Peer Review File

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Reviewer A: In this study, the authors have investigated the effect of silencing ARID1A on the response of gastric cancer cells to oxaliplatin. Although the question is interesting, this has already been investigated using ovarian cancer cells (ref. 20), which reduces the originality of this manuscript.

Major points:
Comment 1: The whole study was an in vitro one based on two cell lines (MGC-803 and AGS), which were not mentioned in the Abstract section, despite the fact that too many details were included in that paragraph.
Reply 1: Thanks for your suggestion. We have modified the "Abstract" section.

Comment 2: The authors concluded that "ARID1A gene silencing is a promoting factor in gastric cancer cells". Does this simply mean that ARID1A behaves as a tumor suppressor? This is a well-known characteristic of this protein (PMID: 24618703).
Reply 2: Thanks for your comments. There was nothing wrong with what you have described, that from another perspective, "ARID1A gene silencing is a promoting factor in gastric cancer cells" could mean that ARID1A behaves as a tumor suppressor. In addition to the verification of the previous results, this paper also proposed that ARID1A gene silencing could reduce the sensitivity of gastric cancer cells to oxaliplatin.

Comment 3: In contrast, ARID1A seems to be needed for the response of gastric cancer cells to oxaliplatin. Although changes observed in these cancer cells were rather modest (Figure 4), this fact has already been described in other cancer cells. Advancing in the understanding of the exact mechanism underlying this behavior would be required to increase the value of the present study.
Reply 3: Thanks for your suggestion. In the discussion, we added the effect of inactivated ARID1A on the entire cell cycle of mutant tumors, which in turn affects the sensitivity of anti-tumor drugs. But as you mentioned, and we have point out in the articles that a further research should be done to explore the mechanism of ARID1A gene silencing to control cell proliferation and apoptosis.

Comment 4: The sentence: "patients are born or acquired resistance to platinum

drugs" is entirely wrong. It is not the patient but the tumor who is resistant to these drugs.

Reply 4: Thanks for your comments. We have modified the sentence into "many cancer patients are resistant to platinum drugs".

Comment 5: The Discussion section is not focused on the results obtained in the present study. Moreover, it contains much material that fits better in the Introduction section. The main point regarding the mechanism of ARID1A-dependent sensitivity to oxaliplatin is very speculative. The authors should have carried out appropriate experimental work to unravel the mechanism of action.

Reply 5: Thanks for your suggestion. For the Discussion section, we deleted some description of the material and focused more on the experimental results, like adding the discussion of effect of ARID1A gene silencing on the entire cell cycle of mutant tumors. And we have pointed out that a further we should verify in the future is that Akt signaling pathway is related to the drug resistance mechanism of gastric cancer cells induced by ARID1A gene silencing.

Comment 6: The authors concluded that their results provide "a theoretical basis for predicting the efficacy of oxaliplatin-based chemotherapy in patients with gastric cancer". However, in this, as well as in many other cases, only the lack of efficacy could be predicted.

Reply 6: Thanks for your comments. As you mentioned, lacking of the ARID1A gene can reduce the sensitivity of gastric cancer cells to oxaliplatin. But from our perspective， the lack of efficacy was also one of the theoretical basis, could equally show the importance of ARID1A gene in gastric cancer cells.

Comment 7: English spelling, grammar, and style must be carefully revised (e.g., line 156, "fluorescencemicroscope" and line 211, "In addition to the conclusions discussed above").

Reply 7: Thanks for your suggestion. We have revised the article carefully as you suggested.

Comment 8: The code of colors used in the figures is confusing because red was used for controls in some cases and in others for ARID1A silenced cells.

Reply 8: Thanks for your comments. We have modified the picture to show ARID1A silenced cells in red.

Reviewer B: The Manuscript by Qing Liu et al. is interesting and well written and

highlights the importance of studying the role of the tumor suppressor gene ARID1A in gastric cancer development and resistance to anticancer drugs. However, some Major and Minor revisions are required in order to improve this Manuscript.

MAJOR Revisions

Comment 1: In Figure 3G & 3H the authors showed that the shARID1A treatment significantly increased the invasion rate of both AGS and MGC-803 cells. Since "Li C. et al. [2017; Neoplasma]" demonstrated that the shRNA treatment improved the invasion ability of neuroblastoma cells through the increase of activity and levels of matrix metalloproteinase 2 and 9, the authors should evaluate if the shARID1A treatment leads to an increase of MMP2 and MMP9 protein levels in both AGS and MGC-803 cells.

Reply 1: Thanks for your comments. As you mentioned, Li C. et al. [2017; Neoplasma] suggested that loss of ARID1A may associate with the promotion of invasion and metastasis of neuroblastoma, we have proved that the shARID1A treatment significantly increased the invasion rate of gastric cancer cells through experiments. The former research was more detailed, and a further study on the activity of matrix metalloproteinase 2 and 9 showed their relationship with the internal mechanism. This was one of our limitations, and we will conduct further research on its mechanism.

