Author's response to reviews

Title: Establishment of using serum YKL-40 and SCCA in combination for the diagnosis of patients with esophageal squamous cell carcinoma

Authors:

Xin Zheng (zhengxin@sysucc.org.cn)
Shan Xing (xingshan@sysucc.org.cn)
Xiao-Min Liu (liuxm@sysucc.org.cn)
Wen Liu (liuwen@sysucc.org.cn)
Dan Liu (liudan@sysucc.org.cn)
Pei-Dong Chi (chipd@sysucc.org.cn)
Hao Chen (chenhao@sysucc.org.cn)
Shu-Qin Dai (daishq@sysucc.org.cn)
Mu-Sheng Zeng (zengmsh@sysucc.org.cn)
Wan-Li Liu (liuwl@sysucc.org.cn)

Version: 6 Date: 20 June 2014

Author's response to reviews: see over
To: Giovanni Corso MD PhD
Associate Editor of BMC Cancer

Re: MS: 5761335971215888

Establishment of serum YKL-40 and SCCA combination for the diagnosis of patients with esophageal squamous cell carcinoma

Jun 20, 2014

Dear Dr. Giovanni Corso,

Thank you very much for your great effort in the handling of our manuscript (MS: 5761335971215888, entitled "Establishment of serum YKL-40 and SCCA combination for the diagnosis of patients with esophageal squamous cell carcinoma").

We are very pleased that the reviewers found the paper to be interesting and thank you for offering us the opportunity to revise the manuscript.

We appreciate the editor and reviewers very much for their positive and constructive comments and suggestions and have revised the manuscript accordingly. Attached please find the point by point response to each of the reviewers' suggestion. With the comments and suggestions from the reviewers, the manuscript has been substantially improved.

We hope that the revised version will satisfy the reviewers and the paper is now considered for publication in *BMC cancer*.

Thank you very much for your kind efforts!

Sincerely yours,

Wanli Liu Ph.D
Department of Clinical Laboratory Medicine
Sun Yat-sen University Cancer Center
651 Dongfeng Road East, Guangzhou 510060, China
Phone: 86-20-87343438; Fax: 86-20-87343199
E-mail: liuwl@sysucc.org.cn
Editorial’s Request:
“We recommend that you copyedit the paper to improve the style of written English…”
Re: We have edited the manuscript thoroughly with the help of a professional language editing service (American Journal Experts) and hope the paper is now considered acceptable for publication.

Reviewers’ Comments to Author/s:
Reviewer: Wei Guo

Comments to the Author
1) As shown in the Methods, the ESCC cell lines Eca-109, Kyse30, Kyse140, Kyse180, Kyse510 and Kyse520 was used to detect YKL-40 expression. However, Figure 1A showed mRNA expression of YKL-40 in Eca-109, Kyse30, Kyse140, Kyse180 and Kyse520, Figure 1B demonstrated ELISA results in Eca-109, Kyse180, Kyse510 cell lines. Why different cell lines were used in the two experiments. Application of the same cell line will make the results more convincing.
Re: Thanks for the thoughtful suggestions. We repeated the Real-time RT-PCR, Western-blotting and ELISA in the same ESCC cell lines including the Eca-109, Kyse 30, Kyse 140, Kyse 180, Kyse 510 and Kyse 520. Higher levels of YKL-40 mRNA and protein were observed in all examined tumor cell lines compared to the immortalized cell line, NE-3. The data have been displayed both in figure 1 and in the line 192-202 of page 9.

2) mRNA expression of YKL-40 in Eca-109 cell line is low, however, protein level of YKL-40 in Eca-109 cell line is relatively high. Please explain the results.
Re: Thanks for your suggestion. Real-time RT-PCR showed that the relative expression level of YKL-40 mRNA in Eca-109 cells was $1.6 \times 10^6$ ($2^{\Delta Ct}$, the mean of three independent experiments), which was 6.4-folds higher than the NE-3 cells ($2^{\Delta Ct} = 2.5 \times 10^7$, the mean of three independent experiments). Compared with NE-3, mRNA expression of YKL-40 in Eca-109 cell line was relatively higher. Meanwhile, the YKL-40 protein level in Eca-109 cell line supernatant (2.5pg/ml, the mean of three independent experiments) was 1.5-folds higher than that of NE-3 cell line (1.5pg/ml, the mean of three independent experiments) detected by ELISA. The mRNA expression level of YKL-40 was somewhat inconsistent with its protein
expression level in Eca-109 cell line. The difference between the level of mRNA and protein may caused by the post-transcriptional regulation of YKL-40 mRNA. However, the mechanism of the post-transcriptional regulation of YKL-40 mRNA in Eca-109 cell line remains elusive. Furthermore, YKL-40 mRNA level in Eca-109 cell line was similar to that of Kyse30, Kyse140, Kyse510 cell lines, whose protein expression levels were also consistent. The results are shown in figure 1A, 1B and 1C.

