Author's response to reviews

Title: Expression and Prognostic Significance of THBS1, Cyr61 and CTGF in Esophageal Squamous Cell Carcinoma

Authors:

zhuqing zhou (s_zqzhou@163.com)
weihua cao (zhw1405@126.com)
jianjun xie (g_jixie@stu.edu.cn)
jing lin (lijingst73@hotmail.com)
zhongying shen (zhongyingshen@yahoo.com)
qingying zhang (qyzhang@stu.edu.cn)
jinhui shen (enmin2007@yahoo.com)
liyan xu (liyanxu1130@yahoo.com.cn)
enmin li (nmli@stu.edu.cn)

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Author's response to reviews: see over
Dear Editor:

We are now submitting the revised version of our manuscript entitled *Expression and Prognostic Significance of THBS1, Cyr61 and CTGF in Esophageal Squamous Cell Carcinoma* for publication in BMC Cancer (MS: 6382342562458000).

According to the comments of the editor and the two reviews, we have made some major revisions. The changes are described detailedly in the *Response to the reviews* following this letter. *Supplementary figures* are also provided to response to the comments.

All authors have read and approve this revised version of the manuscript. No part of this paper has been published or submitted elsewhere. No conflict of interest exists for any of the authors.

We appreciate your reconsideration of our manuscript, and we look forward to receiving comments from the reviewers. Please acknowledge receipt of this manuscript at your earliest convenience, and let us know if you need any further information.

Sincerely,

En-Min Li, Ph.D.
Professor
Department of Biochemistry and Molecular Biology
Medical College, Shantou University
Guangdong Province
Shantou, 515041
China
Tel.: 86-754-88900847
Fax: 86-754-88900847
E-mail: nmli@stu.edu.cn
Response to the reviewers

Reviewer: Giovanni Zaninotto

1. It is hard to see why a worse survival is associated with THBS1, given that THBS1 overexpression is inversely correlated with regional lymph node invasion (virtually all N0 patients, i.e. 94%, but only 72% of N1 patients overexpressed this protein). To justify this claim, the Authors should show separate survival curves for each TNM stage and level of protein overexpression.
   Response: We have analyzed the survival for each TNM stage (I and IIa / IIb and III) and THBS1 expression (Supplementary figure 1).

2. More information should be given on the number of nodes harvested from each patient.
   Response: The TNM stage of the patient in this study was determined according to the TNM classification of 2002 (the 6th Edition), in which regional lymph node was divided into No (no regional lymph node metastasis) and N1 (regional lymph node metastasis). Hence, we don’t think it is necessary to show the exact number of nodes harvested from each patient.

3. The present TNM classification divides Stage II patients into IIA (no nodes involved) and IIB (metastasis to regional nodes): the Authors would be well advised to use the currently-adopted classification.
   Response: The patients have been divided into two groups (I/IIa and IIb/III) according to currently-adopted classification (Table 1).

4. It is also hard to follow the reasoning that patients overexpressing CTFG and Cyr61 show such a poor survival, given that the two markers are almost equally overexpressed in patients in stages I/II and stage III (see point 1).
   Response: TNM (Tumor, Node, and Metastasis) stage is a powerful predictor of survival. However, there are still other factors such as tumor size and histology which are associated with survival. Hence, we supposed that CTGF and CYR61 might be involved in some other clinical characteristics and resulted in a final negative effect on survival.

5. Minor points: The Ms has several misspellings and grammatical errors; in table 1 there are 46 N0 patients and 45 patients in Stages I/II: is that correct?
   Response: We have corrected the misspellings and grammatical errors; in table 1, there are 48 N0 patients and 45 patients in Stages I/IIa, because there are three cases of T4N0, which should be determined as IIb.
Reviewer: Jan Brabender

1. The authors do not specify the surgical procedures that were performed on the patients. Was complete tumor resection R0 achieved in all patients?
   **Response:** We have specified the surgical procedures in *Materials and Methods* and the complete tumor resection R0 was achieved in all patients.

2. What were the inclusion criteria? Were all patients from 1997-2006 included in the study? This needs to be clarified.
   **Response:** Patients (with only surgery) were included in this study if a follow-up was obtained and clinical data were available (80 cases). We have added this information to *Materials and Methods*.

3. How were the patients treated besides surgery? Most of the patients had a locally advanced tumor stage (T3). Neoadjuvant therapy is usually recommended in these patients. Was neoadjuvant therapy applied to those patients? This could change the expression status in the resected specimen. Was the study population treated uniformly? Unless this is the case, the prognostic conclusions have to be drawn very carefully.
   **Response:** Patients with only surgery were subjected in this study. No neoadjuvant therapy was applied to those patients.

4. No information is given concerning tumor stage and prognosis. Unless the authors can provide this information for the study population, the conclusion of the present are not validated
   **Response:** We supplied information concerning tumor stage and prognosis (Supplementary figure 2).

Reviewer: Ahmad Faried

1. The author should add and discuss the review article by Kuwano H., et al (Surg Today 2005; 35: 7-18) in which reviewing the TGFβ/Smad proteins signaling pathways in ESCC.
   **Response:** We have added discussion about TGF-β/Smad proteins signaling pathways in ESCC according to the review article (Surg Today, 2005;35:7-18).

2. For Western blot (WB) results, how many patients sample subjected in this study? Where is the densitometry quantification result (the range of normal and tumor values, statistical differences normal vs tumor, etc)? CTGF and Cyr61 molecular weight? How about THBS1 WB results?
   **Response:** Expressions of CTGF and CYR61 protein in four, randomly picked, paired ESCC samples were analyzed by western blot. We added the densitometry quantification result to the figure (Figure 2). Statistical analysis had not been performed because this result was just used to confirm the over-expression of CTGF
and CYR61. Molecular weight of CTGF and CYR61 were also shown in Figure 2. For THBS1, we did not analyze its expression by western blot because commercial antibody for THBS1 was not available for us.

3. In discussions, could the authors speculate at their best what is the mechanism of discrepancy between their study and others (CTGF and THBS1)? Not only stated that there is diverse role in the tumorigenesis.

Response: The mechanisms for the diverse role in the tumorigenesis of CTGF or THBS1 were provided in the Discussion.

4. Minor comments: In the material methods part is incomplete and need to be clarified. For example; how did the author get consent from all the patients, what the eligibility of the patients include in this study? The author should change word: western blot # Western blot (since this is a person’s name) in the whole manuscript.

Response: The eligibility of the patients include in this study had been described in the Materials and Methods and we changed the word Western blot to western blotting in the whole manuscript.