Name of Journal: World Journal of Gastroenterology

Manuscript Type: REVIEW

Type: Invited manuscript ID 03967085

Dear Editor,

Dear reviewers,

Thank you for your time to revise our Manuscript ID: 03967085, What could microRNA expression tell us more about colorectal serrated pathway carcinogenesis? Authors: Peruhova M, Sekulovska-Peshevska M, Krastev B, Panayotova G, Georgieva V, Konakchieva R, Nikolaev G et Velikova T.
We have incorporated most of the suggestions made by the reviewers. Those changes were highlighted in the manuscript. Please see below, marked in blue, for a point-by-point response to the reviewers' comments. All page numbers refer to the revised manuscript file with tracked changes.

Reviewer #1 No. 05194997

Reviewer #1: Summary: Thank you for inviting me to review the manuscript entitled: "What could microRNA expression tell us more about colorectal serrated pathway carcinogenesis?" by Peruhova M and colleagues. Overall, it is an interesting study addressing an important interest regarding the involvement of the microRNAs in the serrated pathway. The manuscript is well structured. The background evidences concisely and adequately the major points concerning the molecular and histological characteristics of serrated polyps. Concerning microRNAs and serrated pathway in colorectal cancer, the authors evidence the most recent investigations. This field is rapidly advancing, and there have been a variety of recent published reviews on the same argument. However, the authors included some information (i.e. the mucosal immunity) that makes it different from the other published reviews. There are several changes (major and minor revisions) that authors need to address to complete this

manuscript, which has a high potential of interest. Moderate English changes are required.

- ✓ Thank you very much for the overall evaluation of our manuscript.

Major: 1) The title of the review clearly asks if the microRNA expression could tell us more about colorectal serrated pathway carcinogenesis. I would really appreciate if the Authors could better highlight, stress more and critically answer to this question, as well as discuss the potential clinical role of miRNAs in serrated CRC pathway along all the text and particularly in the conclusion. Moreover, they should summarize what we can conclude from the literature search. What do the authors think of summarizing the results within a table (for instance, evidencing microRNAs deregulated expression in specific serrated lesions and when available by comparing it with the one in normal mucosa?);

- ✓ We appreciate your constructive comment. We have corrected the conclusion of our review and highlighted the potential clinical role of miRNAs in serrated colorectal carcinogenesis.
- ✓ It would be a great benefit if the results could have been summarized in a table. However, at the moment, the data related to miRNAs expression in the serrated pathway in the literature is not sufficient for such a presentation of the data.

2) Authors included some information that makes this review different from the other already published reviews. However, adding some recent findings concerning also the role of fecal miRNAs in this field would further distinguish this manuscript from the others.

- ✓ Thank you for your insightful comment. However, we were intended to do this, but according to our search in the literature, there were no data related to fecal miRNAs in the serrated pathway carcinogenesis. Thus, we included the most relevant information concerning the role of fecal miRNAs as a diagnostic and prognostic marker in CRC. The additional information is presented in the section about miRNA-21.

Minor:

1) In Abstract, Core tip and Introduction verify the plural of some words (i.e. replace "non-coding-RNA" with "non-coding-RNAs", or "the pivotal role of miRNAs").

- ✓ Thank you for your valuable remark. We have changed the words in plural form, as you recommended.

2) In "MORPHOLOGICAL ASPECTS OF SERRATED POLYPS":

a. Ascribing 25% of all CRCs to the serrated neoplastic pathway is not completely correct. Based on the literature, the percentage prevalence of serrated pathway bears really a high variability, ranging from 15 up to 30% of all CRCs;

b. "TSAs are extremely rare <1%, while HPs are the most common, comprising approximately 75% of all serrated polyps….". TSA represent 1% of all CRC polyps, not of all serrated lesions. Authors should specify that 1% refers to all CRC lesions, as they did subsequently in the text.

c. Authors should describe SAC.

- ✓ Thank you for pointing out our mistake. We corrected the percentages, and we put additional information about SAC. You can follow-up on our change in the text with Track changes.

3) In "EPIGENETIC AND GENETIC ASPECTS IN SERRATED PATHWAY":

a. "Methylation" as well as "promotors" are not the right terms, replace them with "methylator and "promoters".

b. Along the text "Methylation is an epigenetic process where a methyl group (CH3) is added to the cytosine nucleotide in a CpG dinucleotide settings", authors would say at CpG dinucleotide group?

- ✓ Thank you for your valuable comments. We corrected the terms as you recommended.

4) In "Microsatellite Instability Mechanism in CRC":

a. Authors should also add atypical MSH3 and epCAM mutations, as well as the mutational frequency in HNPCC patients;

- ✓ We appreciate your comment. We added additional information about this issue.

b. MSI tumors can also be subclassified. Authors could briefly describe their subclassification (MSS, MSI-H, MSI-L);

- ✓ We subclassified MSI tumors briefly. You can follow-up on the changes vie Track changes in the text.

c. Authors should better clarify this point. 3–15% of all CRCs are represented by sporadic forms with MSI, and that about 80% of MSI CRCs are characterized by the hypermethylation of MLH1, while 20% of MSI CRCs by mutations in MMR genes.

- ✓ We appreciate your insightful comment. We acknowledge not being entirely correct. We corrected the data according to your comment.

