

Answering to the reviewers

Answering to the reviewer 00672053 :

Thank you for the reviewing of our paper and giving your valuable comments. We have reviewed our manuscript again and again and made some necessary modifications to the manuscript according to the comments. We hope that the revised version of our paper will fulfill the referee's expectations and will be published in *WJG*.

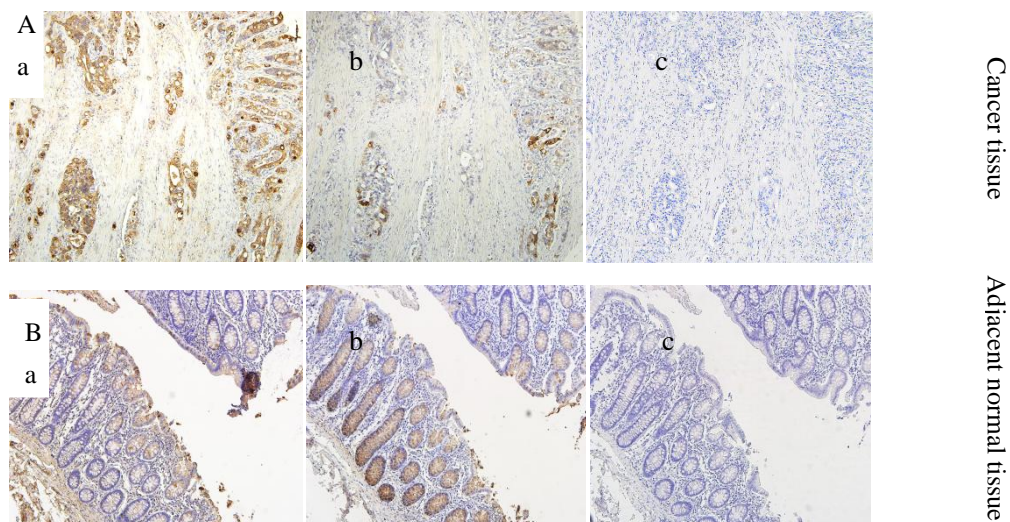
1. Although the TFFs are primarily secreted by the epithelium of the gastrointestinal tract, their exact role in the progress of CRC and their prognostic value have not yet been systematically expounded. There is still no any consensus about if the TFFs are oncogene or anti-oncogene(Perry, J.K et al (2008) Are trefoil factors oncogenic? Trends Endocrinol. Metab. 19, 74–81).

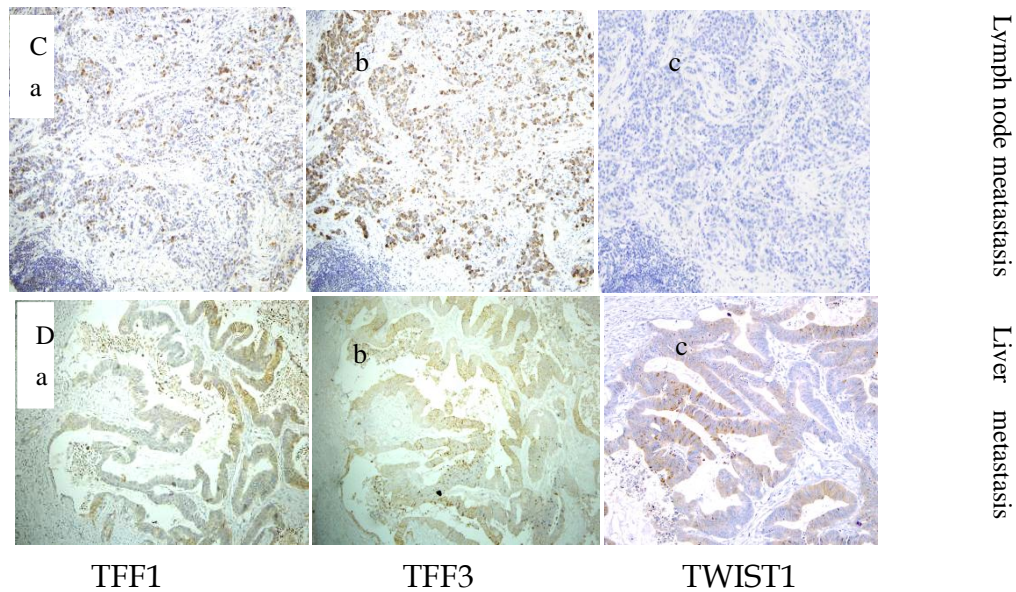
Our study was based on some research results that reported that TFF3 continuously up-regulates the expression of Twist1 in HT29 cell line and Twist1 is a vital transcriptional factor through the course of Epithelial-Mesenchymal Transition(EMT), which indicates the possible involvement of TFF3 in the process of EMT. Inspired by this relations, we hypothesize that TFF3 may play some role in EMT by up-regulating the Twist1. there are few reports about the relation between TFF3 and TWIST1 expressions in CRC and the correlation with survival. Our research group consider that the TFF3-TWIST1 passway we were working on is a promising intervention point in CRC treatment .

