Author’s response to reviews

Title: Altered expression of DLG1-AS1 distinguished papillary thyroid carcinoma from benign thyroid nodules

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Version: 1 Date: 10 Sep 2019

Author’s response to reviews:

Dear Editor, Dear reviewers,

Thank you for your letter. We thank your and reviewers for the time and effort that you have put into reviewing the previous version of the manuscript. Your suggestions have enabled us to improve our work. Based on the instructions provided in your letter, we uploaded the file of the revised manuscript. Accordingly, we have uploaded a copy of the original manuscript with all the changes highlighted by using the track changes mode in MS Word.

Appended to this letter is our point-by-point response to the comments raised by the reviewers.

Reviewer reports:
Klaus Brusgaard, PhD (Reviewer 1): The manuscript is interesting and relevant to the scientific community but needs some editing.
I the introduction the relevance of DLG1-AS1 is stated. The relevance of analysing miR-199a-3p should as well be explained.
Response: We added-
“MiR-199a-3p is a well-characterized tumor suppressive miRNA in cancer biology [12-14]. We observed the inverse correlation between DLG1-AS1 and miR-199a-3p from our preliminary bioinformatics analysis. This study aimed to explore the roles of DLG1-AS1 and miR-199a-3p in PTC and analyze the clinical values of DLG1-AS1 for this disease.”

p.4;l.72: Your write "IHH-4 and MDA-T32 (ATCC, USA) two human PTC cell lines were included" please state why - something like "positive controls of...." Response: We just wanted to repeat the in vitro cell experiments in two different cell lines to obtain more confident data.

p.4;l.74: you write "Negative control (NC) miRNA and miR-199a-3p were from RIBOBIO77 (Guangzhou, China)." could be "Scramble microRNA mimics were designed as negative control microRNA (NC) and miR-199a-3p were both from RIBOBIO77 (Guangzhou, China)." Response: Revised. Thank you.

p.4;l.80: transections should be transfections Response: Revised. Thank you.

p.6;l.110 What you perform are "ROC curve analysis" not "ROC curve"
Response: Revised. Thank you.

p.8;l.158: You write "in this study the expression of DLG1-158 AS1 has only been investigated in tumor" I think it should be "in that study the expression of DLG1-158 AS1 has only been investigated in tumor"
Response: Revised. Thank you.

p.8;l162-163: You write "Therefore, DLG1-AS1 may also regulate gene expression in 163 distant end after trafficking in blood" I think "Therefore, DLG1-AS1 may also regulate gene expression in 163 distant targets after trafficking in blood" would be better.
Response: Revised. Thank you.

p.8;l.170-171: You write "We future studies will include more..." better write "We will in future studies include more...."
Response: Revised. Thank you.

Martyna Borowczyk (Reviewer 2): The authors of the paper describe new methods of differentiating between benign and malignant thyroid nodules, which is an issue under high interest. Their data are unique - as they represent an analysis of circulating DLG1-AS1, which as far has been investigated only in tumor and adjacent non-tumor tissues. The group is considerably large, includes healthy controls, the methodology of genetic experiments is well written, and statistics is well calculated and convincing. The results are supported by the ROC curve with very high AUC. However, a few points should be corrected or clarified by authors.

In the Abstract section, authors fail to present any description of methodology - it should be rewritten.
Response: We added-“In this study DLG1-AS1 and miR-199a-3p in plasma of both PTC patients and BTN patients were detected by qPCR. ROC curve analysis was performed for diagnostic analysis. Overexpression experiments were performed to analyze the interaction between DLG1-AS1 and miR-199a-3p. CCK-8 assay was performed to analyze cell proliferation.”

The introduction section and the beginning of the Methodology section lag behind the rest of the paper - it is too general and contains linguistic errors - please, correct it. There is a lack of thyroid cancer genetic background brief description and a description of the role of lncRNA and authors’ explanation of a decision to study only one, this particular lncRNA.
Response: We added more information.

At the beginning of the Methodology section - the inclusion and exclusion criteria are too general and
misleading. "No therapies carried out" was described as one of the inclusion criteria. However, it may be confounding if beforementioned is information about patients' surgical treatment. Those criteria should be much more precise. The time from the diagnosis, pTNM of patient's tumors and preferably histopathological details with subtypes of papillary thyroid cancer or other information should be included in this section, preferably with detailed Table showing patients' clinicopathological data. If the group was heterogeneous, it might have biased the results.

Response: We changed to “2) no therapies were initiated before the admission of patients.”

We only included patients at AJCC stage I and II. AJCC stages can reflect TNM stages. For some information we are not confident with we cannot present.

Methods of DNA extraction not described.

Response: We did not include DNA extraction in this paper.

The role of the expression pattern and function of DLG1-AS1 have also been investigated in glioblastoma. In the Discussion section Authors wrongly state that it was studied only in cervical cancer. The phrase needs to be corrected.

Response: We added- “The expression pattern and function of DLG1-AS1 have been investigated in cervical cancer [11].”

Linguistic corrections are needed - e.g., "We future studies will include more patients to further analyze the accuracy".

Response: We carefully revised the whole manuscript.

Additionally, the potential biases of the study are missing.

Response: We added-

“This study only included Han Chinses, which may provide biased results. The values of DLG1-AS1 in the diagnosis of PTC among other populations remain to be further explored. ”

Finally, conclusions are a bit too concise; it is recommended to develop this paragraph.

Response: We added-

“In conclusion, DLG1-AS1 plays oncogenic roles in PTC by downregulating miR-199a-3p to promote cancer cell proliferation. In addition, measurement of the levels of DLG1-AS1 in plasma among the population with high risk of PTC may improve the early diagnosis. ”

We would like also to thank you for allowing us to resubmit a revised copy of the manuscript. We look forward to hearing from you regarding our submission. We would be glad to respond to any further questions and comments that you may have.

Best regards,

Li Yufeng