July 23, 2022

Editorial Office

World Journal of Gastrointestinal Oncology

RE: Transcriptional factor III A promotes colorectal cancer progression by upregulating cystatin A (Manuscript NO: 77566)

Dear Editor:

We thank you and the reviewers for your careful review and the constructive comments. Following the editors and reviewers' comments, we modified some inappropriate elaboration in the previous manuscript and added some relevant contents in the revised manuscript. Meanwhile, we checked and revised the grammar and spelling mistakes in the manuscript. The revised sections are marked in red in the revised manuscript, and the detailed revisions are given in our Point-by-Point Response. We hope our revisions meet the approval of the reviewers.

We are now submitting the revised manuscript. Please contact us if you have any questions.

We look forward to hearing from you.

Sincerely yours,

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Hunan Cancer Hospital & The affiliated Cancer Hospital of Xiangya School of Medicine, Central South

Point-by-point Response

Reviewer 1:

The researchers in this study identified the role of GTF3A, an RNA polymerase III transcriptional factor, in promoting progression of colorectal cancer by upregulating Cystatin. The work is well conducted with appropriate controls and I recommend the paper could be published in the "World Journal of Gastrointestinal Oncology" after the authors address the major and minor points. Addressing the comments will improve the quality of the manuscript and the impact of this research work.

Response: We thank the reviewer's positive comments and constructive comments

Issue Major points: 1. Abstract: It is written as "Human tissue microarrays containing 90 pairs of CRC tissues and adjacent non tumor tissues, and human tissue microarrays containing 20 pairs of CRC tissues, corresponding adjacent non tumor tissues and lymph node tissue" … why the authors have not written "Human tissue microarrays containing 110 pairs of CRC tissues and adjacent non tumor tissues and lymph node tissue". This was confusing to me. Then I checked the M&M and it was somewhat clear. But then in the results section it was mentioned only 90 pairs so again I got confused. The authors need to clarify this and make it clear. Also, the authors have not elaborated the detailed source of these two sources. The authors should be more explicit in providing the details for better clarity.

Response: Thanks! This is a good suggestion. In this study, we detected two sets of CRC human tissue microarrays, one (HCol-Ade180Sur-08) contains 90 pairs of CRC tissues and adjacent tissues, other one (HCol-Ade060Lym-01) has 20 pairs of CRC tissues, adjacent tissues, and lymph node tissue. At first，we tested GTF3A expression of HCol-Ade180Sur-08 microarray (90 pairs of CRC tissues and adjacent tissues) with immunohistochemistry (IHC), and found that CRC cancers had a higher expression than adjacent tissues. To further probe whether metastatic cancers had a higher, and analyze the

association of GTF3A expression with metastasis, we tested HCol-Ade060Lym-01 microarray containing CRC cancer, metastatic tissues, and adjacent tissues. The raw data were carefully checked and analyzed again, the new results were shown in the revised Figure 1B. In these two experiments, 8 of 110 tissue sections in CRC cancer group were chipped off and could not be used, and 4 of 110 adjacent tissues were chipped off and could not be used. After being stained with IHC, the useful samples (102 cases in the cancer group, 106 cases in adjacent the group and 20 cases in metastatic group) were together calculated by gray scanning and scored，and survival time and the survival curve were analyzed. These were stated Page 14 of the revised manuscript.

Issue 2. The expression of GTF3A in HCT116 cells in Fig. 2A is very low (hardly a band is visible) but again expression is observed in Fig. 2B(b). Why such a discrepancy and why did the authors choose this cell line for knockdown experiments? I understand the selection of SW480 cells but not clear about the selection of HCT116 cells. Why was DLD-1 cells not chosen for this experiment, at least they had some basal expression (Fig. 2A)?

Response: Thanks! This is a good question. In the experiments (Fig. 2A) that GTF3A expressions in CRC cell lines including HCT116, SW480, DLD-1, SW620, and HT-29 were detected with Western blotting, 20 μg protein of the samples was used in Western blotting for GTF3A overexpression SW480 good-looking, GTF3A band of HCT116 cells looks like weak. In Fig. 2B(b), 60μg protein of HCT116 and its knockdown cell lines samples was used in western blotting for relative low GTF3A SW480 visible, GTF3A bands display strong .

Actually, all of HCT116, SW480, DLD-1, SW620, and HT-29 had been used to knockdown GTF3A, only in HCT116 and SW480 cells, the shGTF3A expression stable cell lines were gained. We got HCT116-shcramble, -shGTF3A#1, and -shGTF3A#1, and SW480-shcramble, -shGTF3A#1, and -shGTF3A#1. HCT116 had a relative low GTF3A expression, while SW480

had a relative high GTF3A, so we used these two cell lines to investigate the functions and mechanisms of GTF3A in CRC.

Issue 3. In Fig. 5A and discussion section, Vimentin is mentioned but the blot is not shown. Please add.

