September 15, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 4815-review.doc).

Title: IGF-1 induces lymphangiogenesis and facilitates lymphatic metastasis in colorectal cancer

Author: Zhenjun Li, Xiaojiang Ying, Hongliang Chen, Pingjiang Ye, Zhiliang Chen, Gang Li, Huafeng Jiang, Jiang Liu, Shuzhen Zhou.

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 4815

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

For Reviewer #1:

Thank you very much for your carefully reading of the manuscript. We very much appreciate your valuable suggestions and kind advice.

(1) The present manuscript should be checked by a native English speaker who is a medical scientist.

Answer:

Thank you very much for your kind advice. The manuscript has been checked by a native English speaker engaging in medical research and edited by the English language editing companies: Jing-Yun Ma Editorial Office.

(2) Abstract

Line 8: "Lovo cell…" should be "LoVo cell……" Please use abbreviation consistently.

Answer:

We have corrected the mistakes according the kind advice.

(3) Introduction Line 15: "IN a recent…" should be "In a recent….".

Answer:

We have corrected the mistakes according the kind advice.

(4) Statistical analysis Line2: "hi-square" should be "chi-square"

Answer:

We have corrected the mistakes according the kind advice.

(5) Discussion Since histologic confirmation of lymphatic vessel invasion by tumor cells has prognostic value in various malignancies, please address this point in the Discussion.

Answer:

We have added the information in paragraph 3 of Discussion in the revised manuscript.

(6) Tables 1 and 2. Subjective data have no meaning in two decimal points. Please give

data with one decimal point in Tables.
Answer:
It is a very good question.
We have corrected the data according the kind advice.

(7) Figure Figure 2b shows lymphatic vessel invasion. You may take a photo showing much more apparent lymphatic vessel invasion.
Answer:
Thank you very much for your kind advice.
We have added this information in Figure 2C in the revised manuscript.

For Reviewer #2:
(1) Abstract/ Introduction
① The research question is not clear and expressed in the introduction
a. They wish to show that IGF-1/IGF-1R induce CRC lymphangiogenesis but did not explain clearly how they IHC study and the mouse study serves to answer the specific questions
Answer:
Thank you very much for your critical comments.
We have added the following information in Introduction in the revised manuscript:
In the present study, through immunohistochemistry (IHC) assay, we found that IGF-I and IGF-1R were significantly over-expressed in CRC tissues compared with adjacent normal tissues. Using D2-40 staining, a specific lymphatic endothelial marker [3, 13], we found lymphatic vessel density (LVD) was significantly higher in CRC tissue. The level of IGF-I, IGF-1R and LVD were all correlated with lymphatic metastasis. And, a positive correlation was found between LVD and IGF-IR. These results indicated that IGF-I/IGF-1R axis might promote lymph node metastasis of CRC by the induction of lymphangiogenesis. To further explore its role in lymphangiogenesis, the LoVo cell (human colon cancer cell line) xenograft model was employed, showing that IGF-1 could indeed increase the level of LVD in xenografts. Together, our findings suggest that IGF-1/IGF-1R can also induce lymphangiogenesis in CRC and may facilitate lymphatic metastasis in these patients.

(2) MATERIALS AND METHODS
① It is not clear and vague how this population is selected especially in a retrospective study.
a. Some more information will add value; paint a clear train of thought on selection to assure the audience there is no inherent bias for selection of this population
Answer:
The patients were randomly chosen from the CRC patients who underwent surgical resection without preoperative neoadjuvant chemoradiotherapy between January 2011 and June 2011. We have added the information in the revised manuscript.

② IHC
a. Two independent investigators scored each sample without any prior knowledge of each patient's clinical information and outcome.
i. Please elaborate if the 2 investigators are pathologists or how are they qualified to provide a good

objective assessment as a lot of the findings will be based on this staining assessment.

Answer:

The sections were examined by pathologists without any prior knowledge of each patient's clinical information and outcome. We have added the information in the revised manuscript.

③ The establishment of cell xenograft model and IGF-1 treatment

i. Please provide more details of how the mouse model was optimized

1. Why intraperitoneal injection chosen as a route?

Answer:

It is a very good question.