5) In "BRAF / KRAS Gene Mutations":

a. Concerning the title "BRAF / KRAS Gene Mutations," authors should evidence that they are overviewing these mutations in relationship with the serrated pathway. It could be replaced with "BRAF / KRAS Gene Mutations in serrated CRC";

- ✓ Thank you for your comment. We have changed the title of the section as you recommended.

b. Add reference of the work of Catherine E. Bond and Vicki L. J. Whitehall (2018, Gastroenterology Research and Practice). This is a comprehensive and an interesting review that clearly summarize the role of BRAFV600E mutation in CRC;

- ✓ We appreciate your comment. We have discussed this interesting review under the number 56th in the main text, as you suggested.

c. Authors should also discuss the conflicting results that recently emerged on the association between BRAF mutation and female sex among serrated adenomas (Ref 44 of the manuscript, Travaglino et al. 2019 hystopathology);

- ✓ Thank you for your insightful comment. Travaglino et al., in their publication, did not confirm the association of BRAF mutation with the female sex. However, their study has several limitations, such as an insufficient number of patients included in the analysis. Controversy to the study of Travaglino et al. (new ref. No 48), Sinicrope et al. (ref. No 57) showed the correlation between female gender and BRAFV600E mutated tumors.

d. Authors should better clarify the difference between serrated tumors driven by BRAF or KRAS mutation. For instance, serrated polyps emerging from the KRAS mutant pathway evolve into carcinomas that are characterized by low levels of CIMP.

- ✓ We appreciate the insightful comment. We clarified the difference between serrated tumors driven by BRAF or KRAS mutation in the legend below Figure 2.

6) In "The Role of miRNA-31 in Carcinogenesis":

a. Replace the title with "The Role of miRNA-31 in Carcinogenesis of serrated pathway of the colorectum" or "… serrated CRC carcinogenesis";

- ✓ We think this is an excellent remark. We changed the title of the section, as you recommended.

b. Authors should cite the paper of Aoki et al on miR-31 in serrated progression (World J of Gastr. 2014) and the recent paper of Nobuhito Kubota et al. (Oncology Letters 2020) in which upregulation of miR-31 is associated with poor prognosis in patients with advanced colorectal cancer.

- ✓ Thank you for your constructive comment. We added in the review relevant information about the expression of miRNA-31 in serrated progression, from the papers of Aoki et al. and Kubota et al. (ref. No 71 and 77, resp.).

7) In "The Involvement of miRNA-21 in CRC":

a. Recent investigations on miR-21 as novel non-invasive biomarker for early detection and prognosis of CRC patients should be cited and/or discussed (i.e. Ghareib et al. Journal of Gastrointestinal Cancer 2020, or the one of Monteleone et al. scientific report

2019). Moreover, what the authors think about the recent findings on faecal miR-21, miR-92a and their combination as promising non-invasive biomarkers for faecal-based CRC screening (scientific reports 2019, Tung on Yau et al.)?

b. Add reference. The down-regulation of PTEN protein by miR-21 in CRC xenografts nude mice has been also demonstrated by Wu Y and collaborators in Cell Physiol Biochem, 2017.

c. The oncomiR-21 predicts recurrence and poor survival in patients with CRC. Authors should include this information (Chen et al Onco Targets Ther. 2016)

- ✓ Thanks for your valuable remarks. We took into account your comments. Thus, we summarized and added the information and all the suggested references in the text.

8) Concerning the title "The Role of miRNA-181a-2 in Cancer Development", should be more appropriated to specify the correlation of this miRNA with serrated CRC. Authors are not refferring to the role of the miRNA-181a-12 overall in cancer. It could be modified as: "The Role of miRNA-181a-2 in the development of serrated pathway in CRC"

- ✓ Thanks for your valuable remark. We have corrected the subtitle. The changes that we made can be followed-up via Track changes.

9) In "HUMAN GUT MICROBIOTA, MUCOSAL IMMUNITY, AND miRNA IN SERRATED PATHWAY", authors should discuss the recent investigation of Nakanishi et al (Immunity 2018) and cite the recent and interesting review of the same author (Trends cancer 2019). Moreover, they should also discuss the particular capacity of SAC in avoiding the immune response.

- ✓ Thank you for providing us with these papers. We have cited them to clarify the particular capacity of SAC in avoiding the immune response.

Figures general comment: verify abbreviations at the end of the legends;

1) Figure 1:

a. “CD” stands for cytological dysplasia. Miss the abbreviations in the legend (as well as for “WHO”);

b. Authors could also add the % of each subtype and in addition range HP subtypes from the higher to the lower %.

- ✓ Thanks for your valuable remarks. We have corrected the title. The changes that we made can be seen by Track changes.

2) Figure 2:

a. Describe briefly your scheme on the serrated pathway progression in the legend;

b. Add the color-code explaination in the legend;

c. Authors could also specify that TSA tumors with KRAS mutations could be caused by MGMT loss.

- ✓ We made a brief description of the schematic serrated pathway progression below Figure 2.

3) Figure 3:

a. Authors should clarify the color-code of the figure in the legend.

b. Why the direction of the figure goes from the left to right and not vice versa?

- ✓ Thank you for pointing out the mistake. We added the color explanation of the legend, below figure 3. We decided to put the picture of the colon in the right side of the figure, to underline the proximal predominance of serrated lesions in the colon.