

Prof. Dr. Yuan Qi,

Science Editor

Email: y.qi@wjgnet.com

Date: Oct. 28th 2016

Dear Editor Yuan Qi,

We would like to take this opportunity to express our appreciation of your kind efforts and to thank the anonymous reviewers for their valuable suggestions and comments.

Please find attached the revised manuscript, and Revision Notes. The manuscript has been subjected to revision according to the reviewers' and editors' suggestions. We hope that the revised version will now be acceptable for publication in *World Journal of Gastroenterology*.

Sincerely, yours,

Jun Zeng, Ph.D

zengjun_2012@163.com

Revision Notes

1. "Scientific Research Process"

q1: Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO:

Manuscript Type: ORIGINAL ARTICLE

Title:

Authors:

Correspondence to:

- 1 What did this study explore?
- 2 How did the authors perform all experiments?
- 3 How did the authors process all experimental data?
- 4 How did the authors deal with the pre-study hypothesis?
- 5 What are the novel findings of this study?

This file must be signed by the corresponding author and provided in a PDF format.

Answer: Thanks for your critical comments and kind advices. Following your suggestions, "scientific research process" was added. The content is " **In this study, we explored the expression level of IL-6 in colorectal cancer tissues and its clinicopathological significance in patients with CRC. We collected 50 colorectal cancer tissue specimens and 50 matched adjacent mucosa specimens. The expression of IL-6 in the clinical samples was examined by immunostaining. The correlation between IL-6 expression and clinicopathological factors was analyzed by statistical analysis. The data were analyzed by SPSS 16.0 for Windows (SPSS Inc). Pearson χ^2 Test and Fisher's exact test were used to compare qualitative variables, and the quantitative variables were analyzed by the t test. The correlation between clinical factors and IL-6 expression was evaluated by the Spearman test for non-parametric variables. When the P value was less than 0.05, the results were regarded as statistically significant.**

Previous studies have demonstrated that IL-6 is a critical tumor promoter during early CRC tumorigenesis. We hypothesized that IL-6 might also play an important role in CRC progression. Therefore, we examined the expression level of IL-6 in CRC tissue specimens and matched adjacent mucosa specimens. There have been few studies regarding the expression levels of IL-6 and its clinicopathological significance in CRC. To the best of our knowledge, this is the first analysis of the expression levels of IL-6 in resected CRC samples using immunohistochemistry combined with biostatistics. The results showed that the expression levels of IL-6 were correlated with TNM stage and histodifferentiation. Furthermore, IL-6 in tumor cells showed a stronger immunoreactivity as tumor cells invaded more deeply. These data indicate that IL-6 might be used as a potential target in patients with CRC." The title of the revised manuscript is " **Overexpression of IL-6 in colorectal cancer tissues and its clinicopathological significance in patients with CRC** ". The running title part is changed from "Running title: IL-6 expression was upregulated in CRC " to " **Zeng J et al. IL-6 expression was upregulated in CRC**". The author names is changed from " Jun Zeng, Zhonghua Tang, Shuang Liu, Shanshan Guo " to " **Jun Zeng, Zhonghua Tang, Shuang Liu, Shanshan Guo** ". The affiliation is " **College of Life Sciences, Chongqing Normal University, Chongqing, 401331, P. R. China**". Correspondence to Jun Zeng, PhD, College of Life Sciences, Chongqing Normal University, Chongqing, 401331, P. R. China. Zengjun_2012@163.com. Tel: + 86-18723179063.

This file has been signed by the corresponding author and provided in a PDF format.

2. "Author contributions"

q2: **for example: Author contributions:** Jethwa P and Naqvi M performed the majority of experiments; Hardy RG, Hotchin NA, and Roberts S provided vital reagents and analytical tools and were also involved in editing the

manuscript...

