Author’s response to reviews

Title: Association between ERCC1 and TS expression levels and disease free survival in colorectal cancer patients in response to oxaliplatin and fluorouracil (5-FU) adjuvant chemotherapy

Authors:

Sheng Li (shengli34t@hotmail.com)
Liangjun Zhu (LiangjunZhu5@163.com)
Li Yao (LiYao5h@163.com)
Lei Xia (leixia4h@163.com)
Liangxi Pan (LiangxiPan3@163.com)

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Author’s response to reviews: see over
Dear Dr. Tonilynn Manibo,

Manuscript ID 7583331891188427 entitled "Association between ERCC1 and TS mRNA levels and disease free survival in colorectal cancer patients receiving oxaliplatin and fluorouracil (5-FU) adjuvant chemotherapy" has been revised. The authors would like to thank Reviewers for their careful review of our manuscript and providing us with their comments and suggestion to improve the quality of the manuscript. The point-by-point responses to these comments are listed below. We would like to re-submit this revised manuscript to “bmc gastroenterology” and hope it is acceptable for publication in the journal. Please do not hesitate to contact us with additional questions.

Our response to Editorial Board Comments:

Reviewer: 1

Comment 1: How many patients with CRC were seen during the 5 years or this study? The authors have not responded to this question.
Response: Thanks for your kind suggestion. We were sorry for misunderstanding your meaning. A total of 390 patients with CRC were treated by surgery during the five years. Among them, there were 202 patients with stage II - III CRC. After excluding the patients who did not treated by chemotherapy after surgery, 115 patients were remained. Finally, total 112 patients were investigated in this study after excluding three patients in whom the mRNA levels of TS and ERCC1 was not successfully detected.

Comment 2: The authors have also not responded to power calculation. This is particularly pertinent in a negative study where there may not have been sufficient power to detect a difference. This is important to include.
Response: Thanks for your careful review. The power calculation has been performed using PS Power and Sample Size Calculation, version 3.0.43 (Vanderbilt University, Nashville, TN, USA). The results ($\alpha = 0.05$; TS: power $1 - \beta = 0.511$; ERCC1: power $1 - \beta = 0.656$) showed that the sample size was small for reliably accessing the association between TS or ERCC1 expression and DFS. We have considered this limitation in the discussion.

Comment 3: I had suggested replacing the terms “chemotherapy 1” with “FOLFOX” “chemotherapy 3” with “oral XELOX” etc. This has not been done and the authors continue to use the terms chemotherapy regimen 1, regimen 2 etc. It would be preferable to have the labels be descriptive and informative.
Response: Thanks for you kind suggestion. We were sorry for misunderstanding your meaning. We have revised the terms in the current manuscript as follows: “chemotherapy 1” to “standard FOLFOX-4”, “chemotherapy 2” to “modified FOLFOX”, “chemotherapy 3” to “oral XELOX” and “chemotherapy 4” to
“Conventional intravenous drip infusion”.

**Comment 4:** If the mRNA expression was not significant on univariate analysis, why was it included in the multivariate model?

**Response:** Thanks for you kind suggestion. Although there was no significant on the univariate analysis in this study, the mRNA expressions of TS and ERCC1 were found to have significant association with the survival of patients treated by chemotherapy in other relevant studies. Hence, we further analyzed it by multivariate model.

**Comment 5:** They haven’t answered the question on quantification of TS and ERCC1 expression. Are there biologically relevant thresholds? Was a spline analysis performed?

**Response:** Thanks for you kind suggestion. We have described the quantification method of TS and ERCC1 expression in the manuscript. The comparative Ct method using β-actin as an endogenous control was used to quantify the relative expression of TS and ERCC1.

**Comment 6:** The authors have not clarified the terms in the multivariate model. What does “Stage” refer to? What is the reference category? What does “tumor location” refer to?

**Response:** Thanks for you kind suggestion. We have clarified the terms in the multivariate model in the revised manuscript. The reference category for each categorical variable has been provided in the results of multivariate analysis and Table 2.

**Comment 7:** There continue to be significant language and grammar issues. Please do consider consulting a professional scientific English writing service to improve the readability of this work.

Page 3: “The ERCC1 (encodes excision cross-complementing 1) gene is coding for a nucleotide excision.” Should be “ERCC1 gene codes for...”

Page 4: “colorectal cancer patients who were treated at Jiangsu tumor hospital” should be “...who were treated”.

Page 6 “The mRNA expression levels of TS and ERCC1 in 112 samples was all detected” this sentence is difficult to interpret. Please clarify what this means.

Page 8 “Many previous studies intimated that ERCC1 and TS related with the tumor response to the oxaliplatin or 5-FU-based adjuvant chemotherapy” this sentence is poorly written and needs to be changed.

Page 8 “Shirota Y et al” normally we don’t list the initial when using the phrase et al.

Page 8 “In this study, the patients were at the stage II-III of colorectal cancer. However, the patients in the study of Shirota Y et al were at the stage IV” there should be no “the” preceding stage.

Page 8 “The mRNA expression levels of ERCC1 and TS may be varied based on different stage of cancer.” should be “may vary”

Page 9 “was not proved to associate...” is not accurate phrasing.
Page 10 “the primary tumors were not counted as events in the DFS analysis” they normally are not counted as events. So this is not a limitation.

Response: Thanks for you kind suggestion. We have revised the manuscript according to your suggestion. Meanwhile, we seek help from coworkers who have better English to revise the language and grammar issues of this manuscript.

We appreciate very much for your time in editing our manuscript and the referees for their valuable suggestions and comments. We are looking forward to hearing from your final decision when it is made.

With kindest regards,
Sheng Li