Manuscript NO.: 87150, Basic Study

Title: Association and potential molecular mechanisms between HSF4 methylation and colorectal cancer risk: a bioinformatics study

On behalf of my co-authors, we thank you very much for giving us an opportunity to revise our manuscript, we appreciate editor and reviewers very much for their positive and constructive comments and suggestions on our manuscript entitled "*Association and potential molecular mechanisms between HSF4 methylation and colorectal cancer risk: a bioinformatics study*". Those comments are all valuable and helpful for revising and improving our paper, as well as the important guiding significance to our researches. We have studied comments carefully and have made correction which we hope meet with approval. Revised portion are marked with yellow color in the revised manuscript.

REVIEWER'S COMMENTS

Reviewer #1:

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Minor revision

Specific Comments to Authors: The present study was proposed to investigate the correlation between HSF4 methylation and CRC risk, and to uncover the underlying molecular mechanisms. Actually, the current proposal is interesting and well-written. Therefore, I recommend that the current study be published after minor revisions as follows:

1- Please add a diagrammatic figure to propose the possible mechanistic pathway for these findings

Reply: Thank you very much for your comment, it's an important element that we had previously overlooked. Combined with the results of our previous research, we have drawn diagrammatic figure. For details, please see revised Figure 9.

2- Please discuss whether TCGA data is important to study the complex interaction within the tumor microenvironment of cancer as well as cancer cells. reference: SnapShot: TP53 status and macrophages infiltration in TCGA-analyzed tumors. Int Immunopharmacol. 2020 Sep;86:106758. doi: 10.1016/j.intimp.2020.106758.

Reply: In fact, the immune microenvironment is not part of our research. However, after we read that literature, this guided our future research. Therefore, with reference to your suggestion, we cited that literature and the analysis of the immune microenvironment as a flaw and prospect of this study. For more information, please see lines 311-316. Thanks again for your comment, it's vital to the improvement of our articles.

Reviewer #2:

Scientific Quality: Grade B (Very good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Minor revision

Specific Comments to Authors: Congratulations on the manuscript. Well written, and conclusions are consistent with findings. Please add limitations in the manuscript.

Reply: Thank you very much for your recognition, it encourages the motivation of our research. In addition, with reference to your suggestion, we have added the limitations that still exist in this study in the Discussion section. For more information, please see lines 302-316. Thanks again for your comment, it's vital to the improvement of our articles.

Reviewer #3:

Scientific Quality: Grade E (Do not publish)

Language Quality: Grade B (Minor language polishing)

Conclusion: Rejection

Specific Comments to Authors: From this study, methylation status of HSF4 did not correlate to the prognosis of CRC. However, a previous study showed the methylation correlated with the prognosis, according to the authors' description. I recommend them to study the subtype of CRC that is correlated with the prognosis. Also, the methodology may have problem. Alteration of the method will save the data of this vigorous study. Line 29, page 2 It's not appropriate to stress China data, if the journal is international.

Reply: Your comments are much appreciated. "a previous study showed the methylation correlated with the prognosis, according to the authors' description "should refer to the fact that some of the combinations of gene methylation we describe were shown to correlate with CRC prognosis and were approved by the Food and Drug Administration as commercially available biomarkers (lines 61-63). In this study, we found that *HSF4*, like most single-gene markers [1-4], has an ordinary diagnostic and prognostic value for its methylation level in colorectal cancer. This is most likely due to the small sample size or insufficient accumulation of methylation in a single gene. This is the essential limitation of single-gene methylation in prognostic studies. As you mentioned, it may be more meaningful to subsequently explore the correlation of *HSF4* methylation with different colorectal cancer subtypes or the combination of *HSF4*-associated gene methylation. Therefore, we describe them as limitations in the discussion.

Furthermore, the finding that HSF4 methylation is not associated with CRC prognosis is discouraging. However, the present study identifies the molecular mechanisms associated with *HSF4* methylation from another perspective. This is of value for subsequent studies on *HSF4* methylation in colorectal cancer. Therefore, this study is not without significance. Hopefully, our viewpoints will be recognized by you.

Also, thank you so much for the reminder. As you say, it is inappropriate to emphasize the Chinese data after mentioning the WHO data. Therefore, we have removed the China data and retained only the WHO data. For more information, please see lines 99-102. Thank you again for your review, which was crucial to the refinement of our manuscript.

[1] Shao C, Dai W, Li H, et al. The relationship between RASSF1A gene promoter methylation and the susceptibility and prognosis of melanoma: A meta-analysis and bioinformatics[J]. PLoS One, 2017, 12(2): e0171676.

