Author’s response to reviews

Title: microRNA-21 promotes breast cancer proliferation and metastasis by targeting LZTFL1

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Version: 2 Date: 11 Jan 2019

Author’s response to reviews:

Dear Editors,

Thank you very much for providing us an opportunity to revise the manuscript. We appreciate reviewers for their positive comments and constructive critiques. We revised the manuscript and listed our point-to-point response to reviewers’ comments in the rebuttal letter.
We would like to submit this revised manuscript to BMC Cancer, and hope it is acceptable for publication. If you have any question, please do not hesitate to contact me at the address below. Thanks again and I am looking forward to hearing from you soon.

Sincerely,

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Point by Point Response

Reviewer 2:

1. The total number of breast cancer patient’s samples are double the size of controls (as it needs to be equal or more than for better accuracy). Author must explain the reason for taking less number of control samples.

Answer: Thank you for your comment. At the beginning of our study, the size of healthy controls and breast cancer patient’s samples were equal. However, with the progress of our project, we had a chance to get access to more breast cancer patient’s samples and benign breast cancer samples. So the sample size is not equal in the present study. Nevertheless, we will try to collect more healthy control samples.

However, according to our data, plasma miR-21 levels were significant higher in breast cancer patients compared with either healthy controls or benign breast cancer patients. Even though the number of control samples was not equal with the number of breast cancer samples, the data is still reliable and solid to indicate that plasma miRNA21 level is up-regulated in breast cancer.
2. It looks like there is some kind of confusion or typing error due to which source of RNA for human samples is not clear e.g. they collected plasma samples (in materials and methods) but extracted from serum (in RNA extraction and QRT-PCR section)

Answer: We are sorry for the typing error. RNAs were isolated from human plasma samples, but not from serum. We corrected “serum” to “plasma” in the first sentence of Method/RNA extraction and QRT-PCR section in line 17, Page 6.

3. Correlation analysis between miR-21 expression and LZTFL1 expression can also be performed and a graph can be added.

Answer: Thank you for your comment. Since the regulation of miR-21 on LZTFL1 is possibly based on a post-transcriptional mechanism, we analyzed the correlation of miR-21 level and LZTFL1 protein level in this manuscript. We inhibited or overexpressed miR-21 in breast cancer cells, then detected the protein level of LZTFL1 in each corresponding treatment in Fig 3F and Fig 5A-B. The results showed that the miR-21 mRNA level and LZTFL1 protein level were negatively related. However, it is hard to draw a graph indicating the correlation between miR-21 mRNA level and LZTFL1 protein level based on our present western blot data, because we only had two or four groups of treatment in Fig 3F and Fig 5A-B and the data points are not enough to make a correlation analysis.

4. The present submission contains several textual overlaps throughout with other previously published works.

Answer: Thank you for your suggestion. We have made great change to the sentence structure in Abstract and Discussion parts. The details of changes are list below (underline indicates the changed sentence or paragraph):

Abstract/Background part, line 3-8, Page 2: “Breast cancer is one of the most common female cancers. Since MicroRNAs play vital roles in breast cancer progression, this study aimed to explore the clinical value and mechanism of miR-21 in breast cancer diagnosis and development” was changed to “Breast cancer is the most common cancer type in female. As microRNAs play vital role in breast cancer, this study aimed to explore the molecular mechanism and clinical value of miR-21 in breast cancer.”

Background section, line 32, Page 3
paragraph 1: “Breast cancer is one of the most common female cancers, and many patients have recurrences and eventually die due to metastasis.” was changed to “Breast cancer is the most common cancer type in female, and many patients are suffered from recurrences and metastasis”.

“Recently, several studies focusing on the discovery of new biomarkers have demonstrated that miRNAs and their targeted genes are involved in determining the aggressiveness of cancers” was changed to “Recently, several studies indicated the promising role of miRNA in the diagnosis and outcome prediction in several cancers”.

Line 44-49, Page 3, Paragraph 2: “miR-21 is upregulated and promotes metastasis in several cancers (13-20), including one of our previous studies on patients with large B-cell lymphoma among the Chinese population (21).” was changed to “miR-21 is upregulated and promotes metastasis in several cancers (13-20). Our previous study also proved that plasma levels of miR-21 were upregulated in large B-cell lymphoma patients in China (21).”

