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Dear Editor,

Please find enclosed the edited manuscript in Word format and a clean copy for your review.

Title: Intestinal stem cells and the colorectal cancer microenvironment
(Previously: "*Tumor stem cells and colorectal cancer*")

Author: Bryan A Ong, Kenneth J Vega, Courney W. Houchen

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 6260

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

1. Format has been updated

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

Reviewer 00367509

1.) *The title doesn't attract enough attention to this important research field.*
Thank you. We have changed the title as suggested. Though it is still somewhat conservative, we think it more accurately reflects the content of the manuscript.

2.) *The first paragraph needs to be entirely rewritten, because the case numbers from the different continents are confusing.*
We appreciate your suggestion regarding the statistics quoted in the manuscript introduction. We have simplified the prose for simplicity and clarity. Notably, we have omitted the specifics regarding mortality in the United States and United Kingdom.

3.) A.) "*the past 25 years": in five years this number refers to another period, better: Since e.g. 1985 Fearon and Vogelstein's Model for Colorectal Carcinogenesis.*
Your suggestion about avoidance of using "the past 25 years" is well taken. We have adjusted the sentence to reflect the actual date of Fearon and Vogelstein's review article (1990) in which they introduced their CRC model. We have also restructured the introduction for logic and flow purposes, and by doing so have shortened it slightly.

B.) *The listed genetic aberrations are not CRC-specific. It's unclear whether Fearon and Vogelstein first discovered this aberrations in CRC or whether these mutations have already been identified and then also found in CRC.*
The listed aberrations had been identified prior to Fearon and Vogelstein discussing them and proposing their stepwise colorectal carcinogenesis model. We did not intend to imply that Fearon and Vogelstein had discovered these aberrancies themselves. We

have thus re-worded the passage and restructured the paragraph accordingly.

4.) *"Some aspects of Fearon and Vogelstein's stepwise model…": This paragraph is not easy to understand.*

Thank you for your advice. As with the previous point, we have rewritten the whole section and made it substantially shorter while keeping the same discussion points as before. We think the changes have improved flow, clarity, and succinctness.

5.) *Normal Intestinal Stem Cells: Nearly eight pages about normal intestinal stem cells are too much in a review titled "Tumor Stem Cells and Colorectal Cancer" ? please be shorter and be more concise.*

Thank you for your advice. The intestinal stem cell section has been shortened.

6.) *Intestinal Stem Cell Niche ? The term "bacteria and epithelial cell-derived chemicals" is ambiguous, better: natural enteric flora and epithelial cell-derived soluble factors*

We appreciate your wording suggestion. We have revised the sentence accordingly.

7.) *The second part of this review "Intestinal Tumor/Cancer Stem Cells" is by far better written as the first part, very well structured and easy to follow, especially the part "Epithelial Mesenchymal Transition: Prevailing Metastatic Program?". Even the second part would benefit a lot of a shortening.*

Thank you for your concern. Revision has been made to second the half of the paper in response to other reviewer's comments as well as from significant grammatical simplification and flow corrections. We hope that you find the changes acceptable.

Reviewer 02446041

1.) *In "Core Tip": The authors state "CRC stem cells remain attractive targets for anti-tumor therapy." They also state "The coexistence of two epithelial stem cell types (normal SC, CRC-SC) questions whether intestinal cells are singularly-derived." If they cannot distinguish them, how can they target CRC-SC? They should reconcile and modify the logic flow with clarity.*

You are correct, our Core Tip lacked cohesive structure and a lack of emphasis on colorectal cancer stem cells which are the topic of the paper. Our comment regarding the two stem cell types does not contribute to establishing a solid take-home message and has been removed. In response to logic problems, the Core Tip has been rewritten.

2.) (A) *Paragraph 2, the authors state, "Growing evidence suggests that individual CRC cells differ in functional and proliferative capacity to the point that separate cells may serve unique roles.[5, 6]" However, their citations are not specific for CRC and they should have specified the CRC-related evidence as the sentence stands. Here, "separate cells" – what do they refer to?*

The lack of specificity afforded by the statement in question is likely related to the awkward way we organized the sentence and introduction as a whole. In response to this issue it has been rewritten. The sentence in question has been removed because it was too much of an assertion without sufficient supporting evidence.

