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Dr. Jing Yu
Science Editor
World Journal of Gastroenterology

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Dear Editor,

Enclosed is a revised version of our manuscript entitled "Recent advances in mass spectrometry-based proteomics of gastric cancer". I would like to take this opportunity to thank the reviewers for their thoughtful comments to help us improve our manuscript. As suggested by the reviewers, the title has been changed and our manuscript has been edited by a commercial professional English-editing service. We have been able to address all the comments raised by the reviewers, and our point-by-point responses are detailed below. All the revisions addressing to the reviewer comments, but not mere English editing changes, are highlighted in the revised manuscript. I hope the manuscript is now suitable for publication in the *World Journal of Gastroenterology*. If there are any matters that require further clarification, please do not hesitate to contact us.

Sincerely yours,

J. Eugene Lee, Ph.D.
Principal Research Scientist
Corresponding author

Responses to the reviewer comments

Reviewer code: 03572722

1. *It is not really a summary of the article, and do not include key findings of these hot topic. I suggest rephrasing the abstract by highlighting the novelty, advances and importance of proteomic research in the field of gastric cancer. For example, it was mentioned "characterization of thousands of proteins in gastric cancer cells", but proteomics can characterize proteins not only from cells, but also from serum.*

Response: Abstract is now modified in the three highlighted sentences on lines 32-36 of page 2.

2. *Rephrase the introduction to be more effective, clear and well organized. The first paragraph with genomics tools is too large for a proteomic review; one phrase with genomics should be enough.*

Response: The first paragraph of Introduction is shortened to one highlighted sentence on lines 52-53 of page 3.

3. *There is a large part in Introduction in which is not mentioned references.*
Response: New references 3, 4, 8 and 9 are added now on page 3.

4. *Table 1 should be placed in the body of article, not in introduction (where is not so appropriate).*
Response: Table 1 is moved out of Introduction to the next section on lines 79-80 of page 3.

5. *Too many details regarding description of technologies and less information regarding gastric cancer.*
Response: In this revision, technology description is shortened and gastric cancer information is lengthened throughout the manuscript.

6. *It is mentioned "Singhal et al.[13] " and in references list it is "13. Cai XZ, Huang WY, Qiao Y, Du SY, Chen Y, Chen D, et al."*
Response: Thanks. Paper information for reference now #14 is corrected to Singhal et al's on lines 455-458 of page 15.

7. *Could be mentioned other platforms, like SELDI-TOF.*
Response: Two paragraphs describing MALDI imaging and SELDI-TOF are added to lines 110-123 of page 5.

Reviewer's code: 03508701

1. *First, it would be better to summarize the role of proteomic analysis in the study of gastric cancer in the INTRODUCTION, such as identifying novel biomarker or elucidating molecular mechanism.*
Response: A phrase "... that can help identify protein biomarkers and elucidate the molecular mechanisms of gastric cancer" is added to the Introduction section on lines of 73-74 of page 3.

2. *Additionally, it might be not appropriate to place TABLE 1 in INTRODUCTION.*
Response: Table 1 is now moved from the Introduction section to the next section on lines 79-80 of page 3.

3. *Second, TABLE 1 and TABLE 2 could be merged into one as it would be more concise and informative. And providing more information about whether the differently expressed proteins identified from the proteomic analysis are validated by IHC or WB in the following step in each study would be helpful.*
Response: Because merging the two already big tables would make the merged table too big, they remain separate. As suggested, however, an additional column is added to Table 2 of pages 26-27, indicating whether the altered proteins have been validated using immunohistochemistry or western blot or have not been. This added information would be very useful - Thanks.

4. *Third, I am not sure if the authors have performed comprehensive literature retrieval as there are some publications which are not included in this article (Song, D., et al. (2016). "Diagnostic and prognostic role of serum protein peak at 6449 m/z in gastric*

adenocarcinoma based on mass spectrometry." Br J Cancer 114(8): 929-938.). I guess this manuscript was submitted before the publication time of the above article?

Response: We now surveyed the literature again and add five more papers, including the one mentioned, to this revision. The five are highlighted in Table 1 of pages 23-25.

