

Prof. Lian-Sheng Ma  
Science Editor, Company Editor-in-Chief, Editorial Office  
Baishideng Publishing Group Inc  
World Journal of Gastroenterology

Dec. 25, 2021

RE: World Journal of Gastroenterology Manuscript NO: 72475 – Manuscript revision  
Differential DNA methylation analysis provides novel insights into colorectal cancer prognosis  
prediction in Taiwan

Dear Professor Ma,

Thank you for your invitation to contribute an article to the World Journal of Gastroenterology (Invited Number ID: 03005388) We have taken into account the reviewer's in-depth comments and have carefully and extensively revised our manuscript according to the reviewer's comments. We have highlighted amendments we made in red font.

Our specific responses are as follows:

Reviewer #1:

**Scientific Quality:** Grade D (Fair)

**Language Quality:** Grade B (Minor language polishing)

**Conclusion:** Major revision

**Specific Comments to Authors:** The manuscript titled, 'Differential DNA methylation analysis provides novel insights into colorectal cancer prognosis prediction in Taiwan', by Jing-Quan et al., has investigated the DNA methylation of three genes in a local cohort of CRC patients from Taiwan. This is an interesting study and of late, role of epigenetic factors including DNA methylation in causation and progression of cancers has become an active and expanding area of research. The few comments regarding the present manuscript are:  
a) Title of the research is rather vague given that only three genes have been investigated for DNA methylation. It will be good to modify the title according to the work undertaken.

**Responses:**

**We agreed with the reviewer's comments and revised the title to put more emphasis on the research conducted. Please see the modified title on page 1.**

b) Background section in the abstract is too general, should be re-written to focus more on the research work conducted.

**Responses:**

**We appreciated the reviewer's comments. We modified the abstract to close to the work**

undertaken. Please see the revised manuscript in Background section of the abstract on page 3.

c) Conclusion sub-section of the abstract states 'fundamental observations.....', which again is too broad a conclusion and should be re-written to describe the findings.

**Responses:**

**We appreciated the reviewer's comments and adjusted statements to describe the findings of this study. Please see the revised manuscript in Conclusion section of the abstract on page 4.**

d) Primers sequences and PCR reaction parameters can be summarized in a Table. This will improve the flow of the experimental procedures described in the methods section. At present, these lengthy details make it hard to follow the procedural details.

**Responses:**

**We appreciated the reviewer's comments and organized primers sequences and PCR reaction parameters into a table. Please see the revised manuscript in Methylation-specific polymerase chain reaction and EpiTYPER DNA methylation analysis section of the Materials and Methods on page 10 and table on page 31.**

e) The method by which DNA methylation was quantified at the sites of interest should be clearly detailed. At present, it is not clear how authors have quantified the DNA methylation at the selected regions of the target genes.

**Responses:**

**We were grateful for the reviewer's comments and added sentences to provide more details about the quantification of DNA methylation. Please see the revised manuscript in Methylation-specific polymerase chain reaction and EpiTYPER DNA methylation analysis section of the Materials and Methods on page 11.**

f) Results sections should show the results of the PCRs i.e. the amplified product and gel-pictures with relevant controls.

**Responses:**

**We showed the methylation-specific polymerase chain reaction (MS-PCR) results of PXDN gene in Figure 2 and described in this manuscript on page 10.**

g) There is no mention of controls for the PCRs undertaken, they should be included too in the methods section.

**Responses:**

**We appreciated the reviewer's comments and added sentences to supplement more information about controls for the PCRs undertaken. Please see the revised manuscript in Methylation-specific polymerase chain reaction and EpiTYPER DNA methylation analysis section**

of the Materials and Methods on page 10.

Reviewer #2:

**Scientific Quality:** Grade C (Good)

**Language Quality:** Grade B (Minor language polishing)

**Conclusion:** Minor revision

**Specific Comments to Authors:** This study investigated the association of changes in DNA methylation of candidate genes in tumor tissue and adjacent normal tissues to evaluate colorectal cancer (CRC) prognosis. The methylation on CpG sites in certain genes has been shown to be significantly associated with CRC recurrence, progression, and survival. This is an interesting study and has clinically significant implications for researchers targeting epigenetics, but supplementation of the following points will be required.