2. Our study definitely is descriptive or correlative, which is also a basis of our

continuous further research work. The amount of samples of CRC patients and metastatic tissues are relatively less than that we need. This could result from the following reasons; (1) some of original samples were damaged during the Immunohistochemistry test; (2) A portion of samples were stained poorly, so were eliminated from the data. The remnant samples and data are quite authentic. We also reviewed the manuscript again and again and gave some necessary revision to the parts of the introduction and conclusion for highlighting the novelty.

3. We have previously performed the IHC test on the same areas of samples of cancer tissue、lymph node metastasis and liver metastasis for this three proteins , Unfortunately, the same areas of cancer tissue and metastatic tissues were not stained positively for TWIST1 protein. We also tried IHC test again according to the reviewer's advice and replaced the figure of Twisit1 expression on liver metastatic tissue. But the Twist1 expression of the same area of cancer tissue and lymph node metastatic tissue still showed the negative outcome (figure shown below)

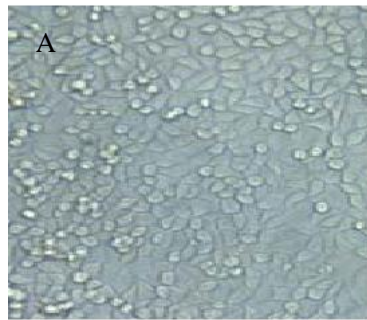




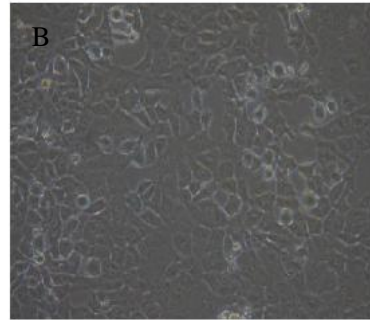
4. We failed to obtain the clear blotting for Snail protein when we performed the western blot assay during cell experiment. So the western blot result of Snail protein was not demonstrated in figure3. We will continually make up for the result of the relevant experiments in the future.
5. Grammatical errors were corrected and abbreviations are explained in the manuscript properly, and article has already been sent to the professional language editing agency for further language correction.
6. additional supportive material and data for the article are attached below.

we have made a form contrast to the four kinds of cell lines. It can be seen that the morphology of the cell lines from normal intestinal epithelial cell(HIEC) to Lovo cell showed a trend of varying from the shape of rotundity or similar to funicular one in the order of HIEC,HT29,SW680 and Lovo , which is accorded with the cell transformation during the EMT process.

