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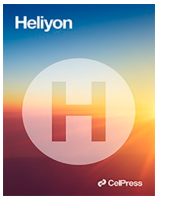

Research article

# Pro-tumorigenic activity of PYCR1 in gastric cancer through regulating the PI3K/AKT signaling

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ABSTRACT

*Background:* The primary objective of this investigation was to assess the impact of pyrroline-5-carboxylate reductase 1 (PYCR1) on the progression of gastric cancer (GC), specifically focusing on tumor growth and metastatic potential.

*Methods:* Surgical specimens from patients with different stages of GC were assayed for PYCR1 expression using immunohistochemistry. PYCR1 expression was manipulated by depletion or overexpression approaches in GC cells, and these cells were applied to explore the functional roles of PYCR1. Expression of apoptosis– and metastasis–related markers was quantified through quantitative real-time PCR and Western blot.

*Results:* Higher PYCR1 expression was ascertained in surgical specimens from patients with GC as compared to noncancerous adjacent tissues. Additionally, PYCR1 overexpression in GC tissues was linked to adverse clinical outcomes. The depletion of PYCR1 in GC cells resulted in a pronounced reduction in proliferation, the induction of apoptosis, and the attenuation of invasion and metastasis. Conversely, its ectopic expression notably augmented proliferation, restricted apoptosis, and stimulated invasion and metastasis. In addition, the knockdown of PYCR1 resulted in a significant elevation in the activation of caspase 3, a key protein involved in apoptosis. This depletion also led to a decrease in the activation or expression of proteins associated with metastasis, such as phosphorylated (p)-phosphatidylinositol 3-kinase (PI3K), p-AKT serine/threonine kinase (AKT), and snail family transcriptional repressor 1 (Snail). Additionally, it resulted in an upregulation of E-cadherin expression. Conversely, the overexpression of PYCR1 notably increased the levels of p-PI3K, p-AKT, and Snail, while simultaneously reducing E-cadherin expression.

*Conclusion:* PYCR1, by activating PI3K/AKT signaling, assumes a crucial role in governing malignant characteristics of GC cells, including proliferation, apoptosis, and metastasis. These findings underscore the promising potential of PYCR1 as a diagnostic biomarker and a target for tailored therapeutic interventions in patients with GC.

## 1. Background

Among various malignancies, gastric cancer (GC) ranks fifth in terms of incidence and third in terms of its impact on cancer-related mortality [1]. Despite the availability of comprehensive treatment modalities such as surgical procedures, molecular-targeted therapy,

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chemotherapy, and radiotherapy, GC poses significant challenges in terms of management, with frequently unfavorable prognoses prevailing. Current interventions yield disappointing outcomes, including a 5-year survival rate below 10% and a median overall survival (OS) of 1 year [2,3]. Over the past decade, therapeutic targets for advanced GC have mainly included programmed cell death 1 (PD-1) and HER2, followed by the introduction of immunotherapies and HER2 inhibitors into clinical practice, and these treatments are becoming the new standard of treatment for various tumors [4–6]. Several molecularly targeted agents have exhibited encouraging clinical effects in GC treatment, with trastuzumab used as the first-line therapy for receptor tyrosine-protein kinase erbB-2-positive patients, and ramucirumab as the second-line therapy. However, treatments targeting other specific markers, such as tyrosine-protein kinase Met epidermal growth factor receptor, and mammalian target of rapamycin, have not demonstrated significant improvements in survival outcomes [7–9]. Therefore, it is urgent to elucidate additional core pathogenic genes in GC, clarify its underlying mechanisms, and identify promising favorable therapeutic targets.

