ANSWERING REVIEWERS

March 29, 2015

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 16204-review.docx).

Title: Circulating RNAs as new biomarkers for detecting pancreatic cancer

Author: Takahiro Kishikawa, Motoyuki Otsuka, Motoko Ohno, Takeshi Yoshikawa, Akemi Takata, Kazuhiko Koike

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 16204

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
Please see the point-by-point response to the reviewers' comments in the next page

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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Reviewer #02533276:

Comments to Authors:

The aim of this review is to summarize current knowledge about circulating RNAs in pancreatic cancer focusing specially on the possible clinical use of these RNAs as biomarkers for this cancer. The manuscript is interesting and in general is well written. However, I consider that the following points should be addressed for the authors

Response:

We thank Reviewer #025333276 for his/her valuable comments. To address the concerns, new references have been included in Tables 1, 2 and 3. Changes to the text are highlighted in yellow.

1. The abstract should be modified in order to summarize the main points discussed in this review.

Response:

We have revised the abstract, adding a summarized description of "other non-coding RNA" and a "current issues and future directions" section.

2. Authors should focus more specifically on pancreatic cancer and discuss more deeply the possible diagnostic and/or prognostic value of circulating RNAs for this neoplasia. In literature there are other general reviews describing circulating RNAs in cancer.

Response:

We thank the Reviewer for pointing out the insufficiency of the discussion of pancreatic cancer. In fact, there are currently few reports of circulating RNAs other than miRs, and there are particularly few reports of non-coding RNAs (ncRNAs), in cases of pancreatic cancer. Therefore, we have listed the circulating small ncRNAs and lncRNAs that are deregulated in other types of cancers (Table 2) and that may also be applicable in pancreatic cancer. For future exploration to discover novel biomarkers, we consider the following three issues to be crucial: obtaining pre-cancer samples, utilizing genetically engineered mouse models, and performing high-throughput RNA sequencing, which are additionally described in the "Current issues and future directions" section. We have added new references to the "Circulating RNA", and "Long ncRNAs" sections and to Table 1, 2, and 3, about pancreatic cancer.

3. In my opinion, it would be useful for the readers that this review includes a section describing the different types of circulating RNAs

Response:

We thank the Reviewer for pointing out this issue. According to this suggestion, we have added the new sections "Small ncRNAs" and "Long ncRNAs", and have added brief explanation of the classical definition and functions of snoRNA, snRNA, piRNA, and lncRNA in the "Other circulating ncRNAs and cancer" section.

4. Authors should indicate if their own results are included in this review.

Response:

We thank the Reviewer for this suggestion. Although we have no published data about circulating ncRNAs in pancreatic cancer cases, we have unpublished data about the alternations in comprehensive miR expression levels that occur over the course of oncogenesis that were obtained using our genetically modified mouse models of pancreatic cancer. These findings may lead to the discovery of circulating biomarkers present during the early stage of carcinogenesis. Some comments about these data have been added to the "Current issues and future directions" section.

5. Some new references should be included, Rachagani S et al, Adv Drug Deliv Rev 2015 Jan; 81C:16-33. doi: 10.1016/j.addr.2014.10.020. Epub 2014 Oct 23. Frampton AE et al, Expert Rev Mol Diagn 2014 Apr; 14(3):267-71. doi: 10.1586/14737159.2014.893192. Epub 2014 Feb 28.

Response:

We thank the Reviewer for the kind recommendation about these references. We have included the suggested references with comments in the "Biological functions of circulating RNAs" section (ref. #154). Because the latter reference is a letter pertaining a previous original article (Ma MZ et al, J Exp Clin Cancer Rez. 2013 Sep 28; 32:71. Doi: 10.1186/1756-9966-32-71), we have included it in the "Circulating microRNAs and pancreatic cancer" section (Ref. #67).

Reviewer #02731744:

Comments to Authors:

The authors described the review of RNA circulating RNAs as new biomarkers for detecting Panc Ca. This paper is almost well-written, however, I would recommend some revision before publication. Please describe more discussion section, and more depth. This is important point for our readers.

Response:

We thank Reviewer #02731744 for his/her valuable comments. To address these concerns, we have thoroughly revised the "Current issues and future directions" section, adding a discussion of the difficulties in detecting pancreatic cancer by the currently available modalities and suggesting additional approaches, including obtaining pre-cancer samples, capitalizing genetically engineered mouse models, and performing high-throughput RNA sequencing. In addition, we have completely revised the text to discuss this information in more depth.

Reviewer #00503512:

We thank the Reviewer #00503512 for his/her valuable comments. To address these concerns, new references have been included in Tables 1, 2 and 3. Changes to the text are highlighted in yellow.

