Response to reviewer comments

Colorectal Cancer: The Epigenetic Role of Microbiome

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Reviewer comment	Author Response	Comments
- There is a recent review written on the same topic (see: ncbi.nlm.nih.gov/pmc/articles/PMC6087872/). This was not referenced in the current manuscript. Authors should make reference where relevant and try to avoid areas for overlap.	This reference was taken in consideration.	Citation inserted in its appropriate place See ref. #17
–Please avoid the use of superfluous and imprecise language in scientific writing. Descriptions such as "Gut microbiota is an enhancer to our second brain; the intestine" and "With millions of proteins expressed by the microbiota's arsenal, human could make use of various kinds of dietary ingredients, that otherwise will be rubbish-in/rubbish-out" can be tautological; they are redundant 'padding' words that add no meaning to the sentence; or your meaning might be clearer by using a verb instead of abstract nouns.	All the mentioned phrases were paraphrased so that the meaning became more precise and clearer.	Highlighted in the conclusion section
- Please change "in inducing CRC will help attaining reliable strategies to precisely diagnose and treat this fatal disease" to "in the	Changed	The sentences were paraphrased accordingly

pathogenesis of CRC will help in diagnosis and management".		
- I am not really sure how understanding the role of the microbiome will help in diagnosis. Please make this link to diagnostic strategies clearer in your article.	Several researches have indicated that CRC-associated microbiota is altered compared to healthy people, suggesting a useful role in early diagnosis and treatment of CRC. Furthermore, metagenomic analysis of fecal) microbial communities provide alternatives for the noninvasive diagnosis of CRC. Others reported that the quantification of Fusobacterium nucleatum could be used as a valuable putative microbial biomarker for early diagnosis of CRC. This paragraph has been added to the article (highlighted)	*Some articles:* *Nakatsu, G., et al., Gut mucosal microbiome across stages of colorectal carcinogenesis. 2015. 6: p. 8727.* *Feng, Q., S. Liang, and H. Jia, Gut microbiome development along the colorectal adenoma-carcinoma sequence. 2015. 6: p. 6528.* *Baxter, N.T., et al., Microbiota-based model improves the sensitivity of fecal immunochemical test for detecting colonic lesions. Genome Med, 2016. 8(1): p. 37.* *Zackular, J.P., et al., The human gut microbiome as a screening tool for colorectal cancer. Cancer Prev Res (Phila), 2014. 7(11): p. 1112-21.* *Mangifesta, M., et al., Mucosal microbiota of intestinal polyps reveals putative biomarkers of colorectal cancer. Scientific Reports, 2018. 8(1): p. 13974.*

- Please change "most prevailed" to "most prevalent".	Changed	Highlighted
- Please change "It is well established that gut microbiota plays critical role in the progression of CRC either via their metabolites or interaction with their host intestinal epithelial cells" to "It is known that gut microbiota plays a role in the progression of CRC either via their metabolites or interaction with their host intestinal epithelial cells." I would not use the word "critical" as genetics may probably plays a more significant role.	Changed	Highlighted
- What is "a hasty shift in the gut microbiome"? Please be more scientific and specific.	Paraphrased to **rapid**	
- Dietary polyphenols, which are often indigestible, may also positively influence gut microbiota (citation: ncbi.nlm.nih.gov/pubmed/30248988).	The mentioned reference **(A Meta-Analysis of the Clinical Use of Curcumin for Irritable Bowel Syndrome (IBS))** does not contain anything related to Dietary polyphenols, which are often indigestible, may also positively influence gut microbiota	No action taken
In Sprague Dawley rats with hepatic steatosis (induced by a high-fat diet), curcumin not only restored intestinal barrier integrity (increased expression of tight junction proteins ZO-1 and occluding), it markedly altered the overall composition of the gut microbiota, towards that	The mentioned reference **Modulation of gut microbiota contributes to curcumin-mediated**	No action taken

