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PEER-REVIEW REPORT

Name of journal: *World Journal of Gastroenterology*

Manuscript NO: 89415

Title: Fusobacterium nucleatum-induced imbalance in microbiome-derived butyric acid levels promotes the occurrence and development of colorectal cancer

Provenance and peer review: Unsolicited manuscript; Externally peer reviewed

Peer-review model: Single blind

Reviewer's code: 00004700
Position: Peer Reviewer
Academic degree: N/A

Professional title: N/A

Reviewer's Country/Territory: Portugal

Author's Country/Territory: China

Manuscript submission date: 2023-10-31

Reviewer chosen by: Jia-Ru Fan

Reviewer accepted review: 2023-11-28 11:22

Reviewer performed review: 2023-12-15 09:03

Review time: 16 Days and 21 Hours

Scientific quality	[ ] Grade A: Excellent [Y] Grade B: Very good [ ] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish
Novelty of this manuscript	[ ] Grade A: Excellent [Y] Grade B: Good [ ] Grade C: Fair [ ] Grade D: No novelty
Creativity or innovation of this manuscript	[ ] Grade A: Excellent [Y] Grade B: Good [ ] Grade C: Fair [ ] Grade D: No creativity or innovation

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Scientific significance of the conclusion in this manuscript	[Y] Grade A: Excellent [ ] Grade B: Good [ ] Grade C: Fair [ ] Grade D: No scientific significance
Language quality	[ ] Grade A: Priority publishing [ ] Grade B: Minor language polishing [Y] 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

Comments to authors In this manuscript by Wu et al., the authors report that Fusobacterium nucleatum shows increased prevalence in the microbiome of colorectal cancer patients and suggest that F. nucleatum leads to an inhibition of butyric acid production by the colon microbiome, leading to conditions that favor tumor development. Using colorectal cell models, they further provide evidence that butyrate acts through the AMPK signaling pathway to induce inhibition of cell proliferation. The paper is based on solid evidence combining patient-derived sample analysis, mouse models for fecal microbiome inoculation, and cell culture models for mechanistic studies. The initial identification of F. nucleatum is based on the analysis of 39 fresh clinical tissue samples (including 24 colorectal cancer samples, 10 normal tissue samples, and 5 paracancerous tissue samples) which gives an important impact to the result. Nevertheless, the manuscript requires major revision due to the concerns raised below. 1) The title is misleading because it is not the “F. nucleatum-derived butyric acid” that promotes tumorigenesis but the F. nucleatum-induced imbalance in microbiome-derived butyric acid levels 2) the corresponding sentence in the abstract is also of unclear

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wording: “We discovered that when we gavaged mice with F. nucleatum, the butyrate-producing bacteria in the treatment group reduced, showing that F. nucleatum can regulate the quantity of butyric acid, the intestinal metabolite.”. Less ambiguous would be “showing that F. nucleatum can compete with butyrate-producing bacteria leading to deregulated quantity of butyric acid, the intestinal metabolite” 3) page 11: “Fusobacterium was found in high abundance”. What Fig 1A shows is rather a low abundance compared to other bacteria families; however, the relative difference between normal and cancer may be high. However, this cannot be appreciated in the figure due to its scale; the author should add a zoom-in area, which magnifies the scale for the case of fusobacteria and clostridia. 4) p 12: “It indicates that F. nucleatum is crucial to the occurrence and progression of colorectal cancer.” I suggest to modify to “is strongly associated with “ 5) Legend to Figure 2: the authors need to explain what is shown in part A and B of the Figure. 6) Legend to Fig 3: the authors need to explain what each bar stands for; each individual mouse that was treated? 7) p 13: Shown is the change in the amount of total short chain fatty acids (SCFAs) in the F. nucleatum-treated group, but then the further analysis focusses only on butyrate; the authors should state or show whether the other SCFAs are also diminished to the same extent 8) p 14: please comment on the choice of HCT116 and DLD-1 cells as an in vitro model to test the effects of butyrate 9) p 14 and 15: “sodium butyrate's inhibitory action diminished when sodium butyrate co-treated with F. nucleatum, showing that F. nucleatum can suppress the efficacy of sodium butyrate”. The authors need to explain how this experiment was performed. Were bacteria added to the cells’ culture medium? How can you exclude that culture medium exhaustion by the rapidly growing bacteria was not the cause of the observed effect? Later, for the description of Figs 6, 7 and 8, the same issue applies, but here the authors mention the use of F. nucleatum supernatant. This needs to be well explained in the text and the figure legends. 10) p 18, In the discussion, the authors