[Introduction] The theoretical background of how the three selected genes (SUMF2, ADAMTS5, and PXDN) are related to the progression of colorectal cancer is missing.

**Responses:**

**We agreed with the reviewer's comments and added sentences to describe how the candidate genes are correlated with the progression of colorectal cancer. Please see the revised manuscript in Introduction section on page 7.**

[Methods] 1. A specific description of how to gather specimens in patients including adjacent normal regions to analyze gene methylation is missing.

**Responses:**

**We appreciated the reviewer's comments. We added sentences to explain how to gather specimens in patients. Please see the revised manuscript in Patient and specimen collection section of the Materials and Methods on page 7-8.**

2. The description of most steps for extracting DNA from a biopsy sample was omitted (Any processes before using the extraction kit).

**Responses:**

**We valued the reviewer's comments. We added sentences to tell more details for extracting DNA from a biopsy sample. Please see the revised manuscript in Patient and specimen collection section of the Materials and Methods on page 9.**

Reviewer #3:

**Scientific Quality:** Grade B (Very good)

**Language Quality:** Grade B (Minor language polishing)

**Conclusion:** Minor revision

**Specific Comments to Authors:** 1 Title. Does the title reflect the main subject/hypothesis of the manuscript? Yes. 2 Abstract. Does the abstract summarize and reflect the work described in the manuscript? Yes. 3 Key words. Do the key words reflect the focus of the manuscript? Yes. 4 Background. Does the manuscript adequately describe the background, present status and significance of the study? Yes. 5 Methods. Does the manuscript describe methods (e.g., experiments, data analysis, surveys, and clinical trials, etc.) in adequate detail? Yes. 6 Results. Are the research objectives achieved by the experiments used in this study? What are the contributions that the study has made for research progress in this field? Yes. 7 Discussion. Does the manuscript interpret the findings adequately and appropriately, highlighting the key points concisely, clearly and logically? Are the findings and their applicability/relevance to the literature stated in a clear and definite manner? Is the discussion accurate and does it discuss the paper's scientific significance and/or relevance to clinical practice sufficiently? Yes. 8 Illustrations and tables. Are the figures, diagrams and tables sufficient, good quality and appropriately illustrative of the paper contents? Do figures require labeling with arrows, asterisks etc., better legends? Yes. 9 Biostatistics. Does the manuscript meet the requirements of biostatistics? Yes. Please analyze the chi-square statistics in Table1 and provide the p value for each column.

**Responses:**

**We were grateful for the reviewer's advice. We analyzed the chi-square statistics and provided the p value for each column. Besides, we further added sentences to describe what we observed after the statistical analysis. Please see the revised manuscript in Patient characteristics section of the Results on page 12 and the revised table on page 32.**

10 Units. Does the manuscript meet the requirements of use of SI units? Yes. 11 References. Does the manuscript cite appropriately the latest, important and authoritative references in the introduction and discussion sections? Does the author self-cite, omit, incorrectly cite and/or over-cite references? Yes. 12 Quality of manuscript organization and presentation. Is the manuscript well, concisely and coherently organized and presented? Is the style, language and grammar accurate and appropriate? Yes. 13 Research methods and reporting. Authors should have prepared their manuscripts according to manuscript type and the appropriate categories, as follows: (1) CARE Checklist (2013) - Case report; (2) CONSORT 2010 Statement - Clinical Trials study, Prospective study, Randomized Controlled trial, Randomized Clinical trial; (3) PRISMA 2009 Checklist - Evidence-Based Medicine, Systematic review, Meta-Analysis; (4) STROBE Statement - Case Control study, Observational study, Retrospective Cohort study; and (5) The ARRIVE Guidelines - Basic study. Did the author prepare the manuscript according to the appropriate research methods and reporting? Yes. 14 Ethics statements. For all manuscripts involving human studies and/or animal experiments, author(s) must submit the related formal ethics

documents that were reviewed and approved by their local ethical review committee. Did the manuscript meet the requirements of ethics? Yes.

