Format for ANSWERING REVIEWERS

December 25, 2012

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 5364-review.doc).

Title: Death decoy receptor overexpression and increased malignancy risk in colorectal cancer

Author: Liang Zong, Ping Chen, Da-Xin Wang

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 5364

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
Comments 1:
1)   The title does not reflect well the context of whole manuscript. It should be revised.
Answer: The title was exchanged with "Death decoy receptor overexpression and increased malignancy risk in colorectal cancer".
2)   Patient information: What were the inclusion and exclusion criteria? Was this study approved by any ethical committee?
Answer: The inclusion and exclusion criteria were introduced in the patient information section and the information of ethical committee was added in this part.
3)   Supporting references are required in many places, including the scoring system for HER2 staining approved by FDA.
The supporting reference was added through the manuscript.
4)   How were the patients followed up in this retrospective study? What was the range (minimum, maximum) of the follow-up period?
Answer: The range of follow-up period was added in the follow-up section.
5)   The statistical analysis method for the correlation between the two proteins should be described.
Answer: The statistical analysis method for the correlation between the two proteins was described in statistical analysis section.
6)   Figure 1. The author should specifically indicate the positive signal by using arrows. In addition, I would suggest that authors perform double staining immunohistochemistry in those 36 patients positive for both HER2 and DcR3 if this is technically feasible.
Answer: We added the arrows for positive signal and tried to perform the double staining immunohistochemistry. Unfortunately, the staining colors of HER2 and DcR3 are similar, and difficult to differentiate. Therefore, these figures were not added.
7)   Figures 2-4. *P* values should be added in the figure or described in the figure legends.
Answer: The *P* values was added the figure legends.

8)   In the text, the authors should cite tables in the correct order, which means the Table 1 should be cited first. Table 1 is likely mistakenly labeled as Table 2 in the section "Correlation of molecular markers…..". Actually, Table 2 can be deleted as the data can be described in detail in the text.

Answer: We corrected the order of tables and deleted the Table 2.

9)   Two important groups were excluded in the survival analysis when HER2 and DcR3 were combined, which are HER(-)/DcR3(+) and HER(-)/DcR3(-). It is likely that HER(-)/DcR3(+) has the similar survival to HER(+)/DcR3(+), and HER(-)/DcR3(-) has the similar to HER(+)/DcR3(-). The author may include them two groups in the analysis and added the results in Figure 4. If my estimation is correct, then, the conclusion "Patients with both HER2 and DcR3 overexpression are associated with a pooper survival..…" is not valid, and the conclusion would be "Patients with DcR3 overexpression are associated with a pooper survival……"

Answer: We totally agree with reviewer's opinion, so we improve the conclusion with "DcR3 overexpression is highly correlated with lymph node metastasis and poor overall survival".

10) Discussion section should focus on the major findings of the study. The first, second, fourth, and fifth paragraphs are simply a literature review only. Please significantly incorporate with the discussion on your major findings where appropriate. Otherwise, delete them.

Answer: We delete the the first, second, fourth, and fifth paragraphs in the discussion part.

11) Reference should be in uniform format. Some have both volume and issue numbers, whereas others are not. Moreover, the authors should provide complete page numbers in the references, e.g. Refs. 11-16.

Answer: We revised the reference to be in uniform format.

12) There manuscript needs extensive editing, as there are numerous grammatical errors, inappropriate expressions of phrases and sentences, and inappropriate use of abbreviations.

Answer: We invited a native English speaker to help us revise the manuscript.

Comments 2:

Major points:

1)   *Question:* Big parts of the introduction and discussion are very similar as in one manuscript of the same group (Li et al., 2001; BMC Cancer; reference 28) and as in Lang et al. 2009 (doi: 10.1631/jzus.B0920077). The latter should be cited if this manuscript would be accepted.

Please change this since it really has the bad taste of plagiarism.

Answer: It was a serious problem like the reviewer pointed, so we decided to delete these parts and revised them.

