

Dear Professor Ma,

We would like to thank the editors and reviewers' work devoted to our manuscript and we are very grateful for their valuable suggestions.

I modified the entire manuscript according to the opinions of the editor and reviewer. We have fully addressed each concern and hope that this revised manuscript is now acceptable. Please see below for our detailed responses. Each concern is discussed in detail below.

Company editor-in-chief:

Comment 1. Please authors are required to provide standard three-line tables, that is, only the top line, bottom line, and column line are displayed, while other table lines are hidden. The contents of each cell in the table should conform to the editing specifications, and the lines of each row or column of the table should be aligned. Do not use carriage returns or spaces to replace lines or vertical lines and do not segment cell content.

Response 1. As your mentioned, We have modified the format of the tables as required, please refer to 74288-Table File.

Comment 2. Before final acceptance, the author(s) must provide the English Language Certificate issued by a professional English language editing company.

Response 2. We have uploaded the English Language Certificate in the system, please check it.

Reviewer 1

Dear Authors, thank you for preparing your manuscript with attention to detail - the overall presentation is solid and the research is interesting.

Response. We thank the reviewer for their appreciation of the content of the manuscript and put forward valuable suggestions for us.

Comment 1. In the you wrote that there were 18 samples of patients with benign intestinal polyps, as well as 3 samples from patients with ulcerative colitis. The total samples that was mentioned afterwards was 20. Just to make sure, shouldn't it be 21 (18 + 3), or maybe I misunderstood something?

Response 1. We carefully searched the original data of the experiment and found that there were 17 fecal samples from patients with benign intestinal polyps and 3 fecal samples from patients with ulcerative colitis, totaling 20 samples. I have made modifications in the "Subject" subsection of "Materials and Methods".

Comment 2. The first table in the manuscript (page 12) is not numerated or named. This is the small table with only reagents, but in my opinion such objects should still be considered as a table. Moreover, in the first column of this table, there is "colume"

- shouldn't it be "column" or "columns" by any chance?

Response 2. I numbered the table on page 12 as Table 1 and arranged all the tables in the manuscript in ascending order according to the order before and after they appeared. There were 13 tables in total. In addition, I have changed "column" into "columns" in Table 1.

Comment 3. Please add space when appropriate, examples are in table "Clinical data analysis of CRC patients" [people(%) is without space] and table "Comparison of pathogenic mutation sites in preoperative stools vs. tumor tissues" [there is no space between some % values and brackets in the "tumor tissue" column]; same applies to "postoperative stool" column of table "Comparison of pathogenic mutation sites in preoperative stools vs. Postoperative stool".

Response 3. We thank for reviewer for this helpful comment. As for the space you mentioned, I have revised the whole manuscript.

Comment 4. Please clarify, if the "enon5" and "enon7" (page 24, the beginning of section "Distribution of mutation sites of "unknown clinical significance"") are obvious typos of "exon5" and "exon7"?

Response 4. The "exon" at the beginning of the section "Distribution of mutation sites of "unknown clinical significance" was misspelled and I have corrected it. I really appreciate your careful reading of the manuscript.

Comment 5. Please change "kD" to "kDa" in the sentence beginning with "The 21 kD protein encoded by KRAS" (page 28).

Response 5. According to your reminder, we have checked the relevant requirements of the magazine on the use of symbols and made corrections. Thank you for pointing out the mistakes for us so carefully.

Comment 6. In the table "Clinical data analysis of CRC patients", I would move the main features groups like age, tumor location, tumor size etc. a little bit to the left, similar as in table "Fecal gene mutation results in the control groups".

Response 6. We consulted the requirements of the magazine on the form format and made unified modifications to the two forms "Clinical data analysis of Colorectal cancer patients" and "Fecal gene mutation results in the control groups".

Comment 7. Please standardize the referencing to tables in the main text - example is in section "Gene mutations in various samples", where table 2 is referenced as "Tab. 2" while table 3 as "Table 3". Please double-check the rest of the manuscript.

Response 7. Your suggestion is very important. I have replaced "Tab. 2" with "Table 2", and the rest of the text has also been modified.

Comment 8. Change "database" word near the html link to Clinvar (page 16) so it is not italicized.

Response 8. Thanks for your advice. I have linked Clinvar database into word document.

Comment 9. Last but not least, can the Authors discuss the topic of miRNA in CRC or the impact of diet on CRC? These are the aspects that I missed during the lecture.