**Responses: Thank you very much.**

Reviewer #4:

**Scientific Quality:** Grade C (Good)

**Language Quality:** Grade A (Priority publishing)

**Conclusion:** Minor revision

**Specific Comments to Authors:** In this original article the authors focused on the association between CRC prognosis and the status and level of differential DNA methylation of selected genes. The methylation status of SUMF2, ADAMTS5, and PXDN in tumor tissue and tumor-free adjacent areas were evaluated via MS-PCR, and the methylation degrees of SUMF2 and ADAMTS5 were assessed using EpiTYPER DNA methylation analysis. The relationships of gene methylation with RFS, PFS, and OS were evaluated. They found that CpG\_3+CpG\_7 hypermethylation of SUMF2 from tumor tissue is associated with significantly shorter PFS and OS compared with CpG\_3+CpG\_7 hypomethylation. Contrary, CpG\_2 and CpG\_13 hypermethylation of ADAMTS5 from normal tissue is associated with a significantly longer RFS compared with CpG\_2 and CpG\_13 hypomethylation. The study is well designed, the used methodology is adequate, the results are clear, and the discussion is moderate, critical, and logical. One aspect needs more explanation: - the selection of the 3 candidate genes is understandable, but it must be visualized what other genes were amongst the (at least) TOP10 candidates.

**Responses:**

**We appreciated the reviewer's comments. We modified the abstract to give more information about other candidate genes. Please see the revised manuscript in Gene selection and DNA extraction section of the Materials and Methods on page 9.**

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

**(1) Science editor:**

The authors have analyzed 208 patients with colorectal cancer and found that Significantly shorter PFS and OS were associated with the CpG\_3+CpG\_7 hypermethylation of SUMF2 from tumor tissue compared with CpG\_3+CpG\_7 hypomethylation [hazard ratio (HR) = 2.24, 95% confidence interval (CI) = 1.03-4.85 for PFS, HR = 2.56 and 95% CI = 1.08-6.04 for OS]. By contrast, a significantly longer RFS was associated with CpG\_2 and CpG\_13 hypermethylation of ADAMTS5 from normal tissue compared with CpG\_2 and CpG\_13 hypomethylation [HR (95% CI) = 0.15 (0.03-0.71) for CpG\_2 and 0.20 (0.04-0.97) for CpG\_13]. I believe the manuscript is well written and the study is well designed

Language Quality: Grade B (Minor language polishing)

Scientific Quality: Grade B (Very good)

**Responses: Thank you very much.**

*(2) Company editor-in-chief:*

I have reviewed the Peer-Review Report, the full text of the manuscript, and the relevant ethics documents, all of which have met the basic publishing requirements of the World Journal of Gastroenterology, 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. Please provide decomposable Figures (in which all components are movable and editable), organize them into a single PowerPoint file. 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.

**Responses: Thank you very much and we all done.**

We sincerely thanks for reviewer's comments and your editorial efforts on our manuscript. We believe that the revised manuscript is significantly improved for scientific merits.

Sincerely yours,

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Dear Professor Ma,

Thank you for your invitation to contribute an article to the World Journal of Gastroenterology (Invited Number ID: 03005388, NO.: 72475) We have taken into account the reviewer's in-depth comments and have carefully and extensively revised our manuscript according to the re-reviewer's comments. We have highlighted amendments we made in red font. Our specific responses are as follows: SPECIFIC COMMENTS TO AUTHORS  
The authors have revised the manuscript substantially based on the comments. Few grammar and syntax errors are still to be corrected e.g. 'mo' in methods section has not been elaborated as 'months' in the manuscript.  
Responses: Thank you for your comments. We have replaced 'mo' with 'months' in the methods and materials section. Thank you very much. In Figure 2, it is not clearly mentioned that what is the difference between 'negative control' and 'sterile water' as both are referred to as 'negative control' in the methods and materials section. Responses: We appreciated the reviewer's comments. Please see the revised manuscript in the methods and materials section, and in figure legends. Thank you very much. We sincerely thank for reviewer's comments and your editorial efforts on our manuscript. We believe that the revised manuscript is significantly improved for scientific merits.

Sincerely yours,

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