Peer Review File

Article Information: https://dx.doi.org/10.21037/tlcr-22-500

Reviewer A

The authors demonstrated A novel Cuproptosis-Related LncRNA Signature to Predict Prognosis and Immune Landscape of Lung Adenocarcinoma.

Comment 1: I recommend adding more comprehensive and clinical implication of cuproptosis in the introduction.
Reply 1: We thank the reviewer for this suggestion. We added the literature that reported the importance of copper and cuproptosis in the Introduction section.
Changes in the text: see Page 5, line 98-108

Comment 2: Since cuproptosis is not familiar to clinicians, please explain in more detail why you should study cuproptosis-related biomarkers.
Reply 2: We appreciate the reviewer's attention to detail. We think this comment is similar to the previous one to some extent. In recent years, more and more studies have focused on molecular diagnosis and treatment. Considering the great potential of copper and cuproptosis in cancer treatment, cuproptosis-related genes are promising to be novel therapeutic targets. In addition, various studies have shown programmed cell death-related genes could be used as prognostic markers to predict the response to immunotherapy and patient outcomes (mentioned in Page 6, line 119-122). The identification of cuproptosis-related genes may also provide new prognostic and therapeutic methods in cancer treatment. We added some information in the Introduction part to explain more clearly.
Changes in the text: see Page 6, line 104-113

Comment 3: You described the evaluation of the risk model. I think that the criteria between the high and low risk patients should be added.
Reply 3: Thanks for the reviewer's suggestion. We are sorry for the unclear description in our manuscript. We established the 10-CuRLs signature based on stepwise multivariate Cox regression analysis. Each patient's risk score was obtained using the formula (mentioned in Page 9, line 213).
The criteria between high and low-risk patients were the median risk score. This approach is widely used in similar articles about the risk model establishment based on prognostic biomarkers (1,2). In this study, based on the median risk score, patients in TCGA or GEO meta-cohort were classified into high-risk or low-risk groups (see Page 10, line 222-223; Page 10, line 237). We have made minor changes to make it clear.
Changes in the text: see Page 10, line 222-223

Comment 4: Table S1.
In TCGA-LUAD, stage III and IV patients are only 21.8% and in GSE31210, there is no stage III and IV patient. I think this is a big limitation of this study.
I suggest that the authors address this point in detail.
Reply 4: We greatly appreciate the reviewer's attention to detail and thanks for giving us the opportunity to correct this problem. The RNA sequencing data of III and IV LUAD patients is actually limited in the GEO datasets. To address this to the extent possible, three eligible LUAD cohorts, including GSE31210, GSE37745 and GSE50081, were integrated into a GEO meta-cohort in the revised version. After excluding patients with other pathological types and survival time less than 30 days, 458 LUAD patients were included for validation. The revised Table S1 showed the basic characteristics of patients in the TCGA-LUAD and GEO meta-cohort. We modified the flow chart, and text in the Methods and Results parts accordingly.
Changes in the text: see Page 8, line 166-172; Page 16, line 370-397; Table S1; Figure 1; Figure 5

Comment 5: There are many drugs that are not prescribed for lung cancer in Fig. 9.
I recommend showing the data about drugs which are clinically used for lung cancer.
Reply 5: We greatly appreciate the reviewer's suggestion. This is really something that we didn't think about when we wrote the manuscript. According to the reviewer's suggestion, we adjusted the displayed data about drugs. The sensitivity of 15 drugs that are currently in clinical use or in preclinical trials for lung cancer showed significant differences between low-risk and high-risk groups ($p < 0.05$, Figure 9A). Among drugs commonly used in the treatment of non-small cell lung cancer clinically, the sensitivity of Cisplatin, Docetaxel, Gemcitabine, Gefitinib, and Paclitaxel was higher in patients with low risk (Figure 9B-9G), suggesting that these drugs may be more effective in high-risk patients. However, patients in the low-risk group might benefit more from Imatinib. The added Figure S4 showed the sensitivity of other drugs which had the potential for lung cancer treatment.
Changes in the text: see Page 20, line 467-475; Figure 9; Figure S4

Comment 6: The font size of text in the figures is quite small and the resolution is low, so it is difficult to recognize the text exactly. You need to adjust the font size and resolution.
Reply 6: We appreciate the reviewer's attention to detail. We have adjusted the quality of our figure images to meet the required resolution. Fonts have been enlarged when possible, with the exception of the numbers in the interior of the plots as making these any bigger would overcrowd the plot.
Changes in the text: see figures in the attached file.

