Reviewers' and editor's comments and responses

Comment: When you send back, please provide the format of doc, not the pdf. Thank you!
Response: Sorry for this. Doc format has been provided.

Comment: Please offer the grant approval. Thank you!
Response: We are providing the approval letters for NIH 1R21CA175916, the Department of Veteran Affairs (I101BX001927) and MDREF grants to Dr. Majumdar.

Comment: Conflict of interest statement: please offer signed pdf file.
Response: Signed and updated conflict of interest pdf according to the guidelines has been provided.

Comment: My only suggestion to help this lovely review would be that the authors add a Table or two to list the bacteria that have been linked to CRC or healthy controls as well as the stem cell changes - this will help provide a simplified view of the information contained in the manuscript to the reader.

Response: Manuscript has been edited

"A summary depicting bacteria, whose presence has been shown to have or probably has a positive or negative association with colorectal cancer in African-Americans is shown in table 1."
and Table 1 incorporated.

Comment: Section of Figure2-legend; Authors should indicate each comments for Figure2-A and B, otherwise, remove Figure2-A.

Response: The legend for figure 2 has been edited to add specific comments for Figure2-A and B

"2A

Human APC gene with β-catenin (green and blue bars) and Axin(yellow circles) binding sites. Red bar represents conserved sequence of APC gene. Forward(F) and Reverse(R) primers were designed to demonstrate mutation in APC gene

2B

Agarose gel electrophoresis of PCR products showing higher rate of APC gene mutation (Mut:175 bp) in the colonic mucosa of AAs without adenomas than their CA counterparts."

Comment: Although the section of microbiota is very well organized, the section of CSC is not so great; the relation between microbial dysbiosis and prevalence of CSC in colorectal cancer is obscure and the author's conclusion is based on preliminary and unpublished data (for example Figure 2).

Response: We agree with the concern that some of the conclusions are based on unpublished data. The area of racial disparity in colorectal cancer particularly in relation to CSCs and microbiota is a new area of investigation and has not been studied in greater details. Therefore, there is a paucity of information on this subject. In view of this, we felt that it would be best to provide recent observations from our ongoing studies, which we believe to be relevant for this important topic. In addition, we believe that this information would be of interest to your readers.

We have also included the following paragraph in conclusion to strengthen the relationship between microbial dysbiosis and prevalence of CSC in colorectal cancer:

"It is well known that butyrate induces differentiation of colon cancer cells [78,79]. Forced cell differentiation has not only been shown to deplete Cancer Stem Cells (CSCs) in colon cancer but also to sensitize colon cancer cells to chemotherapy [80, 81]. When these findings are viewed in light of the observations of lower amount of butyrate in stool from African-Americans with colon cancer than other racial groups (*see section on dietary regulation of microbiota and racial disparity*), it provides an interesting link between racial disparity, CSCs and colorectal cancer. "

Comment: The legend for Figure 2 is insufficient.

Response: The legend for figure 2 has been edited to add specific comments for Figure2-A and B

"2A

Human APC gene with β-catenin (green and blue bars) and Axin(yellow circles) binding sites. Red bar represents conserved sequence of APC gene. Forward (F)

and Reverse(R) primers were designed to demonstrate mutation in APC gene

2B

Agarose gel electrophoresis of PCR products showing higher rate of APC gene mutation (Mut:175 bp) in the colonic mucosa of AAs without adenomas than their CA counterparts."

Comment: Page 13, second paragraph. "Preliminary data on miR-21 levels in normal-appearing colonic mucosa of AAs with adenomas" should not be included or should be cited after publication.

Response: We sincerely acknowledge the concern that this is unpublished data. However, these observations are from ongoing research in our lab and we included it as we feel that this is an important topic and our observations would be of interest to readers.
We have also edited the manuscript to clarify as follows:
"Ongoing studies (unpublished data) from our lab revealed that miR-21 levels in normal- appearing colonic mucosa of AAs with adenomas were significantly higher than their CA counterparts."

Comment: Page 12, the 7th line from the bottom. Ref. 53 must be 54.

Response: We thank the reviewer for the astute observation. Infact, the reference should be 54 there and we have changed it as per the suggestion. All other references have also been rechecked.

Comment: It is known that forced cell differentiation leads to depletion of CSCs in brain tumors and colon cancer and that butyrate induces differentiation of colon cancer cells. Is there any possibility that the racial disparity is dependent on such microbial metabolites?

Response:

We thank the reviewer for an excellent point very pertinent to our review. It is entirely possible that the difference in microbial metabolites could contribute to

racial disparity through CSC pathways and differentiation of colon cancer cells. Accordingly, we have included the following statements in the manuscript:
"It is well known that butyrate induces differentiation of colon cancer cells [77,78]. Forced cell differentiation has not only been shown to deplete Cancer Stem Cells (CSCs) in colon cancer but also to sensitize colon cancer cells to chemotherapy [79,80]. When these findings are viewed in light of the observations of lower amount of butyrate in stool from African-Americans with colon cancer than other racial groups (*see section on dietary regulation of microbiota and racial disparity*), it provides an interesting link between racial disparity, CSCs and colorectal cancer. "