Reviewer's code: 00679634

1. *The manuscript begins with a helpful summary of current protein mass spectrometry methods. However, the rest of the manuscript consists of an uncritical summary of selected individual studies. A good review should provide expert insights in the topic and should not be limited to a list of summary statements from published work. This review lacks critical and analytical perspectives of the studies cited. The authors also do not address several points which are pertinent to the future development and utility of protein mass spectrometry. For example, the review should address the importance of sampling handling and pre-analytical processing, the relatively low throughput of protein mass spectrometry compared to genomic methods, reproducibility of putative biomarkers and the barriers to clinical adoption of protein mass spectrometry in precision medicine.*

Response: We appreciate these suggestions for perspectives of the individual studies and future developments. These are, however, beyond the scope of this review, in which we aim to introduce a variety of mass spectrometry-based quantitative proteomic techniques that have been and can be used for gastric cancer research, so that readers can understand pros and cons of the tools and researchers can select appropriate tools suiting for their individual purposes.

2. *Studies summarized in Tables 1 and 2 are very incomplete. Numerous studies have not been included (e.g. PMID 21165559; PMID15649254; PMID 22204653; PMID 17022644; PMID 17154271; PMID 22015459; PMID 22533479; and other studies)*

Response: After a new survey, five more papers, including the four mentioned here (PMID 21165559, PMID 22204653, PMID 22015459, PMID 22533479), are added to this revision, as highlighted in Table 1 of pages 23-25. However, excluded are PMID15649254 that did not adopt mass spectrometry-based proteomics; and PMID 17022644 (2006) and PMID 17154271 (2007) that were reported more than 5 years ago.

3. *The gastric cancer proteomics literature should be summarized according to the different types of biological samples i.e. separate tables for (a) gastric cancer cell lines; (b) gastric cancer tissues; (c) gastric cancer secretomes; (d) serum biomarkers; (e) biomarkers in gastric juice/gastric fluid; (f) gastric cancer interactomes; (g) patient-derived gastric cancer xenotumors.*

Response: The categories (a), (b), (c), (d), and (f) are shown in Table 1, although Table 1 is not divided into separate tables according to these categories. As another reviewer (code 03508701) suggested merging rather than dividing the tables, these two opposite suggestions are compromised and the two tables are not merged or further divided. Within Table 1, however, the order of studies is changed to comply with the suggestion. Studies are now listed according to sample types and measurands. Studies with tissue samples are listed first and followed by those with serum samples and then those with cell lines. Thanks, this revised order makes a better sense than the unrevised. Furthermore, we focus on diversity of mass-spectrometric tools for discovering protein biomarkers rather than the list of all biomarkers including the categories (e)

and (g) for gastric cancer. Such biomarkers can be found in many other recent reviews of the *World Journal of Gastroenterology* and other journals.

4. *Several expressions and words are unclear to readers of standard English. Page 3: "A couple of mass analyzers are used in current proteomics." Page 3: "… 29 proteins were in different cellular levels." Page 6: "… can attribute to the protein." Page 6: TYPES OF MEASURANDS Page 7: "… cancer tissues differs from that from normal tissues." Page 8: Misspelling – "triple-quadruple" Page 8: "… than conventional discovery base mass spectrometry".*

Response: The whole manuscript is now edited for English. Especially the mentioned are corrected as the followings.
Page 4, line 90: "Two major types of mass analyzers are used in current proteomics technology."
Page 4, lines 101-102: "... the cellular levels of 29 proteins differed."
Page 8, line 237: "... can be matched to the protein."
Page 9, line 251: "MEASURANDS OF MASS SPECTROMETRY"
Page 10, lines 284-285: "... cancer tissues differs from the proteins that are secreted from normal tissues."
Page 11, line 324: "triple-quadrupole"
Page 11, lines 329-330: "... than conventional discovery-based mass spectrometry ..."

5. *Page 4: Singhal et al. is wrongly cited (not reference 13). Singhal et al. did not analyze biopsy samples.*

Response: The information for reference now #14 is corrected on lines 455-458 of page 15. Singhal et al. indeed examined a total of 183 tissue biopsies that were collected from 126 patients with or without oesophago-gastric malignancy.

Reviewer's code: 00679634

1. *Since it is entitled "Recent Advances in Proteomics of Gastric Cancer", there are still other methods in proteomics, such as protein chips, this manuscript only focus on mass spectrometry, the title is inappropriate.*

Response: The title is now changed to "Recent advances in mass spectrometry-based proteomics of gastric cancer.".

2. *If the disadvantages or limitations of mass spectrometry are mentioned, and comparison to other methods is made, the manuscripts will be more comprehensive.*

Response: A phrase "Despite the inherent low sensitivity and undersampling suffered by mass spectrometry ..." is added to the Introduction section on lines 61-62 of page 3.