Peer Review File

Article information: https://dx.doi.org/10.21037/jgo-23-628

Reviewer A

1) First, please indicate the experiment design of this study in the title, i.e., an in vitro or vivo study.

Reply:This study has been marked as an in vitro study in the title, see page 1 line 3-4.

2) Second, the abstract needs further revisions. The background did not indicate the potential clinical significance of this research focus and limitations of prior studies on the pathophysiological mechanisms. The methods need to describe the purposes of these research procedures. The results did not quantify the findings by reporting statistics and accurate P values such as the expression levels. The conclusion needs to have comments on the possible clinical implications of the findings.

Reply: According to the above suggestions, the background, methods, results and conclusions of the abstract have been revised. For details, see page 1-2 line 1-57.

3) Third, the introduction of the main text is inadequate. The authors need to have an overview on what has been known on the pathophysiological mechanisms in CRC, the known biomarkers involved, why the miRNAs are important and understudied, and importantly, what the potential clinical significance of this study is.

Reply: According to the above opinions, the text citation has been supplemented and improved. See page 3 line 73-84 and page 3 line 96-99.

4) Fourth, in the methodology of the main text, please have an overview of the experiment procedures and the questions to be answered by them. In statistics, please consider to assess the normality of the outcome variables and ensure $P<0.05$ is two-sided.

Reply: The relevant description of the statistical method has been revised, as detailed on page 7 line 217-221.

5) Finally, please consider to review and cite several related papers: 1. Jiang J, Zhu F, Zhang H, Sun T, Fu F, Chen X, Zhang Y. Luteolin suppresses the growth of colon cancer cells by inhibiting the IL-6/STAT3 signaling pathway. J Gastrointest Oncol 2022;13(4):1722-1732. doi: 10.21037/jgo-22-507. 2. Zhang X, Wang H, Yu M, Ma K, Ning L. Inhibition of autophagy by 3-methyladenine promotes migration and invasion of colon cancer cells through epithelial mesenchymal transformation. Transl Cancer Res 2022;11(8):2834-2842. doi: 10.21037/tcr-22-1736. 3. Guo Y, Zhou Y, Gu X, Xiang J. Tripartite motif 52 (TRIM52) promotes proliferation, migration, and regulation of colon cancer cells associated with the NF-κB signaling pathway. J Gastrointest Oncol 2022;13(3):1097-1111. doi: 10.21037/jgo-22-317. 4. Du Q, Ye X, Lu SR, Li H, Liu HY, Zhai Q, Yu B. Exosomal miR-30a and miR-222 derived from colon cancer mesenchymal stem cells promote the tumorigenicity of colon cancer through targeting MIA3. J Gastrointest Oncol 2021;12(1):52-68. doi: 10.21037/jgo-20-513.

Reply: The above articles have been cited in the article, see the reference section for details.

Reviewer B

The paper titled “miRNA-369-3p inhibits the malignant biological behavior of colon cancer cells by reducing the level of TCF4” is interesting. MiR-369-3p could inhibit cell proliferation, invasion and oxidative stress in CRC cells by binding TCF4 mRNA and suppressing its protein expression. However, there are several minor issues that if addressed would significantly improve the manuscript.

1) In the introduction of the manuscript, it is necessary to clearly indicate the knowledge gaps and limitations of prior study and the clinical significance of this study.

Reply: Relevant descriptions have been supplemented in the introduction, as shown in page 3 line 73-84 and page 3 line 93-99.

2) It is recommended to increase the expression of miRNA-369-3p in patients with colon cancer and compare the clinical pathology.

Reply: The expression of miRNA-369-3p in colon cancer patients was increased, as shown in page 4 line 104-111 and Fig1A.

3) There are many miRNAs that regulate the colon cancer. Why did the author choose miRNA-369-3p for research? Please describe the reason.

