ANSWERING REVIEWERS

November 26, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (manuscript revision NO: 6140).

Title: Approaches that ascertain the role of dietary compounds in colonic cancer cells

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Name of Journal: *World Journal of Gastrointestinal Oncology*

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

Reviewer ***00753027*** suggested: The figure legends (Fig 1 and 2) should be more informative.
Answer: We augmented the contents of the figure legends.

Reviewer ***00556405*** suggested: The various approaches, some better than others, and their individual advantages and disadvantages is also comprehensive. Perhaps a better, more critical, review of the in extensive in vitro literature as to the role of butyrate in colon carcinoma differentiation and apoptosis would have improved this manuscript. This should be included as a minor revision.
Answer: The purpose of the review was not to discuss butyrate's functions in colon cancer cells, but to describe approaches that can ascertain better the effects of diet on colon cancer cells. Thus, exposing colon cancer cells to fecal water, digesta from TIM-2 or other colonic simulations and evaluating the effects on signaling pathways is one approach that is rarely applied today. We pointed out that this approach can be applied to unravel the combined effects of butyrate and polyphenols on colon cancer cells, but this was done only to illustrate the possibilities of the strategy (analyzing complex digesta instead of individual compounds).

Reviewer ***01213174 :*** This is a review of methodology to identify diet-derived bioactive compounds that are involved in colorectal cancer (CRC) development. The authors claimed that there are limitations in the conventionally used in vitro models because the quality and amount of bioactive compounds contained in feces are changed by colonic milieu. They presented a new in vitro model (TIM-2) that enabled simulation of human colonic conditions with computer control. This reviewer agrees with the authors' claims but could not read this review with significant interest. The reviewer felt that the description in this manuscript was superficial overall. It is well understood that the TIM-2 model enabled fine analysis of metabolites contained in fecal water under artificial colonic conditions. However, it was not clear how the results could be linked to a better understanding of CRC development. The authors should cite cases showing the inadequacy of the conventionally used in vitro model and the advantages of the new model in unearthing new findings that would facilitate understanding of the molecular mechanisms of CRC development. In this regard, this reviewer thinks the present manuscript has little to contribute to the international readership and therefore, does not

recommend its publication in World Journal of Gastrointestinal Oncology. SPECIFIC The reviewer could not sufficiently understand Figure 2. A more detailed explanation is required in the Figure legend

Answer: The reviewer misunderstood the aim of the review: we do not want to "identify diet-derived bioactive compounds that are involved in colorectal cancer development". The review discusses strategies that will allow evaluating **better** the effects of complex diet-derived digesta on colon cancer cells. Such strategies will facilitate the formulation of dietary recommendations for colon cancer prevention.

We agree that TIM-2 is well-known model; however, it is not being used frequently to analyze the combined effects of complex diet-derived digesta. One reason for that is the lack of knowledge among the researchers and the research-funding institutions. Currently, NIH reviewers (U.S.) believe that the use of TIM-2 or other sophisticated colonic simulations is not necessary, as it is expensive, and it is offered by foreign institutions (as it is in the TIM-2 case).Most NIH reviewers have also admitted that they have not been aware of the TIM-2 model prior to reading grant proposals that include TIM-2 as an analytical tool. Thus, a major goal of the present review was to inform the wide scientific audience of these new strategies.

The second goal of the review was to indicate that the colonic digesta obtained from such simulators, rather than the individual diet-derived compounds, should be studied for their effects on colon cancer cells. The reviewer states: "However, it was not clear how the results could be linked to a better understanding of CRC development." We have stated all advantages of the new strategies in the review. We believe that evaluating the effects of colonic digesta from different diets, rather than analyzing individual diet-derived compounds at physiologically unachievable concentrations, will allow for the proper conclusions on the association between diet and colon cancer (in terms of cancer prevention). The current roadblocks on the way of clarifying the association between diet and colon cancer prevention were cited and discussed honestly in our review. Discussions on what impedes the progress of defining dietary recommendations against colon cancer should be more frequent and should be encouraged, as such discussions will put a stop to studies utilizing single diet-derived compounds at physiologically unachievable concentrations. Studies utilizing colonic simulators will also take into account the activity of the colonic microbiota and its impact on the preventive role of diet. We think that this alone very clearly points to the advantages of the proposed strategy over "the conventionally used in vitro model", as the reviewer states.

Finally, we did introduce more information in the figure legends (as also required by the first reviewer).

Dear editor, thank you again for considering our manuscript for a publication in the *World Journal of Gastrointestinal Oncology!*

Sincerely yours,

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