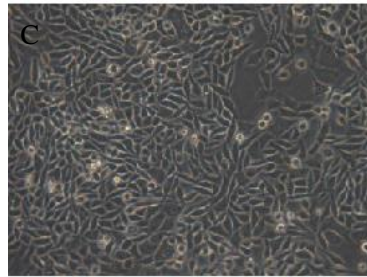
(1) Form contrast of the four kinds of cell lines



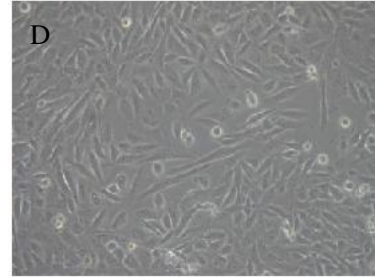
HIEC



HT29



SW620

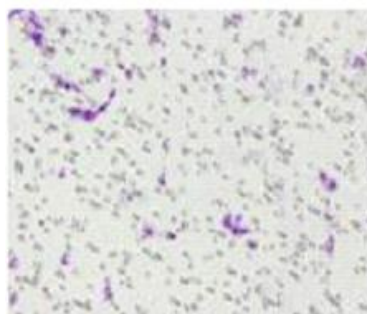


LOVO

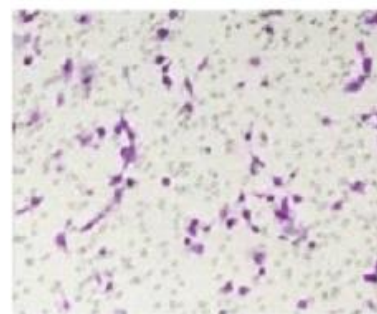
Form contrast of four kinds of colon cell lines

A. HIEC (normal intestinal epithelial cell line for control) B. HT29 (from the moderately differentiated colon cancer) C. SW620 (from the metastatic lymph node) D. LOVO (from the metastatic supraclavicular lymph node)

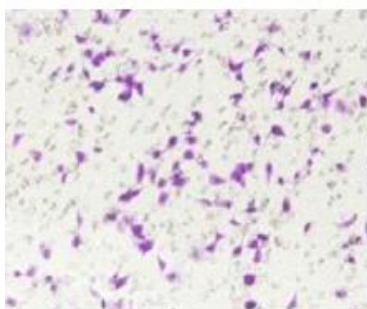
(2) Transwell assay



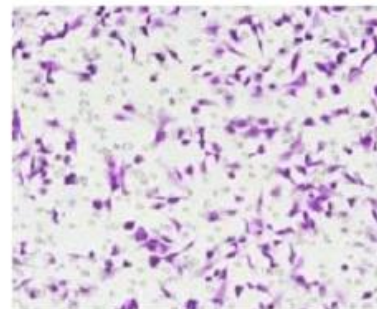
HIEC



HT29

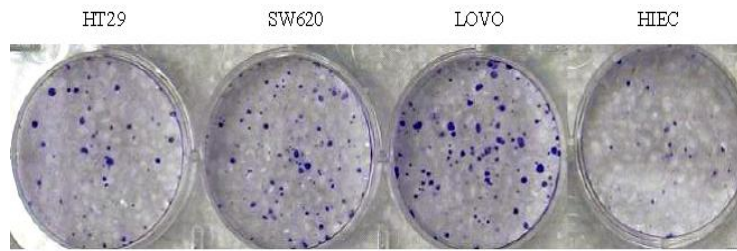


SW620



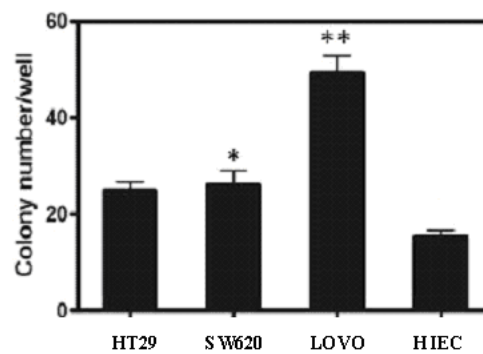
LOVO

(3) plate clone formation assay



(2)and (3): Comparison of invasive potential of four cell lines

The transwell test and plate clone formation assay showed that the invasive potential of the cell lines increased gradually in the order of HT29, SW680 and Lovo



Comparison of Plate clone formation abilities of four cell lines

* P < 0.05 ** P < 0.01 vs HIEC