Thomas Jefferson University
Sidney Kimmel Medical College
Kimmel Cancer Center

Scott A. Waldman, M.D., Ph.D., F.C.P., F.A.H.A.
Samuel M.V. Hamilton Professor and Chair,
Department of Pharmacology and Experimental Therapeutics
***Professor*, Department of Medicine**
***Director*, MD-PhD Program**
***Director*, GI Program, Kimmel Cancer Center**

***Editor-In-Chief*, Clinical Pharmacology & Therapeutics (Wiley)**
132 S. 10th Street, 1170 Main
Philadelphia, PA 19107
T 215.955.6086; F 215.955.5681
scott.waldman@jefferson.edu

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Dr. Ya-Juan Ma
World Journal of Gastroenterology

Dear Ya-Juan Ma:

Thank you for the Reviewers' comments and suggestions regarding the manuscript **The GUCY2C signaling axis and colon cancer prevention (25923**) for consideration for publication in **World Journal of Gastroenterology**. We appreciate their time and thoughtful consideration with respect to this manuscript. Detailed responses to those comments are provided below.

Reviewer 1

1. It would be helpful to know more about the two hormone ligands (guanylin, uroguanylin) that activate GUCY2C? How are they normally regulated, what is their known role in GI tract diseases and is there much known about why their levels decrease in early stages of CRC. Two recent papers from the Gustafsson lab in Trondheim might be included in the review, see Brenna et al, 2015 and Brenna et al., 2016. The 2016 paper describes the use of ISH to localize guanylin and uroguanylin to specific cell types in the intestinal tract. The 2015 paper describes the down-regulation of both genes in IBD.

References have been expanded to include more information regarding the two endogenous hormone ligands, including the papers suggested by the reviewer. There are currently no published reports describing mechanisms underlying ligand loss in early CRC. Additionally, a sentence indicating the need to identify the mechanism by which these hormone ligands are regulated has been added to the introduction. See Introduction, paragraph 2, sentence 4.

2. There should be more information about GUCY2C and its signaling cascade in human CRC. Are there any somatic mutations in GUCY2C reported (e.g., in TCGA data)? Any evidence of epigenetic regulation? Is loss of GUCY2C signaling more prevalent in certain subtypes of CRC, e.g., MSS vs MSI, left side vs right side, serrated, flat or tubulovillus adenomas, etc? A statement in the review indicates that down-regulation of the hormone ligands "often times accompanies APC loss" – so, is there evidence that hormone down-regulation is more prevalent in APC-dependent, CIN, MSS CRC vs MMR-dependent, MSI CRC? Further, in Fig. 1 genomic instability is shown to be a consequence of loss of hormone ligands, leading to tumorigenesis. Is this genomic instability strictly chromosomal? Finally, is there clinical data on the loss of the hormone ligands? For example, is loss of this signaling cascade associated with worse prognosis?

The reviewer raises several interesting questions, all of which are currently under investigation in this laboratory. Unfortunately, there are no published studies investigating somatic GUCY2C mutations and colorectal cancer prevalence, association between ligand loss and CRC subtype or prognosis, or mechanism underlying genomic instability.

3. There are numerous instances of statements where the intent is fairly clear but the wording is not precise. It is suggested that the authors go through the wording of every sentence very carefully and revise the text where necessary.

   Some examples:
   Second paragraph of introduction starts out " Colorectal tumorigenesis is widely accepted as a disease of both sporadic and inherited genomic instability…and chromosomal instability". Customarily, we refer to CRC as having sporadic and inherited forms of the disease, and that genomic instability of some kind is characteristic of all CRC, and certainly not just chromosomal instability.

   Another example is referring to mutant APC as "stabilizing beta catenin" – generally, the most common APC mutations do not produce proteins that physically interact with beta catenin protein, thus causing a failure of beta catenin protein degradation, resulting in excess nuclear localization etc. Therefore, it is somewhat inaccurate to imply a function of the mutant APC protein that it does not possess, it is more accurate to say that the absence of wildtype APC protein stabilizes beta catenin levels or leads to excess beta catenin levels.

   At several points the statement is made that GUCY2C is "inactivated" – yet, it is also claimed that levels of GUCY2C do not change in CRC, only levels of their hormone ligands. Now, it is understood that what is meant is that the GUCY2C signaling cascade is inactivated in the absence of hormone ligands, but that is not what is stated at several junctures – which can lead to confusion.

   Another, "for several years, CRC has been implicated as the gold standard for investigating…". Indeed, this has been true for more than two decades, not several years.

   The text has been revised to improve clarity, as recommended.

Reviewer 2

1. The main idea of the third part, "Genetic pathways for colorectal cancer", has not been shown very clear. The relationship between genetic factors and GUCY2C signaling axis should be clarified and those well known genetic pathways could be written more briefly. It is better to discuss the mechanism of hormone loss in the development of CRC in detail.

   As noted above in the response to comment #1 from the first reviewer, there are currently no published reports regarding the mechanism of hormone loss in the development of CRC. However, the authors agree that this is an important area of research and we have added a sentence indicating the need to identify this mechanism.

2. Information in the forth part, "GUCY2C signaling axis and colorectal cancer", is put together without a good logical order. It is better to use a few more subheadings to show the

mechanism of GUCY2C related colorectal tumorigenesis more clearly, e.g., GUCY2C signaling axis and metabolism, GUCY2C signaling axis and proliferation, GUCY2C signaling axis and stem-like properties, etc.

Subheadings have been modified to improve the flow of the manuscript.

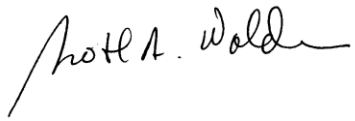
3. There are some typos in this manuscript. E.g., "Intestinal homeostasis maintenance by the GUCY2C signaling axis"; "... cGMP-dependent protein kinase II (PKGII) leading to PKGII-dependent phosphorylation ..."; "... leads to consequent microsatellite instability ..."; "... including cyclin D1, pRb and B-catenin ..."..

All misspelling and incorrect grammar was corrected in the revision of this manuscript.

We hope these revisions and clarifications are satisfactory for publication of this manuscript in ***World Journal of Gastroenterology***. Of course, please let us know if there are any questions or if there is other information we can provide.

Thank you in advance for your consideration.

Sincerely,



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Samuel M.V. Hamilton Professor and Chair,
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