SUNY DOWNSTATE Health Sciences University

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College of Medicine
School of Graduate Studies
College of Nursing
School of Health Professions
School of Public Health

Dear Editorial staff,

Re: Manuscript ID 69856

We thank the reviewers for their thoughtful comments and for taking the time to review the manuscript. . Each comment was taken under consideration and improvements were made to the review based on the recommendations.

Reviewer #1

Specific Comments to Authors:

- A few tips on the graphic presentation: please make the references' position uniform before punctuation.

We have corrected the reference format.

- Table 1 is complete for what regards contents, anyway it is quite difficult to read and not immediate. I suggest to simplify the table, unifying common concepts (e.g., common bacterial machineries) and inserting authors as references to leave space to concepts.

We have attached additional schematic Figures as a complement to the Table to help the reader follow the information more easily. If Reviewers and Editors prefer, Table 1 could be done in landscape format.

- In page 9, I read "S. gallayticus": do you intend S. gallolyticus? Correct the spelling or expand on S. gallayticus.

Thanks for making note of the misspelling, we have corrected it.

- Overall, I appreciated reviewing the paper, as it belongs to my fields of interest. It is well-written and complete, anyway I suggest some minor revisions. In the title, authors focus on the concept of racial differences, while in the text the concept of racial difference in gut microbiome is expressed but marginally. It could be interesting to expand this concept is the authors think it to be important for the aims of the paper. Epidemiologic data, studies focusing on racial differences in gut microbiome and eventual correlation with the incidence of CRC and adenomas could be added.

We greatly appreciate these comments, as authors we believe that a fundamental point of our review is to describe and discuss the possible differences in the oral and gut microbiota

between racial/ethnic groups, especially considering that there is a disparity in colon cancer incidence and mortality for African American patients compared to otherpatient populations. We reviewed the literature again and found newly published papers and we have added relevant information throughout the text (see sections X, Y, Z), but unfortunately this research area is understudied so the information available is limited.

"Analysis of the gut microbiota by 16S in 1,673 participants in the US reported 12 microbial genera and families that vary by ethnicity. This suggests that the gut microbiota could be inherited and associated with human genetic variation.[21]"

"Hester et al[72] compared bacteria and SCFA in stool samples of AA and CA in a small pilot study. They found lower acetate, butyrate, total SCFA content and a higher pH in AA compared to the other racial groups. Similar results reported in another study where AA had increased levels of SCFAs in stool than other racial/ethnic groups and significantly lower intake of non-starchy vegetables.[73]"

"A study comparing microbiota from more than 1,000 fecal samples including 416 pairs of twins identified numerous microbial taxa whose abundance was influenced by host genetics. In the case of monozygotic twins, a more similar microbiota was observed than in dizygotic twins. However, it is unclear whether the host's genetic variation shapes and interacts with the gut microbiome to affect the host's phenotype.[89] The analysis of gut microbiota in stool of 2,084 participants in the Healthy Living in an Urban Environment Study described that people who live in the same city tend to show similar gut microbiota with other people of their ethnic origin[90]. Ethnic differences in alpha diversity and inter-individual differences were independent of metabolic health and were only partially explained by ethnic characteristics, including sociodemographic, lifestyle, or dietary factors. Therefore, the ethnicity of individuals may be an important factor to consider in the research of microbiome and cancer colorectal cancer.[90] "

"F. nucleatum levels have been found to be significantly higher in AAs[22] and has been shown to induce a series of tumor-specific molecular events, including the CpG island methylating phenotype (CIMP), microsatellite instability (MSI), and genetic mutations in BRAF, CHD7, CHD8 and TP53.[82]"

- Moreover, the relationship between oral microbiome and gut microbiome is not so clear through the paper. If the authors retain the concept important from a diagnostic or mechanistic point of view, the concept should be expanded. How are the two microbiomes related? Are there any possibilities to use information about oral microbiome to select groups at risk of CRC? The paper develops the mechanisms of action of various bacteria, but does not expand on clinical applications of those concepts.

We have added additional information about the relationship between oral and gut microbiome and clinical applications.

"F. nucleatum is one of the most prevalent species found in extra-oral sites. This bacterium regulates biofilm organization and interacts with the host cells by producing various adhesins and associates

with other bacteria through cross-feeding and metabolic interactions.[18] As such, *F. nucleatum* has been suggested to be a "driver bacterium" with pro-carcinogenic characteristics that contribute to tumor development by facilitating "passenger bacteria" to continue the progression of colorectal cancer.[19]"

"When combining testing for oral bacteria *F. nucleatum* with FIT, the combination showed superior sensitivity than FIT alone in detecting CRC, and additionally increased the performance of adenoma detection, suggesting the potential of bacterial biomarkers as more useful diagnostic tools over current diagnostic strategies.[108] In addition, the ratio *F. nucleatum/Bifidobacterium* showed superior sensitivity of 84.6% and specificity of 92.3% for diagnosing CRC in comparison with the use of a single fecal bacterial biomarker candidate.[109]"

"The deficit in the number of butyrate-producing bacteria can have detrimental consequences in the progression of the disease, hence the screening of SCFA and microbial-derived metabolites have potential as biomarkers and diagnostic tools for CRC."

- Finally, I appreciated the conclusive paragraphs in which the authors try to connect available knowledge to clinic in terms of the possible utilizations of microbiome and its changes to adjuvate therapy. These concepts should be stressed and expanded, as literature is becoming rich in data of basic research, but is still lacking of practical aspects drawn from available studies.
We took into consideration this comment and we have added a few paragraphs related to utilizations of microbiome and its changes to adjuvant therapy in the Conclusion section.

Reviewer #2

Specific Comments to Authors: a deeper understanding of the association between oral and intestinal bacterial profile, in addition to identifying prevalent bacteria in patients with CRC and the differences observed in ethnicity / race, could be the key predicting incidence, prognosis, and the development of new treatments. The manuscript is well, concisely and coherently organized and presented.
Thank you for your kind comments, we truly appreciate it.

Reviewer #3

Specific Comments to Authors: This is a good topic, a large reading of literature, with full discussion and basis for the content involved. But the content is too fragmented, more like a book than an article, suggesting thin space, such as increasing schema diagrams, to increase readability and rationality.

Thank you for taking to time to read our manuscript, we have taken into consideration your comments and we have added schematic diagrams (Figures) to increase readability and rationality of the information shared in the paper.

EDITORIAL OFFICE'S COMMENTS

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

(1) Science editor and (2) Company editor-in-chief:

This is a well written paper on a great topic. A more in-depth discussion on racial difference in gut microbiome should be included to reflect title. Moreover, the relationship between oral microbiome and gut microbiome should be discussed in terms of how they are related, and possibilities to use information about oral microbiome to select groups at risk of CRC, etc. Please add a figure to increase readability.

Thank you for your comments, as we mentioned, we have added additional information in the text about the relationship between oral and gut microbiome and schematic diagrams (Figures) to improve the comprehension of the text.

Sincerely,

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