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Fusobacterium nucleatum-Mediated Alteration in Expression of *VEGF* and *CCL3* Genes and *KRAS* Mutation in Colorectal Cancer Patients

Hataw Jalal Taher, fouad kamal hussain

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About this report

This open peer review (OPR) report shows the public comments made on this article during peer review, by the reviewers, the associate editor and the editor-in-chief, for every revision round, together with the authors' replies. Comments appear only with the consent of the people who wrote them. Reviewers who chose to sign their report are named; the others are shown as "Referee". Comments are edited for language only, and confidential remarks to the editors are not included.

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1 Original submission

1 comment · 24 May 2023

Fatemeh Behnam Rassouli Reviewer

MINOR REVISION 24 May 2023

The introduction needs to be expanded regarding the significance of CCL3 and VEGF in CRC.

The sex ratio of the patients must be defined, as must, of course, the correlation between CCL3 and VEGF expression and the sex ratio.

As described in the methods, some samples were collected during surgery. Thus, it is crucial to specify whether patients had received chemotherapy and/or radiation before the operation.

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Revision 1

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

Dear Reviewers,

Thank you for taking the time to review our manuscript entitled [**Fusobacterium nucleatum-mediated alteration in VEGF and CCL3 genes expression and KRAS mutation in colorectal cancer patients**]. We sincerely appreciate your insightful comments and suggestions, which have undoubtedly contributed to the improvement of our work. In response to your valuable feedback, we have carefully considered each comment and have made revisions to address the issues raised. In the following sections, we provide detailed responses to each of your comments and describe the specific changes we have made to the manuscript.

Reviewer 1:

1. The introduction needs to be expanded regarding the significance of CCL3 and VEGF in CRC.

   Response: Thank you for your comment. We appreciate your suggestion, and we expanded the introduction to provide a more comprehensive explanation of the significance of CCL3 and VEGF in colorectal cancer (CRC) (Lines 48-66). We highlighted their roles in tumor progression, metastasis, and angiogenesis, as well as their potential as therapeutic targets. Please

2. Sex ratio of patients must be defined, and of course, the correlation between CCL3 and VEGF expression with sex ratio.

   Response: We apologize for the oversight. In the revised manuscript, we included the sex ratio of the *F. nucleatum* patients and analyze the correlation between CCL3 and VEGF expression with the sex (Figure 3 is added with figure caption (Lines 177-184) and (lines 162-166) added as text in results). We presented the data in a clear and concise manner, including appropriate statistical analyses (Lines 134-136).

3. As described in methods, some samples were collected during surgery. Thus, it is crucial to define whether patients have received chemotherapy and/or radiation before the operation.

Response: Thank you for bringing this to our attention. We ensured that the patients were not underwent any chemotherapy or radiotherapy. In the revised methods section, we included a statement (Lines 86 & 87) clarifying that the patients did not undergo any chemotherapy or radiation treatments before the operation.

Reviewer 2:

1. The subject of the paper is interesting.
2. The novelties of work have to be highlighted. What has been done in this research that we cannot find in previous studies.

Response: We appreciate your positive feedback regarding the subject of our paper. In the revised manuscript, we will emphasize the novelties of our work. Specifically, we will highlight the following aspects that differentiate our research from previous studies:

- Novel insights into the association between CCL3 and VEGF expression in CRC with the presence of Fusobacterium nucleatum.
- Relation of presence of positive Fusobacterium nucleatum with mutation of (6144A>AT) at exon 2 of KRAS gene.

Background:

1. More explanation is required, and this section has to be extended. For example, the basic information about Fusobacterium nucleatum is required.

Response: Thank you for your feedback. We agree that the background section needs further expansion. We provided a more comprehensive explanation of Fusobacterium nucleatum, including its basic information, its association with CRC (Lines 33-37).

Methodology:

2. Some of the methods (e.g., DNA extraction) are not explained properly. Summary of some methods and their short details have to be added.
3. The name of key equipment and materials with the name of the manufacturer is also needed.

   Response: We appreciate your suggestions regarding the methodology section. In the revised manuscript, we provided a more detailed explanation of the DNA extraction method, including a summary of the steps involved and their specific details (Line 94-98). Additionally, we included the main key equipment and materials used, to ensure the reproducibility of our study.

   Results:
4. Did gel images were obtained? If yes, it would be better to be presented.

Response: Thank you for your comment. Gel images were indeed obtained during our study. In the revised manuscript, we included representative gel images to provide visual support for our results (Figure 4 with figure caption in Lines 95-97) and refereeing to the figure inside the result test in Lines 188&189). This image will aid in the interpretation and understanding of the obtained data.

2 Revision 1

0 comments

No public comments in this round.