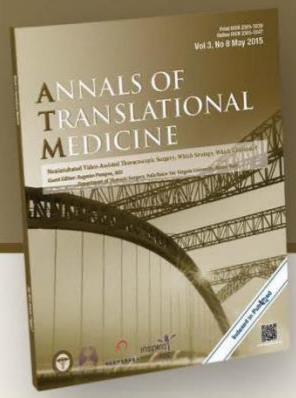
ANNALS OF TRANSLATIONAL MEDICINE



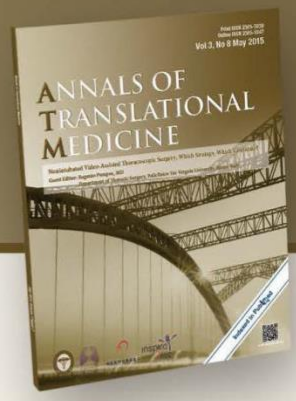
Peer Review File

Article information: http://dx.doi.org/10.21037/ atm-20-1113

Reviewer A:

Comment 1: Line 11: Authors must describe what JC-1 is?
Reply 1: We are very grateful to you for this important suggestion. JC-1 (5, 5', 6, 6'-Tetrachloro-1, 1', 3, 3'-tetraethyl-imidacarbocyanine) is a fluorescent lipophilic carbocyanine dye has become widely used to measure MMP based on its characteristics of MMP-dependent aggregation. When the mitochondrial membrane potential is high, JC-1 gather in the matrix of the mitochondria to form polymers (J-aggregates), which can produce red fluorescence (585 nm); when the mitochondrial membrane potential is low, JC-1 cannot aggregate in the matrix and are monomer at this time, which can produce green fluorescence (488 nm). Therefore, we can use flow cytometry to detect the green (PI tunnel) and red fluorescence (FITC tunnel) values of cells, and quantify MMP by the JC-1 aggregates positive rate or the ratio of JC-1 aggregates to monomers.
Changes in the text: we have added the describe of JC-1 in our text as advised: "The potential of the mitochondrial membrane was detected by the JC-1 (5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimi- dazolylcarbocyanine iodide) mitochondrial membrane potential kit (Thermo Fisher Scientific, MA, USA). JC-1 is a fluorescent lipophilic carbocyanine dye that has become widely used to measure MMP due to its characteristics of MMP-dependent aggregation. JC-1 monomers (green fluorescence, 488 nm) in the cytoplasm relies on the polarity of the mitochondrial membrane to enter the mitochondria and form a polymer (JC-1 agggregate, red fluorescence, 585 nm)". (see Page 6, line 119-124).

Comment 2: The authors should write the statistical significance in the results text.
Reply 2: Thank you very much for this suggestion. It is necessary to describe the statistical significance in the results text. So, we have added the description of the statistical significance as advised.
Changes in the text: we added the statistical significance as advised:
"($P < 0.05$)" (see Page 9, line 196,198; Page 10, line 205,207,210,211,222; Page 11, line 245; Page 12, line 251,253,267; Page 13, line 269);
"no statistical significance" (see Page 11, line 206);
"The JC-1 aggregate percentage was reduced by 36.3% in the MMQ cells and 13.4% in the REFs ($P < 0.05$) (Fig. 4F). The JC-1 aggregate–monomer fluorescence ratio decreased by 2.4 in the MMQ cells, but only decreased by 0.3 in the REFs ($P < 0.05$) (Fig. 4G)." (see Page 11, line 240-243).



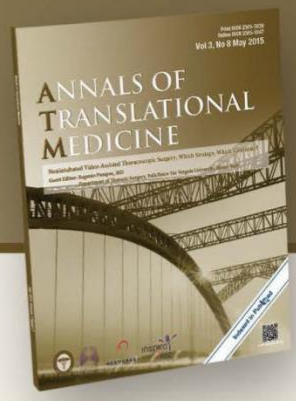
"There were no statistically significant changes in basal respiration or ATP production in REFs, but was a slight decrease in maximum respiration capacity ($P < 0.05$)" (see Page 11-12, line 246-247)

Comment 3： The figures should be sharper and clearer because figures 2A, 2C, 4 A, B, C, D, and F and figure 6 are especially blurred.
Reply 3: We are so sorry for that. We had submitted the original clear figure files, it may be because the figures were compressed during the conversion to the PDF version, resulting in a blurry image. If the reviewer wants to view the clear figures, you may be able to obtain it from the editors. Meanwhile we used the ICC profiles to save the figures so that it can display the original colors on different devices.
Changes in the text: There is no Change in the text.

