Format for ANSWERING REVIEWERS

April 1, 2015

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 16105-review.doc).

Title: Conjugation of TLR7 agonist to gastric cancer antigen MG7-Ag exerts antitumor effects

Author: Wang Xiaodong, Gao Ningning, Diao Yuwen, Liu Yu, Gao Dong, Li Wang, Wan Yanyan, Zhong Jingjing, Jin Guangyi

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 16105

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) Monoclonal gastric cancer 7 antigen (MG7-Ag) is discovered by a research group of Dr. Fan Daiming (the Fourth Military Medical University, Xi'an, China), who also offered guidance and assistance in our study. Dr. Fan group has done plenty of research for new tumor-specific antigens for gastric cancer. Through immunization of mice with human gastric cancer cells, they prepare 14 different strains of hybridomas producing mAbs against gastric cancer (referred to as MG series). It is noteworthy that one of these antibodies, mAb MG7, has the highest positive rate in detecting corresponding antigen (MG7-Ag) in gastric carcinoma tissue sections and sera of gastric cancer patients [1]. Using the immuno-PCR assay, positive MG7-Ag detection rate of 82.8% is obtained in gastric carcinoma patients with low pseudopositivity [2]. Although failing to clone the gene encoding MG7-Ag, they apply phage-display technology to identify MG7-Ag epitope mimics. Here, we chose the most deeply-investigated mimotope sequence (KPHVHTK) in our study, which has been used in previous development of DNA vaccines by Dr. Fan [3-4]. In addition, although MG7-Ag is originally identified in human cells, Dr. Fan has verified its existence in mouse EAC cells using mAb MG7 by western blot and immunofluorescence. EAC cells have also been applied in the evaluation of MG7-Ag DNA vaccines [3-4]. We also proved the expression of MG7-Ag in EAC cells by western blot and the result has been mentioned in the text.

[1] Xu L, Jin BQ, Fan DM. Selection and identification of mimic epitopes for gastric cancer-associated antigen MG7 Ag. Mol Cancer Ther 2003; 2: 301-306
[2] Ren J, Chen Z, Juan SJ, Yong XY, Pan BR, Fan DM. Detection of circulating gastric carcinoma-associated antigen MG7-Ag in human sera using an established single determinant immuno-polymerase chain reaction technique. Cancer 2000; 88: 280-285
[3] Chen Y, Wu K, Guo C, Liu C, Han S, Lin T, Ning X, Shi R, Shi Y, Fan D. A novel DNA vaccine containing four mimicry epitopes for gastric cancer. Cancer Biol Ther 2005; 4: 308-312
[4] Lin T, Liang S, Meng F, Han Q, Guo C, Sun L, Chen Y, Liu Z, Yu Z, Xie H, Ding J, Fan D. Enhanced immunogenicity and antitumour effects with heterologous prime-boost regime using vaccines based on MG7-Ag mimotope of gastric cancer. Clin Exp Immunol 2006; 144: 319-325

(2) We chose mouse breast cancer 4T1 cells as negative control and confirmed the absence of MG7-Ag expression by western blot. After immunizations with T7, MG3, T7+MG3, T7-MG3 and PBS as control, BALB/c mice were challenged with 4T1 cells. No palpable tumor burden reduction was detected, indicating MG7-Ag specificity of the observed effects in EAC cells. Besides, after immunizations with vaccines, determinations of ADCC and CTL activities were also repeated using 4T1 cells as target cells for negative control. No obvious ADCC and CTL activities were shown of our vaccines to 4T1 tumor cells. All of the above results have been mentioned in the text.

(3) As the reviewer mentioned, dendritic cells are important target cells for TLR7 agonists. Therefore, we replenished the cytokine release assays in mouse BMDCs and the levels of two related cytokines (TNF-α and IL-12) were determined. The outcomes proved the activations of DCs upon exposure to our TLR7 agonist and conjugations. On the other hand, mouse spleen lymphocytes (an element in innate immunity) could also be activated after treatment by our TLR7 agonist. Consequently, results of cytokine assays have been completed in both BMDCs and lymphocytes.

3 References and typesetting were corrected

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

Sincerely yours,

Wang Xiaodong

Wang Xiaodong
School of Medicine
Shenzhen University
Shenzhen
China
Fax: +86-755-86671906
E-mail: wangxiaodong@szu.edu.cn