Comment 2: In Figure 4E-4J the authors showed that the shARID1A treatment led to a decrease of apoptosis rate and necrosis rate in MGC-803 cells in "MGC-803(KD)" and also in "MGC-803(KD)+oxaliplatin" samples. I think that this is one of the most important result obtained by the authors and they must perform the same experiments also in AGS cells, in order to exclude that the reduction of oxaliplatin pro-apoptotic effect is limited to only one gastric cancer cell line, i.e. MGC-803 cells.

Reply 2: Thanks for your comments. We completely agree with your point of view, but due to the global impact of the Corona Virus Disease 2019, many experiments cannot be carried out as scheduled due to some factors. We are sorry for the inconvenience.

Comment 3: Regarding the results of Figure 4, the authors should evaluate if the apoptotic process induced by oxaliplatin and decreased by shARID1A treatments is caspase-dependent, through the evaluation of caspase 3 activity levels in both AGS and MGC-803 cells in Control cells and after +shARID1A; +oxaliplatin; +oxaliplatin+shARID1A treatments.

Reply 3: Thanks for your comments, providing us such an idea to prove our point, we

will also explore its mechanism in future research.

Comment 4: In the Discussion section, the authors cited the research of "Luo B. et al. [2008, PNAS]", writing that "…. a previous study about Jurkat leukemia cells showed that the FAS-mediated apoptosis was decreased after knocking out the ARID1A gene". As the death receptor Fas is involved in the activation of caspase 8 and in the induction of the extrinsic pathway of apoptosis, the authors should evaluate also the caspase 8 activity levels in both AGS and MGC-803 cells in Control cells and after +shARID1A; +oxaliplatin; +oxaliplatin+shARID1A treatments.
Reply 4: Thanks for your comments. Regarding your suggestion, we believed that caspase 8 activity levels in both AGS and MGC-803 cells in different groups could further clarify the influence of ARID1A gene on oxaliplatin. Thank you very much for providing us with such good ideas. We also understood that this was a limitation of this study.

MINOR Revisions
Comment 5: In the Abstract please replace "After treated with oxaliplatin" with "After treatment with oxaliplatin".
Reply 5: Thanks for your suggestion. We have replaced "After treated with oxaliplatin" with "After treatment with oxaliplatin"in the Abstract.

Comment 6: In the Abstract please replace "a promoting factor in gastric cancer cells" with "a factor promoting proliferation of gastric cancer cells".
Reply 6: Thanks for your comments. The sentence "a promoting factor in gastric cancer cells"has been replaced with "a factor promoting proliferation of gastric cancer cells"in the Abstract.

Comment 7: In the Introduction, please replace "cisplatin chemosensitivity reduction and cell apoptosis" with "cisplatin chemosensitivity and also cell apoptosis reduction".
Reply 7: Thanks for your suggestion. We have replaced "cisplatin chemosensitivity reduction and cell apoptosis" with "cisplatin chemosensitivity and also cell apoptosis reduction"in the Introduction section.

Comment 8: In the "Western blot analysis" section of "Materials and methods", are the 1:5000 dilution of primary ARID1A polyclonal antibody and the 1:1000 dilution of secondary anti-mouse antibody correct? Because the primary antibody has been used at a very low concentration and the secondary antibody has been used at a very

high concentration.
Reply 8: Thanks for your comments. It was our input error, the correct concentration was 1:1000 dilution of primary ARID1A polyclonal antibody. This error has been modified in the manuscript. We are sorry for the inconvenience.

Comment 9: Concerning Figure 1, I think that it should be used as Supplementary Figure 1, because it represents only a characterization of the gastric cancer cell lines AGS and MGC-803.
Reply 9: Thanks for your suggestion. The original picture has been adjusted.

Comment 10: In Figure 2G&2H the authors showed that the shARID1A treatment led to a remarkable decrease ($p<0.001$) of ARID1A protein levels in MGC-803 cells, but Figure 2H shows that there was only a 50% reduction of ARID1A protein levels, so which is its exact p value?
Reply 10: Thanks for your comments. Actually, We can't show you the specific data, we used the GraphPad Prism 7.04 to calculate the P value, and when the value was less than the 0.001, the system will automatically display $P<0.001$. We are sorry for the inconvenience.

Comment 11: In Figure 3D, the % G1 values of MGC-803 cells are missing.
Reply 11: Thanks for your comments. The G1 values of MGC-803 cells have been added.

Comment 12: In Figure 3E&3F, please replace “shADIR1A(KD)” with “shARID1A(KD)”.
Reply 12: Thanks for your comments. I'm sorry that was a mistake when we input the words. It has been revised in Figure 3E&3F.

Comment 13: In Figure 4I&4J, please use “MGC-803” instead of “MGS-803”.
Reply 13: Thanks for your suggestion. I'm sorry that was a mistake when we input. “MGS-803” has been replaced with “MGC-803”in Figure 4I&4J.

Comment 14: In the Conclusions, please replace “….and it is the first time to verify that ARID1A gene silencing” with “… and we verified for the first time that ARID1A gene silencing”.
Reply 14: Thanks for your suggestion. We have changed the whole sentence as you suggested.