Referring to the following reports, we chose intraperitoneal injection as a route.

a. Rinaldi C, Bott LC, Chen KL, Harmison GG, Katsuno M, Sobue G, Pennuto M, Fischbeck KH. Insulinlike growth factor (IGF)-1 administration ameliorates disease manifestations in a mouse model of spinal and bulbar muscular atrophy. Mol Med 2012; 18: 1261-1268.

b. Murali SG, Brinkman AS, Solverson P, Pun W, Pintar JE, Ney DM. Exogenous GLP-2 and IGF-I induce a differential intestinal response in IGF binding protein-3 and -5 double knockout mice. Am J Physiol Gastrointest Liver Physiol 2012; 302: G794-804.

c. Zacharakis E, Demetriades H, Pramateftakis MG, Lambrou I, Zacharakis E, Zaraboukas T, Koliakos G, Kanellos I, Betsis D. Effect of IGF-I on healing of colonic anastomoses in rats under 5-FU treatment. J Surg Res 2008; 144:138-144.

d. Zacharakis E, Demetriades H, Kanellos D, Sapidis N, Zacharakis E, Mantzoros I, Kanellos I, Koliakos G, Zaraboukas T, Topouridou K, Betsis D. Contribution of insulin-like growth factor I to the healing of colonic anastomoses in rats. J Invest Surg 2007; 20:9-14.

We have added the above information in the revised manuscript (References 16-19).

(3) Results

As shown, IGF-1 and IGF-1R were weekly or moderately stained in the well-differentiated CRC tissues

a. Spelling mistake

Answer:

The word "weekly" was corrected as "weakly" in the revised manuscript.

b. Table 1: what are the units for the staining and pls provide p values as a column.

Answer:

The IHC results were scored according to previous study (References 14). Total staining of IGF-1 and IGF1R were scored as the product of the staining intensity (on a scale of 0–3: negative=0, weak=1, moderate=2, strong=3) × the percentage of cells stained (0 = zero, 1=1–25%, 2 = 26–50%, 3=51–100%), which resulted in a scale of 0–9. The staining results have no unit. And, following your advice, we have added p values in Table 1and 2 in the revised manuscript.

c. The IGF-1 and IGF-1R, LVD parameters- do they correlate with clinical outcome e.g. OS, DFS

Answer:

As stated above, the tissues samples were obtained from the patients undergoing surgical resection between January 2011 and June 2011. The follow-up period is too short for adequate analysis of OS and DFS. So, the relationship between LVD and OS or DFS was not assessed in the manuscript.

(4) Discussion

(1) “MLVD”.

a. What is this acroymn for?

Answer:

We are very sorry for our negligence of spelling mistake. The word “MLVD” was corrected as “LVD” in the revised manuscript.

b. VGEF- all these need to be spelled in full before acronyms can be used subsequently.

Answer:

We have added the information in the revised manuscript.

(5) General comments

① In the limits a small study, the efforts are commendable and paper is generally well written with good and relevant review. However, it lacks focus and tends to generalize its findings.

Answer:

We have improve the manuscript and made some changes in the manuscript. We have marked the changes in red in revised paper.

② There is a need for some correction in grammar and syntax. Authors may consider rephrasing some of their statements. Statements made should be backed up with more references and they need to be more specific.

Answer:

The manuscript has been checked by a native English speaker engaging in medical research and edited by the English language editing companies: Jing-Yun Ma Editorial Office. We have added more references in revised paper.

③ Discussion is sound and broad-based but too brief and lacks depth on each of their findings.

Answer:

Thank you very much for your critical comments. We have improve the manuscript and made some changes in the manuscript. And here we did not list the changes but marked in red in revised paper. We hope that the correction will meet with approval.

For Reviewer #3:

This article demonstrated some relationship between IGF-1/IGF-1R and lymphangiogenesis in CRC. Although the paper is well written, there is a need for some correction in grammar and syntax.The present manuscript should be checked by a native English speaker who is a medical scientist.

