Cover Letter

Dear Editors and Reviewers,

Thank you very much for your efficient work and thank you for your letter and comments. Accordingly, we have revised the manuscript entitled *"* **A novel long non-coding RNA LINC02532 promotes gastric cancer cell proliferation, migration, and invasion *in vitro." (Manuscript NO: 43472)***,* and would like to resubmit it for your consideration. We have addressed the instructive comments raised by the reviewers, and the amendments are highlighted in red in the revised manuscript. Point by point responses to the reviewers' comments are listed for your consideration. We would like to express our sincere thanks to the editors and reviewers for the constructive and meaningful comments.

We are so grateful that you have offered us this opportunity to resubmit our manuscript. We hope that the revised version of the manuscript is now acceptable for publication in your journal.

Please address correspondence to: Dong-Qiu Dai, Professor, Chief Physician. Department of Gastroenterological Surgery, the Fourth Affiliated Hospital of China Medical University, Shenyang 110032, China. E-mail: daidq63@163.com.

We shall look forward to hearing from you at your earliest convenience.

Yours sincerely,

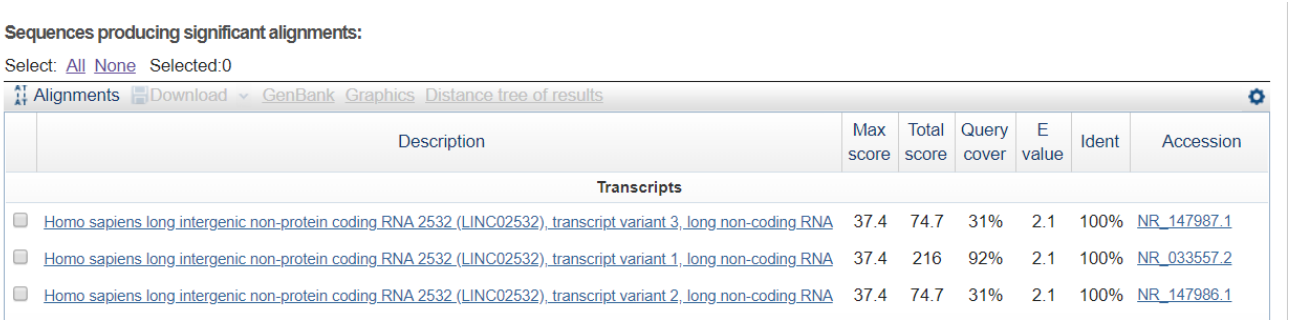
Dong-Qiu Dai

Response to Reviewers

Reviewer # 1 (Number ID: 02545023)

Comment 1: It has been reported that Linc00483, which is upregulated in GC, also functions as ceRNA to promote gastric cancer cell proliferation, invasiveness and metastasis in vitro and in vivo by absorbing endogenous tumor suppressor miR-30a-3p in gastric cancer (Li et al, J Cell Mol Med, 2018). In the siRNA targeting LINC02532 knock-down study, how were the changes, if any, of Linc00483 in these GC cell lines? Was there any compensation involved?

Response 1: Thank you for your meaningful comments. In present study, the primers sequences of siRNAs were specially designed for silencing LINC02532 expression by the GenePharma (Shanghai, China). Furthermore, the specificity of siRNAs sequences was confirmed by the tool of BLASTN program [1], and the results showed that the identity scores for LINC02532 are 100% (See figure below). So, the three siRNAs would not have a knock-down effect on Linc00483. Hopefully, this may address your concerns.



Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Transcripts						
Homo sapiens long intergenic non-protein coding RNA 2532 (LINC02532), transcript variant 3, long non-coding RNA	37.4	74.7	31%	2.1	100%	NR_147987.1
Homo sapiens long intergenic non-protein coding RNA 2532 (LINC02532), transcript variant 1, long non-coding RNA	37.4	216	92%	2.1	100%	NR_033557.2
Homo sapiens long intergenic non-protein coding RNA 2532 (LINC02532), transcript variant 2, long non-coding RNA	37.4	74.7	31%	2.1	100%	NR_147986.1

Comment 2: In "MATERIALS AND METHODS", under the "Wound healing assay" section, it says "The cells continued to be cultured with serum-free medium for 48 h". Please clarify whether it is complete serum-free or low serum, such as 0.5% or 1% FBS.

Response 2: We are so grateful for your meaningful comments. As described in the "Wound healing assay" section, we confirm that the medium used for wound healing assay is complete serum-free. Hopefully, this may address your concerns.

Comment 3: In the "Transwell migration and invasion assays" section, "A total of 2.0 × 104 cells with 200 μL of serum-free medium" should be written as "A total of 2.0 × 10(4) cells with 200 μL of serum-free medium".

Response 3: Thank you for your meaningful comments, and we fully understand and agree with you. We have revised it according to your suggestion (See in the "Transwell migration and invasion assays" section). We hope this may address your concerns.

Comment 4: In the "RESULTS", under "Functional and KEGG pathway enrichment analyses of target genes" section, the sentence "Identifying the function of these target genes can also benefit the efforts targeting the study of the underlying LINC02532 molecular role" seems hard to understand. It may read better to change to "Identifying the function of these target genes may reveal the novel molecular roles of LINC02532 in GC.".

Response 4: Thank you for your meaningful comments, and we fully understand and agree with you. Accordingly, we have revised it (See in the "Functional and KEGG pathway enrichment analyses of target genes" section).

Comment 5: For the authors' affiliation of "Fourth Affliated Hospital of China Medical University", there is a typo for "Affliated", which should be corrected as "Affiliated".

Response 5: We are so sorry for the spelling mistake and we have corrected it. Thank you for your meaningful comments.

Reviewer # 2 (Number ID: 02742218)

Comment 1: In this study the authors have identified LINC02532 was significantly overexpressed in GC. Analysis showed that patients with higher LINC02532 expression had poorer prognosis than those with lower expression. The correlation analysis between expression and clinicopathological features revealed that the high expression of LINC02532 was associated with a high TNM stage and poor differentiation grade. Functional assays supported the finding that LINC02532 promoted GC cells proliferation, migration, and invasion. According to the bioinformatics analysis, LINC02532 may sponge downregulated miR-129-5p and miR-490-5p and participate in transcriptional misregulation in cancer, cell cycle, and TGF-beta, and mTOR and p53 signaling pathways. Overall the study was well designed and proves that LINC02532 acted as an oncogene in GC and may be a promising target for the therapy and prognosis management. However, use of only bioinformatics to analyze the relationship of LIN02532 with miR and then with mTOR or p53 pathways is a limitation, it is suggested if authors can show western blotting data to support their bioinformatics data. Also, authors have commented cell cycle misregulation, a cell cycle analysis in knock-down samples could support this statement.

Response 1: Thank you very much for your meaningful comments. We agree with you that it is not enough only with bioinformatics analysis results. However, the bioinformatics results can provide directions for our further study. KEGG pathway analysis showed that LINC02532 may participate in transcriptional misregulation in cancer, cell cycle, TGF-beta, mTOR and p53 signaling pathways. Considering there are about 5 pathways from the results, we cannot perform experiment one by one in our present study. Besides, the prediction of miRNAs which LINC02532 may sponge and gene functional enrichment analysis are aimed at understanding the potential mechanisms of LINC02532. We will pay more attention to these bioinformatics results in future. We are fully appreciative for your understanding and approval to our study. Hopefully, this may address your concerns.

Reference:

1 Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 1997; 25(17): 3389-3402.