The enzymatic function of pyrroline-5-carboxylate reductase (PYCR) is responsible for facilitating the ultimate phase in the transformation of 1-pyrroline-5-carboxylate (P5C) into proline in almost all organisms [10,11]. Proline is a nonenzymatic antioxidant that suppresses apoptosis [12], whereas P5C can produce reactive oxygen species that stimulate apoptosis [13–15], inferring that PYCR holds the potential to regulate apoptosis. PYCRL, PYCR1, and PYCR2 are the three human PYCR isoenzymes that have been discovered [11]. PYCR1 exhibits the capability to protect cells from mitochondrial fragmentation induced by oxidative stress [16]. Furthermore, investigations have revealed that PYCR1 overexpression and increased activity have been observed in various carcinoma types, encompassing lung, prostate, colon, liver, and breast cancers [17–21]. PI3K/AKT pathway plays an important role in inducing cell malignant transformation, tumor angiogenesis and apoptosis [22]. Moreover, AKT1 and PIK3mRNA levels in gastric cancer tissues were positively correlated with PYCR1 [22,23]. However, whether PYCR1 regulates the occurrence and development of GC by regulating the PI3K/AKT pathway remains unclear. In this study, the relationship between PYCR1 expression and GC prognosis was analyzed. We found that overexpression of PYCR1 in GC tissues was related to decreased survival time and that PYCR1 could promote the migration and proliferation of GC through the phosphatidylinositol 3-kinase (PI3K)/AKT serine/threonine kinase (AKT) signaling.

## 2. Methods

### 2.1. Specimens and immunohistochemical analysis

GC specimens from 280 patients at the First Hospital of Jilin University (Changchun, China) were analyzed. Prior to the inclusion of surgical specimens in this study, informed consents were obtained from all patients following the guidelines approved by the Ethics Committee of the First Hospital of Jilin University. The histologic evaluation was conducted using the streptavidin-peroxidase assay. The tissue sections were probed with a primary antibody targeting PYCR1 (diluted at 1:100; Cat. No. 13108-1-AP, Proteintech, Chicago, IL), and the staining intensity was evaluated by two independent pathologists. The scoring system was based on the assessment of the staining intensity of each specimen (0, light yellow; 1, yellow; 2, yellow-brown; or 3, red-brown), and the proportions of cells displaying positive staining (0; 0%; 1, 0–20%; 2, 20–60%; or 3, 60–100%). Samples with total scores of 0–1 were considered negative, while samples with scores of 2–6 were considered positive.

### 2.2. Database analysis

This research investigated the progression and OS of 641 and 876 patients diagnosed with GC, stratified based on their PYCR1 mRNA levels (high and low). This analysis involved the construction of Kaplan-Meier curves by utilizing the online Kaplan_Meier Plotter tool (http://www.kmplot.com). Furthermore, hazard ratios, accompanied by their corresponding 95% confidence intervals, and log-rank $p$ values were computed to determine the significance, with a significance level set at $p < 0.05$.

### 2.3. Cell culture

Human GC cell lines (MGC80-3, AGS, SGC-7901, and MKN28) and a human embryonic kidney cell line (HEK293T) were acquired from the Cell Bank of the Chinese Academy of Science (Shanghai, China). MGC80-3, SGC-7901 and MKN28 cells were cultured in Roswell Park Memorial Institute-1640 medium (Hyclone, Logan, UT), AGS cells were cultured in Ham's/F12 medium (Hyclone), and HEK293T cells were cultured in Dulbecco's modified Eagle's medium (Hyclone). To optimize the growth conditions, all culture media were enhanced with 10% fetal bovine serum (FBS) sourced from Biological Industries (Kibbutz Beit, Israel). All cell lines were maintained at a temperature of 37 °C in a 5% $CO_2$ atmosphere.

### 2.4. Lentiviral infection

The short hairpin RNA (shRNA) interference vector pFH-L was obtained from SBI company (CA, USA). This vector comprises several components, including a green fluorescent protein, an H1 upstream promoter, the pCDH cDNA cloning and expression lentiviral vector, and the lentivirus packaging vectors. The pFH-L vector was modified to incorporate specific shRNAs against PYCR1 (target sequences: CACAGTTTCTGCTCTCAGGAACTCGAGTTCCTGAGAGCAGAAACTGTG, shPYCR1) or negative control (shNC). Additionally, human PYCR1 cDNA (produced by Genewiz, Suzhou, China) was incorporated into the EcoRI and XmaI sites of the pCDH vector (oePYCR1). Subsequently, the shRNA or overexpression vector, along with the lentiviral packaging plasmids, was introduced into 293T cells through transfection for the purpose of producing the lentivirus particles. Further, both MGC80-3 and AGS cells were

transduced with either shPYCR1 or shCon constructs. These cell lines were then plated into 6-well plates at a density of 80,000 cells/well for MGC80-3 and 200,000 cells/well for AGS. Lentivirus was introduced to the cells at a multiplicity of infection (MOI) of 60 for MGC80-3 and an MOI of 50 for AGS, respectively. For PYCR1 overexpression, MGC80-3 and MKN28 cells, seeded into 6-well plates at 100,000 and 120,000 cells/well, respectively, were transduced with oePYCR1 or oeCon. Cells were analyzed and imaged using a fluorescence microscope (BX50, Olympus, Tokyo, Japan) after transduction for 96 h. Infection efficiency was evaluated using PYCR1 Western blot and quantitative real-time polymerase chain reaction (qRT-PCR) analysis.

