

Peer Review File

Article information: <http://dx.doi.org/10.21037/atm-20-2002>

Reviewer A

1. The manuscript by Jin et al. described the potential of circRNA–miRNA–mRNA network as prognostic biomarkers for radiotherapies with X-rays and carbon ions in non-small cell lung cancer (NSCLC). In general, this study is meaningful in future for the development of prognostic biomarkers for radiotherapies with X-rays and carbon ions in NSCLC, but there are still some problems needing explanations or corrections.

Firstly, to investigate the differentially expressed circRNAs, the authors conducted next-generation RNA sequencing to explore circRNA expression profile in radiosensitive A549, acquired radioresistant A549-R11 and intrinsic radioresistant H1299 cell lines, the differentially expressed circRNAs were taken at the intersection of the sets of A549-R11 and H1299 compared to A549 respectively. However, when identifying the differentially expressed miRNAs and mRNAs, only A549-R11 and A549 cell lines were tested and compared. Why the H1299 cell line was not involved?

Reply 1: During the preparation stage of this manuscript, we have noticed this problem. In the set of H1299 cells, 380 differentially expressed miRNAs were found. However, if we match the OS-related differentially expressed 9 miRNAs obtained from the A549-R11 set to the H1299 set, there were only 3 miRNAs in the H1299 set. They were hsa-miR-142-3p, hsa-miR-449a and hsa-miR-584-5p, respectively. Moreover, the expression of hsa-miR-449a was inconsistent in the two sets (upregulated in the A549-R11 set and downregulated in the H1299 set). Then, the 16 differentially expressed mRNAs, which were predicted in the downstream of hsa-miR-142-3p in the A549-R11 set, were irrelevant to the prognosis of patients with lung cancer. The similar result was observed in hsa-miR-584-5p. So, we couldn't complete our paper if the differentially expressed miRNAs and mRNAs were taken at the intersection of the sets of A549-R11 and H1299. In addition, we considered

that the A549-R11 and A549 cells were the same origin but with obviously different radiosensitivities. This avoids the influence of confusing factors such as genetic and inherent variation, allowing for the clarification of molecules that are involved in radioresistance. Therefore, only the A549-R11 set was used to construct the circRNA-miRNA-mRNA network correlated with the prognosis of NSCLC.

Change in the text: -

2. Besides, from Line270 to Line273, A549-R11 cell line had a similar sensitivity to carbon ions compared with its parental A549 cell line, why did the authors mentioned that different expressions of circRNAs between the two cell lines might be potential biomarkers to distinguish the radiation response after irradiation with carbon ions.

Reply 2: The differentially expressed circRNAs from A549-R11 might regulate the radiosensitivity to X-rays, but they would have no effect on NSCLC cells after exposure to carbon ions since A549-R11 cells were resistant to low- linear energy transfer (LET) X-rays compared with A549 cells and these two cell lines showed a similar sensitivity to high-LET carbon ions. These circRNAs are the biomarkers which we would like to acquire, because they could be used to distinguish the patients who are suitable for X-ray radiotherapy (the expression of the circRNAs is normal) or carbon ion radiotherapy (the expression of the circRNAs is differed). We also developed other radioresistant cell lines (A549-R6, R8 and R9), which showed similar radioresistances to X-rays and carbon ions. We considered the differentially expressed circRNAs from these cell lines might regulate the radiosensitivity both to X-rays and carbon ions rather than only to X-rays. This is also very interesting, but in the next story.

Change in the text: -

3. Thirdly, this manuscript needs grammar review.

Additional more specific comments follow.

- 3.1. Line 82, "...have been reported to involve in the regulation of NSCLC progression."— should be "...to be involved in ..."

Reply 3.1: We have corrected it in our revised manuscript.

Change in the text: ... have been reported to be involved in the regulation of NSCLC progression. (see Page 6, line 82)

3.2. Line161, “however, a A549-R11 cells” --the word “cells” doesn’t match the article “a”.

Reply 3.2: We have deleted ‘a’ in our revised manuscript.

