ANSWERING REVIEWERS

January 24, 2014

Dear Editor,

Please find enclosed the revised manuscript in Word format with changes highlighted in red to facilitate revision (file name: 6643_revised_highlighted.doc). Notice that we are sending other version of the manuscript without marks (file name: 6643_revised.doc) to j.l.wang@jgnet.com

Title: Proteomics for discovery of candidate colorectal cancer biomarkers

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Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 6643

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

1 Format has been updated

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

Please notice that pages are referred to as in the highlighted manuscript (file name: 6643_revised_highlighted.doc).

Reviewer 1

1. **Rather than "potential", the authors could use the word "candidate" for biomarkers before being validated. The title could be changed in this respect.**

   The authors agreed with this suggestion and thus the title was modified. Now it reads "Proteomics for discovery of candidate colorectal cancer biomarkers"

2. **The authors mentioned in the introduction: "More recently, the technique of choice is the LC-MS/MS (liquid chromatography-tandem mass spectrometry) as it is much more rapid and sensitive." Please, develop here with two sentences the powerful shotgun LC-MS/MS approach.**

   Following the suggestion of Reviewer 1, we included a brief paragraph (page 6) to describe the powerful shotgun LC-MS/MS approach.

3. **The authors reported the interest in analyzing the secretome of human colon adenocarcinoma cell lines. This is a hot topic and probably will be fruitful as secreted proteins can be easily accessible for biomonitoring. Recently some drawbacks have been presented in the literature for such analysis from cell lines and the authors could mention the recent work by Malard and his co-workers on "Analytical constraints for the analysis of human cell line secretomes by shotgun proteomics" published in Journal of Proteomics.**

The authors agreed with this suggestion and thus this work was cited in the text (page 26), mentioning the main drawbacks related to the analysis of human cell line secretomes by proteomics techniques.

4. **The authors cited 119 references. Among them, some old references appear useless (17, 44, 45, …) and could be advantageously replaced by citing a unique historical review on this matter. Indeed, the readership of the World Journal of Gastroenterology will rather read other accessible reviews on this topic rather than the original works.**

   Following the suggestion of Reviewer 1, we replaced references 17, 44 and 45 by citing two historical reviews (references 14 and 43).

Reviewer 2

1. **In page 13 the sentence beginning with "SELDI-TOF-MS combines two powerful technologies, liquid chromatography (LC) and mass spectrometry (MS)…." Should be corrected as "SELDI-TOF-MS combines two powerful technologies, chromatography and mass spectrometry (MS)……".**

   Thank you for your comment. This sentence has been changed to "SELDI-TOF-MS combines two powerful technologies, chromatography and mass spectrometry (MS)……."

2. **In page 20 the last word of the sentence below should be "markers" Therefore, in order to reach a greater diagnostic accuracy it can be more useful to use a panel of several makers.**

   Thank you for your observation. The typing mistake has been corrected in the revised version.

3. **In Figure 1 the words written as "quantificacion" and "identificacion" should be corrected as "quantification" and "identification", respectively.**

   Thank you very much for your comment. These words have been corrected to "quantification" and "identification".

Reviewer 3

1. **The aim of the review is not entirely clear. In the abstract and in the introduction part, the authors put our attention in to "the discovery and validation of new protein markers for CRC", but later on there are few examples of biomarkers in each of the methodological sections. In addition, almost all of the examples concern the difference in between the tumour and normal tissue, but not the discovery and validation of biomarkers for CRC treatment. In this context, it is not clear if the article aims to characterize the methods, recently used for diagnosis and prognosis of CRC or the biomarkers itself**.

   Following the advice of the Reviewer 3, we have modified the abstract and the last paragraph of the introduction (page 6) in order to clarify the aim of this review, which is to describe the different proteomic techniques as well as the different biological samples used for the identification of candidate biomarkers for CRC.

2. **The long explanatory part of the protein methods is more suitable for a Molecular Biology textbook than for a journal review paper with such a title and aim. I would present only the**

advantages and disadvantages of protein methods, avoiding the methodological details. The paper should be significantly shortened, which will make it more suitable for the targeted reader audience.

As we have mentioned above, the aim of this review was clarified in both the abstract and the Introduction section. Our intention with this work is to describe the different proteomic tools used for the identification of candidate markers for CRC. Therefore, we consider that it is worthy to include a methodological description of the most common techniques employed in this area.
In addition, we contacted the Editor and asked for his opinion regarding the extension of the manuscript, also pointing out that Reviewers 1 and 2 did not make any comments concerning the methodological description of the protein methods nor extension of the proteomic techniques section.

3. **A table would be appreciated, presenting the recently found CRC protein biomarkers. The biomarkers should be clearly divided to: diagnostic, prognostic, therapeutic, etc. In the same table, the different detection methods should be shown. Are there biomarkers, discovered by only one approach and others by several??? Are they found in blood, tissue, stool…. or cell lines??**

   As suggested by the reviewer, we have included a new table with the information about the candidate protein biomarkers identified by proteomic techniques most frequently reported in the literature. According to the reviewer´s indication, we have included in the table for each candidate protein biomarker the gene, sample used for the identification, its potential clinical value in CRC, and the proteomic technique used for its identification.

4. **The reference list is not up to date. For example the authors cited the Cancer Statistics for 2010, but not for 2013: Cancer statistics, 2013. Siegel R, Naishadham D, Jemal A. CA Cancer J Clin. 2013 Jan;63(1):11-30.**

   According to the request, we updated this reference. We also reviewed and confirmed the rest of the references.

5. **The title could be changed from "Proteomics for discovery of potential colorectal cancer biomarkers" to "Proteomics for the discovery of potential diagnostic, prognostic and therapeutic biomarkers in colorectal cancer"**

   In our opinion, considering that a detailed description of the different candidate biomarkers for CRC is beyond the scope of this review, we did not follow the reviewer´s suggestion regarding the title. We changed the title according to the suggestion of Reviewer 1 from "Proteomics for discovery of potential colorectal cancer biomarkers" to "Proteomics for discovery of candidate colorectal cancer biomarkers."

6. **Finally, to remain consistent with quality of articles in this journal and the targeted readership audience, this article would greatly benefit from additional review by someone with strong scientific English, in order to correct some grammatical errors. For example: The following sentence is unclear and should be revised: "In addition, by analysing the tumour itself there is no doubt about the origin of altered proteins, and the concentration of a putative marker is higher in tumour tissue than in blood."**

Following the suggestion of Reviewer 3, the manuscript has been reviewed by someone with strong scientific English, and the grammatical mistakes have been corrected. Regarding the sentence pointed out by the reviewer, this has been modified. Now the paragraph reads "This approach has several advantages over serum or plasma analysis. The first one is that not all the proteins altered in the tumour, and therefore marker candidates, are secreted to the blood from the tumour cells. Therefore, the concentration of a putative marker is higher in tumour tissue than in blood. In addition, since tumour samples are analysed, there is no doubt that the altered proteins identified originate in the tumour itself."

3 References and typesetting were corrected.

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

Sincerely yours,

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