Line 49-54, Page 3, “The epithelial–mesenchymal transition (EMT) is an important mechanism of tumor metastasis by which epithelial cells lose their cell polarity and cell adhesion ability (22-23).” was changed to “The epithelial–mesenchymal transition (EMT) is a process that epithelial cells lose their cell polarity and cell adhesion ability, which will lead to cancer metastasis (22-23).”

Line 54-59, Page 3, “Recently, researchers have shown that miR-21 is involved in the induction of the EMT process and promotes cancer metastasis (24-31). However, few studies have focused on the association between miR-21 and the EMT, and the essential genes targeted by miR-21 that are involved in the EMT process remain to be identified and further investigated.” was changed to “Although miR-21 was indicated to play a crucial role in the metastasis of lung cancer, ovarian cancer and head and neck cancer though several signaling pathways, the molecular mechanism of how miR-21 regulates the EMT process in breast cancer is not clear (24-31).”

Line 2-17, Page 4, Paragraph 3 was totally changed to “Leucine zipper transcription factor-like 1 (LZTFL1) is one of the key genes which regulate cancer metastasis (32-35). Previous study found that LZTFL1, acting as a tumor suppressor, was down-regulated in gastric and lung cancer (34-35). Mechanically, LZTFL1 was reported to regulate β-catenin signaling which then activated the EMT in several cancers (35). In our study, we will explore the new target gene of miR-21 and investigate the mechanism of miR-21 in regulating breast cancer metastasis, in order to provide new insights and strategies for breast cancer therapy.”

Discussion section

Line 39-59, Page 14, We changed the first paragraph to “Although studies have already revealed the importance of miR-21 as an oncogene, its new target genes, precise molecular mechanisms and clinical potential are still needed further exploration (13-21). In order to confirm the clinical value of miR-21 in breast cancer, we detected the plasma miR-21 levels in several groups of patients. We found that plasma miR-21 levels were significant higher in breast cancer patients’ samples, compared with healthy controls and benign breast cancer patients’ samples. These findings were also approved by other studies (39-40). Moreover, plasma miR-21 levels of breast
cancer patients tended to decline following surgery, and plasma miR-21 levels were correlated with lymph node metastasis and the TNM stage in breast cancer. According to these results, plasma miR-21 could be a promising biomarker in the diagnose and outcome prediction of breast cancer. In this sense, its novel targets and mechanisms involved in breast cancer metastasis need to be discovered.

Paragraph 2:

Line 10-15, Page 15, “LZTFL1, a new target gene of miR-21, was identified by a screening based on four prediction databases and then confirmed by luciferase reporter and western blot assays.” was changed to “In our study, LZTFL1, a new target gene of miR-21, was identified by a screen based on four prediction databases. Then we confirmed the regulation of miR-21 on LZTFL1 by luciferase reporter and western blot assays.”

Line 17-19, Page 15, “Studies have shown that LZTFL1 is significantly downregulated in several tumors, and a low expression level is associated with inferior survival in patients with lung cancer (32–33).” was changed to “Studies have shown that LZTFL1 is significantly downregulated in several type of cancers, which associated with shorter overall survival of patients (32-33).”

Line 20-22, Page 15, “Analysis of the data from the TCGA breast cancer database also suggests that low expression of LZTFL1 predicts a poor outcome.” was changed to “Analysis based on TCGA database also suggests that low expression of LZTFL1 predicts a poor outcome in breast cancer.”

Line 24-27, Page 15, “Therefore, we selected LZTFL1 for further investigation.” was changed to “According to these data, we selected LZTFL1 as novel target of miR-21 for further investigation.”

Line 31-34, Page 15, “Therefore, the ability of miR-21 to promote breast cancer proliferation and metastasis is due, in significant part, to its suppression of LZTFL1.” was changed to “Therefore, the function of miR-21 in promoting breast cancer progress is due, in significant part, to its suppression on LZTFL1.”

Paragraph 3:

Line 36, Page 15, We added “However” in the first sentence.