Answer:

The manuscript has been checked by a native English speaker engaging in medical research and edited by the English language editing companies: Jing-Yun Ma Editorial Office.

For Reviewer #4:

(1) Page 7, Line 4-11: The authors described the effect of lymphangiogenesis by in vivo experiments, which showed LoVo cells injection induced tumor growth. Tumor growth has different mechanism from those of lymph node metastasis via lymphangiogenesis. Tumor growth is mainly affected by angiogenesis, while lymph node metastasis is induced by lymphangiogenesis. The authors seem to be confused on two basic concepts.

Answer:

Thank you very much for your critical comments. We have deleted the Line 4-11 of Page 7 in revised paper.

(2) In Table 2, CRC tissues with lymph node metastasis had lower expression of IGF-I and IGF-IR compared to those without lymph node metastasis. However, in the text, the authors described the positive correlation between higher expression and lymph node metastasis.

Answer:

Expression of IGF-I and IGF-I R in CRC tissues were described in Table 1. We are very sorry for the negligence and mistake in the Table. We have corrected it in revised paper.

(3) The authors emphasized the importance of IGF-1/IGF-1R signaling system in lymphangiogenesis and lymph node metastasis. To support the fact, double staining of IGF-1/IGF-1R should be done.

Answer:

IGF-1R is the only receptor of IGF-1. It is well known that receptor is only activated by the binding of its ligand. IGF-1 and IGF-1R is in the same signaling system. And so, the double staining of IGF-1/IGF-1R is not done.

(4) The detailed surgical information should be indicated in such study including lymph node metastasis and lymphangiogenesis analysis. How about the degree of lymph node dissection (D1 or D2) and the number of dissected lymph node?

Answer:

Following your advice, we have added the information in the revised manuscript.

(5) Why not insert the result of immunostained well-differentiated samples by IGF-1/IGF-1R in Figure 1?

Answer:

The difference between adjacent normal tissues, well-differentiated and moderately-differentiated was very small. It is difficult to find out the difference among the three groups in light microscopy without counting. So, the result of immunostained well-differentiated samples by IGF-1/IGF-1R was not inserted in Figure 1.

(6) Table 1 indicated that IGF-1/IGF-1R expressions were inverse correlation with lymph node metastasis. Did this means IGF-1/IGF-1R axis suppress lymph node metastasis?

Answer:

We are very sorry for the negligence and mistake in Table 1. We have corrected it in revised paper.

(7) The detailed statistical results should be shown between IHC results and clinicopathological features of clinical samples, such as P-value, lymphatic invasion and depth of tumor invasion? The Table.1 looks too simplified.

Answer:

Following your advice, we have added the information in Table 1 and 2 in the revised manuscript.

(8) Is it true that there was a significant difference between LVD in tumor tissue with or without lymph node metastasis? The difference of LVD values between two groups looks very small.

Answer:

Just as you said, the difference of LVD values between the two groups is very small. We have checked the data and we believed it is true.

(9) The author states importance of IGF-1 in CRC carcinogenesis in Discussion. Is there the rational evidence about it?

Answer:

We have deleted the statement about CRC carcinogenesis in Discussion in the revised manuscript.

(10) It is very hard to comprehend Fiugre.3, because I can't find which is before treatment and after 48h and which is the control and IGF-1 group.

Answer:

Thank you very much for your critical comments. We have corrected the legends of the figure in the revised manuscript.

(11) It doesn't look significant difference between pictures in Figure.4. Do the authors intend to suggest IGF-1 group have more lymphatics?

Answer:

Although, it doesn't look significant difference between the pictures in Figure.4. The difference was found by counting with light microscopy. LVD was significantly higher in IGF-1 group than that in control group (10.7±3.3 vs 6.4±2.9, $P<0.05$).

(12) Grammatical errors and unfamiliar expressions are found. The manuscript should be reviewed by natives or proofreaders.

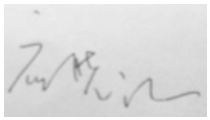
Answer:

The manuscript has been checked by a native English speaker engaging in medical research and edited by the English language editing companies: Jing-Yun Ma Editorial Office.

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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