ANSWERING REVIEWERS

March 10, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 8438-review.doc).

Title: Predictive proteomic biomarkers for colitis-associated cancer; where are we now in era of the next generation proteomics?

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Name of Journal: *World Journal of Gastroenterology*

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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 reviewer

(1) **Reviewer I**

This is an interesting review with some new evidence. However, and since proteomics in IBD are not extensively studied it is questionable a review with such limited literature.

→We have tried to include as much as literatures, with many outcomes from our research group publication as well as very recent outcomes in this revised review article.

In addition, there are several points requiring correction or clarification, in order the m/s will be suitable for publication, such as:

1. There are many errors in English grammar/ syntax. The authors should read carefully, word by word, all the text and correct. Moreover, most of the sentences are very long and difficult to read.

→ Professional English correction was done in this revised manuscript

2. The introduction should be more focused on biomarkers. The sections "Colitis-associated cancer; pathogenesis and biomarker for prediction" and "Molecular pathogenesis of CRC and CAC" should be incorporated in the general introduction.

→ Yes, according to your suggestion, in this revised manuscript, we have rearranged the whole paragraphs. All arrangements of section and subtitles were done in this revision.

3. The application to the topic of specific chemical/biochemical techniques should be more explained. The reader of the journal is not interested to the possible biomarkers that could be recovered by applying such methodology, but what means the presence or absence of a putative biomarker.

→ Yes, in this revised manuscript, we have generated new chapters describing the implication of biomarkers in IBD and CAC as well as accompanying technologies according to your suggestion. You can find whole changes of section rearrangement in this revised manuscript.

4. The fig. 1 legend (8 patients with UC, 8 patients with CD, and 8 patients with CAC) differs with the text (8 patients with UC, 8 patients with CD, and 8 patients with irritable bowel syndrome).

→ Yes, we corrected those in this revised manuscript. 8 patients with UC, 8 patients with CD, and 8 patients with irritable bowel syndrome is correct.

5. It is not correct to describe proteoglycans as "filling" substances of the extracellular matrix. Most of the proteoglycan molecules participate in specific interactions, regulate growth factors' activity, or provide the milieu for cell spreading and proliferation, depending on their structure.

→ In this revised manuscript, we corrected description regarding proteoglycans (PRG) as follows; "PRG is a major component of the animal extracellular matrix and has been shown to be involved in the differentiation process across the epithelial-mesenchymal axis. It is one of potential biomarker inferred principally through their ability to bind growth factors and modulate their downstream signaling since malignant tumors have their individual characteristic PRG profiles closely associated with their differentiation and biological behavior. PRG2 has further been implicated as a biomarker ….."

(2) **Reviewer II**

This is a worthwhile review, although this field of proteomics in IBD is still in its infant stages and thus is there is not really much literature on which to base a review! Comments for authors to please address:

1. The review is difficult to read, as in many sentences throughout the manuscript there are errors in English grammar/ syntax which detract from the paper. A native English speaker or writing assistant will be needed to correct this issue prior to publication.

→ Yes, revised manuscript is completed with native English editor.

2. The introduction focuses on IBD and its molecular diagnosis, but the review, and therefore the introduction, should be more specific and directed to biomarkers for colitis-associated cancer, rather than IBD overall. A brief discussion of IBD in general in the first paragraph of the introduction should

then be followed by a more focused introduction of the topic of colitis-associated cancer and proteomics associated with this entity.

Yes, in this revised manuscript, we have done whole rearrangement.

3. Equally, the next two sections under subheadings "Current status of biological markers in IBD" and "Classic serological or fecal marker in IBD" again are too broad and these biomarkers have been reviewed in detail (over and over!) elsewhere. These sections add nothing to the topic of proteomics or colitis-associated cancer either as introduction or providing perspective, and thus should be removed/ drastically shortened from this review article.

→ In this revised manuscript, besides of rearrangement of subtitled paragraphs, we have made whole description in concise and core-telling way.

4. The sections under subheadings "Colitis-associated cancer; pathogenesis and biomarker for prediction" and "Molecular pathogenesis of CRC and CAC" are more appropriate as introductory paragraphs than the current introduction and thus should be incorporated in or replace the current introduction.

→ Yes, in this revised manuscript, we have rearranged totally the subtitles according to reviewer's comments.

5. The section beginning on page 5 under subheading "Proteomic biomarker in IBD" could then be moved to more logically follow the above paragraphs as mentioned in point (4) above. Also, for a non-molecular biologist like myself, passing reference is made to the techniques of "Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) and "surface-enhanced laser desorption/ionization (SELDI)-TOF MS" but there is insufficient explanation of their application with reference to the topic of the review in simple to understand language for the clinician reader (like myself!). Why are these techniques worthy of mention in this field and how will they advance our understanding of molecular prediction of colitis-associated cancer in IBD? These questions should be addressed rather than the rather non-explanatory sentence in this paragraph [quote] "With these applications, analyses of serum or plasma by MALDI-TOF MS provide new information mainly about small proteins and peptides with high molar abundance suggests value for applications such as assessment of IBD, UC or CD, respectively, candidate for potential disease markers."

→ Yes, in this revised manuscript, we have rearranged the subtitles according to suggestions of both reviewers.

6. The paragraph with subheading "Label free quantification analysis to pull out potential biomarkers predicting CAC risks in 16 patients with IBD" somewhat appears out of the blue and there needs to be explanation of why this technique and how this study mentioned here relates to the preceding discussion and studies mentioned in the previous paragraph entitled "Proteomic biomarker for CAC in IBD". This will greatly improve the flow of the article here.

→ Our study shown in this review article was aimed to pull out potential biomarkers predicting the risk of CAC in patients with IBD using both label free quantitation analysis and iTRAQ analysis of labeled protein quantification analysis.

7. The Figures are nicely presented but I am confused by the apparent discrepancy between the sentence on page 10 "Applying label free quantification analysis to discover proteomic biomarkers in patients with different type and different stage of 16 IBD patients, comparative analysis was done in 8 patients with UC,

→ As repeated in below answer, biomarkers for CAC risk were drawn from 16 patients with IBD compared with 8 patients with IBS.

8. 8 patients with CD, and 8 patients with irritable bowel syndrome (IBS)" versus the Figure 1 legend which suggests the analysis was done on "8 patients with UC, 8 patients with CD, and 8 patients with CAC." The latter makes more sense to me – which one is it?

→ You are right. In this revised manuscript, we all corrected this.

9. Also, the proteomics analyses appear to have been done on the basis of the conferred risk of known clinical risk

→ Absolutely, new set of experimental works are undergoing in our department, of which outcomes will be submitted soon in following article. The aim of the current review was to stress the potential and value of proteomic biomarker in IBD diagnosis and treatment in addition to colitic cancer risk prediction.

3 References and typesetting were corrected

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

Sincerely yours,

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