Peer Review File

Article information: https://dx.doi.org/10.21037/gs-22-736

Reviewer A

Comment 1: First of all, my major concern for this study is the arbitrary statement on "therapeutic biomarker" in the title and elsewhere of this paper, because the authors did not provide empirical evidence on this focus. The authors need to revise the whole paper.
Reply 1: Thanks for your generous suggestion. We completely agreed with your opinion and removed conclusions that HELLS is therapeutic biomarker/target for ACC in the whole paper. In fact, we simply wanted to express the possibility that HELLS could be a therapeutic target, but ignored the validity of the conclusions, so we removed this overstated claim. And we have revised the whole paper to express the right meaning. The title has been adjusted to "Identification of a ferroptosis regulator HELLS with prognostic value for adrenocortical carcinoma based on integrated analysis and experimental validation" and other contents have also been revised in the text.
Changes in the text: We have modified our text as advised (see Page 1, line 3-5; Page 3, line 61-63; Page 5, line 123-126; Page 16, line 440).

Comment 2: My second concern is that HELLS is only one of the three prognostic biomarkers in the nomogram, so it is very arbitrary to have comments on the prognostic and predictive value of HELLS. The authors need to revise related statements and sentences accordingly. The title also did not indicate the research design of this study.
Reply 2: Thanks for your generous suggestion. As you suggested, we have changed "prognostic biomarker" to "with prognostic value" in the title. And we have revised related statements in the whole paper. Moreover, we found that the expression of HELLS in ACC was highly positive correlated with the enrichment scores of E2F targets, G2M checkpoint, MYC targets, MTORC1 signaling and DNA repair pathways, while it was negative correlated with bile acid metabolism and unfolded protein response pathways. In contrast, MT1G and ATF4 showed only a very weak correlation with enrichment scores of these pathways. Therefore, we inferred that HELLS might be a more crucial gene among these three genes in ACC.
Changes in the text: We have modified our text as advised (see Page 2, line 30; Page 11, line 286-292).

Comment 3: My third concern regarding this study is the poor predictive accuracy of the nomogram in the validation sets, with one-year AUC of 0.693. Second, the abstract needs some revisions. The background did not indicate the clinical significance of and research gap on this research focus. The methods need to describe the identification of predictors, the establishment of the nomogram, and the validation of the predictive accuracy of the nomogram. The results need to quantify the findings by reporting statistcis and accurate P values such as the expression levels and HR values. The

conclusion needs more detailed comments for the implications of the findings.
Reply 3: Thanks for your generous suggestion. We have carefully revised the abstract as your suggestions. As a data in the GEO database validation set, the 8-year of ACU was only 0.693, and we speculated that the reason might be related to small samples with a survival period of over 8 years in this dataset. While AUC in other datasets were more than 0.7, these results indicated that there was a certain prognostic value for this signature.
Changes in the text: We have modified our text as advised (see Page 2-3, line 25-63).

Comment 4: Third, the introduction of the main text needs to briefly review what has been known on the prognostic biomarkers in ACC and their prognosis predictive accuracy, have comments on the limitations and knowledge gaps of prior studies, and explain why HELLS deserves to be studied. The last paragraph should indicate the clinical significance, hypotheses, and objectives of this study, not to repeat what has been done and found in this study.
Reply 4: Thanks for your generous suggestion. Besides, the underlying mechanisms of ACC are complex and heterogenous; therefore, the biological process associated with ACC progression requires further analysis. We have readjusted the content of the introduction in accordance with your suggestion. Here, the thinking about HELLS and the prognostic value of ACC, ferroptosis agonists drug treatment are added.
Changes in the text: We have modified our text as advised (see Page 3-5, line 68-126).

Comment 5: Fourth, the methodology of the main text needs to have an overview of the research procedures of this study and the questions to be answered by them. Please also provide the threshold values of AUC for assessing the predictive accuracy in both the training and validation datasets.
Reply 5: Thanks for your suggestion. We already have provided the flowchart of this study in Figure 1A and we have added the threshold values of AUC in the method.
Changes in the text: We have modified our text as advised (see Page 7, line 168-170).

