**Dear Editors and Reviewers:**

On behalf of my co-authors, we thank you very much for giving us the constructive comments and suggestions on our manuscript entitled "Prognostic and predictive value of vascular endothelial growth factor receptor 1 and class III β-tubulin in long-term prognosis of non-metastatic rectal cancer" (**ID: 40131**). These comments are all valuable and helpful for revising and improving our paper. We have studied comments carefully and made corrections in order to meet your expectations. Revised portions are marked with an underscore in the paper. The main corrections in the paper and the responds to the reviewer's comments are as flowing:

**Reviewer #1:**

**Concern #1:** *Why choose determine the "mRNA" of VEGFR1 and TUBB3 rather than others?*

**Response:** Thanks for this important comment. We had tested a total of 14 genes in our preliminary experiments and found that only these two genes were significantly associated with the prognosis. We aimed to explore the prognostic value of genes expression, so we directly detected the mRNA expressions to avoid the effects of post-translational

modification on protein expression.

**Concern #2:** *The result indicated that the expressions of VEGFR1 and TUBB3 were positively correlated. The authors should discuss possible underlying mechanisms.*

**Response:** We would like to thank the editor for this comment. Paradiso et al. [1] had investigated the combination of TUBB3 and VEGFR1 in advanced breast cancer before. Hypoxia in tumor microenvironment promotes angiogenesis, and VEGFR1 is known to be related to angiogenesis [2]. TUBB3 was found to be involved in an adaptive response to low oxygen levels and poor nutrient supply in solid tumors [3, 4]. Therefore, we speculated that the underlying mechanism of the two correlations might be related to anoxic environments. We have added the related details in the 'Discussion' (page 12, paragraph 2).

**Ref.**


1 Paradiso A, Mangia A, Chiriatti A, Tommasi S, Zito A, Latorre A, et al. Biomarkers predictive for clinical efficacy of taxol-based chemotherapy in advanced breast cancer. *Annals of oncology : official journal of the European Society for Medical Oncology*. 2005;**16 Suppl 4**:iv14-9.

2 Huang H, Shen J, Vinores SA. Blockade of VEGFR1 and 2 suppresses pathological angiogenesis and vascular leakage in the eye. *PloS one*. 2011;**6**(6):e21411.PMID.PMC3120882

3 Raspaglio G, De Maria I, Filippetti F, Martinelli E, Zannoni GF, Prislei S, et al. HuR regulates beta-tubulin isotype expression in ovarian cancer. *Cancer research*. 2010;**70**(14):5891-900.

4 Raspaglio G, Filippetti F, Prislei S, Penci R, De Maria I, Cicchillitti L, et al. Hypoxia induces class III beta-tubulin gene expression by HIF-1alpha binding to its 3' flanking region. *Gene*. 2008;**409**(1-2):100-8.

**Concern #3:** *The results indicated that a favorable OS in both low expression of VEGFR1 and TUBB3 was noted as compared to others. The authors should discuss possible underlying mechanisms.*

**Response:** We're sorry that the discussion was not clearly stated. According to previous studies, we had speculated that the underlying mechanism of the two correlations might be related to anoxic environments. We have added the possible underlying mechanisms in the 'Discussion' (page 12, paragraph 2).

Special thanks to you for your good comments.

**Reviewer #2:**

**Concern #1:** *This study showed the correlation between the expressions of VEGFR1 and TUBB3, and those markers had positive correlation (P = 0.006, r = 0.315). Isn't it weak correlation by the Spearman's correlation test?*

**Response:** Thanks for your comment. Though the correlation is weak according to our results, the trend might arouse our attention for further verification with a larger sample size.

**Concern #2:** *In the Discussion section, page 10, "Tsai et al. reported that*

*the overexpression of VEGF is a significant negative predictor of early postoperative relapse in stage I–III colorectal cancer patients, leading to poor OS" had a vague expression. I think that the overexpression of VEGF is a positive predictor of early postoperative relapse, instead of negative predictor. A "negative predictor of overall survival" is a right expression, but "negative predictor of early relapse" is vague.*

**Response:** We are very sorry for our incorrect expression. We have revised the manuscript according to reviewer's comment (page 10, paragraph 3).

**Concern #3:** *In the Discussion section, page 10, "A previous study evaluated the VEGF expression of 117 colorectal adenocarcinoma patients, and confirmed that lymph node metastasis (negative vs. positive, P < 0.001) and TNM stage (stage III vs. I/II, P < 0.001) were related to increased VEGF expression" had also a vague expression. The "positive vs. negative" is a comfortable expression than "negative vs. positive" when matched with "TNM stage (stage III vs. I/II, P < 0.001)".*

**Response:** We apologize for our negligence. We have corrected the mistakes according to reviewer's comment (page 11, paragraph 1).