### 2.5. qRT-PCR analysis

TRIzol reagent (manufactured by Invitrogen) was utilized for the extraction of total RNA from GC cells. Subsequently, the extracted RNA underwent reverse transcription, converting it into cDNA by applying the M-MLV Reverse Transcriptase (Promega, Madison, WI). Following this, specific primer sequences were used for amplification: PYCR1–forward (F): 5′-GAAGATGGGGGTGAAGTTGA-3′; PYCR1–reverse (R): 5′-CTCAATGGAGCTGATGGTGA-3′. β-actin–F, 5′-GTGGACATCCGCAAAGAC-3′; β-actin–R, 5′-AAAGGGTGTAACGCAACTA-3′. The qRT-PCR was carried out using an ABI7300 cycler (Applied Biosystems, Foster City, CA).

### 2.6. Western blot analysis

Following cellular collection, cells were subjected to lysis utilizing a solution known as sodium dodecyl sulfate (SDS) lysis buffer, which consisted of components such as 100 mM Tris-HCl (pH 6.8), 10 mM ethylenediaminetetraacetic acid, 4% SDS, and 10% glycine. Equivalent amounts of proteins (20–30 μg) were meticulously loaded for SDS–polyacrylamide gel electrophoresis. Following this, proteins were safely transferred to specialized polyvinylidene fluoride membranes supplied by Millipore (Billerica, MA). To prepare the membranes for further analysis, they were appropriately blocked utilizing 5% skim milk in Tris-buffered saline. Afterwards, the membranes underwent a subsequent incubation stage at 4 °C overnight with primary antibodies against various proteins: PYCR1 (#13108-1-AP; 1:1000, Proteintech), cleaved caspase 3 (#19677-1-AP; 1:1000, Proteintech), SNAI1 (snail, #13099-1-AP; 1:1000, Proteintech), E-cadherin (#20874-1-AP; 1:1000, Proteintech), phosphorylated (p)-PI3K (#4228; p85 (Tyr458)/p55 (Tyr199), 1:1000, Cell Signaling Technology [CST], Danvers, MA), PI3K (#4249; 1:1000, CST), p-AKT (#4060; Ser473 1:1000, CST), AKT (#4685; 1:1000, CST), as well as the loading control glyceraldehyde-3-phosphate dehydrogenase (GAPDH; #10494-1-AP; 1:500000, Proteintech). Then, the Tris-buffered saline was later utilized to rinse the membranes before incubation with a horseradish peroxidase-conjugated secondary goat anti-rabbit IgG antibody (#SC2054; Santa Cruz Biotechnology, Dallas, TX). Protein signals were detected with a Tanon 4200 Chemiluminescent Imaging System (Tanon Science and Technology, Shanghai, China).

### 2.7. Viability assay

The viability of MGC80-3 and AGS cells, transduced with shCon– and shPYCR1, was assessed utilizing the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Following a 72–h infection, the cell lines were trypsinized, suspended, counted, and plated in 96-well plates at 2000 and 2600 cells/well, respectively, for a duration of 1–5 days. In each well, an MTT solution was introduced for a 4–h incubation period, followed by the addition of acidic isopropanol and overnight incubation. To evaluate cell growth, the absorbance measurement at 595 nm was triplicated.

### 2.8. Cell cycle entry analysis

MGC80-3 and AGS cells transduced with shCon or shPYCR1 were trypsinized, centrifuged at 2500 g for 5 min, rinsed with cold phosphate-buffered saline (PBS), and fixed with 75% ethanol. The cells were subsequently washed with PBS and incubated with 5 μL of RNase (200 U/mL, DNase-free) for 5 min. Then, 10 μg/mL propidium iodide (Sigma) was added. Cell cycle status was evaluated on a FACS Calibur instrument (BD Biosciences, San Jose, CA) in triplicate, and the analysis was facilitated using the Modfit LT 4.1 (Verity Software House, Topsham, ME).