Reply: First, studies have found that miRNA-369-3p plays a tumor suppressor role in various cancers such as liver cancer and thyroid cancer. Secondly, studies have shown that miRNA-369-3p induces epigenetic reprogramming and inhibits the malignant phenotype of human colon cancer cells. In addition, our research confirmed that the expression of miRNA-369-3p was significantly decreased in CRC tissues and cells. Therefore, the purpose of this paper is to explore the target and mechanism of miRNA-369-3p in the development of CRC, in order to provide a new effective target for the diagnosis and treatment of CRC.

Changes in the text: Please see page 3 line 84-99.

4) What are the relevant characteristics of the tumor microenvironment of colon cancer? What is the correlation between miRNA-369-3p and the tumor microenvironment? What are the possible goals of future drug development? It is recommended to add relevant content to the discussion.

Reply: I’m sorry, because this study does not involve the tumor microenvironment of colon cancer, it is not suitable to increase the relevant content in the discussion.

5) The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as “The anti-tumor effect of miR-539-3p on colon cancer via regulating cell viability, motility, and nude mouse tumorigenicity with CDK14 inhibition, J Gastrointest Oncol, PMID: 33209486”. It is recommended to quote the article.

Reply: The article has been cited, see References.

6) It is suggested that increasing the functional impact of miRNA-369-3p on apoptosis of colon cancer cells may enrich the study.
Reply: The functional effect of miRNA-369-3p on the apoptosis of colon cancer cells has been provided. See page 6 line 184-189, page 8-9 line 264-268 and Fig 3C.

7) It is recommended to increase the study of lncRNA regulating the miRNA-369-3p/TCF4 axis, which may make the whole study more complete.
Reply: Thank you for your advice. Our next research plan is to find lncRNAs that regulate the miRNA-369-3p/TCF4 axis, and to explore the mechanism of lncRNA/miRNA-369-3p/TCF4 axis in colon cancer. Therefore, the study of lncRNA regulating the miRNA-369-3p/TCF4 axis is not involved in this study.
Changes in the text: None.

Reviewer C

1. The authors mentioned "studies...", while only one reference was cited. Change "Studies" to "A study" or add more citations. Please revise.

A growing number of studies have shown that miRNAs play an important role in promoting or inhibiting tumor cell proliferation, invasion, apoptosis and drug resistance by regulating oncogenes or tumor suppressor genes (6).

Recent studies have shown that miR-369-3p plays a tumor suppressor role in several cancers. For example, miR-369-3p inhibits the viability and motility of hepatocellular carcinoma (HCC) cells by binding to paired Box 6 (7).

Studies have confirmed that the increase in morbidity and mortality of patients with colon cancer is related to changes in the interaction of lifestyle, diet, age, obesity, genetic and environmental factors (exposure to carcinogens and smoking, etc.) (13).
Reply: Revised.

2. Figure 3

mimic-pcDNA-TCF4 or mimic+pcDNA-TCF4? Which one is correct? Please check and unify.
Reply: mimic+pcDNA-TCF4 is correct and we have modified the figure legend.

