Author's response to reviews

Title: Elevated IGFIR expression regulating VEGF and VEGF-C predicts lymph node metastasis in human colorectal cancer

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Author's response to reviews: see over
Dear Dr. Marshall:

Thank you very much for your mail along with the Reviewers’ comments on our manuscript (2145346225302296). We are very grateful to the reviewers’ careful reviewing. Following the reviewers’ comments and suggestions, we have performed some additional experiments, added some data in the manuscript, and made careful revisions to our manuscript. Our responses to the reviews are appended below point by point.

Finally, we hope that our responses provide a satisfactory answer to the reviewer’s concern and our manuscript is now suitable for publication in the journal *BMC Cancer*. We are looking forward to hearing your decision.

Sincerely yours,

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Response to Reviewer Markus Moehler

1. The presentation of the statistical analyses as well as the results within the abstract and through the full manuscript are still weak. The abstract does not mention any details of the paper, i.e. the number of patients, the patients characteristics and the clinical status of the patients. Clinical pathological features are not clearly defined in the abstract.

Response: According to the suggestion, we rechecked all the statistical analysis in the manuscript, added some statistical data on the survival curve, and made proper revision to strength the statistical analysis in the revised manuscript. In addition, the patient details were added in the Abstract and Methods sections.

2. Beginning with the background, many citations and important references to lymphangiogenic pathways (also like VEGF-D) are missing.

Response: According to the suggestion, we added a paragraph in the Background section to demonstrate the lymphangiogenic pathways as follows:

The regional lymph nodes draining primary tumors are generally the first, and by far the most common, site of metastasis for some of the major human malignancies, and tumor cell dissemination to the regional lymph node was generally believed to be a passive process. Recent evidence suggests that tumor-derived VEGF-C and VEGF-D can stimulate de novo formation of intratumoral lymphatic capillaries (lymphangiogenesis), which raised the possibility that cells within primary tumors can contribute actively to lymphatic dissemination through the induction of lymphangiogenesis. When overexpressed in breast cancer MCF-7 cells, VEGF-C promoted tumor lymphangiogenesis and tumor metastasis. Notably, IGF-I has recently been demonstrated to be a positive regulator of VEGF-C expression through IGFIR signaling and implicated in the control of lymphatic metastasis.
3. VEGF is always mentioned, but not clearly defined, whether it means really VEGF-A.

Response: VEGF in our manuscript is equal to VEGF-A, we made a correction in the revised manuscript to clearly demonstrate this concept.

4. Is the interest of the authors only to analyse the markers on their prognostic value? However evidently, the data are made retrospectively. This should be mentioned. Could it be even predictive for a treatment intervention? This is not analysed or answered.

Response: We addressed this issue to demonstrate the predictive and prognostic value in the Conclusion as follows:

Overall, combined analysis of IGFIR and VEGF or VEGF-C will prove to be invaluable for predicting lymph node metastasis in human colorectal cancer. Inhibition of IGFIR/IGF-I based on this mechanism may develop an effective treatment for human colorectal cancer. This study would provide a novel insight into diagnosis of lymph node metastasis and therapeutic strategy of tumor in human colorectal cancer.

5. The results are not satisfying. Here the expression of corresponding VEGF-Rs are not analysed or discussed.

Response: According to suggestion, we added the descriptions in the Discussion to analyze the expression of corresponding VEGF-Rs.

6. In the results, the most important part, the survival data and the treatment of these patients and their correlation with the markers is missing.

Response: According to the reviewer’s suggestion, we added the survival data in
Fig. 3 and Fig. 4 in the revised manuscript, and related description were added in the Results as follows:

The prognostic impacts of IGFIR, VEGF and VEGF-C expression were analyzed by using Kaplan-Meier survival curves. Patients who had strong IGFIR, VEGF and VEGF-C staining showed a significantly lower survival rate compared with patients who had low staining (Fig. 3, \( P < 0.001 \)). Survival rates also were evaluated according to combinations of IGFIR/VEGF and IGFIR/VEGF-C co-expression. Patients who were both high expression of IGFIR/VEGF and IGFIR/VEGF-C had unfavorable prognoses, and patients who were negative or one of high expression of these molecules had more favorable prognoses (Fig. 4, \( P < 0.001 \)).