### 2.9. Cell apoptosis measurement

MGC-803 or AGS cells in the presence or absence of PYCR1 knockdown were gathered after exposure to the lentiviruses for 96 h and replanted in a 6-well plate ($2 \times 10^5$ cells/well). After approximately 40 h, the cells were collected utilizing trypsin and rinsed twice with cold PBS and binding buffer. The harvested cells were subsequently re-introduced in a staining buffer and then stained by applying Annexin V-allophycocyanin (APC) staining solution in the light-restricted environment for a duration of 10–15 min at ambient temperature. A FACS Calibur equipment was utilized to conduct the flow cytometric analysis, and the acquired data were Assessed using FlowJo7.6 software (FlowJo LLC, Ashland, OR).

### 2.10. Transwell migration assay

MGC80-3, MGC80-3 (shPYCR1), MGC80-3 (oePYCR1), and MKN28 (oePYCR1)–transduced cells were placed in serum-free culture medium at specific cell densities of 150,000, 80,000, 80,000, and 100,000 cells/well, respectively. These cells were then seeded into the upper insert of the Transwell plate. On the other hand, the lower insert was introduced with 500 μL of culture medium, and it

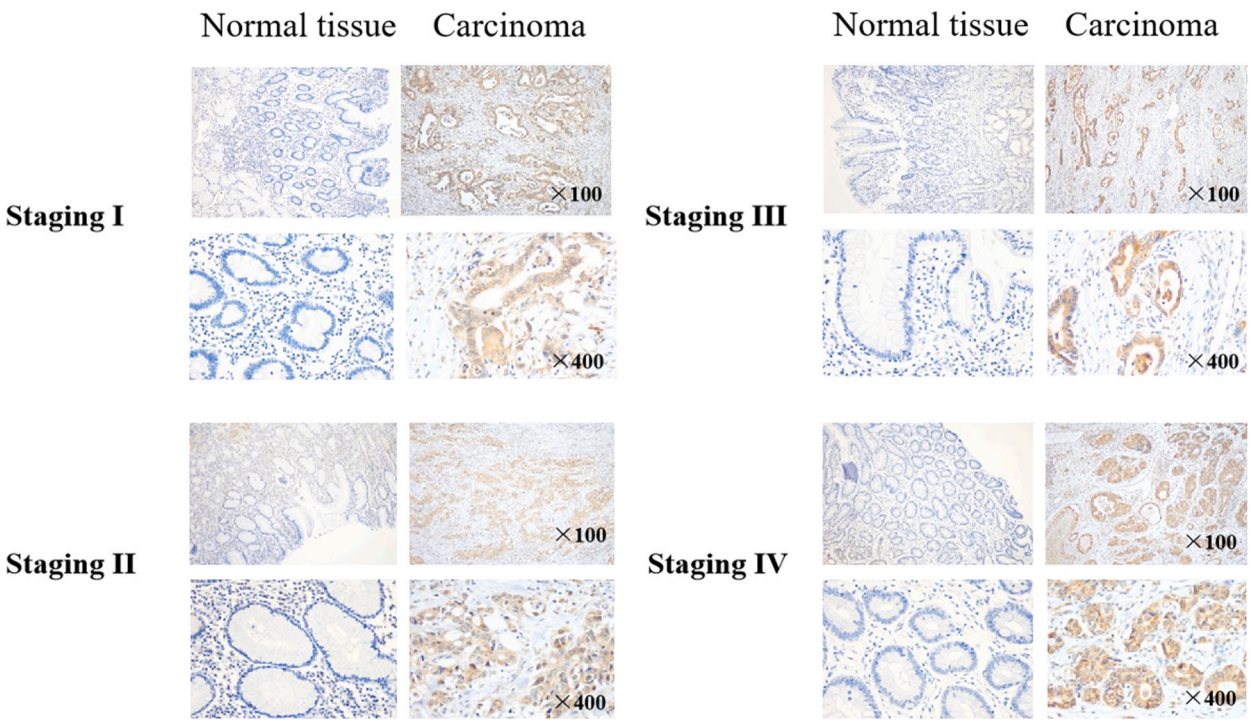


**Fig. 1.** Histologic examination of specimens obtained from patients with gastric cancer (GC) using the streptavidin-peroxidase assay.