Response: Thanks! This is a good question. The vimentin expression had no difference between GTF3A knockdown and not, such as the vimentin in HCT116-shcramble had not been different with HCT116-shGTF3A#1 or HCT116-shGTF3A#1, SW480-shcramble had no different vimentin compared with SW480-shGTF3A#1, and SW480-shGTF3A#1. And when knockdown CSTA, the vimentin expression had also no difference. So, vimentin had not been focused in this manuscript. The vimentin was deleted in the revised manuscript.

Issue 4. The manuscript has many typographical errors. Units have sometimes space and sometimes not. No uniformity. No space before reference at many places. Spelling mistakes in GTF3A name itself, cell lines names and other words at many places. The authors are seriously requested to look into this aspect thoroughly.

Response: We think the reviewer for comment and suggestion. We had carefully checked and revised the manuscript, and asked for the native language company (Editage English Language Editing Company) to modify. The manuscript had thoroughly been revised.

Comment 5. At few places, English needs to be improved (especially the titles of results section) for clarity and understanding (few are suggested above). The company that provided English language certificate has not done an excellent job.

Response: We think the reviewer for this suggestion. The manuscript had again been modified by the native language company (Editage English Language Editing Company). The revised manuscript should meet the Journal approval.

Minor points: 1. Expand “Csta gene” in abstract 2. Abstract: “were examined

for the GTF3A expression" instead of "were examined the GTF3A expression" 3. Abstract: "Functionally, knockdown of the Gf3a gene". The gene name is misspelled. It should have been "Gtf3a". 4. Abstract: "GTF3A might upregulate the expression cystatin A (CSTA)". "of" is missing in the sentence and Cystatin A abbreviation expansion should have been earlier in the abstract and it should be in italics in this sentence. 5. "Progress" cannot be a keyword. Please delete.

Response: Thanks! 1. "Csta gene" was expanded in the revised abstract; 2. In the revised abstract, "were examined the GTF3A expression" had been instead for "were examined for the GTF3A expression". 3. "Gf3a gene" had been revised as "Gtf3a" in the revised manuscript. 4. In the revised Abstract, "of" had been added, and CSTA had been explanted as Cystatin A. 5. "Progress" in keyword was deleted. All these are shown in Page 3, 4 of the revised manuscript.

Issue 6. The coretip last sentence should have been the abstract last sentence.

Response: Thanks! It was corrected at Line 84 in Page 4 of the revised manuscript.

Issue 7. Please write "5S rRNA" instead of "5SrRNA" wherever applicable in the manuscript.

Response: Thanks! "5SrRNA" had been revised as "5S rRNA" in the revised manuscript.

Issue 8. Please write this sentence as suggested here. "GTF3A gene is present in all the organisms. Human …"

Response: Thanks! It had been changed in at Line 98 in Page 5 of the revised manuscript the revised manuscript.

Issue 9. Expand "RNP".

Response: Thanks! "RNP" had been explanted at Line 102 in Page 5 of the revised manuscript the revised manuscript.

Issue 10. Please write this sentence as suggested here. "and the complex functions as a NES to transfer" instead of "and the complex functions as a

nuclear export signal to transfer" as NES is already abbreviated earlier in the introduction.

Response: Thanks! It had been revised at Line 103 in Page 5 of the revised manuscript.

Issue 11. Please rewrite this sentence as English is NOT correct and typographical error is present. "So far, several studies suggested that the 5S rRNA bound with L5 and L11 to form the 5S RNP complex, further regulating the MDM2 p53 checkpoint[9-12]."

Response: Thanks! It had been revised at Line 104 in Page 5 of the revised manuscript .

Issue 12. Please rewrite this sentence as English is NOT correct. "Other cysteine protease inhibitors, cystatin SN (CST1) and cystatin S (CST4) are type 2 cystatin proteins; they the enhance the metastasis of various malignant tumors and contribute to the poor survival of patients [20, 21].". The authors can write as "Other cysteine protease inhibitors, cystatin SN (CST1) and cystatin S (CST4), are type 2 cystatin proteins, which enhance the metastasis of various malignant tumors and contribute to the poor survival of patients [20, 21]."

Response: Thanks! It had been revised at Line 118 in Page 6 of the revised manuscript.

Issue 13. Please rewrite this sentence as English is NOT correct. "In the present study, we showed that GTF3A was highly expressed in CRC, and that GTF3A bound to the promoter of Csta to facilitate Csta transcription, which regulates EMT markers and promotes CRC progression.". The authors can write as "In the present study, we showed that GTF3A was highly expressed in CRC, and it bound to the promoter of Csta to facilitate Csta transcription, which then regulated EMT marker expression and promoted CRC progression."

Response: Thanks! The above sentences were revised in the revised manuscript.