(B) *Paragraph 3, "The aim this review is primarily to reappraise current evidence" should be "The aim for this..."*

Thank you for your suggestion. We have corrected the grammatical error.

3.) *Page 8, "Theories on CRC population dynamics – which are not explored in Fearon and Vogelstein's model – have also subsequently been proposed." It needs reference.*

We agree that the sentence as it stands needs evidence and believe it seems that way because it is a poorly-worded bridge to the next manuscript section. We have

since removed it because it contributed little to the passage overall.

4.) A.) *Page 10, "that Bmi1+ cells restored the intestinal epithelium following radiation injury sufficient to eliminate Lgr5+ stem cells." How do Bmi1+ cells eliminate Lgr5+ stem cells?*

In the cited experiment, Bmi1+ cells did not eliminate Lgr5^{+} stem cells. Radiation was used to ablate the entire intestinal epithelium of all cells except Bmi1+ cells. We recognize that the wording was confusing and have simplified the sentence. We have also revised wording through the rest of the passage for clarity as well.

B.) *Human relevant? Lgr5+, Bmi1?*

Thank you for bringing to our attention this important point. Both Lgr5 and Bmi1 have been identified among mouse and human intestine. We have now added mention to this at the beginning of the "Normal Intestinal Stem Cells" passage with appropriate references.

5.) *Page 10, "An evolving model of normal intestinal stem cell behavior" – Can the authors integrate these three options into one in a summary sentence or paragraph? For example, they said, "the composite behavior of various semi-mutable cells contained within the intestinal crypt" – what timeline in what space does such an event exists? What is the sequential event for "various semi-mutable cells?"How does it relate to the Wnt signaling pathway with Bmp agonist on page 13 (Ref. 19, 32)?*

After review of this passage we definitely agree that this section was redundant and lacked simplicity. Because of the points we hoped to make in this passage we were not able to reduce it down to one paragraph, but the passage is has assuredly been made much simpler and specific.

6.) *Page 14, Can they clarify the difference between CRC-supporting ISEMFs and normal stem cell-supporting ISEMFs?*

We appreciate you having raised a very important question. To our knowledge, not much is understood about the exact differences in origin and role of ISEMFs between the normal intestine and CRC. Given your attention to the issue, we have included a brief mention to at least the suspected known functional differences in the two ISEMF types and provided citation to Dr. Timothy Wang's 2011 review on stromal fibroblasts in digestive cancer. We have also clarified that Vermeulen et al. used *normal* colonic myofibroblasts and updated the passage to reflect this important point.

7.) *Page 14, if "Paneth cells are only found in the small intestine," what is the equivalent in other segments? Do they share the same biomarkers if they serve the same function?*

In our passage discussing Paneth cells, we have already addressed the possible existence of a separate population of CRC crypt cells analogous to small intestinal paneth cells. We have re-worded the text out of concern that we have not conveyed this information clearly in the text.

8.) *Page 15, "Uncertainty remains as to whether all intestinal epithelial cells are equally prone to developing cancer." Is there any single cell study? It's hard to believe all intestinal epithelial cells are equal for any given function or status.*

Your concern about our subject sentence regarding the susceptibility of intestinal cells to cancer is well taken. We agree that not all cells have equal potential for carcinogenesis, and have discussed it in the associated passage. In retrospect, we think the subject line fails to address the correct issue. We have changed it to: "Is there a population of cells in the intestinal epithelium serves reliably as the source for most, if

not all colorectal cancers?"

9.) *Page 16, "Wnt-constitutive non-stem cells in the intestine can de-differentiate and re-acquire stem cell properties in a NF- B dependent manner, ultimately leading to tumorigenesis". In the context of Wnt is essential for normal stem cells, can the authors elaborate Wnt is a switch? In what conditions does Wnt go to cancer instead to maintain normal stem cell stage.*

Thank you for suggesting this interesting point of discussion. We have added to the passage to reflect discussion regarding response to extrinsic triggers (position in the crypt or response to inflammation).

10.) *Page 17, They state: "Very few, if any, markers are both specific to CRC stem cells and ubiquitous among all CRCs.[8]" They state Dclk1; however, it'll be helpful to have a table to list these biomarkers.*

We appreciate your suggestion. We have provided a table listing putative stem cell markers based on previous literature.