[2] Laugsand E A, Brenne S S, Skorpen F. DNA methylation markers detected in blood, stool, urine, and tissue in colorectal cancer: a systematic review of paired samples[J]. Int J Colorectal Dis, 2021, 36(2): 239-251.

[3] Vedeld H M, Nesbakken A, Lothe R A, et al. Re-assessing ZNF331 as a DNA methylation biomarker for colorectal cancer[J]. Clin Epigenetics, 2018, 10: 70.

[4] Gogna P, King W D. The relationship between colorectal cancer risk factors and LINE-1 DNA methylation in healthy colon tissue[J]. Epigenomics, 2020, 12(13): 1087-1093.

Reviewer #4:

Scientific Quality: Grade B (Very good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Minor revision

Specific Comments to Authors: Manuscript title: Association and potential molecular mechanisms between HSF4 methylation and colorectal cancer risk: a bioinformatics study Article type: Original Research Authors: Wenjing Zhang, Kelin Yue, Jingzhai Wang, Yu Zhang Journal: World Journal of Gastrointestinal Oncology Comments and Suggestions for Authors Please see several comments and my observations for improving the manuscript which I consider potentially suitable for World Journal of Gastrointestinal Oncology. In my opinion, the publication of this interesting work is recommended. However, improvements are necessary.

a) The work, which is a complex computational study, is detailed, well conducted, and well organized. The experimental design is strong, given the numerous methodologies/bioinformatic analyses applied. An undoubted limitation is the lack of experimental validation of the data being obtained in silico on HSF4. Methods and results are well written and described. Figures are highly explicative and clear. The discussion supports the main study findings.

Reply: Thank you very much for your affirmation, it encourages the motivation of our research. As you mentioned, an undoubted limitation is the lack of experimental validation of the data being obtained in silico on HSF4. Therefore, we have included that element as one of the flaws of this study in the discussion section. For more information, please see lines 306-311. Thanks again for your comment, it's vital to the improvement of our articles.

b) Abstract (but also discussion), “hub gene” should be plural

Reply: Thank you very much for the reminder, we have checked the full text for relevant content and made corrections. Please see lines 70, 244, 275, 300, 358 for more information. In addition, our manuscripts will be edited by professional native

English speakers to ensure accuracy and clarity of language. Looking forward to your re-review.

c) More recently published supporting references should be included on CRC and methylation defects, as example: 1. https://clinicalepigeneticsjournal.biomedcentral.com/articles/10.1186/s13148-023-01518-5 2. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6266092/ 3. https://clinicalepigeneticsjournal.biomedcentral.com/articles/10.1186/s13148-023-01516-7 4. https://pubmed.ncbi.nlm.nih.gov/33762255/ 5. https://pubmed.ncbi.nlm.nih.gov/37400791/ 6. https://www.nature.com/articles/s41598-023-35631-5

Reply: Thank you very much for your advice. This literature has opened our horizons. With reference to your suggestion, we have conducted a description of the research flaws and the corresponding literature was cited in the discussion section. For more information, please see lines 302-307.

d) Acronyms such as, FDA etc..WHO should be explained for non-expert readers. Please check the work for the presence of additional, unexplained, abbreviations.

Reply: Thank you so much for the reminder. We have checked the full text and fully described the first occurrence of abbreviations.

e) Introduction. “DNA methylation is a process of …… information and is widely applied in cancer prediction and diagnosis [8,9].” I recommend including these two additional references on DNA methylation and cancer as a support (https://jamanetwork.com/journals/jamadermatology/article-abstract/2524840 and https://www.frontiersin.org/articles/10.3389/fgene.2019.01150/full)

Reply: Thank you very much for your advice, which is crucial to the refinement of our manuscript. These studies have allowed us to learn more about tumor-associated methylation. Therefore, we have made the appropriate citations. Please see lines 109-110 for more details.

f) Limitations and conclusions should be reorganized in order to improve the reading/quality of the discussion. Limitations should be moved from the conclusive paragraph and placed before conclusions. Conclusions should instead improve by giving a succinct description of the main study findings and future applications. Moreover, authors are also encouraged to include more details on the validation experiment that can be conducted.

Reply: Thank you very much for your valuable comments. Referring to your suggestion, we separated the limitations and conclusions, and made an order adjustment. For more information, please see lines 302-326.

g) Methods, section2.3 Please include this additional, recently published, reference for the PPI network and the use of Cytoscape PMID: 37436928 h) GO enrichment, have the GO analyses for cellular components and biological function been performed?

Reply: Thank you very much for your comment. In fact, we have previously cited the latest literature from the makers of the Cytoscape software. For more information, please see line 184. In addition, previous GO enrichment analyses have included cellular components and biological function. Please see Figure 6D for more information. Thanks again for all your comments, they are very meaningful.