April 3, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 9079-review.doc).

Title: HIF-1α induces VE-cadherin expression and modulates vasculogenic mimicry in esophageal carcinoma cells
Authors: Na-Na Tang, Hong Zhu, Hong-Jie Zhang, Wei-Feng Zhang, Hai-Lin Jin, Lu Wang, Pin Wang , Gui-Jun He, Bo Hao, Rui-Hua Shi
Name of Journal: *World Journal of Gastroenterology*
ESPS Manuscript No: 9079

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 reviewers

Reviewer 555673:
(1) The authors do not provide the right information to really discriminate what level the absence of HIF-1alpha or VE-cadherin is affecting cell functionality in general.

Response: In the supplemental data, we have demonstrated the efficiency of HIF-1α or VE-cadherin RNA interference and alterations of cell functions such as proliferation and apoptosis.

(2) It is not clear if the deficiency of forming capillary-like structures in Matrigel is just a result of defects on basic cellular machinery as proliferation and migration; maybe the alteration of vasculogenic mimicry is just the consequence of the proliferative and migratory defects.

Response: We fully agree with your comments. The mechanism of vasculogenic mimicry still remains unclear. The formation of vasculogenic mimicry may be the consequence of the proliferative and migratory defects and it may be associated with the hypothesis of "a tumor as a superorganism".

(3) Immunohistochemistry in Figure 3B is not clear and it is difficult to do any interpretation.

Response: We have offered clearer immunlohistochemical image in Figure 3B. Xenograft VM structure can be observed in Eca109 group and Eca109/NeoHIF group, while it is reduced significantly in Eca109/shHIF group. It indicated that HIF-1α knockdown may inhibit the VM structure *in vivo*.

(4) In Figure 3C, while the down-regulation of the protein is clear, the PCR data do not show any changes. Quantitative PCR would be more valuable.

Response: Thank for the valuable suggestion. We used quantitative PCR to detect HIF-1α,

EphA2, VE-cad and MMP2 mRNA again.

Reviewer 2563046:
(1) The introduction and discussion section appear not yet complete and both are to some extent rather short: As the authors address with their data aspects of tumor microenvironment, hypoxia, and metastasis, which strongly relates to intra-tumor heterogeneity and clonal cancer development, they should discuss their data along recent progress in the field as reviewed in Grunewald TG et al. (J. Transl. Med. 2011) and Merlo LM et al. (Nat. Rev. Cancer 2006).

Response: We have re-written the sections of Introduction and Discussion according to your suggestions.

(2) The authors should expand their discussion on the stability of HIF1-alpha referring to recent articles in the field such as that of Chen K and Chen S et al. (Biology of the Cell, 2013), as well as to recent progress in terms of methodology to assess endothelial structures detailed in Prigozhina NL et al. (Biol Cell, 2011).

Response: We have extended our discussion on this topic according to your suggestions.

(3) Vasculogenic mimicry might be part of the EMT program of cancer cells, which is induced by epigenetic alterations such as aberrant miRNA expression and changes in barrier functions by altering expression of VE-cadherin and other important proteins as reviewed in Bullock MD et al. (Biol Cell 2012) and Guelte AL (Biol Cell 2011).

Response: We fully agree with your comments. In the present study, we investigated the alterations of VE-cadherin, EphA2, LN5γ2 and MMP-2. We will probe into other important proteins and other regulators such as miRNAs in the future studies.

(4) The manuscript could benefit in certain passages (especially in the results section) of proofreading by a native English speaker to enhance grammar and readability.

Response: We have our manuscript proofread by native English speaking expert from Jing-Yun Ma Editorial Office. The editorial certificate is provided.

Reviewer 61678:
Title need to be modified as it is too long.

Response: We have modified the title according to your suggestion and requirement of the journal.

3. References and typesetting were corrected

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

Sincerely yours,

Rui-Hua Shi, PhD
Department of Gastroenterology, the 1st Affiliated Hospital of Nanjing Medical University
300 Guangzhou Road, Nanjing 210029, China
Fax: +86-25-83674636
E-mail: ruihuashi@126.com