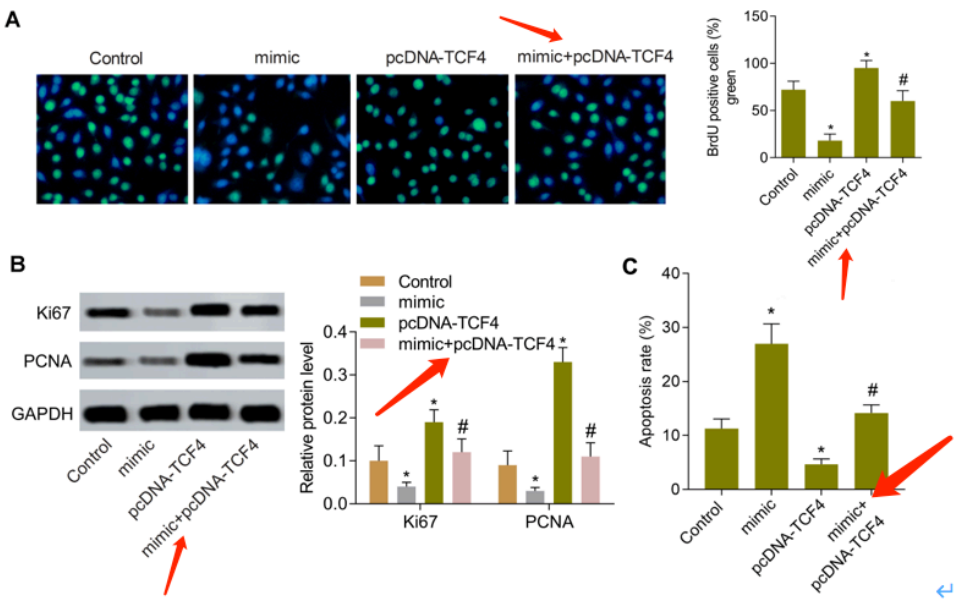

Figure 3 miR-369-3p inhibits cell proliferation by modulating Ki67 and PCNA protein expression. (A) Cell proliferation analysis was evaluated in SW480 cells treated with control mimic, miR-369-3p mimic, pcDNA-TCF4, and miR-369-3p mimic-pcDNA-TCF4 by BrdU assay. Photograph showed at 200×. (B) Evaluation of relative protein levels of Ki67 and PCNA by western blotting in SW480 cells treated with control mimic, miR-369-3p mimic, pcDNA-TCF4, and miR-369-3p mimic-pcDNA-TCF4. GAPH

3. Figure 4

mimic-pcDNA-TCF4 or mimic+pcDNA-TCF4? Which one is correct? Please check and unify.

Reply: The same as above.