Comments to Authors:

The authors of this review article have summarized current knowledge on circulating RNAs in pancreatic cancer. The manuscript is interesting for this Journal and well presented. I would recommend to accept it, pending the following changes:

1) Please carefully revise the writing to ensure that you remain consistent throughout the text: the tense changes too frequently

Response:

We thank the Reviewer for pointing out this issue, and we apologize for our inconsistent description. We have reviewed and revised the manuscript, particularly focusing on tense. In addition, we have had the manuscript edited by a professional English language editing company to correct inconsistencies in tense throughout the text as much as possible.

2) In the section on "other non-coding RNAs" the authors quote snoRNAs, lncRNAs etc but they do not introduce their formal definition. That should be changed

Response:

We thank the Reviewer for pointing out this issue. We have added descriptions of snoRNAs, snRNAs, piRNAs, and lncRNAs to the "Small ncRNAs" and "Long ncRNAs" sections.

3) In the same section, the authors state that the function of ncRNA is still subject to "many questions". Even if this partially true, the authors should quote some seminal papers that clearly define the functional role of lncRNAs and other RNAs in human cancer and in the human transcriptome (e.g. PMID: 25599403, 25619839, 24346158)

Response:

We thank the Reviewer for the kind recommendation of these references. We have deleted the phase "many questions about their expression and function" and have added information to "The biological functions of circulating RNAs" section, including the suggested reference (Ref. #145, #106,#105), as the Reviewer recommended.

Reviewer #00227592:

Comments to Authors:

Circulating RNAs have been investigated as potential biomarkers for many cancers including pancreatic cancer. The main purpose of this manuscript was to review circulating RNAs as new bio markers for detecting pancreatic cancer. However, the authors failed to discuss it in depth.

Response:

We thank the Reviewer #00227592 for his/her critical comments, and we sincerely apologize for our inadequate description in the original manuscript. To address the Reviewer's concerns, we have considerably revised the manuscript; in particular, we have provided a more in-depth discussion and new references. Although few studies have been performed evaluating the use of circulating RNAs as biomarkers in pancreatic cancer cases, as we mentioned in the responses to other Reviewers, we believe that this manuscript now provides a concise and adequate review of the current status and problems with these RNAs. We believe that this review article will be of interest to the broad readership of WJG, and again, we thank the Reviewer for the critical comments.

Reviewer #00001832:

We thank Reviewer #00001832 for his/her valuable comments. To address these concerns, new references have been included in Tables 1, 2 and 3. Changes to the text are highlighted in yellow.

Comments to Authors:

The authors specifically focus on circulating micro RNAs as well as on small non-cording RNAs (ncRNAs) or long ncRNAs. This review contains interesting information and is in general well written. However there are some concerns with the manuscript that should be dressed.

1. The abstracts reads more like short introduction section and should be modified to better describe what the authors have analyzed and what they have found during their literature review.

Response:

According to the Reviewer's valuable suggestions, we have revised the abstract by adding a summarized description of "other non-coding RNA" and "current issues and future directions" to reflect the manuscript more precisely.

2. The authors should include somewhere the search strategy. As of now is not clear where there

was a selective literature search or a systematic search to include all available data especially on circulating RNAs in pancreatic cancer. This information should be provided.

Response:

We thank the Reviewer for the suggestions about the search algorithm. We searched references on PubMed website using relevant search terms and subsequently excluded inappropriate articles that were not about pancreatic cancer or RNA markers in addition to those that did not assess human samples. Although we consider that this manuscript includes almost all of the currently available data about circulating RNAs in pancreatic cancer, we did not apply a particular protocol for the systematic search conducted in the review process during the preparation of this manuscript. In this revised manuscript, to assist the readers' understanding and according to the Reviewer's suggestion, we have added information on the search algorithm used to identify the cited references to each table legend.

3. As a general comment: Most of the manuscript deals more with aspects of circulating RNAs in cancer in general and not specifically with pancreatic cancer.

Response:

We thank the Reviewer for pointing out the insufficiency in the discussion of pancreatic cancer. There are currently few reports about circulating RNAs other than miRs, and there are particularly limited reports of ncRNAs, in pancreatic cancer compared with other cancers. Therefore, in the revised manuscript, we have listed circulating small ncRNAs and lncRNAs that are deregulated in other types of cancers (Table 2), which may be applicable to pancreatic cancer as well, although additional studies are needed. We consider the following three issues to be important for the future development of novel biomarkers specific to pancreatic cancer: 1) obtaining pre-cancer samples, 2) capitalizing genetically engineered mouse models, and 3) performing high-throughput RNA sequencing. Thus, we have added this information to the "Current issues and future directions" section of the revised manuscript.

Altogether it is a nicely written manuscript regarding circulating RNAs and cancer. I would advise the authors to include the search algorithm and to point out whether the data concerning pancreatic cancer are the result of a systematic search or a general search.

Response:

We thank the Reviewer for the encouraging comments. Regarding the search algorithm, please see our response to comment #2. We sincerely thank the Reviewer for his/her comments, which have helped us to improve our manuscript.