November 12, 2014

Dear Editor,

Jing Yu, Science Editor, Editorial Office **Baishideng Publishing Group Inc**

Please find enclosed the edited manuscript in Word format (file name: 12721 revised manuscript).

Title: Novel CD9 targeted therapies in gastric cancer

Author: Yoko Murayama, Kenji Oritani, Shusaku Tsutsui

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript No: 12721

1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer1 and reviewer2.

3 References and typesetting were corrected

Thank you for inviting me to submit a review article in WJG 20th Anniversary Special Issue：Gastric Cancer 2014.

We appreciate the valuable comments of the reviews and have extensively revised the manuscript according to their suggestions. We are now submitting a revised version for publication.

We listed the revised portions on accompanying sheets of paper with point-by-point responses to the comments of the reviewers.

We believe the manuscript has been satisfactorily revised.

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

Sincerely yours,

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Responses to the reviewer 1

1. Although a review should tell us everything about the research for CD9, the paper should make the conclusion more clearly, even some conclusions are controversial. For example, the author should tell us the common conclusions about the level of CD9 in most common types of cancer, especially in gastric cancer. Does all research support the author's opinion?

In the submitted version of this review, we tried to describe everything about the researches for CD9. However, we agree with the suggestion of the reviewer 1 that we should make a decision more clearly. Majority of manuscripts have indicated that the expression of CD9 suppressed the development of gastrointestinal cancers although some researches reported opposite effects. Thus, in general, CD9 is believed to act as a tumor suppressor. We also have same opinion and changed our text to introduce the consensus functions of CD9. We now believe that this version of our review clearly describes the conclusion that CD9 expression inhibits tumor development of gastrointestinal cancers and that enhancement of CD9 function is a possible novel treatment. The major changes are listed below.

We have described our opinion in ABSTRACT "Indeed, the reduced CD9 expression is generally related to venous vessel invasion and metastasis as well as poor prognosis." (page 2, line 8-9).

We added the sentence "Although diverse physiological functions (clinical data) of CD9 have been suggested ..... As mentioned above, many investigators have believed that CD9 is a suppressor of tumor development" in CD9 AND CANCERS (GASTRIC CANCER) (page 5, line 13-26 in the revised manuscript).

2. The discussion about mechanism of CD9 also keeps controversial. Page 6, in CD9 AND CANCERS (GASTRIC CANCER), It was found that increased CD9 expression correlated inversely with patients' disease-free survival and overall survival Page 9, in FUTURE PROSPECTS, It was previously reported that CD9 gene transduction could downregulate VEGF-A expression, which was essential for angiogenesis. Can we get the conclusion: in Gastric Cancer,

increased CD9 may promote the development of gastric cancer through a no-angiogenesis pathway. Is it right?

Many investigators including we believe that the reduced CD9 expression is associated with a poor prognosis in various types of cancers. Thus, the report that increased CD9 expression correlated inversely with patients' disease-free survival and overall survival is minor and opposite to general opinion. As the reviewer 1 pointed out in #1, to make the conclusion more clearly, we changed this sentence to "Although diverse physiological functions (clinical data) of CD9 have been suggested, -------". (page 5, line 13-16 in the revised manuscript). In addition, we added data telling positive relationship between CD9 expression and prognosis in gastric and esophageal cancers (page 5, lines 16-22 in the revised manuscript).

As mentioned above, CD9 is a negative regulator of tumor development. Data that CD9 gene transduction could downregulate VEGF-A expression, which was essential for angiogenesis is thought to be one mechanism for the suppression of tumor development by CD9. Thus, our conclusion is that increased CD9 can "suppress (not promote)" the development of gastric cancers through a no-angiogenesis pathway.

3. Page 10, in CONCLUSION, Ab ligation of CD9 is a powerful tool to enhance CD9 functions, enhance may not be suitable here, which should be change or decrease.

The treatment of cells with ALB6 or PAINS-13 enhance, but not inhibit CD9 function. These anti-CD9 antibodies are ligand-mimic and activate CD9. We are afraid that the reviewer 1 may misunderstand in this regard. To avoid misunderstanding, we explain that these are ligand-mimic antibodies (page 5, line 3 in the bottom of the revised manuscript) and that ligation of CD9 with these antibodies enhances CD9 functions (page 5, line 2 in the bottom of the revised manuscript, page 6, line 1 in the revised manuscript). In addition, as the reviewer 1 suggested, we changed "enhance" to "change" (page 8, line 5 in the bottom of the revised manuscript).

4. About POSIBILITY OF CD9-TARGETED THERAPY IN GASTRIC CANCER,

most evidences are the research from the author's institute. It is not enough especially when the mechanism is not very clear. The author should find more evidences.

