Peer Review File

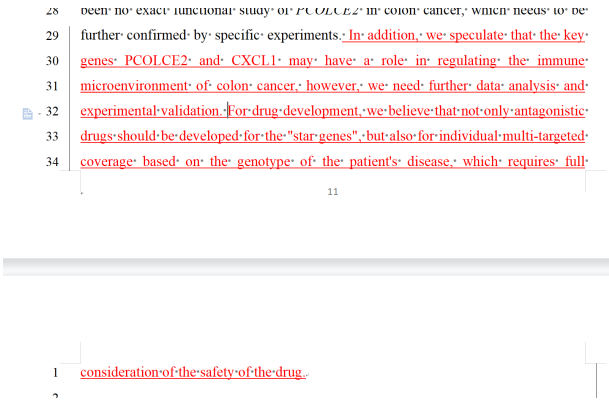
Article information: https://dx.doi.org/10.21037/jgo-23-49

Review Comments-Reviewer A

The paper titled "Role of epithelial cell-mesenchymal transition regulators in molecular typing and prognosis of colon cancer" is interesting. In this study, 22 prognostic genes were screened out from 200 EMT-related genes, and then the PCOLCE2 and CXCL1 molecules were finally focused on through the combination of the NMF molecular typing model and machine learning screening feature genes, suggesting that PCOLCE2 and CXCL1 may have good application potential. However, there are several minor issues that if addressed would significantly improve the manuscript.

1) What is the relationship between EMT-related genes and tumor microenvironment? What are the possible goals of future drug development? It is suggested to add relevant content to the discussion.

Response: Thank you for your comments. We've added that to the discussion.


been no exact functional study of PCOLCE2 in colon cancer, which needs to be further confirmed by specific experiments. In addition, we speculate that the key genes PCOLCE2 and CXCL1 may have a role in regulating the immune microenvironment of colon cancer, however, we need further data analysis and experimental validation. For drug development, we believe that not only antagonistic drugs should be developed for the "star genes", but also for individual multi-targeted coverage based on the genotype of the patient's disease, which requires full

11

consideration of the safety of the drug.

2) It may be more meaningful to add functional research on key genes.

Response: Thank you for your comments. We will strengthen the functional study of key genes in the next step.

3) How to construct the EMT- related gene pair prognostic signature? Can it be used to identify patients with high recurrence risk of phase II colon cancer? It is suggested to add relevant contents.

Response: Thank you for your comments. A prognostic model for colon cancer can be constructed using a combination of multiple genes. Secondly, we did not fully consider the effect of staging on colon cancer in this study, which is a major shortcoming of ours, so now we do not have sufficient evidence to answer whether we can identify patients with high risk of recurrence of stage II colon cancer, so we did not include this part in this study. We will fully consider your suggestion in the next phase of the study.

4) The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as "Prognostic impact of mitofusin 2 expression in colon cancer, PMID: 36388028", "Epithelial-mesenchymal transition classification of circulating tumor

cells in lung and colon cancer patients: potential role in clinical practice, PMID: 35117274". It is recommended to quote the articles.

Response: Thank you for your comments. We have already added that part.

various stages of metastasis are still unclear. Recent literature also reported that the decreased expression of EMT-related gene MFN2 in colon cancer tissues was negatively correlated with the prognosis of colon cancer patients, and further studies suggest that MFN2 is a promising predictive biomarker and therapeutic target for colon cancer(17). Additional findings also suggest that circulating tumor cells with a mesenchymal phenotype or mixed epithelial/mesenchymal phenotype may be potential biomarkers for monitoring tumor progression in lung or colon cancer, respectively(18).

5) This study is based on bioinformatics analysis. It is recommended to increase in vivo and in vitro experimental studies, which may be more meaningful.

Response: Thank you for your comments. We will strengthen the functional study of key genes in the next step.

6) There have been many studies on colon cancer. What is the difference between this study and previous studies? What is the innovation? These need to be described in the introduction.

Response: Thank you for your comments. We've added that to the introduction.
The present study focused on the colon cancer signature genes PCOLCE2 and CXCL1 from 200 EMT genes using multiple bioinformatics techniques, which suggests that these two genes represent the functional nature of a portion of EMT genes, which is the biggest difference between the present study and others.
Second, the PCOLCE2 gene identified in this study has not been studied in colon cancer, which suggests that functional studies targeting the PCOLCE2 gene have the potential to unravel new mechanisms of EMT regulation of tumors, which is the most innovative point of this study.

tumor metastasis process. Using random forest algorithm (RFA) to further analyze EMT DEGs, our study revealed that PCOLCE2 and CXCL1 genes are characteristic genes for clinical prognosis of colon cancer, which means that this study focused on PCOLCE2 and CXCL1, characteristic genes of colon cancer, from 200 EMT genes, among which PCOLCE2 gene has not been studied in colon cancer, using multiple bioinformatics techniques, suggesting that functional studies targeting PCOLCE2 gene have the potential to unravel new mechanisms of EMT regulation of tumors.

