ANSWERING REVIEWERS

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 7849-revised.doc).

Title: Down-regulated γ-catenin expression is associated with tumor aggressiveness in esophageal cancer

Author: Wang-Kai Fang, Lian-Di Liao, Wei Gu, Bo Chen, Zhi-Yong Wu, Jian-Yi Wu, Jian Shen, Li-Yan Xu, En-Min Li

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 7849

The manuscript has been improved according to the suggestions of editors and reviewers:

1 Format has been updated.

2 Highlighted content has been added to the revised manuscript.

3 Revision has been made according to the suggestions of the reviewer (Changes are highlighted in red color in the marked version of the manuscript)

Reviewer #1

Comments to authors: This is a well written manuscript and merits publication. The results are interesting and provide insights into esophageal cancers.

Response: Thank you for your prompt review to our manuscript.

Reviewer #2

Comments to authors: Wang-Kai Fang, et al investigated the expression of γ-catenin in 95 tissue specimens of esophageal squamous cell carcinoma (ESCC). They found that the γ-catenin mRNA expression was significantly associated ESCC survival. The multivariate Cox regression analysis demonstrated that γ-catenin was an independent prognostic factor for survival. In vitro experiments showed that silencing γ-catenin caused defects in cell-cell adhesion and concomitant increase in cell migration in both KYSE150 and TE3 ESCC cells. Knocking-down γ-catenin expression resulted in a significant decrease of E-cadherin along with reduced β-catenin and E-cadherin membrane localization in ESCC cells. They conclude that the γ-catenin is a tumor metastasis suppressor of ESCC and its expression may serve as a prognostic marker. Dysregulated expression of γ-catenin may play important roles in ESCC progression. The results are novel and English writing is good.

Response: Thank you for your prompt review to our manuscript.

Reviewer #3

Comments to authors: I general this is a well written and clearly presented manuscript. However, I have a couple of points:

Comment 1: One of the principal findings of the investigators is the association between reduced gamma catenin expression with tumour grade and adverse prognosis. This association with survival remained significant on multivariate testing. However, I note that neither tumour grade nor N stage were associated with survival duration. How do the authors explain these findings?

Response: In our cohort, the tumor stage is based on the TNM scoring system which takes into account the size of the tumor, its depth of penetration into the esophageal wall, the presence of lymph nodes with cancerous cells,

and whether the tumor has spread to other organs. The depth of invasion and lymph nodes (LN) status in ESCC has been proved to be prognostic factors in a retrospective study for more than 20 years. Factors based on node such as the LN-involved numbers, and the LN metastatic ratios in ESCC patients have also been proved to be independent prognostic factors in some publications. Our recent study has confirmed that the factors of the depth of invasion, LNs' status are independent prognostic factors for patients with ESCC (Wu ZY, et al. Dis Esophagus. 2010; 23: 40-5).

Comment 2: Expression of tumour catenin was described as up-regulated or down-regulated according to levels of differential mRNA expression when compared with adjacent non-neoplastic tissue. However, there are no actual values given. It is possible that the adjacent 'normal' tissue expression levels were also elevated and when compared with the tumour levels those samples may be labelled as 'down-regulated'.

Response: With regard to the evaluation of mRNA expression using RT-PCR assays, the method has been widely used in many publications. Therefore, we chose this traditional calculation method to evaluate the differential mRNA expression of gamma-catenin in esophageal squamous cell carcinoma. Evidence from clinical studies suggests that reduced expression of γ-catenin in human cancers is associated with increased tumor progression and adverse clinical outcome. Our findings are consistent with earlier immunohistochemistry studies revealing that decreased γ-catenin expression was significantly associated with poorer prognosis (*World J Gastroenterol* 2004; **10**: 3235-3239).

Comment 3: Another major conclusion from the authors is that gamma catenin is associated with loss of cellular adhesion and therefore predisposes to tumour invasion and metastasis. However, there was no association between tumour catenin expression and depth of tumour invasion or nodal metastases. This needs to be commented on in the discussion.

Response: As the reviewer mentioned, in our clinical investigation, expression of γ-catenin does not correlate with the degree of lymph node

metastasis. However, Lin et al. have reported that reduced protein expression of γ-catenin is associated with lymph node metastasis in human esophageal squamous cell carcinoma (*World J Gastroenterol* 2004; **10**: 3235-3239). It seems that this confusing result conducted from the statistical analysis might due to the insufficient ESCC specimens, or the discordance between protein and mRNA expression levels of γ-catenin. We have added the discussion in the revised manuscript (Page 15, paragraph 2 of revised manuscript)

Comment 4: There is a typo in the methods section of the abstract ('detest' should read 'detect')

Response: We have rewritten the manuscript and amended the mistakes in grammar, syntax, and sentence structure.

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

Sincerely yours,

En-Min Li, Ph.D.
Professor
Department of Biochemistry and Molecular Biology
Medical College, Shantou University
Guangdong Province
Shantou, 515041
China.