PEER-REVIEW REPORT

Name of journal: *World Journal of Gastroenterology*

Manuscript NO: 74288

Title: Fecal Gene Detection Based on Next Generation Sequencing for Colorectal Cancer Diagnosis

Provenance and peer review: Invited Manuscript; Externally peer reviewed

Peer-review model: Single blind

Reviewer's code: 06195974

Position: Editorial Board

Academic degree: MD, PhD

Professional title: Assistant Professor

Reviewer's Country/Territory: United States

Author's Country/Territory: China

Manuscript submission date: 2022-01-16

Reviewer chosen by: AI Technique

Reviewer accepted review: 2022-01-16 17:20

Reviewer performed review: 2022-01-16 17:29

Review time: 1 Hour

Scientific quality	[Y] Grade A: Excellent [ ] Grade B: Very good [ ] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish
Language quality	[ ] Grade A: Priority publishing [Y] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection
Conclusion	[ ] Accept (High priority) [ ] Accept (General priority) [ ] Minor revision [Y] Major revision [ ] Rejection
Re-review	[Y] Yes [ ] No

Peer-reviewer statements	Peer-Review: [Y] Anonymous [ ] Onymous
	Conflicts-of-Interest: [ ] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

In this study the authors aimed to to screen specific genes or gene combinations in fecal DNA that are suitable for diagnosis and prognostic prediction of Colorectal cancer (CRC) and to establish a technological platform for CRC screening, diagnosis and efficacy monitoring through fecal DNA detection. Next Generation Sequencing (NGS) was used to sequence the stools of patients with CRC, which were compared with normal control and benign intestinal disease groups, as well as the tumor tissues of CRC patients. They found that high mutation frequencies of TP53, APC and KRAS were detected in the stools and tumor tissues of CRC patients prior to surgery. On the contrary, no pathogenic mutations of the above 3 genes were noted in the postoperative stools, the normal controls, or in the benign intestinal disease group. This indicates that the tumor-specific DNA was detectable in the preoperative stools of CRC patients. Compared to the postoperative stools and the stools in the two control groups, the pathogenic mutation frequencies of TP53 and KRAS were significantly higher for the preoperative stools thus suggesting that fecal TP53 and KRAS genes can be used for CRC screening, diagnosis and prognostic prediction. Among CRC patients, the pathogenic mutation sites of TP53 occurred in 16 of 27 preoperative stools, with a true positive rate of 59.26%, while the pathogenic mutation sites of KRAS occurred in 10 stools, with a true positive rate of 37.04%. The sensitivity and negative predictive value for the combined genetic testing of TP53 and KRAS were 66.67% (18/27) and 68.97%, respectively, both of which were higher than the TP53 or KRAS mutation detection alone, suggesting that the combined genetic testing can improve the CRC detection rate. The mutation sites TP53 exon4 A84G and EGFR exon20 I821T (mutation start and stop

positions were both 7574436 for the former, while 55249164 for the latter) were found in the preoperative stools and tumor tissues. They concluded that NGS-based fecal genetic testing can be used as a complementary technique for the CRC diagnosis. Fecal TP53 and KRAS can be used as specific genes for the screening, diagnosis, prognostic prediction and recurrence monitoring of CRC. Moreover, the combined testing of TP53 and KRAS genes can improve the CRC detection rate.   The study is of interest with novel findings potentially supporting new treatment strategies.   I only would suggest discussing some relevant topics to further improve the manuscript. -Introduction: 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).   - 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). -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). -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).

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Reviewer's code: 05776245

Position: Peer Reviewer

Academic degree: BSc, MSc

Professional title: Academic Research, Research Scientist, Teaching Assistant

Reviewer's Country/Territory: Poland

Author's Country/Territory: China

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Review time: 2 Days and 15 Hours

Scientific quality	[ ] Grade A: Excellent [Y] Grade B: Very good [ ] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish
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Re-review	[Y] Yes [ ] No

SPECIFIC COMMENTS TO AUTHORS

Dear Authors, thank you for preparing your manuscript with attention to detail - the overall presentation is solid and the research is interesting. I have only minor comments, which are the following: (1) In the "Subject" subsection of "Materials and Methods" 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? (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? (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". (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"? (5) Please change "kD" to "kDa" in the sentence beginning with "The 21 kD protein encoded by KRAS" (page 28). (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". (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. (8) Change "database" word near the html link to Clinvar (page 16) so it is not italicized. (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.

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SPECIFIC COMMENTS TO AUTHORS

The authors improved the manuscript