7) What is the key role of EMT in the treatment of drug resistance? What is the importance of EMT as a human cancer treatment target? It is suggested to add relevant contents.

Response: Thank you for your comments. We've added that to the discussion.

consideration of the safety of the drug. Finally, because EMT is one of the important phenotypes of tumor cells, and although the causal relationship between EMT and tumor metastasis is not fully understood, yet the role of EMT in drug resistance has been repeatedly confirmed, this study suggests that treatment targeting EMT has the potential to reverse drug resistance, possibly by a mechanism that alters signaling pathways or affects cell proliferation to reverse tumor drug resistance.

Review Comments-Reviewer B

This is an excellent article, but the sample size is small. Also, double-blind, randomized trials and meta-analyses are needed to prove your aims.

Response: Thank you for your comments. Next, we will proceed to clinical trials.

Review Comments-Reviewer C

1. Authors should double check whether detailed information of the settings in each analysis has been provided.

Response: Thank you for your comments. We have carried out checks.

2. Validation of the data is crucial for such study, and more discussion should be provided regarding the experimental or bio-informative analysis from other groups about similar topics and targets. Are results from this study the same or different from others? In which cancer type?

Response: Thank you for your comments. We will study it in detail in the next study.

3. Table S1 should not just provide a name, there should be other information, such as gene ID, or synonyms, reference, etc.

Response: Thank you for your comments. We have corrected it.

15

16 **Table S1** 200 EMT-related genes information

gene ID
ABI3BP
ACTA2
ADAM12
ANPEP
APLP1

4. The quality of the figures is poor. Many were elongated shapes and the texts within the figures were also affected.

Response: Thank you for your comments. We have corrected it.

Review Comments-Reviewer D

1. Please clarify “what is known” and “what is new” respectively in the box.

What is known and what is new?

Despite advances in screening, diagnosis, chemotherapy, and targeted therapy for colon cancer, the prognosis remains poor once distant metastasis or local recurrence occurs. In this study, 22 factors were screened out from 200 EMT-related genes with prognostic value in

Response: Thank you for your comments. We have corrected it.

Key findings

PCOLCE2 and CXCL1 have good potential for clinical translational application in colon cancer.

What is known and what is new?

- Once distant metastasis or local recurrence of colon cancer occurs, the prognosis is still poor, and new markers are needed to predict the prognosis and treatment of colon cancer.

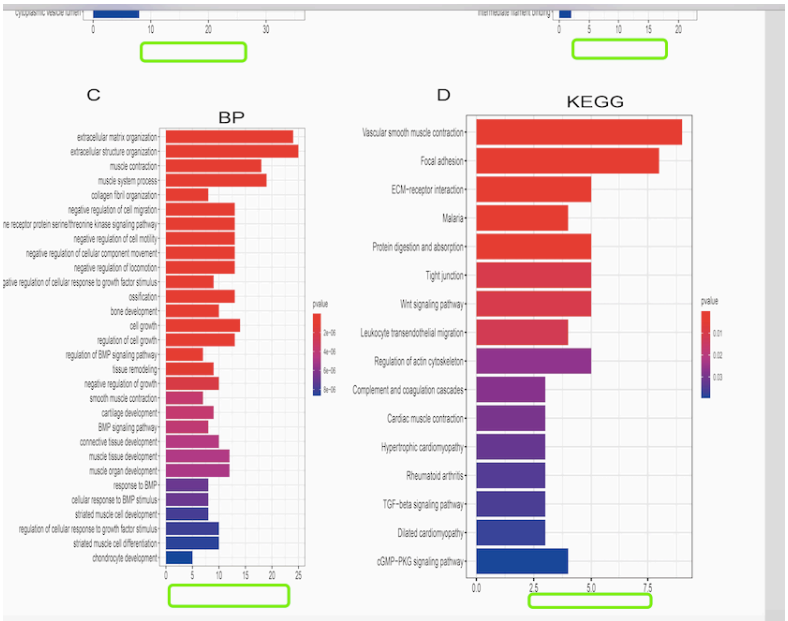
1

In this study, PCOLCE2 and CXCL1 can be used as novel molecular markers for colon cancer.

2. Figure 2D: Please check here and revise.

Response: Thank you for your comments. We have corrected it.

3. Figure 4: Please add the descriptions of X-axis for below figure.



Response: Thank you for your comments. We have corrected it.