11.) *Page 19, Among CRC SC biomarkers, like "Lgr5+-high CRC cell fraction", " low to no Lgr5", "DCLK1+", CD133+, CD133- cell fraction, did anybody check if all these biomarkers exist in human CRC? The authors cite, "Nakanishi et al.[71] did not find DCLK1 among all tumors in their mouse experiments," however; it's well known that human is different from mouse.*

It is true that humans and mouse model systems are different. However, much known about CRC stem cells and normal intestinal stem cells has been gleaned from animal model systems—particularly mouse. Your point is well taken, and we have added a sentence in the next paragraph addressing this concern.

12.) *Page 21, "Given that colorectal cancer is of monoclonal origin," what is the evidence to support this notion? Elsewhere, they discuss about heterogeneity. Is that cancer is of monoclonal origin only assumption?*

We have provided citation to Fearon and Vogelstein's paper documenting restriction fragment analysis indicating that cancer is likely monoclonal.

13.) *Page 23, the sentence of "MET leads to growth of distant cancer foci by reverting CRC cells to a highly-proliferative epithelial stem cell phenotype" should be expanded to illustrate such a process. Is this epithelial stem cell phenotype overlapped with that of CRC stem cells? Do they share biomarkers?*

In response to this issue, we have created a figure demonstrating the EMT/MET process we propose in relation to cancer metastasis. We have also provided citation to an article in support of EMT association with cancer stem cell phenotype. In particular, Wnt pathway mediators, such as beta-catenin are expressed in both EMT studies and cancer stem cell studies. This suggests that that the EMT phenotype does in fact overlap with cancer stem cells. We since have made reference to this in the manuscript.

14.) *Page 24, "fate reprogramming and cellular plasticity may potentially rewrite the foundations upon which both current stem cell theory and cancer stem cell theory are based." What the authors discuss thus far doesn't justify this statement. If they want to keep this, they should expand iPSC-related CRC area. Some specifics (intestine iPSC) are appreciated.*

On review of our paper, we agree that we have not sufficiently delved deeply into this particular issue. We have removed the contentious statement and revised our conclusion.

15.) *In the cover letter, the authors state "Our review has a few minor but key limitations, largely reflective of the current state of the field. Admittedly, we did not find sufficient evidence to quell*

any current controversies regarding normal intestinal stem cell identity, or the existence of colorectal cancer stem cells. Much of the reviewed literature is based on animal model and in vitro observations, both of which may have limited generalizability to human disease. Furthermore, our review does not serve to clarify any specific targets for tumor therapy." *They should have addressed with their insight into all of these issues, because only thoroughly addressing these issues helps the field move forward. Some balanced view like Stem Cells. 2013 Jul 8. doi: 10.1002/stem.1475. [Epub ahead of print] will be appreciated. Some comment on therapies (J Cell Biochem. 2011 Jan;112(1):10-29. doi: 10.1002/jcb.22952) is appropriate. Indeed, it is interesting to connect this CRC draft up to the cancer genome atlas that has arisen of trying to understand tumor genesis and come up with novel cancer therapies.*

Thank you for your constructive feedback. Seeing as this document would now be our cover letter, we have considered your comments and instead incorporated them into our manuscript, especially the points regarding discussion of EMT-directed therapy, for which examples (including your suggested cetuximab citation) have been included with appropriate citations.

16.) *Given so much text for so many complicated issues, some tables and schematic diagrams will be greatly appreciated for enhancing clarity.*

We have made one table listing putative cancer stem cells and three illustrative figures that complement our manuscript text. Hopefully these figures serve to solidify the key concepts expressed in our article.

Reviewer 02446748

1.) *The article is really interesting and well written. The discussion is consistent, although it could be semplified. I suggest minor language polishing.*
Thank you, we appreciate your kind words. We have corrected the entire manuscript, simplifying sentences and revising logic for clarity and flow.

Reviewer 00503561

1.) *I love this article. Is it possible to add a table on potential stem cell markers proposed so far in colorectal cancer; or the scheme of the concept of colorectal cancer stem cells.*
Thank you for your suggestion. We have included a table on potential cancer stem cell markers as well as figures illustrating the aspects of both normal intestinal stem cells and cancer stem cells from the viewpoint of niche and lineage.

3. References and typesetting were corrected.

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

Sincerely,

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