Dear Editor:

We have carefully studied the valuable comments from reviewers and the editor, and tried our best to revise the manuscript titled "Comprehensive analysis of Distal-Less homeobox family gene expression in colon cancer" (Manuscript NO.: 83086). The amendment has been marked with red. We have made detailed changes throughout the text. The point to point responds to the reviewer's comments are listed in the revised text.

Reviewer #1:

The manuscript entitled: Comprehensive analysis of Distal-Less homeobox family gene expression in colon cancer, presents an interesting and important study on Distal-Less homeobox (DLX), where less information is known at the moment. The following few points are adviced to be addressed before further steps:

-It is important to include abbreviation section.

Response. Thanks for your comments. The abbreviation section was supplemented according to your suggestion.

-Abstract Methods: no need to mention software versions here.

Response. Thanks for your comments. The software was deleted according to your suggestion.

-Introduction In paragraph No 2, more information is needed about Distal-Less homeobox (DLX). Specially to explain the gab in knowledge and its correlations with the cancer and microbial interactions.

Response. Thanks for your comments. The introduction was improved according to your suggestion.

-Methods and results are robust and clear.

Response. Thank you very much.

Reviewer #2:

Chen et al. reported the biological role of the DLX family in COAD. DLX 2/3/4/5/6 were significantly upregulated in COAD patients. The expression of DLX family was associated with M stage, pathologic stage, primary therapy outcome, residual tumor, lymphatic invasion, T stage, N stage, age, perineural invasion, and history of colon polyps. DLX2/5 were independently correlated with the prognosis of COAD in multivariate analysis. The author believed that the DLX gene family can be used as potential diagnostic or prognostic biomarkers and therapeutic targets for COAD. Overall, tables and figures are informative. References are appropriate. My main concern with this work is the real clinical application of this study, because one might wonder if the results are really reliable. In the absence of any convincing independent cohort and associated experimental studies, the results of this study should not be overstated.

Specific comments

1. The method of the abstract should be rephrased. A long sentence is very unreadable.

Response. Thanks for your comments. We have improved the description according to your suggestions.

2. CBioPortal analysis: What are the principles and criteria for analyzing cohort selection (CaseCCC, PNAS 2015； CPTAC-2 Prospective, Cell 2019)? Is it random?

Response. Thanks for your comments**.** The principles and criteria for analyzing cohort selection was as follows: (1) cancer type: colon adenocarcinoma; (2) 2 selected studies: colon adenocarcinoma (CaseCCC, PNAS 2015), colon cancer (CPTAC-2 Prospective, Cell 2019); (3) molecular

profile: mutations and copy number alterations; (4) selection of patients/case sets: all samples (139).

3. The missing of supplementary table is the lack of readability of the manuscript.

Response. Thanks for your comments. Supplementary tables have been added.

4. According to the results, some members of DLX are related to M stage, which brings a problem that DLX may be more related to prognosis than to diagnosis.

Response. Thank you for your comments. In this study, we analyzed whether the DLXs gene family had diagnostic and prognostic value. The results of the analysis showed that DLXs had some diagnostic and prognostic value.

5. According to the above comments, the corresponding diagnostic efficacy of DLX should not be overstated in the abstract and discussion sections.

Response. Thanks for your comments. We have improved the description according to your suggestions.

6. Similarly, in ROC analysis, the word prediction is inappropriate.

Response. We have improved the description accordance to your suggestions.

7. The GO and KEGG results are simply lists, with no interpretation of the corresponding results.

Response. Thanks for your comments. We have improved the description in the discussion Section according to your suggestion.

8. KEGG results showed that DLX was associated with breast cancer, gastric

cancer, melanoma, and basl cell carcinoma, so the diagnostic power of DLX was contradicted.

Response. Thanks for your comments. The KEGG analysis is the result of a comprehensive analysis based on existing databases, and all data analysis is relatively rich. We have modified the description of the results of the analysis in accordance with your suggestions to present as many conclusions as possible that are relevant to this study.

Company editor-in-chief:

I have reviewed the Peer-Review Report and the full text of the manuscript, all of which have met the basic publishing requirements of the World Journal of Gastrointestinal Oncology, and the manuscript is conditionally accepted. I have sent the manuscript to the author(s) for its revision according to the Peer-Review Report, Editorial Office's comments and the Criteria for Manuscript Revision by Authors.

Before final acceptance, when revising the manuscript, the author must supplement and improve the highlights of the latest cutting-edge research results, thereby further improving the content of the manuscript. To this end, authors are advised to apply a new tool, the Reference Citation Analysis (RCA). RCA is an artificial intelligence technology-based open multidisciplinary citation analysis database. In it, upon obtaining search results from the keywords entered by the author, "Impact Index Per Article" under "Ranked by" should be selected to find the latest highlight articles, which can then be used to further improve an article under preparation/peer-review/revision. Please visit our RCA database for more information at: https://www.referencecitationanalysis.com/.

Response. Thanks for your comments. We improved the Introduction Section according to your suggestions.

Uniform presentation should be used for figures showing the same or similar contents; for example, “Figure 1Pathological changes of atrophic gastritis after treatment. A: ...; B: ...; C: ...; D: ...; E: ...; F: ...; G: ...”. Please provide decomposable Figures (in which all components are movable and editable), organize them into a single PowerPoint file. Please check and confirm whether the figures are original (i.e. generated de novo by the author(s) for this paper). If the picture is ‘original’, the author needs to add the following copyright information to the bottom right-hand side of the picture in PowerPoint (PPT): Copyright ©The Author(s) 2022.

Response. Thanks for your comments. We prepared the Figures according to your suggestion.

The reviewer and editor would like to addition of independent cohort validation and associated experimental studies in the revision.

Response. Thanks for your comments. To further verify the accuracy of the TCGA database, we downloaded COAD samples from the GEO database for analysis. The 30 COAD tissues and 30 normal colon tissues contained in GSE74062 were used for DLX gene expression analysis.

Yours sincerely,

Hui Peng