7. Also, IGF1R pathways their interaction with VEGF-C or VEGF is not discussed or analysed.

**Response:** In the Discussion section, we added analysis on the IGFIR pathways their interaction with VEGF or VEGF-C in the Discussion as follows:

PI3K has been identified as a major transducer of the IGF-IR signal in various cellular systems. Among others, its activity was shown to be critical for cell survival, a function mediated through Akt and Bax activation, and it was implicated in mitogenesis, protein synthesis, and differentiation. The degree to which different cells use common pathways to convey the IGF-IR signals may be cell context dependent. Thus, IGFIR signaling elevated VEGF and VEGF-C expression could be mediated by PI3K pathway, which may be possible mechanisms underlying induction of VEGF and VEGF-C by IGF-I in human colorectal cancer cell lines or tumor samples.

8. A blockade of IGF-1 induced VEGF and VEGF-C expression should at least
be presented to highlight the association in the cell culture.

**Response:** According to published data, blockade of IGFIR by using small molecular inhibitor and anti-sense oligonucleotide could efficiently inhibit VEGF expression in cell culture. According to the suggestion, we added some description in Discussion to demonstrate this concept as follows:

Recently, some of *in vitro* experiments indicated that IGFIR can increase VEGF expression and stimulate tumor angiogenesis in pancreatic carcinoma cells. Moreover, inhibiting IGFIR signaling can not only down-regulate the expression of VEGF, but also decrease the number of tumor-related blood vessels, increase cancer cell apoptosis and lose lymph node metastatic ability to distal organs.

**Response to Reviewer Paolo Bruzzi**

1. Page 9 “The frequency of positive IGFIR staining in colorectal adenoma was higher than that in normal tissues, but …. there was no statistical difference between these two groups (P = 0.265). Replace with….but the difference was not statistically significant (P=0.265)

**Response:** According to the suggestion, we replace “but the difference was not statistically significant (P=0.265)”.

2. Last paragraph of page 10 “…with the severity of lymph node metastasis. Patients were divided into three groups according to the number of metastatic lymph nodes: ….”All the analyses in this paragraph are statistically flawed: the association with N positivity (N0 vs N+) was already shown in the preceding analysis, which is repeated here without adding anything. This analysis should have been limited to N+ patients. -Table 3 Useless
Response: According to the reviewer’s suggestion and based on our results, we deleted Table 3 in the revised manuscript.

3. Table 1 and table 2: Add percentages (by row) to facilitate the interpretation, and delete the columns ‘low’

Response: According to the suggestion, we added the percentages in Table 1 and Table 2 to facilitate the interpretation.

Response to Reviewer Yajun Guo

1. The authors should specify the histological grade standard used in this report. Whether Grade I, II and III correspond to poorly, moderately and well differentiated?

Response: The histological grade I, II, III in our manuscript correspond to poorly, moderately and well differentiated. We made some corrections in Table 2 to clearly demonstrate this concept according to the reviewer’s suggestion.

2. The authors should provide a WB image about the expression of IGF-IR in COLO-205 cells.

Response: Because we found significant association of IGF-IR with colorectal cancer, we chose cell lines that expressing high level of IGF-IR for in vitro cell culture analysis. COLO-205 is reported to express high level of IGF-IR according to the published data (Cancer Res 2007, 67: 8856-64).

3. What’s source of IGF-I in tumor tissue or cancer cell lines? Whether the
COLO-205 cell itself produces IGF-I therefore form a positive feedback for supporting cancer cell growth. They need add some discussions about the possible mechanisms underlying induction of VEGF and VEGF-C by IGF-I in human colorectal cancer cell lines or tumor samples.

**Response:** According to the suggestion, we made analysis on possible mechanisms underlying induction of VEGF and VEGF-C by IGF-I in human colorectal cancer cell lines or tumor samples in the Discussion as follows:

PI3K has been identified as a major transducer of the IGF-IR signal in various cellular systems. Among others, its activity was shown to be critical for cell survival, a function mediated through Akt and Bax activation, and it was implicated in mitogenesis, protein synthesis, and differentiation. The degree to which different cells use common pathways to convey the IGF-IR signals may be cell context dependent. Thus, IGFIR signaling elevated VEGF and VEGF-C expression could be mediated by PI3K pathway, which may be possible mechanisms underlying induction of VEGF and VEGF-C by IGF-I in human colorectal cancer cell lines or tumor samples.

4. In the sentence “IGF-I could effectively induce the VEGF and VEGF-C mRNA expression and protein secretion in colorectal cancer cells expressed IGFIR molecules.” in Abstract, the word “expressed” should be “expressing”, and so dose elsewhere in text.

**Response:** We corrected the word “expressed” to “expressing” according to the reviewer’s suggestion.