Response 9. CRC is a multifactorial and complex disease. A good dietary strategy may reduce the incidence of CRC and play a preventive role in CRC, which is really worth discussing. However, the theme of this paper is the value of fecal gene detection in the diagnosis of CRC, so there is not much discussion on diet ary strategies. In the third paragraph of the "INTRODUCTION", we briefly describe the effect of RNA on CRC.

Reviewer 2

The revision of noninvasive methods of colorectal cancer screening are very useful.

Response. We thank the reviewer for their appreciation of the content of the manuscript and put forward valuable suggestions for us.

Comment 1. In my opinion, this section could be improved recalling that colorectal cancer may develop in patients with distinct intestinal diseases such as Inflammatory Bowel Diseases, Microscopic Colitis, and Irritable Bowel Syndrome, as previously described (The Prevalence of Inflammatory Bowel Diseases, Microscopic Colitis, and Colorectal Cancer in Patients with Irritable Bowel Syndrome. Gastroenterol. Insights 2011,3,7-10).

Response 1. As you mentioned, CRC may occur in patients with different intestinal diseases such as inflammatory bowel disease, which we have overlooked. The details have been added in the 3 paragraph of the "INTRODUCTION".

Comment 2. Another important topic worth mentioning is the growing role of microRNA in colorectal cancer development. In my opinion, in the discussion, the authors should mention the potential pathogenetic role of some microRNA together with TME in the development of colorectal cancer, as previously described (Prognostic and Predictive Roles of microRNA-383 in Colorectal Cancer. Gastroenterol. Insights 2016,7,26-29).

Response 2. Your suggestion is very important. At present, many studies have mentioned the effects of microRNA and TME on the occurrence and development of CRC, which is indeed missing in our article. Therefore, according to your comments, we give a brief description in the third paragraph of the "INTRODUCTION" of the article.

Comment 3. In a study assessing markers for diagnosis and prognostic prediction of CRC, in my opinion, it would be appropriate to quote the clinically relevant impact of colorectal screening as not has been reported that a targeted policy of screening and

surveillance by colonoscopy will curb the rising incidence of colorectal cancer (Screening Colonoscopy in Port Harcourt, Nigeria. Gastroenterol. Insights 2019,10,1-4).

Response 3. Currently, major screening methods for CRC include colonoscopy, serum tumor marker testing, imaging examination and fecal detection. Although colonoscopy can improve the early diagnosis of CRC, it also has limitations. Patients need to make full intestinal preparation before colonoscopy examination, and there is a risk of intestinal mucosal injury, intestinal perforation and intestinal bleeding after operation. Given the non-invasive nature, fecal gene detection has the advantages of convenient sampling, few sample demand and continuous dynamic monitoring, which has a good application prospect in early diagnosis, prognosis and recurrence monitoring for CRC. Our manuscript mainly highlights the advantages of fecal genetic detection, and does not give a detailed description of the other detection methods.

Comment 4. The last topic I would suggest to quote is the impact of potential dietary strategy for colorectal prevention potentially impacting microbiota modulation and fecal modifications. It has been recently reported that Allium constituents were shown to modify the risk of colon cancer and reduce the mortality rates associated with this malignancy. Supplementation of garlic or its extracts reduces the number of aberrant crypt foci, which are one of the earliest preneoplastic lesions of colon cancer, and the risk of colorectal adenomatous polyps. The prevention of precursor lesions' (adenomatous polyps, crypt foci) formation seems to be an effective strategy to provide early prevention of colon carcinogenesis, as recently reported (The Potential Application of Allium Extracts in the Treatment of Gastrointestinal Cancers. Gastroenterol. Insights 2021,12,136-146).

Response 4. We thank for reviewer for this helpful comment. CRC is a multifactorial and complex disease. A good dietary strategy may reduce the incidence of CRC and play a preventive role in CRC, which is really worth discussing. However, the theme of this paper is the value of fecal gene detection in the diagnosis of CRC, so there is not much discussion on diet ary strategies.

Thank you for your continued consideration. I hope this revised manuscript will be accepted. If you have any questions, please do not hesitate to contact me at the address below.

Sincerely!

Mei Lin

Clinical Laboratory, Taizhou people's Hospital (Postgraduate training base of Dalian Medical University), Taizhou, 225300, Jiangsu, China

l_mei@163.com