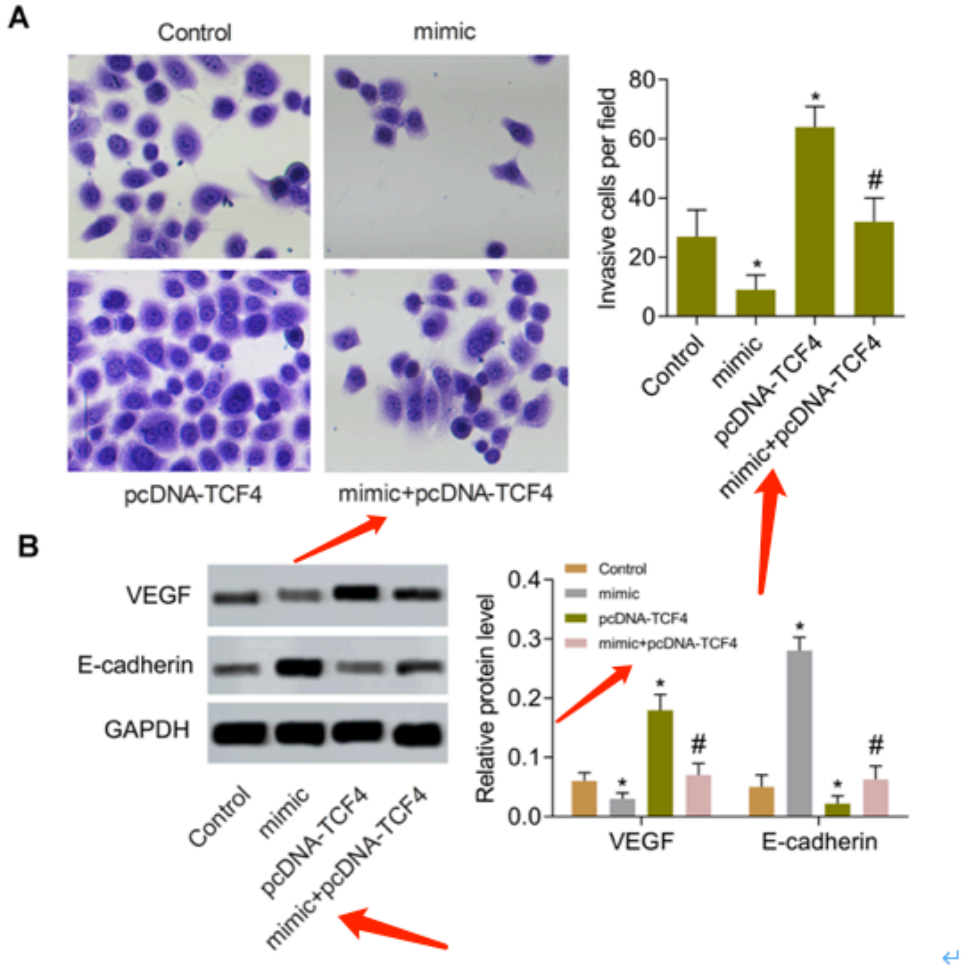

Figure 4 miR-369-3p inhibits cell invasion by regulating VEGF and E-cadherin protein expression. (A) Cell invasion capacity was determined in SW480 cells treated with control mimic, miR-369-3p mimic, pcDNA-TCF4, and miR-369-3p mimic-pcDNA-TCF4 by Transwell assay (crystal violet staining, 200×). (B) Relative protein levels of VEGF and E-cadherin were evaluated in SW480 cells treated with control mimic, miR-369-3p mimic, pcDNA-TCF4, and miR-369-3p mimic-pcDNA-TCF4 by western blotting. *, P<0.05 vs. control group; #, P<0.05 vs. mimic group. pcDNA, pcDNA3.1; TCF4, transcription factor 4; VEGF, vascular endothelial growth factor; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

4. Figure 5

Please check the legend for Figure 5C. Should "LDH SOD" be "LDH"? Please check and revise.

Reply: Revised to "LDH".

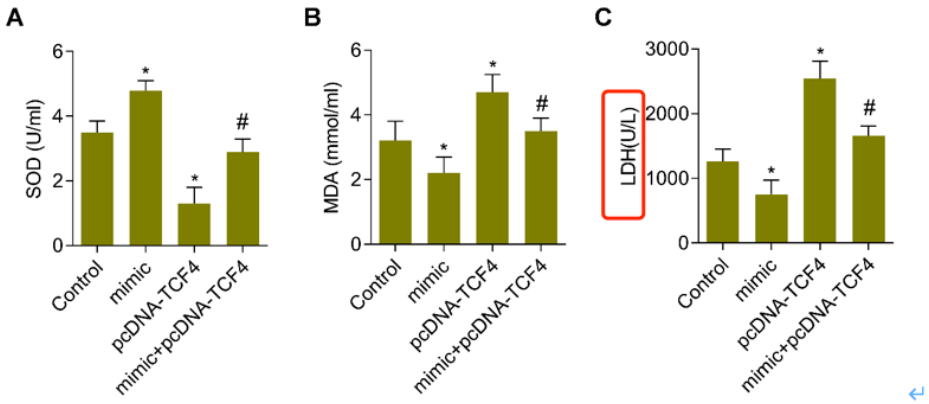

Figure 5 miR-369-3p inhibits oxidative stress by regulating enzymatic activity of SOD and LDH. (A) The activity of SOD was evaluated in cells SW480 treated with miR-369-3p. (B) The content of MDA was analyzed in cells SW480 transfected with miR-369-3p. (C) The activity of LDH SOD was evaluated in cells SW480 treated with miR-369-3p. *, P<0.05 vs. control group; #, P<0.05 vs. mimic group. SOD, superoxide; pcDNA, pcDNA3.1; TCF4, transcription factor 4; MDA, malondialdehyde; LDH, lactate dehydrogenase.