

## ANSWERING REVIEWERS



Apr 02, 2015

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 16747-Edited-revised.doc).

**Title:** Linc00675 is a novel prognostic factor for poor survival and short-term recurrence in patients with pancreatic ductal adenocarcinoma

**Author:** Doudou Li, Zhiqiang Fu, Qing Lin, Yu Zhou, Quanbo Zhou, Zhihua Li, Langping Tan, Rufu Chen, Yimin Liu

**Name of Journal:** *World Journal of Gastroenterology*

**ESPS Manuscript NO:** 16747

The manuscript has been improved according to the suggestions of reviewers:

Reviewer #1:

Comments of reviewer #1:

There are several points that need to be addressed:

Abstract:

Poor grammar and spelling mistakes. The actual fold up-regulation and significance (p-value) should be mentioned in the abstract. Non-descript use of adverbs such as “extremely” to describe the degree of up-regulation in a scientific paper should be avoided at all costs. There is also inconsistent italicization of P when used to indicate P-values.

Results:

The microarray analysis is very underpowered, with only 4 controls and 8 PDAC, with no mention of p-values or correction for false discovery rate. The authors should provide a list of all p-values, including FDR correction and fold-difference in expression. Again, general phrases such as “much obvious expression difference” and “obviously upregulated” should be avoided at all costs in scientific writing, and replaced with fold-change followed by p-value (ideally FDR-corrected).

When reporting the upregulation shown in Figures 1B and C, the authors should state that this is rtPCR, and consider presenting the results in a format other than delta ct values. P-values should also be reported.

The results of the in vitro knockdown assays are fine, however, the authors need to refer to Figures 3C and D when reporting the flow cytometry data on page 10. I do think it's worth pointing out that overall all the figures are very well done.

Discussion:

Again there are several grammatical errors and use of inappropriate general language such as “was extremely higher”, p.13. Although the authors state the functional studies are limited, they do not speculate about any potential mechanistic role for the linc00675 given

that they show its downregulation inhibits proliferation, induces cell cycle arrest and inhibits E-cadherin and Vimentin.

Although the authors allude to a connection with gemcitabine resistance, there is no mention of how this discovery could actually be used to improve patient outcomes. First, since the assay requires tissue (the authors do not mention the possibility of serum), a patient must first be diagnosed, and a biopsy taken either at the time of surgery or by endoscopic ultrasound, before levels can be determined. Then, assuming the level is high, is knockdown in a patient a logical translation from the in vitro experiments? Or would knowing that a high level correlates with poor prognosis be used to modify the patient's existing treatment path. Clarification around these points would be valuable from a lay clinical perspective.

Responses-----

1) Abstract: Poor grammar and spelling mistakes. The actual fold up-regulation and significance (p-value) should be mentioned in the abstract. Non-descript use of adverbs such as “extremely” to describe the degree of up-regulation in a scientific paper should be avoided at all costs. There is also inconsistent italicization of P when used to indicate P-values.

**Response:** The grammar errors and spelling mistakes language have been corrected. The actual fold up-regulation and significance (p-value) have been added to the abstract. The style of P has been consistent. The language of abstract has also been re-checked and improved.

2) Results: The microarray analysis is very underpowered, with only 4 controls and 8 PDAC, with no mention of p-values or correction for false discovery rate. The authors should provide a list of all p-values, including FDR correction and fold-difference in expression. Again, general phrases such as “much obvious expression difference” and “obviously upregulated” should be avoided at all costs in scientific writing, and replaced with fold-change followed by p-value (ideally FDR-corrected).

**Response:** Many thanks to you for finding this mistake. Because of the high cost of the microarray analysis, Commonly used for preliminary screening. Thus, We select a few of chips to use microarray analysis are common. We found that Linc00675 is 672 folds up-regulated in PDAC tissues compared with CP tissues through microarray screening in PDAC tissues compared with CP tissues through microarray screening( $p=3.69 \times 10^{-5}$ ). According to the expression of Linc00675 , we use 90 paired cases of clinical samples to

certificate. We provide a list of all p-values, including FDR correction and fold-difference in expression of lincRNAs through microarray screening (supplemental data). We also utilize appropriate express words to describe fold-change followed by p-value. The Results are authentic.

3) When reporting the upregulation shown in Figures 1B and C, the authors should state that this is rtPCR, and consider presenting the results in a format other than delta ct values. P-values should also be reported.

**Response:** We thank you for bringing this to our attention. We have added explanations that this is RT-PCR. Besides, we add distribution of linc00675 expression from the 25th to 75th percentiles of all patient samplesm with the horizontal lines as describe the results, and P-values also be presented.( figure 1B、figure 1C in the revised manuscript)

4) Results: The results of the in vitro knockdown assays are fine, however, the authors need to refer to Figures 3C and D when reporting the flow cytometry data on page 10. I do think it's worth pointing out that overall all the figures are very well done.

**Response:** Thanks for helping to revise this. The results about Figure3c and 3d has been added. We cite the figures 3C and D when reporting the flow cytometry data.

5) Discussion:

Again there are several grammatical errors and use of inappropriate general language such as “was extremely higher”, p.13. Although the authors state the functional studies are limited, they do not speculate about any potential mechanistic role for the linc00675 given that they show its downregulation inhibits proliferation, induces cell cycle arrest and inhibits E-cadherin and Vimentin.