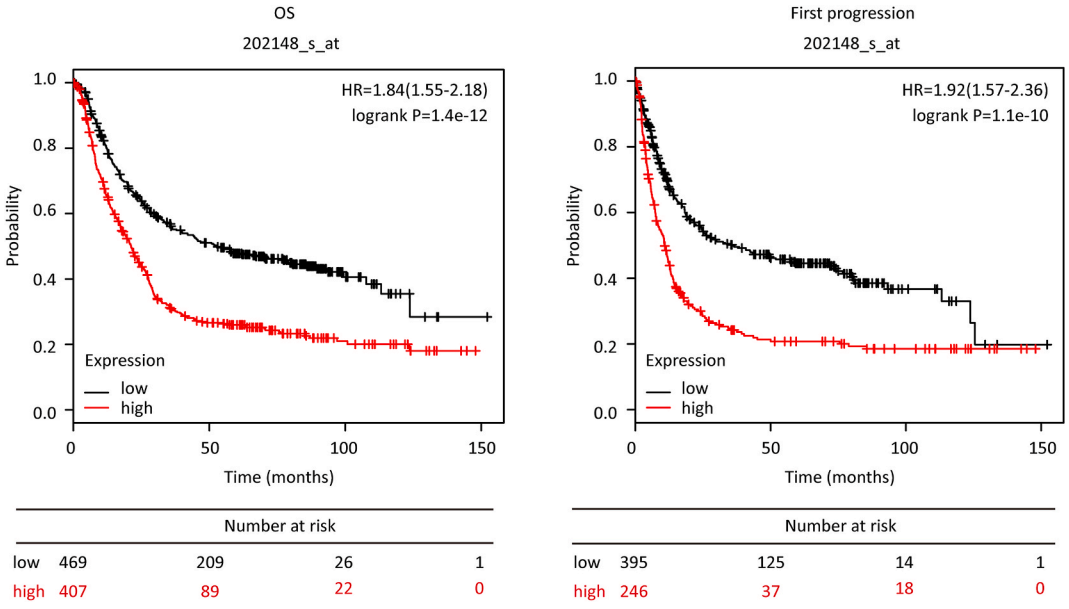


**Fig. 2.** Clinical prognostic relevance of PYCR1 in GC
Kaplan-Meier survival curves illustrate the differences in progression-free survival (PFS) and overall survival (OS) based on the high and low expression of PYCR1.

incorporated a 10% FBS. Following a 24–h of incubation period, a cotton swab was utilized to remove cells in the bottom chamber, and cells were subsequently rinsed twice. After the cells were fixed utilizing 4% formaldehyde for 10 min, and stained with 1 mL/well 0.5% crystal violet for 30 min following three cycles of PBS washing. For cell counting, five random microscopic fields were selected.