Answer: Thanks for your valuable suggestions. Following your advices, we have added a detailed author contribution as below: **Zeng J, contributed to the design of the experiment, data acquisition and analysis, scientific discussion and manuscript revise. Tang Z, Liu S and Guo S substantially contributed to experimental preparation and the writing of the manuscript. All authors approved the final version of the article to be published.**

3. "Supported by"

q3: Please provide the grant application form(s). If you can't provide it, please delete this part.

q4: Please provide the approved grant application form(s) or funding agency copy of any approval document(s)/letter(s). For manuscripts supported by various foundations (i.e., charitable, not-for-profit organizations), the authors should provide a copy of the full approved grant application form(s) or funding agency copy of any approval document(s)/letter(s), consisting of the information section and body section in PDF format. The approved grant application form(s) or funding agency copy of any approval document(s)/letter(s) will be released online together with the manuscript in order for readers to obtain more information about the study and to increase the likelihood of subsequent citation.

Answer: Thanks for your valuable suggestions. Following your advices, we have provided the grant application forms in PDF format..

4. " Institutional review board statement:"

q5-q6: The ethic approval document(s)/letter(s) must be provided in a PDF format, and each statement must also be mentioned as a footnote in the manuscript text. All files must be signed by the corresponding author and provided in a PDF format.

Answer: Thanks for your valuable suggestions. Following your suggestions, Institutional review board statement is "**All colorectal cancer specimens from the patients were acquired after informed consent and ethical permission was obtained for participation in the study**". The ethic approval documents from Sichuan Provincial People's Hospital and all files have be signed by the corresponding author and provided in a PDF format.

5. "Institutional animal care and use committee statement:"

q7: The approval document(s)/letter(s) must be provided in a PDF format, and each statement must also be mentioned as a footnote in the manuscript text.

Answer: Thanks for your valuable suggestions. In this study, no animals were used to study.

6. "Animal care and use statement"

q8: This file must be signed by the corresponding author and provided in a PDF format, and the statement must also be mentioned in the text.

Answer: Thanks for your valuable suggestions. In this study, no animals were used to study.

7. " Conflict-of-interest statement: "

q9: This file must be signed by the corresponding author and provided in a PDF format, and the statement must also be mentioned as a footnote in the manuscript text.

Answer: Thanks for your valuable suggestions. Following your suggestions, we added a sentence that is "**To the best of our knowledge, no conflict of interest exists**". And the file has been signed by the corresponding author and provided in a PDF format.

8. "Data sharing statement:"

q10: This file must be signed by the corresponding author and provided in a PDF format, and the statement must also be mentioned as a footnote in the manuscript text.

Answer: Thanks for your valuable suggestions. In this study, "No additional data are available".

9. "Biostatistics statement:"

q11: This statement must be mentioned in the text, and a certificate of statistical review signed by a biostatistician must be provided in PDF format.

Answer: Thanks for your valuable suggestions. Following your suggestions, "the statistical methods of this study were reviewed by associated professor Gang Cai from the College of Mathematical Sciences, Chongqing Normal University." A certificate of statistical review signed by him has been provided in a PDF format.

10. "Core tip"

WL12: Please write a summary of less than 100 words to outline the most innovative and important arguments and core contents in your paper to attract readers.

q13: **Audio Core Tip** In order to attract readers to read your full-text article, we request that the first author make an audio file describing your final core tip.

Answer: Thanks for your valuable suggestions. Following your advices, we wrote a summary of core contents in our study as follows: **Colorectal cancer (CRC) is the fourth leading cause of cancer-related death worldwide. Previous studies demonstrated that IL-6 was a critical tumor promoter during early CRC tumorigenesis. There were few studies about the expression levels of IL-6 and its prognostic role in CRC. So we explored the correlation between the expression levels of IL-6 and the**

clinicopathological features of colorectal cancer specimens. Till now, to the best of our knowledge, the paper analyzed, for the first time, the expression levels of IL-6 in resected CRC samples by immunohistochemistry combined with biostatistics. Results showed that the expression levels of IL-6 were correlated with TNM stage and histodifferentiation. Furthermore, IL-6 in tumor cells showed stronger immunoreactivity as tumor cells invaded deeply. These data implied that IL-6 might be used as a potential target in patients with CRC. Furthermore, the first author has made an audio file describing our final core tip.

11. References "[1-3]"

q14: Please reformat all the reference numbers like this. Please check throughout. Thank you!

