Response to reviewers

Dear Editor and Reviewers,

Thank you for your guidance! Your suggestions are necessary to improve the quality of my manuscript "Expression of CDK9 is Positively Correlated with the Autophagy Level in Colon Cancer". I appreciated very much the constructive and insightful suggestion from the editor and reviewers. I hope that the revised manuscript can meet the journal's standard and addressed the reviewer concerns.

Point-to-point responses was listed the next pages.

Reviewer #1:

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Major revision

Specific Comments to Authors: The topic of this manuscript is interesting, but there are major defects in results: 1- The figures showing TEM and IHC of autophagy are poor Please provide high quality TEM figures showing clearly the autophagosomes and autolysosomes 2- For IHC, provide better figures with higher magnification showing the expression of studied proteins 3- What is role of ABCG2 in autophagy? 4- Provide a brief discription for the method of TEM and IHC in material and methods section.

1) The figures showing TEM and IHC of autophagy are poor. Please provide high quality TEM figures showing clearly the autophagosomes and autolysosomes.

Answer: Thank you for your guidance! Your suggestion is very necessary to improve my manuscript. High resolution TEM and IHC figures have been provided.

2) For IHC, provide better figures with higher magnification showing the expression of studied proteins.

Answer: Thank you for your suggestion! Higher resolution and magnification of BECN1 and ABCG2 IHC figures have been provided.

3) What is role of ABCG2 in autophagy?

Answer: ABCG2 plays a crucial role in drug transport and cellular defense mechanisms. In vitro experiments have demonstrated that the expression of ABCG2 can enhance cell survival and resistance to adverse conditions, such as amino acid starvation and radiation-associated cell death. These resistance mechanisms are closely associated with ABCG2-dependent autophagy, a

cellular process that helps maintain cell homeostasis and repair damage. The enhancement of autophagy depends on the overexpression of ABCG2 .(Line 367-372)

The positive rate of autophagy in right colon cancer was significantly higher than that in the left colon cancer. We also found that the expression level of ABCG2 in right colon cancer was higher than that in the paracarcinoma tissue, while there was no significant difference between the expression level of ABCG2 in left colon cancer and the paracarcinoma tissue .(Line 372-377)

The expression levels of ABCG2 were associated with tumor N-stage and lymph node invasion.(Line 318-319)

In summary, ABCG2 is an important transporter protein involved in drug resistance, and it may have an influence on the cellular autophagy process, especially under conditions of stress or drug exposure. The exact mechanisms and interactions between ABCG2 and autophagy are complex and can vary depending on the specific cellular context and conditions.

4) Provide a brief description for the method of TEM and IHC in material and methods section.

Answer: Thank you for your reminder! We have added the description for TEM and IHC.

TEM (transmission electron microscope): For TEM, tumor and paracarcinoma tissues from stage IV colon cancer patients were fixed in 2.5% glutaraldehyde, rinsed in 0.1 M phosphoric acid, and then immobilized with 1% osmic acid fixative. Dehydration included sequential steps in ethanol and acetone. Samples were embedded, solidified, and sliced into 70 nm sections. Staining was performed with 3% uranium acetate-lead citrate. Finally, observation and filming were done using the TEMJEOL JEM-1230 at 80KV. (Line 217-222)

IHC (immunohistochemistry): For IHC, paraffin section preparation involved tissue dehydration, transparency, wax immersion, block embedding, and sectioning. Dewaxing included xylene, 100% alcohol, and subsequent

alcohol concentrations with 10-min intervals. Duration varied with temperature. For antigen repair, the tissues were washed, treated with 3% hydrogen peroxide for 10 min and then citric acid buffer before being microwaved for antigen exposure. Serum blocking involved washing with phosphate buffered saline, drying, serum addition, and incubation at 37 °C. The serum was diluted 10 times. Primary antibody was applied, and slides were stored at 4 °C overnight. Secondary antibody was applied after phosphate buffered saline washing. StreptAvidin-Biotin Complex solution was applied for 30 min at 37 °C. The slides were exposed to developer solution, and staining with hematoxylin was completed in 0.5-5.0 min. Dehydration included various alcohol concentrations and xylene. Slides were finally sealed and dried.(Line 223-235)

Reviewer #2:

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Accept (General priority)

Specific Comments to Authors: By combining the analysis of RNAseq data available in the public databases and the translational research performed in house, the group demonstrates relationship between the expression of autophagy-related genes CDK9, ABCG2, and BECN1 and the clinical features and prognosis of colorectal cancer. The data showed that the high expression of CDK9 indicated a poor prognosis in colorectal cancer and possibly the combination of CDK9 inhibitors and autophagy inhibitors could be explored further to enhance the sensitivity of the tumor cells sensitivity to chemotherapy. Overall, the manuscript is well structured and concise, the research is original, and methods are appropriate.

Answer: Thank you very much for your encouragement and affirmation! Your motivation and recognition are the driving force behind my continuous exploration of tumorigenicity!

EDITORIAL OFFICE'S COMMENTS

Authors must revise the manuscript according to the Editorial Office's comments and suggestions, which are listed below:

(1) Science editor:

The manuscript has been peer-reviewed, and it is ready for the first decision.
Language Quality: Grade B (Minor language polishing)
Scientific Quality: Grade C (Good)

(2) 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. The quality of the English language of the manuscript does not meet the requirements of the journal. Before final acceptance, the author(s) must provide the English Language Certificate issued by a professional English language editing company. Please visit the following website for the professional English language editing companies we recommend: https://www.wjgnet.com/bpg/gerinfo/240. 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/. 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) 2023.

Answer: Thank you for the suggestion from company editor-in-chief. I have polished my manuscript by "Filipodia". And the language certificate has been uploaded to the system according the journal required. RCA reference and figures of the manuscript have been rearranged and re-uploaded as required. I hope that the revised manuscript can meet the journal requirements for publication.