3) Do the 20 patients with tissue samples were included in the patients with serum? Please describe in detail the tissue specimen collection. Do normal esophageal epithelial tissues come from the corresponding normal tissues? Tissue samples were too few and suggest increase the number of patients with tissue specimens.

Re: The 20 patients with tissue samples were not included in the patients with serum. The details of the tissue specimen collection were added in the line 124-131of page 6 as following: “The tissue samples were obtained from ESCC patients who underwent surgery at Sun Yat-Sen University Cancer Center in Guangzhou City of China from November of 2012 to December of 2013. After the tumor resection, tissues were formalin-fixed and paraffin-embedded. Normal esophageal tissue specimens were taken from the areas of a standard distance (8cm) from the corresponding resected tumors. Furthermore, normal esophageal tissue sections were histopathologically validated by morphology features and absence of tumor cells.”

Indeed, 20 samples were too few to evaluate the expression level of YKL-40 in ESCC, however, they were enough to investigate which kind of cells expressed YKL-40 in vivo. As the objective of our study was to evaluate the performance of the serum YKL-40 in the diagnosis of ESCC, we had to identify whether the tumor cells expressed YKL-40 protein. YKL-40 was detected in 17 of 20 ESCC samples (85%), in which YKL-40 was found expressing in tumor cells. Together with the fact that YKL-40 is a secreted protein, it suggests that YKL-40 may be suitable to serve as a tumor marker for diagnosis.

4) Real-time PCR should be Real-time RT-PCR. Please describe in detail the
specimens in which were detected mRNA expression.
Re: Thank you for reminding our expression mistake and we have corrected it carefully in the revised version.

Total RNA was extracted from cell lines using the Trizol reagent (Invitrogen, USA) according to the manufacture’s instruction. Reverse transcription of total RNA (2 μg) was done using SuperScript II reverse transcriptase. The information has been included in line 137-146 on page 7 of the revised manuscript. Furthermore, we performed the 6 pairs Real-time RT-PCR tissue samples to reinforce the results. The fresh tissues were collected during surgical procedures and put into the RNA later Solution (Ambion, USA) and stored in liquid nitrogen immediately until RNA extracting. The adjacent normal esophageal tissue specimens’ details have been mentioned in the answer of question 3 above. The information showed in line 126-131 on page 6 of the revised manuscript.

5) The manuscript should be revised by a native English speaker to eliminate the grammar mistake.
Re: We have revised carefully our manuscript completely with the help of a professional language editing service (American Journal Experts). Grammar mistakes have been eliminated through the editing.

Reviewer: Xiaoxia Wang

Comments to the Author

1) The results in Fig. 1 showed that YKL-40 was highly expressed in ESSC cells and tumor tissues. YKL-40 protein in ESSC cells and tumor tissues detected by Western-blot will strengthen the above results.
Re: Thanks for your good suggestion. As showed in paragraph 3 on the page 9 and figure 1 in the revised manuscript, we have detected YKL-40 protein in ESSC cell lines and tumor tissues by Western-blotting. The levels of YKL-40 protein observed in the esophageal cancer cell lines were higher than that of NE-3. Furthermore, 6 pairs of resected tumors and normal tissues showed the similar results. Western-blotting results strengthened the Real-time RT-PCR results.

2) Serum YKL-40 may be a potential diagnosis marker for ESCC. However, if the authors can establish that expression of serum YKL-40 correlates with poor
prognosis, its clinical significance could be further elevated.

Re: Thanks for your constructive advice. As our study was mainly focused on the diagnostic effect of YKL-40, the prognostic data of some patients including in this study are incomplete. Now, the prospective study of the serum YKL-40 in ESCC prognosis is ongoing.