

May, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 17939-reviewedited).

Title: CBP, p300, Butyrate, and Wnt Signaling in Colorectal Cancer

Author: Michael Bordonaro, Darina Lazarova

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 17939

We thank the reviewers for their comments and have made requested revisions to this manuscript; we have made changes in the manuscript in response to those reviewers who had accepted the paper upon revision.

The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated. Suggested changes in writing, spelling, etc. have been made (see below).

2 Revision has been made according to the suggestions of the reviewers

Reviewer one

(1) Reviewer one did not find the paper interesting, did not find the aim of the paper clear, and critiqued the fact that a large part of the review was covering the publications of the authors, with insufficient covering of other work.

This review, which was invited, is meant to be a relatively brief and highly focused summary and analysis of (a) how CBP and p300 modulate Wnt signaling that is upregulated by butyrate and other histone deacetylase inhibitors, and (b) the downstream effects on colonic cell physiology of this modulation. We respectfully disagree with this reviewer; we believe that this review will be of considerable interest to the colon cancer research community because it not only deals with issues of cancer therapeutics but also of cancer prevention, a topic underrepresented in the literature. In addition, and particularly in light of the new material added to the manuscript, we also consider how right-sided colon cancer may differ to other forms to this disease with respect to preventive approaches involving fiber/butyrate.

Given the focused topic matter of this review, it is true that a substantial portion of the discussion focuses on past papers of the authors, since these researchers are those who are doing direct study on these specific topics. That said, we have considerable citation and

discussion of background material of relevance, especially the work of Kahn and colleagues, who did the foundational studies on modulating CBP-Wnt and p300-Wnt activities. We note that the entire section on cancer stem cells focuses on the work of the Kahn group (and not on that of the authors). However, this review is not focused on CBP/p300 in general and/or the roles of CBP/p300 in cancer; those topics have been extensively reviewed elsewhere (for example, the Kahn group has reviewed their work on these topics). The review is, as stated above, focused on CBP/p300 with respect to butyrate, Wnt signaling, and colon cancer.

However, in response to comments by another reviewer, we have added new important text including discussion of other work: for example, discussion on the importance of MSI+ status for butyrate-resistance and p300 expression in right-sided colon cancer, an update on the mechanism by which HDACis may hyperactivate Wnt signaling, identification of an inhibitory agent which is allegedly specific for p300 and acts in colon cancer cells, one important paper from 2013 discussing the positive prognostic impact of p300 expression for CRC patients, another paper discussing the interaction of CBP with thymine DNA glycosylase to promote Wnt signaling and colonic tumorigenesis, several papers focused on CBP/p300 and stem cells/tissue differentiation, and one paper that extends these findings to pancreatic cancer. We also added sentences to the review expanding explanation of the relevance of the Kahn group's work to the later findings on butyrate. This additional material on CBP and p300 in cancer has broadened the scope of, and strengthened, this review.

The reviewer asserted that one figure is insufficient and that the figure present was reproduced from a previous reference.

The original figure (originally designed as part of a grant proposal) was adapted, not copied, from one in the previously published figure (ref. 43), but these figures are not the same. For example, the current figure contains significant differences from the previous one (e.g., separate component for butyrate resistance, no inclusion of E-cadherin or vimentin, and a different figure legend). However, we have added "Adapted from ref. 43" in the figure legend for the sake of clarity.

Finally, as suggested, we have added an additional figure (Fig. 2), which summarizes our hypothesis on p300, butyrate, and right-sided colon cancer (in part based on the new discussion and new cited references in this revision).

Reviewer two

(2) Reviewer two had minor comments on spelling and sentence construction.

We made the suggested changes in spelling ("either") and altered the abstract to eliminate double use of Wnt/beta-catenin in (b).

This reviewer commented on the lack of distinction between MSI and MSS cell lines, and the lack of discussion of the distinct nature of MSI right-sided colon cancer.

We now discuss the importance of MSI+ status for butyrate-resistance and p300 expression in right-sided colon cancer, and the phenotypic consequences in the “What Next?” section.

The reviewer also notes the lack of discussion of other cell line such as SW480 (established from the primary tumor from the metastasis that the SW620 line, which was discussed, was derived from).

We added text on the SW480 cell line and the importance of comparing matched primary and metastatic samples from the same patient. (last paragraph neoplastic progression section). There currently is not any more data on additional cell lines, but we now suggest such studies be done.

The reviewer noted that some of the proposed animal studies should use the Edelmann MMR mouse model.

We agree, and have added such discussion to the last part of the manuscript.

Reviewer three

3) Reviewer three requested some rewriting of the paper to add information on more recent references and in so doing update the information reviewed in our manuscript.

Following the advice of this reviewer, we have included important addition discussion on several more recent papers from 2013-2015 that touch upon issues of relevance to CBP/p300-Wnt and CRC. These include an update on the mechanism by which HDACis may hyperactivate Wnt signaling (crucial 2015 paper cited [new ref. 18] in the first section of our manuscript) identification of an inhibitory agent which is allegedly specific for p300 and acts in colon cancer cells (see “What next?” section), one important paper from 2013 discussing the positive prognostic impact of p300 expression for CRC patients (see neoplastic progression section), another paper discussing the interaction of CBP with thymine DNA glycosylase to promote Wnt signaling and colonic tumorigenesis (see neoplastic progression section), several papers focused on CBP/p300 and stem cells/tissue differentiation (see stem cell section of manuscript as well as end of “What next?” section), and one paper that extends these findings to pancreatic cancer (see end of “What next” section). These additions have enhanced the timeliness of our manuscript, and added important new information supporting the hypotheses within (e.g., p300 associated with better prognosis) and providing the latest information about possible reagents for future studies (i.e., p300 inhibitor). Thus, with these new additions [for example, new references 46,47,50,51, and 54-57] combined with the original material, the text and references are now up-to-date with respect to the topic of this review and some associated issues.

We also note that most of the references of direct relevance to the highly focused topic of our manuscript were in the original submission. For example, all relevant studies on the interaction between butyrate, CBP/p300, Wnt signaling, and downstream effects in CRC cells, were in the cited *Journal of Cancer* papers from 2013-2014, and were discussed in great detail in our original submission. In addition, the essential foundation work for these studies was from the Kahn laboratory, also cited in the original submission.

3 References and typesetting were corrected, if required.

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

Best regards,

A handwritten signature in black ink, appearing to read 'M. Bordonaro', with a stylized, flowing script.

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