Special thanks to you for your good comments.

**Reviewer #3:**

**General Comments**

**Concern #1:** *It seems like the authors do not distinguish sufficient between prognostic and predictive. When applying these definitions in the current study it is important to have in mind that 66 (88%) of the patients have been treated with 5-FU based chemotherapy, which will confound your prognostic data. What you have investigated in your study is mainly the predictive properties of VEGFR1 and TUBB3 in relation to 5-FU based chemotherapy, which will require that the manuscript is corrected.*

**Response:** We are so sorry that we had mixed the concepts of "prognostic" and "predictive". We believed that "prognostic" was more appropriate for our study per reviewer's comment. VEGFR1 and TUBB3 were reported to be related to drug resistance of antiangiogenic agents and taxanes, respectively. However, none of our patients had received the above agents. Without relative therapeutic intervention, there is no role for "predictive". Moreover, as shown in table 3, chemotherapy did not influence the OS in our cohort ($P$=0.572). We have corrected the expressions in the revised manuscript according to the reviewer's suggestion (The title, page 5,9).

**Specific Comments**

**Concern #1:** *Multiplex branched DNA liquidchip (MBL) technology: Please briefly describe how the assays was validated before applied in the current study.*

**Response:** We sincerely apologize for this omission. Multiplex branched DNA liquidchip (MBL) technology is a mature technology for quantitative measurement of the gene mRNA levels in the formalin-fixed paraffin-embedded (FFPE) slides, which had been used in previous studies[5, 6]. Moreover, it had been conducted well in our preliminary experiments, so we applied MBL in the current study.

**Ref.**

5 Han Y, Li G, Su C, Ren H, Chu X, Zhao Q, et al. Exploratory study on the correlation between 14 lung cancer-related gene expression and specific clinical characteristics of NSCLC patients. *Molecular and clinical oncology*. 2013;**1**(5):887-93.[PMID:PMC3915665 10.3892/mco.2013.153]

6 Flagella M, Bui S, Zheng Z, Nguyen CT, Zhang A, Pastor L, et al. A multiplex branched DNA assay for parallel quantitative gene expression profiling. *Analytical biochemistry*. 2006;**352**(1):50-60. 10.1016/j.ab.2006.02.013]

**Concern #2:** *Statistical analysis: The p-values seems to be reported without adjustment for multiplicity. Please explain.*

**Response:** We would like to thanks for the insightful comment. Similar to previous studies[7, 8], as the variables in our study is limited, the adjustment for multiplicity might not be necessary under the consideration of statistician. According to the reviewer's comments, we'll try to conduct adjustment for multiplicity in a larger sample size study with multiple variables in the future.

**Ref.**

7 Chua W, Charles KA, Baracos VE, Clarke SJ. Neutrophil/lymphocyte ratio predicts chemotherapy outcomes in patients with advanced colorectal cancer. *British journal of cancer*. 2011;**104**(8):1288-95.PMID.PMC3078587

8 Deng Q, He B, Liu X, Yue J, Ying H, Pan Y, et al. Prognostic value of pre-operative inflammatory response biomarkers in gastric cancer patients and the construction of a predictive model. *Journal of translational medicine*. 2015;**13**:66.PMID.PMC4343078

**Concern #3:** *Patient characteristics: As the cut-off's for both VEGFR1 and TUBB3 is based on the same patient population as you apply these values on there is a great risk of over fitting. The data you have generated in your study can only be regarded as hypothesis generating, which must be stated in the abstract and the main text of the manuscript.*

**Response:** Thanks for reviewer's suggestion. We have supplemented the details according to the reviewer's suggestion (page 3, paragraph 2 and page 7, paragraph 1).

**Concern #4:** *Discussion: Please discuss the sensitivity and specificity data for your biomarkers. For VEGFR1 the sensitivity is only 44.1% (Figure 1).*

**Response:** Thanks for reviewer's suggestion. The sensitivity of VEGFR1 was low, but the specificity was high with 82.9%. Moreover, the sensitivity would increase by combining with lymph node status. We have added the related details in the 'Discussion' (page 11, paragraph 2).

Special thanks to you for your good comments.

We believe to have properly addressed the comments from the reviewers and editorial board in this letter. They are very important to us and have helped significantly improve the present manuscript. All changes have been marked with the 'Underscore' feature in the revised manuscript.

We hope these corrections will be to your entire satisfaction. We would highly appreciate your kind consideration of the manuscript as a research article in ***World Journal of Gastrointestinal Oncology***.

Please contact me if you have any further questions. Thanks for your cooperation.

Sincerely,

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