of lean rats maintained on a normal diet (citation: ncbi.nlm.nih.gov/pubmed/28341485).	**attenuation of hepatic steatosis in rats** also is not related to our subject	
- It is incorrect to say that "Gut bacteria can attack the intestinal epithelial cells to cause inflammation". Rather, microbial dysbiosis is associated with impaired epithelial barrier, bacterial translocation, decreased regulatory T cells in the gut mucosa and has been shown to promote inflammation (citation: ncbi.nlm.nih.gov/pubmed/24310267).	Sentence changed and citation inserted	See ref. #76
- Please change "the recent researches that addresses causal link between gut microbiota and CRC onset and progression. Meanwhile, the epigenetic changes underlie CRC and its microbial root will be also described" to "the recent studies addressing the causal link between gut microbiota and CRC onset and progression. The epigenetic changes underlying CRC and the host microbiome will also be discussed."	Changed	Highlighted
- Also important to mention would be that the intestinal mucosa is part of a complex enteric immune system and consists of a large variety of immune cells. The intestine mucosal immune system consists of three different mucosal lymphoid structures: Peyer's patches, the lamina propria and the epithelia. The intestinal mucosa is the main site for microbiome-host	A paragraph was added to mention this point and the required citation inserted	

interactions. Exposure to food, bacteria, parasites, and viruses may contribute to sensitization of the enteric immune system and activation of the inflammatory cascade (citation: ncbi.nlm.nih.gov/pmc/articles/PMC6159811).		
- A recent study showed that IgA in the feces significantly increased after treatment with prebiotics, while the expression of pro-inflammatory factor in the mesenteric lymph nodes and Peyer's patches was significantly reduced. Additionally, the IL-10, CXCL-1 and Mucin-6 genes were up-regulated, while the colonic mucosa 4, IFN-γ, GM-CSF and IL-1β genes were down-regulated in the ileum (citation: ncbi.nlm.nih.gov/pubmed/25811034). Quantitative PCR analysis of different bacterial groups revealed significant changes in Lactobacillus population. These results indicate that the gut microbiome affects intestinal mucosal immune balance.	Good study but it is little bit irrelative to the core of our study.	Here is the link to this study
- In Table 1, it is important to explain that H. pylori may also have a hypothetical role in the pathogenesis of CRC, especially cagA-positive strains. One study found an association between cagA seropositivity and an associated increased risk in gastric and colonic cancer. Shmuely et al. reported cag-positive H. pylori strains were associated with a 10.6-fold increased risk of CRC compared to cagA	Study inserted and the paragraph was paraphrased accordingly	This is the link to the study See ref. #167

negative strains (citation: ncbi.nlm.nih.gov/pubmed/11774957).		
- Some present areas of limitations and avenues for future work should be mentioned. For example, it is important to highlight that the strongest evidence for a role of the microbiome in immunity and homeostasis comes from animal studies. There is need for the concepts generated in animal models to be translated to the human in the future. There are also existing gaps in knowledge regarding the interaction between the microbiome and the host in vivo - and the pathway of its metabolites	Future perspectives section was added	Highlighted (section #6)
- and how their metabolites influence the microenvironment. Further mechanistic studies involving "omics" technologies, as adapted from previous studies (citation: ncbi.nlm.nih.gov/pubmed/30056340), might help shed light on these questions.	I went through the provided article, but it seems irrelative	Here is the link to the article
- Please specify if any funding/external grants were received.	Done	At the end of the manuscript

Author response

Dear Editor

Thank you for giving the chance to edit my manuscript

I did respond to all your comments. I need to mention some points:

- I added the missing information of the corresponding author and ORCID
- I added the conflict of interest declaration
- I added the author participations
- I lengthened the abstract as per your request (216 words)
- I added a core tip paragraph
- We have four figures in this manuscript, three of them has been drawn using PPT and the fourth one was drawn using CorelDraw.
- One PPT file will be uploaded containing the four figures along with one CorelDraw file (editable-as per your request)
- For the language editing certificate, I uploaded it on July 26, 2019 during the last round of revision
- For the audio core tip: I also uploaded it on July 26, 2019
- For the PMID and DIO: I added DOI as per your request, but I did not find PMID in the list of items (insertions) in EndNote, Although I am using EndNote X9
- I made all citations superscript
- I checked the list of reference for redundancy and found no redundancies
- I added the full list of authors in the bibliography