

General reply

Many thanks to the reviewer for encouraging our work and for giving useful comments for clarifying, improving and correcting some materials in the paper.

Now we have carefully revised the paper according to your comments, as explained below. All the modifications are highlighted in yellow

Specific reply to comments

Reviewer #1:

Introduction

Point 1

Referee: The authors refer to immune checkpoint therapy in colon cancer, it would be interesting to mention the trials currently in progress. In this regard, the authors could benefit from reading the following article, the contents of which could be useful for improving the manuscript:

PMID: 34638281 DOI: 10.3390/cancers13194797

Reply: Thank you very much for the literature you recommended to us. We will study it carefully and believe that we can get help from it.

How the paper is modified: We read "Cardiac Toxicity Associated with Cancer Immunotherapy and Biological Drugs" carefully. As the authors say, although most immuncheckpoint inhibitors have cardiotoxic potential, their role in fighting against cancer cannot be ignored. Furthermore, previous studies have shown that immuncheckpoint therapy has a good immunotherapeutic effect in patients with colon cancer. Based on this, we believe that further analysis of the relationship between the expression level of genes associated with immune checkpoint inhibitors and risk score is needed. In our article, we listed the most statistically significant genes($p < 0.05$) called PRF1 and TBX2, which were highly expressed in high-risk groups. This suggests that cuproptosis and immune-related risk score have potential predictive value for susceptibility to immunotherapy in colon cancer patients. Patients with high risk scores are more susceptible to immune escape, and they may also be more sensitive to immunotherapy. This may help patients make a better choice between the efficacy of immunosuppressive therapy and its adverse effects.

Point 2

Referee: The authors, at some point, describe copper and its regulation in cancer, this part should be introduced and it should be explained how it is related to the cuproptosis, to clarify the reason for the study.

Reply: Thank you very much for putting forward this valuable suggestion. We agree with your idea very much, and will make corresponding supplement and modification in the article.

How the paper is modified: In the article, **lines 75-82** are revised content. Here, we explained that excessive copper accumulation causes cell death, and this copper death effect is also present in cancer cells. At the same time, studies have shown that in order to resist the toxic effects of copper accumulation, cancer cells can adjust the process of copper metabolism to improve antioxidant capacity, thus achieving cancer metastasis. Based on the correlation between cuproptosis and cancer cells, combined with the previous role of immune cells in colon cancer and the subsequent relationship between cuproptosis and immunity, we conducted this study to explore the effects of cuproptosis and immunity on the prognosis of colon cancer.

Materials and methods

Point 3

Referee: There is a typo in the title.

Reply: Thank you very much for pointing out our careless mistake. In addition to correction, we will also learn from your carefulness and seriousness in the future work.

How the paper is modified: This typo has been corrected in **line 89** to "Materials".

Discussion

Point 4

Referee: The authors should discuss the results in the same order as they are presented in the text, i.e. first talk about CDKN2A and HSPA1A and then about UCN3.

Reply: Thank you very much for your suggestion. It is really something we should pay attention to. We will make adjustments in the text so that the order in which the results are discussed is consistent with the order they are presented in the text.

How the paper is modified: In the third paragraph of the discussion, **lines 184-186** of the text, We adjusted the order in which the three genes appeared in the text, so as to ensure that the subsequent discussion order was consistent with the order in which the text appeared, and such order was also consistent with the order of the three genes in the figures.

References

Point 5 :

Referee: The bibliography needs to be updated. Some relevant papers missing, including:

- PMID: 35965536 DOI: 10.3389/fonc.2022.961213

- PMID: 36313449 DOI: 10.3389/fgene.2022.928105

Reply: Thank you for your valuable suggestion. It is indeed an oversight on our part. And we will modify and improve it according to your suggestion.

How the paper is modified: We roundly updated the references, citing as much as possible the new findings in related studies in the last five years, and modified the format of the references uniformly according to the reference revision guidelines provided by *World Journal of Gastrointestinal Oncology*.

Point 6

Referee: Figure 1 is not mentioned in the text, it should be inserted in Materials and methods section.

Reply: Thank you for your advice, which has helped us a lot. We will improve the article according to your suggestions.

How the paper is modified: The introduction to Figure 1 has been inserted into Materials and methods "2.1. *The idea of this study is shown in Figure 1 in the form of a flow chart.*" in line 90.

Point 7

Referee: Table 1 is not mentioned in the text, it should be inserted in Materials and methods section.

Reply: Thank you for your valuable suggestions. We will revise and improve the article according to your suggestions.

How the paper is modified: The introduction to Table 1 has been inserted into Materials and methods "2.2 *Data collection and handling.*" in line 95-96.

Reviewer #2:

Point 1

Referee: The DOI number does not appear in some references. In the past five years, there are few applications of literature and the overall real-time performance is not strong.

Reply: Thank you very much for pointing out our shortcomings. In fact, we fully agreed with your suggestion and updated the references according to your suggestion.

How the paper is modified: We have roundly updated and modified the references. In order to improve the timeliness of literature, we try our best to quote the latest research in related fields in the last five years, and try our best to ensure that the corresponding DOI number can be found for each literature. In addition, according to the reference modification guide provided by *World Journal of Gastrointestinal Oncology*, the format of the literature has been uniformly modified.

Point 2

Referee: In this paper, the verification is mainly conducted through data sets, but there are limitations in the verification of actual experimental data.

Reply: Thank you for putting forward our deficiency, which will be more conducive to improving our research. Indeed, because this is a study based on publicly available databases, we acknowledge the lack of validation of actual experimental data. Please forgive us for not being able to supplement the experimental verification due to financial and time constraints. On this basis, we tried to make the verification process as scientific as possible, so we selected data from different databases for external verification. It can be seen from the paper that the samples of the training set are from The Cancer Genome Atlas (TCGA), while the samples of the validation set are from the Gene Expression Omnibus (GEO). Nevertheless, we still agree with your point of view. Experimental verification may provide more support for the conclusion of this study, which will also become the direction of our future work.

Firstly, many thanks to the editor-in-chief for encouraging our work and for giving useful comments. And here are our responses.

1. paper page 1, "cuproptosis- " is the dash "-" required after this word? through out the paper some has a dash after and some dose not have, please clarify.

Response: Thank you very much for your question. In this article, we use "cuproptosis" without dash when we are just introducing copper death itself. And "cuproptosis-" usually appears as "cuproptosis- related genes", used to express genes which are associated with cuproptosis. At your prompt, we have checked the article to ensure proper use.

2. "research on " some words have blue underline, please remove.

Response: Thank you very much for your careful review of our article and we have removed the blue underline.

3. "The idea of this study is shown in Figure 1 in the form of a flow chart" this title should be rephrased and more specific, such as"Study flow chart".

Response: We have modified this part, simplified the title and added the purpose of flow chart. The flow chart is used to facilitate readers to understand the idea of the research.

4. All figures' original picutre resolution are too low, it should be improved.

Response: We have resubmitted the higher resolution figures.