Peer Review File

Article information: https://dx.doi.org/10.21037/jtd-23-1171

Reviewer A

Comment: The research manuscript is elegantly written, and experimental work looks impressive and flawless. There are no major issues, since it is well explained, and the findings are of relevance.
Therefore, the article itself in the current form is adequate.
As a comment cisplatin (CDDP) resistance is multifactorial and BRCA1 and other DNA repair proteins are involved in the process.
Although very well explained and documented mechanistically a graphical figure could enhance the relevance and understanding of the findings on EGR4-ZNF205-AS1-mir138-5p and OCT4 interrelations.

Response: We are heartened to see your praise for the quality of our paper. The current study presents the experimental results in a comprehensive manner, and the molecular mechanism of EGR4-ZNF205-AS1-miR-138-5p and OCT4 interrelation is well established in the section of discussion. We read a number of studies on the implication of lncRNAs in the onset and progression of cancer, and find that the graphical figures seem not necessary in adding up to the rationale of the study. We thank you for the suggestion and appreciate your understanding.

Reviewer B

The authors tried to further study the function of ZNF205-AS1/EGR4 positive feedback loop in DDP-resistance in NSCLC based on their previous findings. They use different functional assays and LUAD patient samples to test their hypothesis and stated that miR-138-5p/OCT4 axis is one of the ZNF205-AS1/EGR4 downstream pathways. This regulatory axis may contribute to DDP-resistance in LUAD.

The detailed comments are as follows:

Major

1. The authors used a long introduction to list the literature on lncRNA, miR-138, and OCT4, and describe their previous findings. However, the structure is loose and there is no rationale to support the hypothesis. It looks like the authors proposed a hypothesis from nowhere and tried to find some pieces of evidence to support their idea rather than rigorously test the hypothesis. The authors should streamline the introduction, briefly describe the published findings in a few sentences and emphasize the potential connection among ZNF205-AS1/EGR4, miR-138, and OCT4 to provide a strong rationale.

Response: Thanks for the suggestion. To make it clear, the current study is based on the findings of our previous study, which reported that the level of ZNF205-AS1 was markedly up-regulated in NSCLC cells and tissues to contribute to a dismal prognosis. It also confirmed the existence of the positive feedback loop between ZNF205-AS1 and EGR4, which functions to boost the proliferation of NSCLC cells and could be applied as a promising therapeutic method for NSCLC. Hence, it is highly reasonable to hypothesize that the lncRNA ZNF205-AS1/EGR4 positive feedback loop inhibits the expression level of miRNA-138-5p and thereby diminishing the suppressive effect of miRNA-138-5p on OCT4.

To validate the hypothesis, we performed pilot study by using bioinformatics means, revealing that early growth response 4 (EGR4) is the potential target of lncRNA *ZNF205-AS1*, thereby increasing its promoter activity and activating its transcription. What is more, the ZNF205-AS1 transcript could directly interact with EGR4 mRNA to improve the stability of EGR4 mRNA through RNA-RNA interaction. Therefore, ZNF205-AS1/ EGR4 positive feedback loop could be established. In the current study, we detect the expression patterns of ZNF205-AS1, EGR4, miRNA-138-5p and OCT4 in DDP-resistant A549 cells and non-resistant A549 cells to explore the correlation effects of the ZNF205–AS1/EGR4 positive feedback loop in chemo-resistant NSCLC cancer cells.

While we believe that the hypothesis is solid and rational, the structure of the paper should be improved to make it more concrete. Therefore, following your advice, we streamlined the *Introduction* and elaborates on the molecular mechanism in *Discussion* (line 404-408, and 423-431).

Give that this study elucidates, for the first time, the mechanism of the lncRNA ZNF205-AS1/EGR4 feedback loop in regulating cisplatin resistance of NSCLC and the exact function of the downstream effector of miR-138-5p/OCT4. The previous findings on the interactions between ZNF205-AS1/EGR4, miR-138, and OCT4 are few. We provided detailed background information on these biomarkers (line 404-408, 423-431, 438-442) to shed light on the underlying mechanism. We appreciate your understanding.

2. In Fig. 2 C, D, do the tissues derived from pre-, or post-DDP treatment or from the DDP-sensitive or -resistant LUAD patients? These are two different things. The context and labels in the figure is inconsistent. The authors should specify.

Response: To make it clear, the two groups of tissues, named as non-resistant tissue and DDP-resistant tissue were collected from pre-DDP treatment LUAD patients and DDP-resistant LUAD patients, respectively.

3. The experiment design of the luciferase assay is not clear. Did the authors try to measure the binding of ZNF205-AS1 to miR-138 or vice versa? Please specify.

Response: Thank you for the comment. In the current study, luciferase reporter assay was conducted to examine whether lncRNA ZNF205-AS1 is targeted to miR-138-5p. To that end, we constructed dual-luciferase reporter gene system to establish lncRNA ZNF205-AS1 plasmid vector including wild type (WT) and mutant type (MUT) 3'-UTR. The results of dual-luciferase reporter analysis showed that the luciferase activity of the WT reporter in the lncRNA ZNF205-AS1-WT +miR-138-5p group was significantly decreased, as compared with that of lncRNA ZNF205-AS1-WT+NC group ($p<0.01$) (Figure 3D).