Comment 4: Line 222: Correct the punctuation.
Reply 4: We are very grateful to you for pointing out our mistake and we are very sorry for our carelessness. We have filled in the missing punctuation in the text.
Changes in the text: we have modified our text as advised (see Page 13, line 281).

Comment 5: The figure legends should be more explanatory.
Reply 5: We're sorry that we didn't describe the figures very explanatory. We have modified the figure legends to try to make it more explanatory.
Changes in the text: we have modified all the figure legends of our text as advised (see Page 18-23, line 359-469).

Reviewer B:
Comment 1: ART IC50 of MMQ and REFs were detected by CCK-8 test, but the author did not test the ART IC50 of the corresponding normal cell line, to clarify the toxicity of ART on normal cells.
Reply 1: We are very grateful for this suggestion. In the early stage of the study, we used Rat Pituitary Cell (RPC) line as the corresponding normal cell line, and the results showed that the ART IC50 of RPC was about 130μM. However, because this cell line has been eliminated from the ATCC cell bank which may be due to its unknown origin. Therefore, this cell line was not used in our research anymore. Rat Embryonic Fibroblasts (REFs) is a type of fibroblast prepared from embryonic rat (14 d). In a large number of anti-cancer drug researches (1-3) and our previous pituitary tumor researches (4), it was be used as the corresponding normal cell line and has been widely recognized. So, in our study, we used REFs as the corresponding normal cell line to clarify the toxicity of ART.
Changes in the text: There is no change in the text.

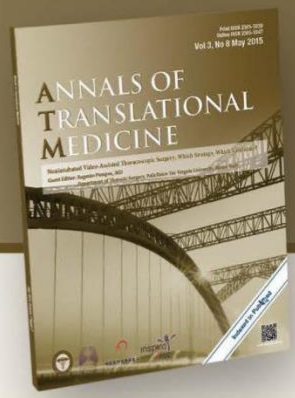


Comment 2: Besides, please confirm whether the legends of FIG. 1 (B) and (C) have the opposite meaning to the pictures.
Reply 2: We are very grateful to you for pointing out our mistake and we are very sorry for our carelessness. FIG.1 (B) and (C) were wrongly placed in opposite positions. We have modified the FIG.1 and corrected these errors.
Changes in the text: we switched the positions of FIG. 1 (B) and FIG. 1 (C) (see FIG. 1).

Comment 3: FIG. 2 (D) does not correspond exactly to FIG. 2 (C). Please check carefully.
Reply 3: We are very grateful to you for pointing out our mistake and we are very sorry for our carelessness. In FIG. 2 (D), The “control 48 h group” was erroneously missing. We have modified the FIG. 1 and corrected these errors.
Changes in the text: We added a bar chart of “control 48 h” in FIG.2D (see FIG. 2).

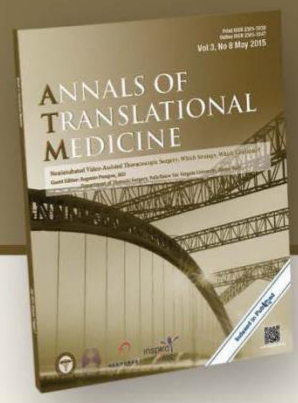
Comment 4: It is suggested that in vivo, the PRL level of each group should be detected so that it can directly reflect the effect of ART on PRL, which finally can be better applied in clinical practice.
Reply 4: we strongly agree with the suggestion. For prolactinomas, the main objective of clinical therapy is to reduce tumor volume, return prolactin (PRL) levels to baseline. Serum PRL is a crucial indicator to evaluate the effect of treatment, and we regret that we did not collect the blood of the mice for further testing. It is a consensus that, serum PRL levels generally parallel tumor size, and the tumor size is another vital indicator used to evaluate clinical treatment (5-7). Moreover, because we are using a subcutaneous tumor model of which tumor anatomy (such as blood supply) is essentially different from prolactinoma. Therefore, the tumor volume may be more valuable as an indicator than serum PRL levels. And compared with serum PRL, the detection of tumor volume is more accurate. Our results show that ART can significantly reduce tumor volume (Fig. 7A-C) and the level of PRL protein in the tumor (Fig. 7D) *in vivo,* which has enough indicated the potential of ART for clinical practice. Also, we are building an in situ models of pituitary tumors in rats. So, in further research, we will use these models and even clinical trials to test the therapeutic effect of ART on prolactinoma.
Changes in the text: We added a missing paragraph describing the: “Moreover, the levels of PRL protein in the tumor were also significantly reduced in the ART group.” (see page13, line 275-276)