Reviewer B

This work by Wang et al focuses on identifying and validating Copper-induced cell death (cuproptosis)- related lncRNA (CuRLs) in lung adenocarcinoma. In this context, Wang et al assembled a novel prognostic signature based on 10 CuRLs, including CARD8-AS1, RUNDC3A-AS1, TMPO-AS1, MIR31HG, SEPSECS-AS1, DLGAP1-AS1, LINC01137, ZSCAN16-AS1, APTR, and ELOA-AS1. This 10-CuRLs risk signature revealed exceptional diagnostic precision integrated with traditional clinical risk factors, and a nomogram was produced for potential clinical translation. In this way, The tumor immune microenvironment was significantly different between different risk groups. The sensitivity of Bleomycin, Embelin, Gemcitabine, Lapatinib, Midostaurin, Paclitaxel, and Pyrimethamine was elevated in low-risk patients, and patients in the low-risk group might aid more from Rapamycin, Roscovitine, and Salubrinal. In the end, Wang et al reaching in the conclusion that the differences in features between diverse risk groups unlock the way to improve patient stratification and investigate novel drugs in various risk groups.

Comment 1: I found this study interesting and relevant in the field.
I found that this study would provide new insight into the knowledge of (cuproptosis)- related lncRNA (CuRLs) in lung adenocarcinoma.
The article is well written.
The methodology is fine and no further control is required.
I found the conclusion to be in line with the evidence and arguments presented.
The figures are fine.
I'm only concerned about Figure 9. The authors should provide Figure 9 in high quality.
Reply 1: Thanks for the reviewer's comment and affirmation of our work. And the other reviewer also had comments about Figure 9. To make the presentation of Figure 9 clear, we displayed the data about drugs that are in clinical use or in preclinical trials for lung cancer. The sensitivity of 15 drugs showed significant differences between low-risk and high-risk groups ($p < 0.05$, Figure 9A). Figure 9B-9G showed the sensitivity of commonly used drugs in the treatment of non-small cell lung cancer. The added Figure S4 showed the sensitivity of other drugs which had the potential for lung cancer treatment.
Changes in the text: see Page 20, line 467-475; Figure 9; Figure S4

Reviewer C

This research did a pure bioinformatics analysis based on the cuproptosis-related LncRNA (CuRLs) in lung cancer from public datasets. They have conducted COX regression, and the LASSO COX regression to find the potential prognosis ability of CuRL signatures and developed a nomogram for patient survival overcome prediction. In addition, they expanded the analyses of CuRLs into functional analysis, immune infiltration as well as drug response prediction. Ten CuRLs were picked as novel prognostic signatures for lung cancer prognosis. It is a comprehensive bioinformatics analysis in terms of currently available datasets, however, I still have some concerns.

Comment 1: Is there any more public datasets available from GEO or somewhere else that can be used as independent validation? Only one independent validation dataset seems insufficient.
Reply 1: We greatly appreciate the reviewer for this point of concern. Another reviewer also raised a similar issue. To address this problem to the extent possible, three eligible LUAD cohorts, including GSE31210, GSE37745, and GSE50081, were integrated into a GEO meta-cohort in the revised version. The risk model based on the 10 CuRLs was validated in the GEO-meta cohort (see Page 16, line 370-397). These results demonstrated the 10-CuRLs signature had a good prognostic value in the GEO-meta cohort.
Changes in the text: Page 8, line 166-172; Page 16, line 370-397; Figure 5

Comment 2: How did the authors decide on the ten CuRLs as prognostic signatures? What's the selection standard? According to Fig 2D, some of the CuRLs didn't show a significant value.
Reply 2: We greatly appreciate the reviewer for raising these issues. The steps for establishing the ten-CuRLs prognostic signature were as follows: firstly, univariate Cox regression analysis was used to screen for prognostic cuproptosis-related lncRNAs. Thirty-six lncRNAs with $p < 0.05$ were further analyzed by LASSO Cox regression; secondly, using the lambda.min as the cut-off threshold, twenty-one lncRNAs significantly correlated to LUAD prognosis; thirdly, stepwise multivariate Cox analysis was utilized to further analyze these lncRNAs. Finally, a model based on ten-CuRLs was established as shown in Fig 2D.
This stepwise variable selection procedure (with iterations between the 'forward' and 'backward' steps) can be applied to obtain the best candidate Cox model. It can be realized by R package 'My.stepwise'. The stepwise approach is based on the Akaike information criterion (AIC) to define the optimal set of variables to retain in each model minimizing the AIC value. A smaller AIC value is considered as an indicator of a better fit of the model to the data (3,4). Although some CuRLs did not show a significant value, the 10-CuRLs model obtained by stepwise multivariate analysis had the smallest AIC value. We are sorry that we did not make it clear in our manuscript. We added relevant explanation in the revised version (see Page 9, line 200-201).
Changes in the text: see Page 9, line 200-201