Change in the text: however, A549-R11 cells possessed a similar sensitivity to high-LET carbon ions like their paternal A549 cells. (see Page 9, line 160)

3.3. Line 294, “...that there was a clinical significance of...” --the word “significance” doesn’t match the article “a”.

Reply 3.3: We have deleted ‘a’ in our revised manuscript.

Change in the text: Recently, several studies showed that there was clinical significance of COL5A2 in patients with adenomas. (see Page 15, line 291)

Reviewer B

In this study, the authors explored the regulatory network of circRNA- miRNA-mRNA in the sensitivity of NSCLC cells to low LET X-rays and high-LET carbon ions by high-throughput sequencing and the mechanism might be helpful to screen potential biomarkers for predicting the CIRT and X-ray radiotherapy responses in NSCLC.

This is a comparative bioinformatics article, which involves less experimental part. Now there are a great number of similar articles in tumors and it is not very innovative. This article would be better if some molecular experiments were added. But I make some suggestions on the form and content of the article.

1. Several sentences contain grammatical mistakes and spelling mistakes. It is advised that you obtain assistance from a colleague who is well-versed in scientific English or whose native language is English.

Reply 1: we have checked the English writing throughout our manuscript and corrected some grammatical and spelling mistakes.

Change in the text: All changes are highlighted using red texts in the revised manuscript.

2. Full names of professional terms should be introduced in the abstract. For example, ncRNAs, NSCLC,

Reply 2: We have revised these abbreviations in our new manuscript.

Change in the text: ...the regulatory network of non-coding RNA (ncRNA)... (see Page 3, line 33)

...in the sensitivity of non-small cell lung cancer (NSCLC) cells... (see Page 3, line 35)

...the carbon ions radiotherapy (CIRT) and X-ray radiotherapy responses in NSCLC. (see Page 3, line 52)

3. There is no significant difference in the Figure 1, 4? This should be mentioned and discussed by marking with *or**. I suggest scale of vertical axis is represented by using equidistance in the figure 1B and C.

Reply 3: In Fig.1, we analyzed our data carefully and conducted statistical analysis using the Student's t-test with the MedCalc software. The mark of statistical significance was added to the figure and the legend. In Fig.4, the gray data were derived from the sequencing results. So there is no error bar in the histogram, and the black data were obtained from three independent experiments. So, we could not analyze the difference significance between the two groups. For the scale of vertical axis in Fig.1B and C, because Hall et al. have suggested that 'Survival curves for mammalian cells usually are presented in the form with dose plotted on a linear scale and surviving fraction on a logarithmic scale.'(Eric J. Hall, Amato J. Giaccia. Radiobiology for the Radiologist Seventh Edition. LIPPINCOTT WILLIAMS & WILKINS, a WOLTERS KLUWER business (ISBN 978-1-60831-193-4), 2012. p37). We followed this suggestion.

Change in the text: Fig.1B and C and the legend (see Page 25, line 487).

4. The flowchart of the construction of circRNA-miRNA-mRNA network is not clear and brief in Figure 2 and some details should be describe in the figure legend. What's the meaning DE Also, Figure 1,2 out of order, they should adjust it.

Reply 4: we have improved the flowchart and explained some abbreviations in the legend. Concerning the order of Figures 1 and 2, we firstly described the process that radioresistant A549-R11 was obtained from its parental cell line A549 in the first part of Methods (Fig.1A), then we depicted the construction process of the network later in Methods (Fig.2). So we think the figures are now in the right order.

Change in the text: Fig.2 and the legend (see Page 25, line 490)

5. I suggest they can delete the Figure 5B. They can show the details about predicted circRNA-miRNA interaction networks by the miRanda in the results.

Reply 5: We have moved the original Fig.5B to the supplementary material of our manuscript.

Change in the text: see line 198, 202, 205, 206, 314, 504, 505 and Fig.S2.