ANSWERING REVIEWERS

June 4, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 3500-review.doc).

Title: Molecular targeting therapy using bevacizumab for peritoneal metastasis from gastric cancer

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Name of Journal: *World Journal of Critical Care Medicine*

ESPS Manuscript NO: 3500

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
(1) 02451548
I greatly appreciate your taking interest in our research and acceptance of our article.
(2) 02493052
Thank you for your useful comments.
I reply for your comments as bellow.
1)I had already reported about the positive correlation between IL-6 , IL8 and peritoneal metastasis in the discussion. About MMP-2 , I add the letters as follow "Degradation of the extracellular matrix is considered to be a prerequisite for peritoneal metastasis, and matrix metalloproteinases (MMPs) are thought to play an important role in this process. There are many reports that highly invasive cancer cells with a high potential for metastasis stimulate the production of MMPs, and MMP-2 is significantly correlated with depth of invasion, lymph node metastasis, and distant metastasis of gastric cancer."
2)About VEGF, I had reported the expression of VEGF protein on the tissue, using immunohistochemical method. The purpose of this study was to approve the effectiveness of bevacizumab for peritoneal metastasis, so I reported only VEGF in in-vivo study. IL-6, IL-8, and MMP-2 were measured using ELIZA kit in in-vitro study, but these proteins were difficult to measure and/or stain immunohistochemically on mouse tissue.
(3) 00069467
Thank you for your useful comments.
I reply for your comments as bellow.
Major concerns

1) The purpose of this study was to approve the effectiveness of bevacizumab for peritoneal metastasis. To analysis the effectiveness of bevacizumab, I established peritoneal dissemination model using MKN-45P cell line which was high frequent peritoneal metastasis cancer cell. And I need to clarify the characteristics of MKN-45P. Therefore, I performed the in-vitro study to compare between MKN-45 and MKN-45P, which was not main research of

this study.

2) The tumorigenesis of MKN-45 was very low. My colleague Dr. Miyagi reported that in MKN-45 group, a small amount of bloody ascites in few cases (*Int J Clin Oncol* 2007; **12:**17-24). So, I think using MKN-45 as control was inadequate.

3) In fact, the volume of ascites in the therapy group was significantly less than that in the non-therapy group ($P = 0.042$), and The mitotic index was significantly higher than that in the therapy group ($P < 0.01$). So, I referred to the vascular permeability effect and cell proliferative activity of VEGF. But I think part of vascular permeability effect of VEGF in discussion is too long as you point out. So I omitted the below part from discussion.
"VEGF (as well as functioning as a growth factor) is able to function as a vascular permeability factor. Increased permeability of blood vessels facilitates the extravasation of protein and the formation of ascites [5, 11, 22]. Kraft et al [23] reported that VEGF may play an important role in tumor progression and the formation of malignant effusion. Mesiano et al [24] found that the neutralization of VEGF activity may have clinical applications in inhibiting malignant ascites formation in ovarian cancer. VEGF levels were also found to be remarkable elevated in malignant ascites [4]."

4) In clinical, hydronephrosis is good indicator and high sensitivity of peritoneal metastasis or recurrence. Other organs is sometimes difficult to evaluate the effective of treatment for peritoneal metastasis. So I analyze the pathology of kidney of nude mice.

Minor concerns

1) This study was approved by Kurume Univerity Institutional Animal Care and Use Committee of Ethnics. So I add the statement to Material and Methods in revise article.

2) All models were recognized peritoneal dissemination using MKN-45P. I stated it in Results in revise article.

3) We determined working concentration of bevacizumab according to Wildiers H et al. report (*Br J Cancer* 2003; 88:1979-1986). I stated it in Materials and Methods in revise article.

4) I have already decided to omit the statement about function of vascular permeability and prliferative activity of VEGF as shown in reply for Major concern (3). Moreover I decide to omit the statement as bellow.
"Bevacizumab was approved by the United States Food and Drug Administration for use in combination with standard chemotherapy for metastatic colorectal cancer in 2004, for non-small cell lung cancer in 2006, and for breast cancer in 2008. It was approved in Japan for combination with anticancer drugs for unresectable advanced or metastatic colorectal cancer in 2007. AVAGASTA was a global, randomized, phase III trial evaluating the efficacy of bevacizumab plus chemotherapy (capecitabine [xeloda]/cisplatin) as first-line treatment for patients with advanced gastric cancer. While the primary endpoint was not met (median overall survival HR 0.87; p=0.1002), there was significant improvement in progression-free survival and in overall response rate, and an acceptable safety profile for bevacizumab + chemotherapy in patients with advanced gastric cancer [32]. However, a subset analysis of the AVAGAST trial, which evaluated the benefit of bevacizumab in advanced gastric cancer, has demonstrated distinct differences in the outcomes according to disease subtype. Shah [33] has reported that the addition of bevacizumab especially to chemotherapy appeared to improve outcomes in patients in non-Asian patients with diffuse or distal gastric cancer. The frequency of peritoneal recurrence in the diffuse type gastric cancer is more than that in the non-diffuse gastric cancer. "

5) I submit revise article which was checked by native speaker again.

Specific points

1) I add arrow which point to tumor nodule on the mesentery in revise article.
2) I'm sorry for missing the title of Figure 2C. I add the title of Figure 2C "Non-therapy group" in revise article. I add arrow on Figure 2F. I omit macroscopic findings of Figure 2A and 2B. Because these figures are difficult to detect the tumor.
3) On figure 3, " Non-therpy group" was control which was given drug-free aline.
   I add arrows which point at mitosis.
4) On figure 4, " Non-therpy group" was control which was given drug-free aline.
   This figure shows clearly that the number of VEGF positive cancer cell in Non-therpy group was more frequently than that in therapy group.
5) I add the number of cases of each group on figure 5 in revise article.

(4) 02493161

Thank you for your useful comments.

I reply for your comments as bellow.

About histpathology and immunohistochemistry, mitotic figures and VEGF immunoreactivity are clearly different between treated group and non-treated group. Bevacizumab is antibody to VEGF protein , not to concern to VEGF receptor. So I assessed VEGF protein only.

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Critical Care Medicine.* .

Sincerely yours

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