Answer: Thanks for your valuable suggestions. Following your advices, we have reformatted all the reference numbers.

12. "COMMENTS"

Wang JL15: Please write the COMMENTS section at here. See the format in the Format.

Answer: Thanks for your valuable suggestions. Following your advices, we have written the COMMENTS section as follows:

"(1) Background

Colorectal cancer (CRC) is the fourth leading cause of cancer-related death worldwide. Previous studies have demonstrated that IL-6 is a critical tumor promoter during early CRC tumorigenesis. However, there were few studies regarding the expression levels of IL-6 and its prognostic role in CRC. The correlation between the expression levels of IL-6 and the clinicopathological features of colorectal cancer specimens has not yet been investigated.

(2) Research frontiers

Previous studies have demonstrated that IL-6 is a critical tumor promoter during early CRC tumorigenesis and that the IL-6/STAT3 signaling pathway plays an important role in the progression of CRC.

(3) Innovations and breakthroughs

To the best of our knowledge, this is the first analysis of the expression levels of IL-6 in resected CRC samples and its prognostic role using immunohistochemistry combined with biostatistics.

(4) Applications

The results showed that the expression levels of IL-6 were correlated with TNM stage and histodifferentiation. Furthermore, IL-6 in tumor cells showed stronger immunoreactivity as tumor cells invaded more deeply. These data indicate that IL-6 might be used as a potential target of postoperative adjuvant therapy in patients with CRC.

(5) Terminology

Invasion depth, also called the depth of tumor invasion, is the most significant histological predictor of lymph-node metastasis in colorectal cancer. Increasing evidence indicates that colorectal cancer with submucosal deep invasion correlates with poor prognosis.

Peer-review"

12. "REFERENCES"

q16: Please add PubMed citation numbers and DOI citation to the reference list and list all authors. Please revise throughout.

Answer: Thanks for your valuable suggestions. Following your advices, we have added PubMed citation numbers and DOI citation to the reference list and list all authors.

12. "Figures"

q17: Please provide the decomposable figure of Figures, whose parts are movable and can be edited. So please put the original picture as word or ppt or excel format so that I can edit them easily.

Answer: Thanks for your valuable suggestions. Following your advices, we have provided the decomposable figures as PPT format.

Other Revision Notes

1. The title is changed into "Overexpression of IL-6 in colorectal cancer tissues and its clinicopathological significance in patients with CRC".

2. In the line 8, the sentence is now "Zeng J *et al.* IL-6 expression was upregulated in CRC".

3. In the line 10, the author name "Shanshaan Guo" is changed into "Shanshan Guo".

4. In the line 12, the affiliation is now " College of Life Sciences, Chongqing Normal University, Chongqing, 401331, P. R. China".

5. In the line 53, we added the information of corresponding author as follows:
Correspondence to: Jun Zeng, PhD, College of Life Sciences, Chongqing Normal University, Chongqing, 401331, P. R. China. Zengjun_2012@163.com
Tel: + 86-18723179063.

6. In the line 89, "**Key Words**" is changed into "Colorectal cancer; IL-6; Invasion depth; Lymph node metastases".

7. In the line 109, we added a sentence "Zeng J, Tang Z, Liu S, Guo S. Overexpression of IL-6 in colorectal cancer tissues and its clinicopathological significance in patients with CRC".

8. In the line 318, we added a paragraph "ACKNOWLEDGEMENTS" as follows:
This work was supported by grants from the Chinese National Natural Science Foundation of China (81502131), the Natural Science Foundation Project of

CQ (cstc2016jcyjA0405), and the Scientific and Technological Research Program of Chongqing Municipal Education Commission (KJ1500332). The authors also thank Professors Ke Xie and Dandan Dong and Technician Fanghua Li at the Sichuan Provincial People's Hospital (Chengdu, China) for providing colorectal tumor tissue samples.

9. In addition, there are some formatting errors, spelling mistakes and punctuation in the figure legends and we have modified them.

10. There are many spelling and formatting mistakes in the whole article, which we have modified and have been highlighted.