

Peer Review File

Article information: <https://dx.doi.org/10.21037/jtd-23-401>

Reviewer A

The Authors of this manuscript entitled, “Dysregulated expression of microRNA involved in resistance to osimertinib in EGFR mutant non-small cell lung cancer cells” have analyzed the miRNAs differences in acquired resistance of non-small cell lung cancer to a third-generation epidermal growth factor receptor tyrosine kinase inhibitor, Osimertinib. They have reported the differential expression of miRNAs in Osimertinib-resistant cell lines.

This is an interesting paper on how miRNAs is crucial for the TKI resistance in non-small cell lung cancer and will open the avenues for future research to overcome the resistance.

The introduction is adequate, and the methods are clearly explained. The results demonstrated the authors goals.

I have few remarks:

- Page 5 (line 162): It is unclear whether authors performed the wound scratch assay or migration assay.

Reply: We performed the wound scratch assay and migration assay.

Changes in the text: Page 5 (line 163) and Page 6 (line 178).

- Page 6, line 177: The author described the method for transwell migration assay but not for transwell invasion assay. They should mention which mat6rix was used for invasion assay.

Reply: We have added the transwell invasion assay in the text and mention which mat6rix was used for invasion assay.

Changes in the text: Page 6 (line 183-188).

- There are many typo errors observed. Please check thoroughly.

Reply: We have corrected some of the typos, others are the name of the drug and the name of the company or software.

Changes in the text: Page 5 (line 144 and 145), Page 8 (line 258 and 259), Page 9 (line 270 ,272 and 278), Page 11 (line 343).

- The authors should give the scale bars in Figs. 1, 6 and 7.

Reply: We have given the scale bars in Figs. 1, 6 and 7.

Changes in the text: Page 17 (line 500), Page 19 (line 535 and 536), Page 20 (line 545)

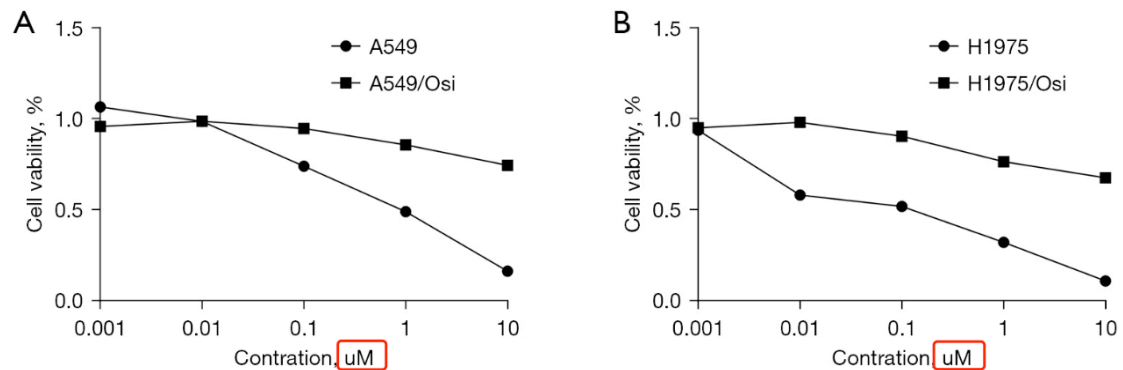
Overall, the manuscript is suitable for publication in the Journal of Thoracic Disease after minor revision.

Reviewer B

1. Figure 2

Please check if the unit of x-axis should be the revise as the legend ($\mu\text{mol/L}$).

concentration: 0.001 $\mu\text{mol/L}$, 0.01 $\mu\text{mol/L}$, 0.1 $\mu\text{mol/L}$, 1 $\mu\text{mol/L}$ and 10 $\mu\text{mol/L}$,



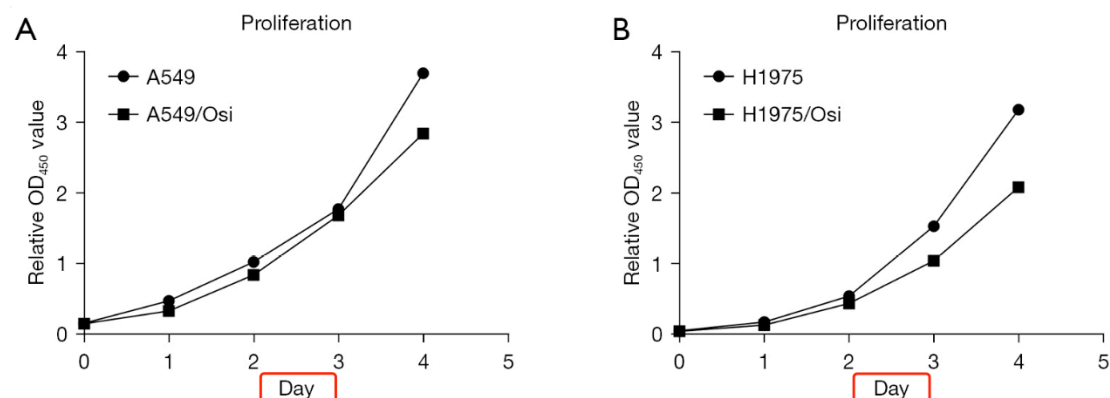
Reply: The unit of x-axis is correct, because the capital M means mol/L.

2. Figure 3

a) Please explain OD in the legend.

b) Please revise the legend as the x-axis.

resistant cells A549/Osi and H1975/Osi increased gradually with the extension of time (24h, 48h, 72h and 96h, respectively), and the proliferation ability of drug-



Reply:

a) We have modified our text as advised.

Changes in the text: see Page 17, line 500-501.

b) We have modified our text as advised.

Changes in the text: see Page 17, line 498-499.

3. Figure 4

Please explain the meaning of ***, ****, and ns in the legend.

Reply: We have modified our text as advised.

Changes in the text: see Page 17, line 510-511.

4. Figure 5

Please explain the meaning of *** and * in the legend.

Reply: We have modified our text as advised.
Changes in the text: see Page 18, line 515-516.

5. Figure 6

Please explain the meaning of * and ** in the legend.

Reply: We have modified our text as advised.
Changes in the text: see Page 19, line 525.

6. Figure 7

Please explain the meaning of ***, ****, and ns in the legend.

Reply: We have modified our text as advised.
Changes in the text: see Page 19, line 533-534.

7. Figure 8

Please explain PC in the legend.

Reply: We have modified our text as advised .
Changes in the text: see Page 20, line 537-540.

8. Figure 10

Please explain BP, CC, MF, and GO in the legend.

Reply: We have modified our text as advised.
Changes in the text: see Page 22, line 552-554.

9. Figure 11

Please explain KEGG in the legend.

Reply: We have modified our text as advised.
Changes in the text: see Page 23, line 559-561.

10. References/Citations

a) References 33 and 35 are the same, please delete one of them and revise both the citation in main text and reference list's order.

Reply: We have modified our text as advised.
Changes in the text: see Page 14, line 453.

b) Please double-check if more studies should be cited as you mentioned “studies”.

prognostic markers in NSCLC (38). A large number of studies have reported the role of miRNAs in cancer progression, such as proliferation, invasion, metastasis, metabolism, and drug resistance (24). Importantly, studies have identified that cancer

Reply: We have modified our text as advised.
Changes in the text: see Page 10, line 309.