Comment 3: Can the authors provide a clearer presentation of the expression level of the CuRLs expression level that compared between tumor and normal samples in both TCGA and GEO datasets? I was wondering which CuRLs are upregulated, and which CuRLs are downregulated. Also, it would be better if the authors can provide a solid regression/machine learning model through the expression level of CuRLs to verify the diagnosis ability in lung cancer instead of a heatmap, and I cannot tell there's an obvious expression level of CuRLs in tumor tissues.
Reply 3: We appreciate the reviewer for raising this point of concern. We added Figure S1 which showed the expression of the 10 CuRLs in normal and tumor samples in detail. Also, we marked the significance on the heatmap (see Figure 2H). RUNDC3A-AS1, TMPO-AS1, ELOA-AS1, LINC01137, DLGAP1-AS1, MIR31HG, and APTR were highly expressed in tumor samples, while the expression of CARD8-AS1 was higher in normal tissues. Although the expression of ZACAN16-AS1 and SEPSECS-AS1 was not significantly different between normal and tumor tissues, the prognostic feature of the 10 CuRLs is still of clinical significance. On the one hand, these 10 prognostic CuRLs were highly

correlated with the overall survival of LUAD patients based on the univariate, LASSO and stepwise multivariate Cox analysis. The 10-CuRLs signature showed good performance for distinguishing low-risk and high-risk patients. On the other hand, the risk model was constructed based on the patients with LUAD. Similarly, some related articles did not examine gene expression in normal tissues (5). In addition, from the perspective of clinical use, we usually send tumor samples for genetic testing only when the patients have a confirmed diagnosis of cancer.
Among the GEO datasets in our GEO-meta cohort, only GSE31210 included gene expression both in normal and tumor samples. The added Figure S1 in the revised version also showed the differential expression of the 10 CuRLs in normal and tumor samples of GSE31210. Consistent with the results of TCGA-LUAD, most of the genes were highly expressed in tumor samples while the expression of CARD8-AS1 was higher in normal tissues.
Changes in the text: see Page 14, line 326-330; Figure 2H; Figure S1

Comment 4: All figures seem to be in low resolution. Please provide a high-resolution version (vector figure). In addition, please pay more attention to providing a detailed description of the figure legend. For example, in Fig 3 "Principal model analysis of low-risk and high-risk groups based on E. whole-genome, F. cuproptosis genes, G. all CuRLs,".
Reply 4: We appreciate the reviewer's attention to detail. We have adjusted the quality of our figure images to meet the required resolution. The figure legends have been rechecked and adjusted.
Changes in the text: see Page 33, line 752-754; Page 32, line 758-760; Page 32, line 768-779; Page 35, line 813-815; figures in the attached file.

Comment 5: This article could benefit from experiment validation (at least RT-qPCR, or cell transfection experiment) to show more clinical translational value. I recommend a wet lab validation of some CuRLs to verify any of the clinical features the article concluded.
Reply 5: Thanks for the reviewer's suggestion. We are sorry that we could not perform experimental research for CuRLs in our established model in the limited time. It is time-consuming to design primers, order reagents, and perform biological experiments. In addition, many articles focusing on cuproptosis-related genes have recently been published. We hope to publish our own findings soon. We would like to add these validation experiments and conduct more detailed experiments in the near future work. And we have added this limitation in the Discussion part in the revised version (see Page 23, line 551-556)
Changes in the text: see Page 23, line 551-556

Reference:

1. Ye Y, Dai Q, Qi H. A novel defined pyroptosis-related gene signature for predicting the prognosis of ovarian cancer. Cell Death Discov 2021;7:71.

2. Yao N, Zuo L, Yan X, et al. Systematic analysis of ferroptosis-related long non-coding RNA predicting prognosis in patients with lung squamous cell carcinoma. Transl Lung Cancer Res 2022;11:632–46.

3. DeBoer MD, Filipp SL, Gurka MJ. Use of a Metabolic Syndrome Severity Z Score to Track Risk During Treatment of Prediabetes: An Analysis of the Diabetes Prevention Program. Diabetes Care 2018;41:2421–30.

4. Zeidan AM, Sekeres MA, Garcia-Manero G, et al. Comparison of risk stratification tools in predicting outcomes of patients with higher-risk myelodysplastic syndromes treated with azanucleosides. Leukemia 2016;30:649–57.

5. Guo Y, Qu Z, Li D, et al. Identification of a prognostic ferroptosis-related lncRNA signature in the tumor microenvironment of lung adenocarcinoma. Cell Death Discov 2021;7:190.