker to improve immunotherapy efficacy in various solid tumors, such as non-small-cell lung (J Clin Oncol. 2018, 36:2995) and colorectal cancers (*Clin Cancer Res. 2019, 25:6141-7*). High-frequency MSI in colorectal cancer is an independent predictor of clinical characteristics and prognosis (*N Engl J Med. 2000, 342:69-77*). TMB is considered a promising pan-cancer predictive biomarker and can guide immunotherapy in the era of precision medicine (*J Clin Oncol. 2018,36:631-2*). MSI is also considered an important biomarker in immune-checkpoint inhibitors (ICI) (*Gastroenterology. 2010,138:2073-87*).

However, few studies have investigated the relationship between the efficacy of immunotherapy and TMB as well as MSI in AML. Our result showed that the expression level of LPCAT3 correlates with tumor immune microenvironment. So, it is necessary to further explore the relationship between LPCAT3 expression and TMB as well as MSI score. Unfortunately, we did not find that the expression level of LPCAT3 was associated with TMB and MSI in AML.

9.In Figure 7, please inform NES.

Reply: We are very sorry for our negligence of the result of NES, which has been added to the Figure 7 (See Figure 7J-L).

10.The data presented based on IC50 in Figure 8 is not clear, it would be appropriate to show LPCAT3 expression in two subgroups, responders and not responders for each drug.

Reply: Thank you very much for your valuable advice. The biochemical half maximal inhibitory concentration (IC50) is the most commonly used metric for on-target activity

in lead optimization, which can also be used as one of the predictors of drug treatment response. In this study, we designed the prediction model on the GDSC cell line dataset using ridge regression and evaluated the satisfactory prediction accuracy using 10-fold cross-validation, and then estimated the IC50 of each sample in TCGA dataset based on the prediction models of these three chemotherapeutic agents. This method has been widely used in many studies (Stem Cell Res Ther 2020,11:457; Front Mol Biosci 2022,8:775700; Front Oncol 2020,10:591254; and etc). The clinical efficacy is primary important for estimation of chemotherapy drug response. However, the accessible clinical data did not contain the result of chemotherapy drug response assessments in TCGA-LAML cohort, so we can't divide patients into responders and non-responders based on IC50 alone.

In addition, we would like to express our sincerest apologies to reviewer. Due to our misunderstanding of IC50, the result of this section was incorrectly described in the original manuscript, which has been corrected in the new manuscript (see Page 13, line 343-348).

11.It is not clear which data allowed the authors to conclude the involvement of LPCAT3 in ferroptosis.

Reply: Thank you for your comments. Firstly, Previous studies have shown that ferroptosis is characterized by intracellular iron overload and lipid peroxides (*J Diabetes Res. 2021, 28;2021:9999612*). Polyunsaturated fatty acid-containing phospholipids are the main substrates of lipid peroxidation in ferroptosis, which is positively regulated by enzymes, such as ACSL4, LPCAT3, ALOXs, or POR. In particular, ACSL4 and LPCAT3 play a key role in promoting ferroptosis by incorporating PUFAs into cellular phospholipids (especially phosphatidylethanolamine) (*Biochem Biophys Res Commun. 2016, 478:1338-1343; Nat Chem Biol. 2017, 13:91-98; Nat Chem Biol 2017, 13:81-90*). Therefore, LPCAT3 is considered to be one of the driver genes that promote ferroptosis.

Secondly, in our study, KEGG pathway analysis indicated that 128 related genes of LPCAT3 were mainly enriched in the phospholipase D signaling pathway, lipid

metabolism, and ferroptosis processes (all adjusted $P < 0.05$) (Figure 7I). Our results also suggest that LPCAT3 may be involved in lipid metabolism and ferroptosis. However, we are sorry for the lack of experimental data to further support this conclusion, which is an important limitation in our study.

12.The title should be adequate to better inform the conclusion of the manuscript. In my opinion, the data shown do not support the conclusion that LPCAT3 is correlated with ferroptosis.

Reply: According to your suggestion, we have revised the title of the manuscript (see Page 1, line 1-2).