**Response:** We thank you for bringing this to our attention. We have corrected several grammar and spelling errors, as well as more accurate expression in the Discussion part. The limitation of this article is lack of depth in mechanism research. But, The main subject of this article is to determine whether linc00675 has an independent prognostic value in PDAC, Our number of cases and statistical results make a authentic results. As the linc00675, was elevated in PDAC tissues reported at the first time, few of the mechanisms can be exploited. Because of the complicated mechanism of lincRNA, we should not disorderly conjecture the mechanism of Linc00675 On the basis of no experimental evidence. Thus, we add some lincRNA content for the discussion of a variety of mechanisms which may response for the reaction of Linc00675. Besides, further study should be conducted to explore the deeply mechanisms of Linc00675. We are looking forward to submitting further research to your journal.

#### 6) Discussion:

Although the authors allude to a connection with gemcitabine resistance, there is no mention of how this discovery could actually be used to improve patient outcomes. First, since the assay requires tissue (the authors do not mention the possibility of serum), a patient must first be diagnosed, and a biopsy taken either at the time of surgery or by endoscopic ultrasound, before levels can be determined. Then, assuming the level is high, is knockdown in a patient a logical translation from the in vitro experiments? Or would knowing that a high level correlates with poor prognosis be used to modify the patient's existing treatment path. Clarification around these points would be valuable from a lay clinical perspective.

**Response:** Theoretically, knockdown of an oncogene can achieve anticancer effects. Our in vitro experiments revealed that linc00675 regulates pancreatic cancer cell proliferation, migration, and invasion, suggesting that linc00675 is a potential oncogene. But, we can not conclude that knockdown of linc00675 in a patient is an anti-cancer strategy based the current evidences we acquired in this study. It still need more in vitro experiments and in vivo experiments.

Our results demonstrated that high linc00675 level correlates with poor survival,

suggesting that patients with high linc00675 level might have high risk of recurrence and poor prognosis. Therefore, close follow-up is required for the early detection of recurrence. In addition, besides Gemcitabine, they could be open to trying another treatment strategies.

Reviewer #2:

Comments of reviewer #2:

Major comments

1. As linc00675 knockdown induced S phase arrest, the authors should add the WB of key marker of G1 and S phase in the figure 3.
2. The authors should clarify the criteria or definition for “predicting tumor progression” and “predicting short-term recurrence in PDAC” in the Results, “Linc00675 is a potential biomarker for predicting recurrence in PDAC patients.

Minor comments

1. More information on the molecular mechanism of lincRNA should be added in the Introduction or Discussion.
2. Some English should be corrected.
  - 2.1 Discussion, page 13, first paragraph, line 10, “these subset of patients showed higher recurrence rate ~~that~~ those with decreased linc00675 expression.”
  - 2.2 Discussion, page 14, line 3, “Some tumor suppressor genes and molecules were reported ~~could to~~ increase gemcitabine sensitivity..” or rewrite.
  - 2.3 Discussion, page 14, line 6, “it is interesting ~~and need~~ to explore whether linc00675...”

Responses-----

(1) Major comments: As linc00675 knockdown induced S phase arrest, the authors should add the WB of key marker of G1 and S phase in the figure 3.

**Response:** Thanks to point out this for us. We have add the western blot of key marker of G1 and S phase in the figure 3E.(figure 3E in the revised manuscript)

(2) The authors should clarify the criteria or definition for “predicting tumor progression” and “predicting short-term recurrence in PDAC” in the Results, “Linc00675 is a potential biomarker for predicting recurrence in PDAC patients.

**Response:** Thanks for pointing out this. “Short-term recurrence” is an unclear and ambiguous description, there is no very exact standard for what is short-term and what is long-term recurrence in PDAC patients. Therefore, we removed the “short-term” and changed the title of this article to “Linc00675 is a novel prognostic factor for poor survival and recurrence in patients with pancreatic ductal adenocarcinoma”. Besides, all the related descriptions in the manuscript were revised.

(3) Minor comments:

More information on the molecular mechanism of lincRNA should be added in the Introduction or Discussion

**Response:** Thanks for helping to revise this. We add some lncRNAs content of molecular mechanism in the introduction and discussion part.(red marked sentences)

(4) Some English should be corrected.

1.1 Discussion, page 13, first paragraph, line 10, “these subset of patients showed higher recurrence rate ~~that~~ those with decreased linc00675 expression.”

1.2 Discussion, page 14, line 3, “Some tumor suppressor genes and molecules were reported ~~could to~~ increase gemcitabine sensitivity..” or rewrite.

1.3 Discussion, page 14, line 6, “it is interesting ~~and need~~ to explore whether linc00675...”

**Response:** Thanks for pointing out these mistakes. We correct the sentence above. Besides, we also revise some spelling mistakes. The language of this manuscript has also been re-checked and improved.

**The manuscript has also been improved according to the suggestions of editors:**

1. A running title has been added.
2. Author contributions has been added.
3. A ethic approval document of PDF format has been provided, and statement has been mentioned in the manuscript text.
4. Institutional animal care and use committee and Animal care and use statement

Response: We did not conduct any animal experiment in this study, therefore, we think we might not need provide the “Institutional animal care and use committee” and “Animal care and use statement”.

5. Data sharing statement of PDF format has been provided.
6. Key words has been provided.
7. Core tip less than 100 words has been provided.
8. The format of reference numbers has been revised.
9. A revised statement of “Statistical analysis” was provided.
10. A “COMMENTS” including section of background, research frontiers, innovations and breakthroughs, applications, and peer review was provided.
11. All authors abbreviation names and manuscript title have been listed in the last of this manuscript.
12. Decomposable figures were provided.

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,



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