2)   *Question:* The authors state that "Scores of 0 or 1+ indicate a tumor negative for HER2 expression …" in the M+M part. However, in the results part, they report that among the 55 HER2 positive samples, 24 were 1+. If this is just a mistake in the M+M part, it is indicative of a shallow preparation of the manuscript. Otherwise (if these 24 cases are not really HER2 positive) it would destroy the basis for the conclusions of the manuscript.

Answer: It was a big careless mistake that 24 cases were stained as weak complete membrane staining whereas 21 cases were moderate complete membrane staining, which all belong to 2+.

1)   *Question:* P values of 0.000 are repeatedly given. Since this is mathematically impossible, please indicate the true values.

Answer: The exact value of these parameters was less than 0.001 and we improved these data.

2)   *Question*: In the discussion, the authors state: "Although there has been a confidently evidence to support DcR3 as an independent prognostic marker, it really represented a poor agent in chemotherapy." How can a prognostic marker be an agent in chemotherapy? Is this just bad use of language?

Answer: It was a poor explanation that made the reviewer confused. After thinking about the importance for discussion part, we suppose this paragraph is not necessary and we decide to delete it.

3)   *Question*: What are the points the authors want to make with the second last paragraph of the discussion? The simple listing of results of other groups (here: DcR3 downregulation of MHC-II and IgG antibodies towards HER2) is not a discussion! This has to be strongly improved throughout the manuscript!

Answer: We totally agree with the reviewer's opinion here, actually we would like to explain why DcR3 can cause the increased risk in colorectal cancer and we improved this paragraph.

4)   *Question:* Finally, the manuscript needs to be seen by a native or at least well-trained English speaker. There are many grammatical and some typing errors that should be addressed throughout the manuscript. Parts of the discussion (point 4 as an example) are indeed not clear since the language is too poor!!!

Answer: We invited a native English speaker to help us revise the manuscript.

5)   *Question:* As the most important prognosticator is still the TNM; the authors have to perform a comparison of their correlations of HER2 and DcR3 expression and the outcome of the patients with the classical TNM classification.

Answer: We have already made a comparison of HER2 and DcR3 expression and the outcome of the patients with pTNM classification (shown in table 1).

6)   *Question:* The final statement: "… and the detection of the coexpression of HER2 and DcR3 in colorectal cancer tissues could be also used in the clinic" does not contain any specificity which would be supported by the data presented. Of course could it be used in the clinic. But this is also true for any other marker – they all <u>could</u> be used …

Answer: Because the last sentence is not meaningful just like reviewer's comment, we deleted this sentence.

7)   *Question:* The examples given in Figure 1 must be expanded. Meaning that exemplary stainings for all the described classifications have to be shown.

Answer: Because of the space, we thought positive staining of HER2 and DcR3 might be enough to convey the information to readers. Therefore, Figure 1 was expanded with HER2 positive (2+ and 3+) and DcR3 +.

8)   *Question:* Why do some of the Kaplan-Meier curves stop in Figure 2 and Figure 4? Please explain. It could be a strong bias?

Answer: This was a retrospective study and all of the patients were followed up after surgery which means it was difficult to keep the patients divided by different criteria in a similar follow up period. But nevertheless, we could observe whether or not there was a difference in the same period.

Minor points:

1)   *Question:* All abbreviations must be introduced when used for the first time. This has for example not been done for DcR3. A similar sloppy style is true for other things, too. Some examples are given in the following.

Answer: We added the full name for the abbreviations when they were used for the first time and this kind of problem was improved through the manuscript.

2)   *Question:* The suppliers of the antibodies used in the IHC are not properly given.

Answer: We add the information of antibodies used in the IHC.

3)   *Question:* "Demic" is not a common word in English. In addition, not one PubMed-listed publication uses this term. Please substitute or introduce properly.

Answer: We revised this paragraph and deleted this term.

4)   *Question:* Please give the range of the follow-up time.

Answer: The follow-up time was given in the follow-up section.

5)   *Question:* References 3 and 33 are double.

Answer: Reference 33 was deleted.

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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