Comment 5: What the statistical difference indicated is not so clear in some of the figures. The authors need to check carefully and mark clearly.
Reply 5: We are very grateful to you for this suggestion and we apologize that we did not clearly show the statistical difference indicators in some of the figures. We have revised the figures to



try to mark the statistical difference indicators more clearly.
Changes in the text: we magnified the markers of statistical difference in the figures as advised (see FIG. 2-7).

Comment 6: It is suggested that some of the references need to be corrected.
Reply 6: We are very grateful to you for pointing out our mistake and we are very sorry for our carelessness. We carefully examined the references and found some errors, and then we corrected these errors.
Changes in the text: we have modified our text as advised:
The text “and studies have reported an increased frequency of valvular heart disease and pulmonary fibrosis with DA treatment (12-13)” and its references were deleted because there is not enough evidence to support it in prolactinoma. (see page 3, line 61-62; page 24, line 490-493).
The reference “24. Buommino E, Baroni A, Canozo N, Petrazzuolo M, Nicoletti R, Vozza A,et al. Artemisinin reduces human melanoma cell migration by down-regulating alpha V beta3 integrin and reducing metalloproteinase 2 production. Invest New Drugs 2009;27(5):412-8.” was deleted because it doesn't match the text. (see page 4, line 73; page 25, line 514-515).
The references of “this characteristic serve as cancer-specific targets to endow certain compounds or drugs with specific targeting effects (45-49).” were corrected to “25. Shi Y, Lim SK, Liang Q, et al. Gboxin is an oxidative phosphorylation inhibitor that targets glioblastoma. Nature 2019;567:341-346. 39. Bernal SD, Lampidis TJ, Summerhayes IC, et al. Rhodamine-123 selectively reduces clonal growth of carcinoma cells in vitro. Science 1982;218:1117-1119. 40. Nadakavukaren KK, Nadakavukaren JJ, Chen LB. Increased rhodamine 123 uptake by carcinoma cells. Cancer Res 1985;45:6093-6099 41. Weinberg SE, Chandel NS. Targeting mitochondria metabolism for cancer therapy. Nat Chem Biol 2015;11:9-15” (see page14, line 300; page 25-26, line 523-524,544-559).
The references of “Currently, for DAs-resistant prolactinomas patients, there are no effective therapeutic drugs (6, 13, 28)” were corrected to “ 6. Pellegrini I, Rasolonjanahary R, Gunz G, et al. Resistance to bromocriptine in prolactinomas. J Clin Endocrinol Metab 1989;69:500-509. 7. Auriemma RS, Pirchio R, De Alcubierre D, et al. Dopamine Agonists: From the 1970s to Today. Neuroendocrinology 2019;109:34-41 8. Maiter D. Management of Dopamine Agonist-Resistant Prolactinoma. Neuroendocrinology 2019;109:42-50. 9. Maiter D, Delgrange E. Therapy of endocrine disease: the challenges in managing giant prolactinomas. Eur J Endocrinol 2014;170:R213-R227” (see page14, line 281-282; page 24, line 479-485).
The references “29. Maiter D, Delgrange E. Therapy of endocrine disease: the challenges in managing giant prolactinomas. Eur J Endocrinol 2014;170:R213-R227” was deleted because it was repeateded with reference 9 (see page 13, line 282; page 25, line 525-526).



References:

1. Shi Y, Lim SK, Liang Q, Iyer SV, Wang H, Wang Z,et al. Gboxin is an oxidative phosphorylation inhibitor that targets glioblastoma. Nature. 2019;567(7748):341-6.
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3. Manago A, Leanza L, Carraretto L, Sassi N, Grancara S, Quintana-Cabrera R,et al. Early effects of the antineoplastic agent salinomycin on mitochondrial function. Cell death & disease. 2015;6(e1930.
4. Mao Z, Zhou J, Wang H, He D, Xiao W, Liao G,et al. Artesunate inhibits cell proliferation and decreases growth hormone synthesis andsecretion in GH3 cells. Molecular biology reports. 2012;39(5):6227-34.