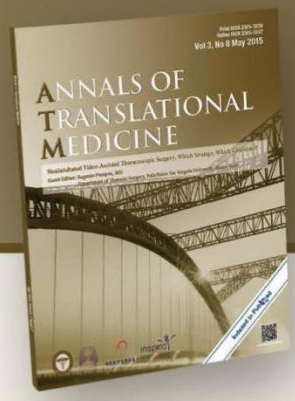
ANNALS OF TRANSLATIONAL MEDICINE



Peer Review File

Article information: http://dx.doi.org/10.21037/atm-19-4402.

Reviewer A

Comment 1: The authors should clarify why they chose AGS and MKN28 for investigation.

Reply 1: Thanks for your kindly advice. We firstly detected the baseline expression of PYCR1 protein in GC cell lines in our pre-experiment in order to select appropriate cell lines for further investigation. We found that PYCR1 expression was high in four GC cell lines (BGC823, AGS, MKN45, SGC7901), but it was relatively low in moderately-differentiated GC cell line MKN28. Thus, AGS (moderately high PYCR1expression) and MKN28 (low PYCR1 expression) were selected to perform further in vitro investigations.

Comment 2: All WB need at least three independent repeats and statistical analysis.

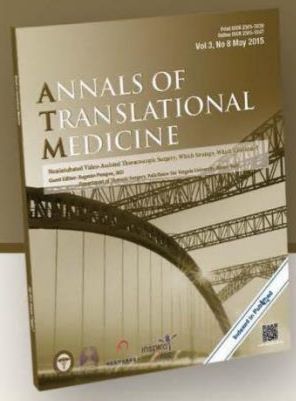
Reply 2: As for the referee's concern, we have added the statistical analysis of WB results based on at least three independent repeats.

Comment 3: PYCR1 overexpression should be performed in GC cells to confirm that PYCR1 promotes GC progression.

Comment 4: In vivo experiments are necessary.

Reply 4: We are appreciative of the reviewer's suggestion. Indeed, it will be more convincing if we get relevant results in vivo. In the present work, we mainly focused on in-silico analysis, clinicopathological analysis with tissue microarray and in-vitro assays, in order to uncover its clinical significance and answer whether proline synthase PYCR1 regulated malignant behavior of gastric cancer cells. On the basis of these results, gene-modified animal model is being applied to investigate its role in gastric carcinogenesis following Correa model, xenograft mice with GC cell lines are being performed to uncover the mechanism behind the promotion of GC mediated by PYCR1 expression. We believe that these results will be shown in our future work. Therefore, we seek for the reviewer's and editor's understanding at present, and we discussed this limitation in the main text.

Comment 5: Figure 6A and 6B, please another two repeats and statistical analysis should be



performed.

Reply 5: Thank you. We apologize for the inaccurate presentation of Figure 6A and Figure 6B. As your suggested, we added the statistical analysis of another two repeats in the gray histogram.

Reviewer B

Comment 1: Although KM analysis revealed the association between different PYCR1 expression and survival, multivariate analysis revealed that PYCR1 was not an independent risk factor for survival. It would be better the authors discuss the possible reason for the inconsistency.

Reply 1: Thanks for your kindly advice. Based on the present results, we considered that the limited sample size of included GC patients for IHC might be the primary reason for this inconsistency. As we all known, there are many risk factors determining the prognosis of gastric cancer patients, therefore a larger sample size might be appropriate and powerful to adjust all those variables of interest to demonstrate and verify the value of PYCR1 in predicting patients' survival in future study.

Comment 2: It would be better the authors included other parameters in Table 1 such as pathology type when they conducted univariate and multivariate analysis (result shown in Table 2)

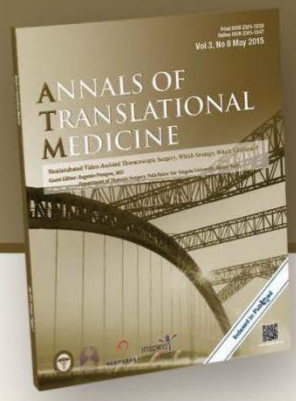
Reply 2: Thanks for your constructive suggestion. Based on the results from KM plotter analysis, it was identified that the degree of tumor differentiation had an impact on survival regarding to PYCR1 mRNA levels. Therefore, In revised Table1, we added the information on the differentiation degree of GC in high and low PYCR1 expression groups in revised Table 1, the rest was kept as previous edition. Thus, we finally included seven parameters [including sex, gender, tumor size, histological type, differentiation degree, clinical stage(TNM) and PYCR1 expression level] into univariate and multivariate analysis.

Comment 3: It would be better the authors provide the value of WB, as there was no obvious change in some of the provided images

Reply 3: As for the referee's concern, we have added the statistical analysis of WB results based on at least three independent repeats.

Comment 4: It would be better the authors provide animal experiments results.

Reply 4: We are appreciative of the reviewer's suggestion. Indeed, it will be more convincing if we get relevant results in vivo. In the present work, we mainly focused on in-silico analysis,



clinicopathological analysis with tissue microarray and in-vitro assays, in order to uncover and its clinical significance and answer whether proline synthase PYCR1 regulated malignant behavior of gastric cancer cells. On the basis of these results, gene-modified animal model is being applied to investigate its role in gastric carcinogenesis following Correa model, xenograft mice with GC cell lines are being performed to uncover the mechanism behind the promotion of GC mediated by PYCR1 expression. We believe that these results will be shown in near future work. Therefore, we seek for the reviewer's and editor's understanding at present, and we discussed this limitation in the main text.

Comment 5: It would be better the language be polished.
Reply 5: The manuscript has been thoroughly revised and edited by a native speaker (provided by Springer Nature), so we hope it can meet the journal's standard. Thanks so much for your useful comments.

Reviewer C
Comment 1: The inhibition of AKT should also be done to show the relationship between PYCR1 and PI3K/AKT and make sure the up-and-down stream of PYCR1, PI3K and AKT.
Reply 1: Thanks for your useful comments. As a core, molecular in this signal pathway, activation of Akt (phosphorylated Akt) is the indicator of PI3K activation. As you suggested, if the expression of PYCR1 was downregulated following Akt inhibition, it would be more solid to make a conclusion that PI3K/Akt regulates PYCR1 expression at upstream. Unfortunately, we cannot perform this assay at present under the influence of COVID-19, because the laboratory was shut down, and colleagues and students were not allowed to return institution, though we have submitted application.

Comment 2: All pictures should be quantified.
Reply 2: As for the referee's concern, we have added the statistical analysis of relevant pictures.

Comment 3: In abstract, method part should be rewritten.
Reply 3: Thanks for your suggestion, we have modified the Method part of abstract in revised version.