To further clarify the results, we divided the two images in the previous Figure 3C into Fig 3C and Fig 3D, for the purpose of showing the results of bioinformatics analysis of ZNF205-AS1 with miR-138-5p, and displaying the results of luciferase reporter assay.

These results convincingly demonstrate that miR-138-5p is the target gene of lncRNA ZNF205-AS1 and establishes the negatively regulation of miR-138-5p expression.

4. "Intriguingly, RNA pulldown assay confirmed that miR-138-5p directly binds to the 3'-UTR of EGR4 and OCT4 (Fig. 5C)." This should be tested by reporter assays using EGF4 and OCT4 3'-UTR constructs.

Response: We appreciate your suggestion. As indicated above, dual-luciferase reporter assay was conducted to establish lncRNA ZNF205-AS1's negative interaction with miRNA-138-5p. A variety of biological functions of lncRNAs are actualized through its interaction with proteins. we performed RNA pull-down assay, a commonly used protocol, to screen and to validate the potential interactive proteins of EGR4 and OCT4. The background information of EGR4 and OCT4 has been elaborated to explain in the section of *Introduction*.

To avoid misunderstanding, we rephrase the sentence as "Intriguingly, RNA pulldown assay confirmed that EGR4 and OCT4 are the interactive proteins of miR-138-5p."

5. The discussion largely repeats the results, the authors should discuss the potential mechanisms in detail and the shortage of this study.

Response: We appreciate your suggestion, and make effort to improve the quality of discussion.
While we explained the meanings of the experimental results, we also elaborated on the information about miR-138-5p, EGR4 and OCT4 to present and discuss the detailed potential mechanism (line 423-431, 438-442).

Meanwhile, the shortage (shortcoming/limitation) of the study is also explained

in the last paragraph (line 480-486):

The potential limitations of the current study should be noted. Firstly, while the vital role of
ZNF205-AS1/miRNA-138-5p axis in regulating DDP resistance was well-established in the study, chemo-resistance is a multifactorial phenotype that involves both internal cellular processes and the microenvironment. Individual cases of NSCLC may present distinct single cell heterogeneity and plasticity in the respective tumor microenvironment(33). Secondly, *in vivo* assay, such as nude mouse xenograft model, could be performed to substantiate the findings of the study in a more comprehensive manner.

Minor

1. In line 244, the lack of an explanation for BESA-2B cell line.
Response: We are sorry for the typo. As a matter of act, BEAS-2B cell line was used. BESA-2B was derived from human lung tissue and has been extensively used as an in vitro non-tumorigenic lung epithelial model in studies associated with lung carcinogenesis. Please refer to line 120.
We have made corrections in line 246, line 248, line 267, as well as in Figure 1 and Figure 2.

2. In lines 245-247, "the results of IHC found that EGR4 and OCT4 expression levels were notably higher in lung tissues of DDP-resistant patient than those without undergoing DDP chemotherapy." Where are the data?
Response: The results of IHC were illustrated in Figure 2C and 2D. It is improper to present the results of IHC in this part, therefore this sentence is removed. We appreciate your meticulousness.

3. Fig. 1C should be the first figure to demonstrate the establishment of DDP-resistant cell lines. The corresponding results should also be rearranged.
Response: Thanks for the suggestion. We have rearranged the images in Figure 1 by presenting 1C in the first place to demonstrate that the establishment of chemo-resistant cell line was successful. Please refer to Figure 1.

4. The lack of labels for Fig. 2C and D.
Response: Figure 2C and 2D presents two representative IHC images (#1 and #2) for detection of EGR4 and OCT4. To clarify the points, we added the label for 2C and 2D. Please refer to Figure 2.

5. In lines 312-313, "along with notably lowered IC50 value to DDP, as seen in the representative images of Fig 4B." Where are the data?
Response: We are sorry for the lack of clarity in wording. The results of IC50 were displayed in Figure 4C. We revised the sentences as" Intriguingly, the

invasion capacity of cells with miRNA-138-5p gain-of-function was significantly suppressed, as seen in the representative images of Fig 4B."

6. In Fig. 4C, the text did not match the data in the figure.
Response: Thanks for the comment. Fig 4C. illustrates the results of CCK8 assay of A549/DDP cells transfected with trmiRNA-138-5p mimic and those transfected with NC.
We rephrase the paragraph as "The results found that the overexpression of miRNA-138-5p significantly suppressed cell viability, in comparison with those without miRNA-138-5p over-expression. The IC_{50} value of A549/DDP cell line with miRNA-138-5p over-expression was significantly lower than that transfected with NC (49.19 ± 7.89μM v.s. 17.58± 1.29μM (*$p < 0.001$).