

## Peer Review File

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### Reviewer A

1. Does the tumor-specific miRNA expression affect the outcome of adjuvant treatment modalities (chemotherapy, or concurrent chemoradiotherapy)?

Does it affect the choice of treatment modalities?

Since the present results have shown correlation with clinical stage, it had better show the usefulness of this extensive microarray study in treatment outcome; otherwise, TNM stage is good enough to explain the major objective proposed by authors. In daily practice, there is no need to do this time-, cost- and labor-consuming microarray analysis if TNM stage is satisfied to predict the prognosis.

Reply: The tumor-specific miRNAs are only associated significantly with N stage, and will change the treatment modalities of ESCC, because they can specifically predict the regional lymph node metastasis of ESCC, guide the selective lymphatic dissection or irradiation of ESCC, and bring great progress in the treatment of ESCC.

Changes in the text: we have modified our text as advised (see Page 7, line 1-5 and Page 17, line 14-18)

### Reviewer B

Abstract:

- Only the main significant findings should be described in the abstract paragraph.

Reply: this was described in “Conclusions” section (see Page 4, line 3-4 highlighted with red color)

- The primary endpoint or aim of this study should be described in the abstract paragraph.

Reply: this was described in “Background” section (see Page 3, line 5-7 highlighted with red color)

Introduction:

- The introduction paragraph is too long, try to focus on the research question and

describe the current literature about miRNA and ESCC.

Reply: The "Introduction" paragraph was revised as advised.( see Page 5-6 with the "Track Changes" function to highlight all changes).

- What is the primary endpoint or aim of this study? This should be described in the last paragraph of the introduction.

Reply: this was described in the last paragraph of the introduction (see Page 8, line 4-5 highlighted with red color).

Methods:

- I doubt whether 4 pairs of ESCC tissues is sufficient to answer the research question with enough power. A power analysis including the primary endpoint, hypothesis and sample size calculation should be included in the methods section.

Reply: This is a sequencing analysis, usually more than three pairs of samples are needed to find some study clues. In this study, we strictly select four pairs of matched ESCC tissues (see Table 1) for microarray analysis and found enough miRNAs which were significant differential expression for present study (see Page 13-14 in "Results" section ).

- It is not clear to me how the 50 samples were chosen for RT-PCR analysis.

Reply: The 50 ESCC samples for RT-PCR analysis included 25 without LNM and 25 with LNM, their clinicopathological characteristics were similar between the two groups, all of these were the basis of our sample selection.(see Table.2, see Page 13 line 6-8 in "Sample selection" highlighted with red color)

Results:

- The results section is well written and contains impressive data presented in nice tables and figures. Well done.

Discussion:

- Start the discussion with the major findings and conclusions of this study. Hereafter, discuss the current available literature about miRNA expression in ESCC. Discuss what's new about this study and describe the clinical relevance of the findings with implications for the current treatment of esophageal ESCC. The describe strengths and

limitations about his study and give a recommendation about the use of the findings from this study in the future. End with the most important conclusions and describe future research which should be performed in detail. By applying this structure, the quality of the discussion paragraph improves significantly.

Reply: In this section, some revisions can be found, and some suggestions from reviewer were described or highlighted in the revised manuscript as follows:

- Start the discussion with the major findings and conclusions of this study (see Page 17, line 14-18). Hereafter, discuss the current available literature about miRNA expression in ESCC (see Page 17, line 8-9 and Page 7, paragraph 2). Discuss what's new about this study and describe the clinical relevance of the findings with implications for the current treatment of esophageal ESCC (see Page 17, line 15-18 and Page 19, paragraph 3). The describe strengths and limitations about his study and give a recommendation about the use of the findings from this study in the future (see Page 21, line 1-6). End with the most important conclusions and describe future research which should be performed in detail (see Page 21, paragraph Conclusions).