Author's response to reviews

Title: The expression of Cytoglobin as a prognostic factor in gliomas: a retrospective analysis of 88 patients.

Authors:

Hong-Wu Xu (hwxu@qq.com)
Yue-Jun Huang (moon_hyj@yahoo.com.cn)
Ze-Yu Xie (xzy3175@yahoo.com.cn)
Lan Lin (lanlin_st@163.com)
Ze-Rui Zhuang (zeruizhuang@163.com)
Wen Zhou (wenzhou_st@163.com)
Mu Li (muli_st@163.com)
Hai-Hua Huang (haihua_st@163.com)
Guo-Jun Zhang (Guoj_zhang@yahoo.com)
Yan-Chun Guo (Guoj_zhang@yahoo.com)
Xin-Peng Lin (Guoj_zhang@yahoo.com)
Kwan Man (Guoj_zhang@yahoo.com)
Xiao-Long Wei (Guoj_zhang@yahoo.com)

Version: 2  Date: 27 March 2013

Author's response to reviews: see over
March 18, 2013

Dear Christna Chap,

Senior Executive Editor
BioMed Central

Thank you very much for providing us an opportunity to revise our manuscript entitled “The expression of Cytoglobin as a prognostic factor in gliomas: a retrospective analysis of 88 patients (manuscript number: 1307189510886922)”. We have revised the manuscript according to reviewers' comments. Please find our responses to each of reviewer shown as below.

To reviewer 1:

Major comments:

1. The authors demonstrate an interesting and potentially clinically relevant new finding. However, the discussion of the data is too speculative; e.g., in the discussion part on Cygb and IL6 and PI3K-pAKT it is not clear what the rationale for their conclusions is. This is particularly difficult when no references are provided to support their claim. The manuscript would benefit from a discussion that is shorter and more focused on the reported data. The authors
claim the existence of a Cygb-PI3K-AKT pathway in gliomas simply based on correlative data. This section should be removed since their data sets do not provide a causal link.

**Response:** Although correlations between Cygb and PI3K/Akt/IL-6 expression in gliomas have been demonstrated in the present study, I agree with you that we did not provide direct evidence to illustrate the correlation between Cygb and PI3K/Akt pathway and how the pathway do promote progression of gliomas.

Thus, we have extensively revised the manuscript and deleted most of the discussion part on Cygb-PI3K/Akt-IL-6 pathway.

2. No negative controls are provided for the immunohistochemical images.

**Response:** In the figure 1 for immunohistochemistry staining, negative controls for both low and high grades are provided. The setup for negative control was also described in the "Results" section (page 12, line 13 to 14).

3. The authors show a gender difference for the Cygb expression. They should provide information as to the gender effect on Cygb expression in different histological grades of glioma.

**Response:** In this particular cohort, 62.2% (23 out of 37) of female
patients was with low grade tumors while about 47% (24 out of 51) of male patients was diagnosed as low grade tumors. The selection bias was found to cause the gender difference in terms of histological grade. Thus, the finding that low expression of Cygb was significantly associated with female gender was most likely because of difference in histological grade. The above discussion was also added to the "Discussion" section (page 18, line 7 to 13).

4. No page and figure numbers are provided.

Response: As suggested, page and figure numbers are provided.

To reviewer 2:

Major comments:

1. Treatment should be included in the multivariate analysis in order to demonstrate that Cygb is an independent prognostic factor.

Response: In this retrospective study, all the enrolled patients had received brain tumor resection. The patients with WHO grade II to IV gliomas received the standard post-operative chemotherapy (detail described in the “Patient description”), while the patients with grade I gliomas did not receive any chemotherapy. The survival comparison between patients with or without chemotherapy is most likely to compare grade I gliomas vs grade II-IV gliomas as shown in the below figure. The
survival difference between the two groups is exactly the same caused by WHO grades. Thus, post-operative chemotherapy was not included in the multivariate analysis.

2. How do the authors plan to further study the role of Cygb in gliomas?

Response: In further study, we will investigate the role of Cygb expression by overexpressing via introducing exogenous Cygb or silencing Cygb protein via siRNA in cultured glioma cells. Moreover, we will try to develop animal models of gliomas either by transgenic models or by subcutaneous injection to characterize the significance of Cygb
overexpression or inhibition. The above discussion was also added to the "Discussion" section (page 18, line 14 to 19).

3. Spearman correlations with other expression data are insufficient in my opinion.

Response: With regard to the correlation of Cygb and other expression data, we considered them as continuous variables, and thus Spearman's correlation coefficient was applied to look at the correlation. Although the correlation analysis is sufficient, Fisher's Chi-square test was also used to see if Cygb expression was associated with other expression data when they were divided into two categories according the median value. We also consulted a statistical expert in Shantou University Medical College here to confirm the sufficiency of statistical analysis.

4. Was the reproducibility of Cygb expression studied, indeed it is an important issue in order to translate Cygb as a biomarker?

Response: Yes, each experiment was conducted 3 times. For example, we subjected 3 continual sections of every case for Cygb immunohistochemical staining. The Cygb expression of immunoreactivity was evaluated with light microscopy by two experienced pathologists without any knowledge about clinical information. If a discrepancy occurred between the assessments of the
two observers, the slides were reassessed in a combined session without information of the previous scores as described in "Materials and Methods". We believe the reproducibility of the Cygb expression studied is credible.

I hope that the revised version will be satisfied to the readers of the journal "BMC Cancer" and is appropriate to publish in the journal. Again, thank you very much for all your consideration. I look forward to hearing from you soon.

Best regards.

Guo-Jun Zhang, MD, PhD