Issue 14. "The knockdown efficiency was determined using RT-qPCR" instead of "The knockdown efficiency was filtered using RT-qPCR".

Response: Thanks! It had been revised at Line 174 in Page 8 of the revised manuscript.

Issue 15. "The lentivirus titers were quantified" instead of "The lentivirus titers were qualified".

Response: Thanks! It had been revised at Line 177 in Page 8 of the revised manuscript.

Issue 16. "Following the manufacturer's instructions, the crude lysate was centrifuged and the supernatant was collected to measure the protein concentration using the BCA Protein Assay Kit (CWBIO, Beijing, China)." instead of "Following the manufacturer's instructions, the cell lysates were obtained by centrifugation and the protein concentration was measured using the BCA Protein Assay Kit (CWBIO, Beijing, China).".

Response: Thanks! It was revised at Line 189 in Page 9 of the revised manuscript**..**

Issue 17. "moving the detached cells". Incorrect English. Please rephrase.

Response: Thanks! It was revised at Line 225 in Page 11 of the revised manuscript..

Issue 18. "enriched longRNA (> 200 nt) was interrupted". Incorrect English. Please rephrase.

Response: Thanks! It was rephrased at Line 239 in Page 11 of the revised manuscript..

Issue 19. "To determine the expression of GTF3A in CRC tissues," instead of "To clarify the expression of GTF3A in CRC tissues,"

Response: Thanks! It was changed at Line 285 in Page 13 of the revised manuscript..

Issue 20. Rephrase the title "Knockdown Gtf3a gene inhibiting CRC cell proliferation" and "Knockdown Gtf3a inhibiting CRC cell motility and invasion" and "GTF3A protein regulating CSTA by binding to the CSTA

promoter" and "GTF3A mediating the CRC cell EMT by through regulating the expression of CSTA" and "GTF3A promoting CRC cell growth in vivo". Incorrect English in all these titles in results section. Please rephrase.

Response: Thanks! The above sentences were rephrased at Line 301 in Page 14, Line 319 in Page 15, Line 328 in Page 15, Line 351 in Page 16, and Line 313 in Page 17 of the revised manuscript.

Issue 21. Please correct the spelling of HCT116 cells in "Gtf3a knockdown SW480 and HC116 cells".

Response: Thanks! It was corrected at Line 320 in Page 15 of the revised manuscript..

Issue 22. Please correct GTF3A instead of GTF3 in this sentence "Next, a dual luciferase assay was carried out to determine whether the interaction of GTF3 with the Csta promoter increase".

Response: Thanks! It was corrected at Line 346 in Page 16 of the revised manuscript..

Issue 23. Please correct "Gtf3a" in this sentence "Both RNA-Seq and RT-qPCR showed that Csta expression was dramatically decreased in Gf3a knockdown cells".

Response: Thanks! It was revised at Line 390 in Page 18 of the revised manuscript..

Issue 24. Conclusion: "increased in the expression of CSTA, enhanced the EMT process" instead of "increased in the expression of CSTA enhanced the EMT process", Please add "," after CSTA in this sentence.

Response: Thanks! It was revised at Line 406 in Page 19 of the revised manuscript..

Issue 25. Please write "Western blotting" instead of "Western-blotting" throughout the manuscript.

Response: Thanks! All of "Western-blotting" were changed as "Western blotting" in the revised manuscript.

Issue 26. "The fluorescence staining of the GTF3A and Csta promoters were

colocalized to a large extent as indicated" instead of "The fluorescence locations of the GTF3A and Csta promoters were approximately coincident, and indicated". ¬ Note: The authors should give page numbers and line numbers in the manuscript.

Response: Thanks! "The fluorescence locations of the GTF3A and Csta promoters were approximately coincident, and indicated" was revised at Line 341 in Page 16 of the revised manuscript.. The page numbers and line numbers were added in the revised manuscript.

Reviewer #2:

Issue: Title reflects the study Abstract is well written Core tip summarizes the study very well Introduction summarizes the study very well/ Materials and methods section: each porcedure is very well explained. the authors should give more detail about the vector for I believe the vector has two reporter Results: The expression of GTF3A was higher in CRC tissues and lymph node metastatic tissues than in adjacent normal tissues. GTF3A was associated with CRC prognosis. Functionally, knockdown of the Gf3a gene impaired the CRC cell proliferation, invasion and motility in vitro and in vivo. Moreover, RNA-Seq analysis revealed that GTF3A might upregulate the expression cystatin A (CSTA), while the luciferase activity assay showed that GTF3A bound to the promoter of Csta gene and increased the Csta transcription. Furthermore, CSTA regulated the expression of epithelial-mesenchymal transition (EMT) markers. Discussion is well written References are up to date.

Response: Thanks! We are appreciated with the reviewer's positive comments. The information about vectors had been added in detail at Line 116 in Page 8 of the revised manuscript. The references were up to date in the revised manuscript.