

January 14, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 1347- Review).

Title: Twist2 is a valuable prognostic biomarker for colorectal cancer.

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Name of Journal: *World Journal of Gastroenterology*

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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 reviewer.

(1) In scoring the Twist2 IHC, criteria of the extent of staining (0 for <15 % positive cells, 1 for 15-30 % positive cells...) is ambiguous. Why the authors used 15%? Is the criteria common used? If yes, it would be good to cite the reference. The method of total scoring (extent + intensity) also looks unusual. Why the authors did not use extent x intensity? Proper references for this point are required.

Question 1: Why the authors used 15%? Is the criteria common used? If yes, it would be good to cite the reference.

Answer : In our department 15% is a common cut off percentage to determine "positive" or "negative" for a new immunochemistry marker, which has been used in our previous studies(two references below). What is more, 15% is suitable for predicting. It is unfavorable to predict the prognosis if we use a lower criteria (10% or 5%), because of the excessive positive rate.

References

1. Jin, G.Z., Li, Y., Cong, W.M., Yu, H., Dong, H., Shu, H., Liu, X.H., Yan, G.Q., Zhang, L., Zhang, Y., *et al.* (2011). iTRAQ-2DLC-ESI-MS/MS based identification of a new set of immunohistochemical biomarkers for classification of dysplastic nodules and small hepatocellular carcinoma. *Journal of proteome research* 10, 3418-3428.
2. Lu, X.Y., Xi, T., Lau, W.Y., Dong, H., Zhu, Z., Shen, F., Wu, M.C., and Cong, W.M. (2011). Hepatocellular carcinoma expressing cholangiocyte phenotype is a novel subtype with highly aggressive behavior. *Annals of surgical oncology* 18, 2210-2217.

Question 2: The method of total scoring (extent + intensity) also looks unusual. Why the authors did not use extent x intensity? Proper references for this point are required.

Answers: To investigating a new biomarker, the two methods (extent + intensity and extent x intensity) are both available. We just used the conventional method which had been adopted

previously(four references below)

References

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2. Rahman, M.A., Dhar, D.K., Yamaguchi, E., Maruyama, S., Sato, T., Hayashi, H., Ono, T., Yamanoi, A., Kohno, H., and Nagasue, N. (2001). Coexpression of inducible nitric oxide synthase and COX-2 in hepatocellular carcinoma and surrounding liver: possible involvement of COX-2 in the angiogenesis of hepatitis C virus-positive cases. *Clinical cancer research : an official journal of the American Association for Cancer Research* 7, 1325-1332.
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4. Zhao, N., Sun, B.C., Zhao, X.L., Liu, Z.Y., Sun, T., Qiu, Z.Q., Gu, Q., Che, N., and Dong, X.Y. (2012). Coexpression of Bcl-2 with epithelial-mesenchymal transition regulators is a prognostic indicator in hepatocellular carcinoma. *Med Oncol* 29, 2780-2792.

(2) Twist2 expression was found to be not correlated with any of the clinicopathological parameters of CRC (bad T, N, M-stage, poor differentiation, vascular invasion) but only associated with OS and DFS. The authors just deduced several possible mechanisms: for example, Twist2 has been proved to be a regulatory factor of EMT. This possibility must be experimentally validated and presented as a 'Result', not just hypothesized in the 'Discussion'.

Interpretation: The reviewer's question was understandable, as Twist2 failed to correlate any of the clinicopathological parameters of CRC but only associated with OS and DFS in our study. Although the mechanism is unclear, we find Twist2 is not alone. Some other prognostic biomarkers share similar feature with Twist2, such as vimentin(Reference 1), α -smooth muscle actin (α -SMA)(Reference 2) and S100A4(Reference 3) for CRC, and osteopontin (OPN)(Reference 4) for HCC.

Supplement: We have added another immunochemistry marker (E-cadherin) to correlate Twist2 with EMT. As expected, Twist2-positive (up-regulation) expression was significantly associated with reduced expression of E-cadherin, which verified the feature of Twist2 as an EMT inducer in CRC.

References

1. Ngan, C.Y., Yamamoto, H., Seshimo, I., Tsujino, T., Man-i, M., Ikeda, J.I., Konishi, K., Takemasa, I., Ikeda, M., Sekimoto, M., *et al.* (2007). Quantitative evaluation of vimentin expression in tumour stroma of colorectal cancer. *British journal of cancer* 96, 986-992.
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combined with CD44, a novel prognostic biomarker for patients with hepatocellular carcinoma undergoing curative resection. *The oncologist* 13, 1155-1165.

3 References and typesetting were corrected.

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

Sincerely yours,

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