According to your suggestion, we added the evidence"The inihibition of cell proliferation in colon carcinoma cells caused by anti-CD9 mAbs PAINS-13 related to the enhanced integrin-dependent adhesion and the increased expression of membrane TNF- Therefore, TNF- partly mediates the anti-proliferative effects of CD9 in this case."(page 6, lines 7-10 in the bottom of the revised manuscript).

5. About the part of PRESENT TREATMENTS FOR PATIENTS WITH GASTRIC CANCER, this paragraph only tell us some Ab can be used for the treatment of gastric cancer, which is somewhat not related to CD9. Would you please make it shorter?

According to your suggestion, we have made this paragraph shorter and rewritten "PRESENT TREATMENTS FOR PATIENTS WITH GASTRIC CANCER" in the revised manuscript.

We have inserted the sentence "Advanced gastric cancer is a very aggressive disease, and the prognosis remains poor. The 5-year survival rate for loco-regional disease is 25%–35% and the median survival ranges from 10 to 14 months in advanced disease (page 8, line 8-10, in the previous manuscript) to (page 3, line 7-9 in the revised manuscript) .

Moreover, we have deleted the sentence "Based on informative benefits of these trials, ramucirumab was approved in United States for advanced gastric or gastricesophageal junction adenocarcinoma patients with progression on fluoropyrimidine or platinum-containing chemotherapy." (page 8, line 2-5, in the bottom of the previous manuscript) and"Thus, these findings validate VEGFR-2 signaling an important therapeutic target[65]. Growing information of somatic mutations in gastric cancer has proposed variable molecular targets." (page 9, line 2-4 in the previous manuscript).

6. Some minor spelling mistakes should be corrected.

Thank you for your pointing out our mistakes. We checked the spelling erros again and corrected. For examples, we have rewritten from "influence" to "influences" (page 2, line 5 in the revised manuscript).

Responses to the Reviewer 2

1. This review discussed the structure, functions of CD9 and the relationship between CD9 and cancers. CD9 has an important role in multiple stages during cancer development and treatment. Enhancing CD9 functions inhibited tumor progression by anti-proliferative, inducing apoptosis, and anti-angiogenetic effect. On the other hand, CD9 may function as an enhancer of malignance because CD9 inhibition could increase the sensitivity to chemotherapies. so the author proposed the possibility of manipulating CD9 as a novel therapeutic strategy in gastric cancer.several question: 1. "For example, the reduced CD9 expression is significantly associated with(more or less?) venous vessel invasion and liver metastasis in patients with colon cancers. However, the increase of CD9 expression was noted during progression of gastric carcinoma" "high levels of CD9 mRNA and protein correlated with(more or less?) tumor stage, vessel invasion, and lymph node metastasis", but "……treatment with anti-CD9 mAb (ALB6), which enhanced CD9 functions, inhibited cell….."----- There are confusions: high or low levels of CD9 expression related with good or poor prognosis? When implementing CD9 target therapy to gastric cancer, we should enhance or weaken CD9 function?

This is the same question of the reviewer 1. Because we tried to describe everything about the researches for CD9 in the submitted version of this review, the conclusion was not clear. Majority of manuscripts have indicated that the expression of CD9 suppressed the development of gastrointestinal cancers although some researches reported opposite effects. Thus, in general, CD9 is believed to act as a tumor suppressor. We also have same opinion and changed our text to introduce the consensus functions of CD9. To make the conclusion more clearly, we changed this sentence to "Although diverse physiological functions (clinical data) of CD9 have been suggested, -------" (page 5, line 13-16 in the revised manuscript). In addition, we added data telling positive relationship between CD9 expression and prognosis in gastric and esophageal cancers (page 5, lines 16-22 in the revised manuscript).

Moreover, we strengthened our meanings as below. "The reduced CD9 expression is significantly associated with more venous vessel invasion and

liver metastasis in patients with colon cancers." (page 5, line 11-13, line 22-24 in the revised manuscript). "The low levels of CD9 expression related with poor prognosis." (page 5, line 10 in the bottom of the revised manuscript). "When implementing CD9 target therapy to gastric cancer, we should come up with various ideas to enhance CD9 functions." (page 9, line 2-3 in the revised manuscript).

2. CD9 specific Ab PAINS 13 could inhibit in vivo tumour growth of colon cancer cells. ----language expression is not clear.

We checked the references and the spelling erros again and corrected. According to your suggestion, we have rewritten from the sentence "CD9 specific Ab PAINS 13 could inhibit in vivo tumour growth of colon cancer cells" to "It has been reported that anti-CD9 mAb PAINS 13 inhibited *in vivo* tumour growth of colon cancer cells." (page 6, lines 22-23 in the revised manuscript).