Reviewer B
For patients with adrenocortical carcinoma, a rare endocrine malignancy with a high rate of mortality and recurrence, it is difficult for clinicians to predict overall survival and choose the most effective treatment. In the manuscript “Identification a ferroptosis regulator HELLS as a promising potential prognostic and therapeutic biomarker for adrenocortical carcinoma based on integrated analysis and experimental validation”, authors identified that ferroptosis-related genes played pivotal roles in ACC progression and could be used as prognostic biomarkers and therapeutic targets for ACC.
Couple questions are required to be answered before it will be accepted.

Comment 1: In the abstract, please supplement the full-name of abbreviation “ACC”, and the methods of experimental validation.
Reply 1: Thanks for your generous suggestion. We have supplemented the full-name

of abbreviation “ACC”, and the methods of experimental validation in the abstract.
Changes in the text: We have modified our text as advised (see Page 2, line 27-66).

Comment 2: It was better to add reference (DOI: 10.21037/tau-22-276) about adrenocortical carcinoma in the introduction.
Reply 2: Thanks for your suggestion. We have carefully read this article (DOI: 10.21037/tau-22-276), but this study was related to the role of ferroptosis in hypertensive nephropathy, not adrenocortical carcinoma. Therefore, we have no idea how and where to add this reference.
Changes in the text: No changes.

Comment 3: What were the roles of ferroptosis in the pathogenesis of ACC? Please state in the introduction.
Reply 3: Thanks for your insightful suggestion. We have added the roles of ferroptosis in the pathogenesis of ACC in the introduction.
Changes in the text: We have modified our text as advised (see Page 4, line 97-102).

Comment 4: What were the functions of immune cell infiltration in the process of ACC? And state in the introduction.
Reply 4: Thanks for your insightful suggestion. We have added the functions of immune cell infiltration in the process of ACC in the introduction.
Changes in the text: We have modified our text as advised (see Page 4, line 100-107).

Comment 5: Please provide references for the formula of risk score.
Reply 5: Thanks for your insightful suggestion. We have added the references for the formula of risk score in the method.
Changes in the text: We have modified our text as advised (see Page 7, line 165).

Comment 6: How to determine the HELLS as a core hub gene in the research? Please state clearly in the results.
Reply 6: Thanks for your generous suggestion. We have added some clarification of the reason that HELLS was a core hub gene among the three risk score members in the part of results. The specific additions are as follows:
Changes in the text: We have modified our text as advised (see Page 11, line 279-286).

Comment 7: The immune cell infiltration was the crucial result data. It was better to test the effects of HELLS on immune cell infiltration by experiments.
Reply 7: Thanks for your generous suggestion. We really agreed the effects of HELLS on immune cell infiltration showed be tested by experiments, but as you know, ACC was a rare tumor type and difficult to obtain clinical specimens and well-established cell lines. To verify the relationship between HELLS and immune cells infiltration levels, we have collected 4 ACC samples as far as possible in our institution. Unfortunately, the expression level of HELLS was very high, while the immune cell markers CD20, CD3, PD1 and PDL1 were basically negative in these samples. This

suggested that these tumor samples may themselves be cold tumors that were less responsive to the immune response. This was also consistent with the results of the negative correlation between HELLS and immune cell infiltration levels. In the future, we plan to continue to collect clinical samples of ACC for related immunohistochemical experiments, and construct primary ACC cell lines by ourselves to verify the regulatory effects of HELLS on ACC cell progression and immune cell infiltration.
Changes in the text: No changes at present.

Comment 8: The HELLS was the crucial topic in the study. How about its biological functions? Please supplement in the discussion.
Reply 8: Thanks for your generous suggestion. We have added some biological functions of HELLS in the discussion. The specific additions are as follows:
Changes in the text: We have modified our text as advised (see Page 15, line 405-409).

Comment 9: What were the correlations between HELLS and immune cell infiltration in ACC? Please state in the discussion.
Reply 9: Thanks for your generous suggestion. We have added some descriptions about correlations between HELLS and immune cell infiltration in ACC in the discussion.
Changes in the text: We have modified our text as advised (see Page 15, line 410-424).