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should use 'we discovered that' instead of "was discovered" to distinguish their results from those reported by others in the literature. 11) In the discussion, it remains unclear whether the author propose that F. nucleatum outcompetes other butyrate-producing bacteria and thus leads to lower butyrate levels, or whether some kind of active degradation or consumption of butyrate by F. nucleatum is involved in the observed results. 12) Language use: Several paragraphs in the method section are incomplete sentences taken from the lab protocol instructions. These need to be adapted to provide a coherent methodological description. Also, several errors such as the lack of capital letter or of a space between words are highlighted in the attached pdf file

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RE-REVIEW REPORT OF REVISED MANUSCRIPT

Name of journal: *World Journal of Gastroenterology*

Manuscript NO: 89415

Title: Fusobacterium nucleatum-induced imbalance in microbiome-derived butyric acid levels promotes the occurrence and development of colorectal cancer

Provenance and peer review: Unsolicited manuscript; Externally peer reviewed

Peer-review model: Single blind

Reviewer's code: 00004700
Position: Peer Reviewer
Academic degree: N/A

Professional title: N/A

Reviewer's Country/Territory: Portugal

Author's Country/Territory: China

Manuscript submission date: 2023-10-31

Reviewer chosen by: Jing-Jie Wang

Reviewer accepted review: 2024-01-23 12:33

Reviewer performed review: 2024-01-23 14:20

Review time: 1 Hour

Scientific quality	[ ] Grade A: Excellent [Y] Grade B: Very good [ ] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish
Language quality	[Y] Grade A: Priority publishing [ ] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection
Conclusion	[ ] Accept (High priority) [ ] Accept (General priority) [Y] Minor revision [ ] Major revision [ ] Rejection
Peer-reviewer	Peer-Review: [Y] Anonymous [ ] Onymous

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statements	Conflicts-of-Interest: [ ] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

In the revised manuscript and in their comments, the authors have satisfactorily addressed the concerns raised by the reviewer. Nevertheless, three issues that are explained in the rebuttal letter should be included into the manuscript for clarification, namely: 1) the choice of HCT116 and DLD-1 cells as an in vitro model (I suggest to add in the results section at the beginning of paragraph: Sodium butyrate blocks the cell cycle in HCT116 and DLD-1 cells). rebuttal answer: Both HCT116 and DLD-1 cells carry k-ras mutations and research has demonstrated that oncogenic activation of the k-ras allele heightens cell sensitivity to butyrate-induced apoptosis. 2) p 14 and 15: The authors need to explain how this experiment was performed. “sodium butyrate's inhibitory action diminished when sodium butyrate co-treated with F. nucleatum, showing that F. nucleatum can suppress the efficacy of sodium butyrate”. (I suggest to add this information to the Material and Method section) rebuttal answer: This experiment entails the concurrent addition of sodium butyrate and Fusobacterium nucleatum to the cell culture medium, followed by the detection of relevant proliferative and apoptotic proteins after 24 hours, when the color of the culture medium had not fully turned yellow, thus enabling us to discount any results attributable to the depletion of the medium. In some experiments, bacterial culture supernatants were utilized to test whether bacteria themselves can influence the detection of ATP, ROS, and membrane potential. Consequently, we shifted our focus to bacterial metabolites and discovered that they, too, can produce a similar effect. 3) In the discussion, it remains unclear whether (..) some kind of active degradation or consumption of butyrate by F. nucleatum is involved in the observed results. rebuttal answer: When DLD-1 colorectal cancer cells were treated with Fusobacterium nucleatum for 24 hours, the culture

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medium revealed a significant decrease in the extracellular concentration of butyrate using nuclear magnetic resonance (NMR) after post freeze-drying. This leads us to hypothesize that Fusobacterium nucleatum actively degrades butyrate. Please also note that in the provided Word format, Figures 1 and 4 are not correctly reproduced or with some formatting error.