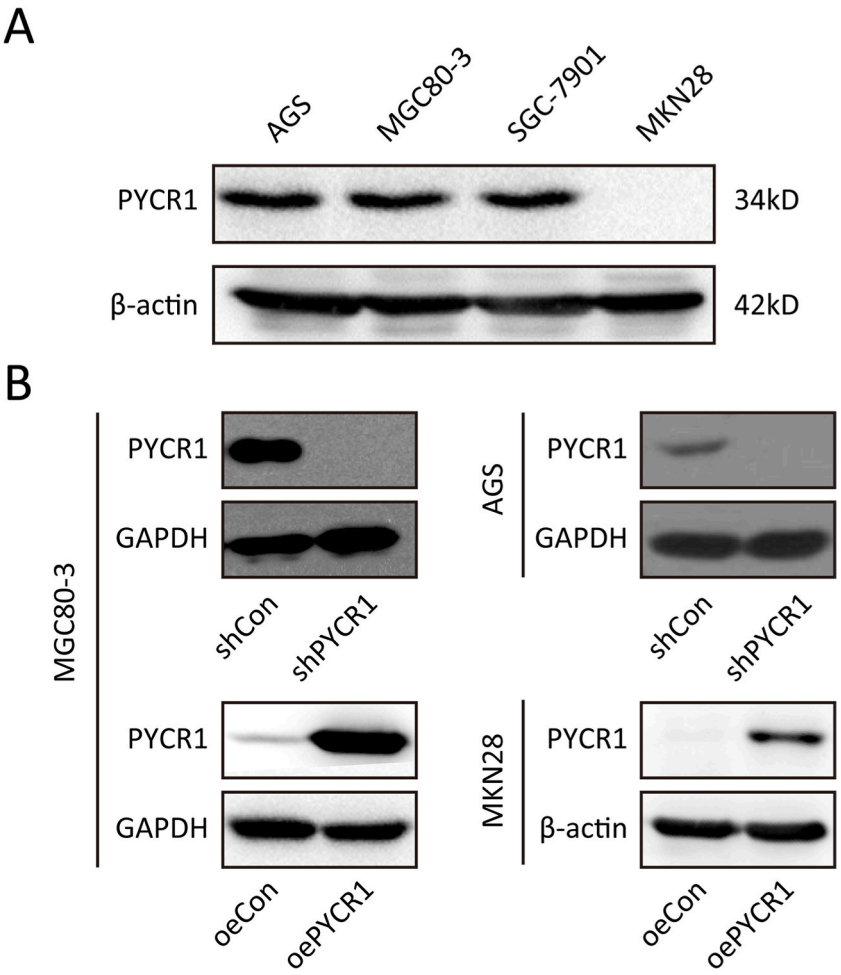


**Fig. 3.** Knockdown efficiency of PYCR1 depletion in GC cells **A**. PYCR1 protein levels in GC cell lines quantified through Western blot, as normalized to β-actin. **B.** Western blot analysis of PYCR1 levels in MGC80-3 cells following transduction with shPYCR1 or shCon, as normalized to GAPDH.

### *2.11. Statistical analysis*

The collected data underwent analysis using SPSS software (version 17.0; SPSS Inc, Chicago, IL) and were displayed as the mean ± standard deviation. The significance of research outcomes was assessed through the application of the student's t-test. The presentation of categorical data was showcased as the number and percentage, with comparisons utilizing the $\chi^2$ test. The Kaplan-Meier method was utilized to produce progression-free survival (PFS) and OS curves, which were analyzed by the log-rank test. *P*-values $<0.05$ meant statistical significance.

## 3. Results

### *3.1. PYCR1 is overexpressed in GC tissues*

In surgical GC specimens, the PYCR1 expression was identified (Fig. 1). Notably, GC tissues at various stages displayed more intense staining than adjacent noncancerous tissues.

### *3.2. PYCR1 overexpression is associated with unfavorable prognosis*

To examine the relevance of PYCR1 expression to the survival outcomes of GC patients, we generated Kaplan-Meier plots of patients categorized as having either high or low PYCR1 levels. Our data revealed a strong correlation between heightened levels of PYCR1 and unfavorable survival outcomes in terms of PFS and OS among GC patients (Fig. 2).

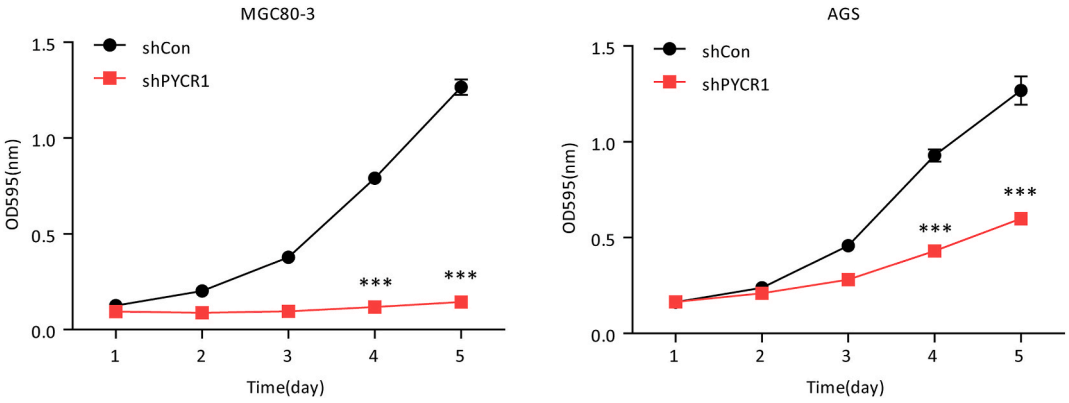


**Fig. 4.** PYCR1 depletion curtails the growth of GC cells
Both MGC80-3 and AGS cells were subjected to transduction with shCon or shPYCR1, followed by analysis using the MTT assay.

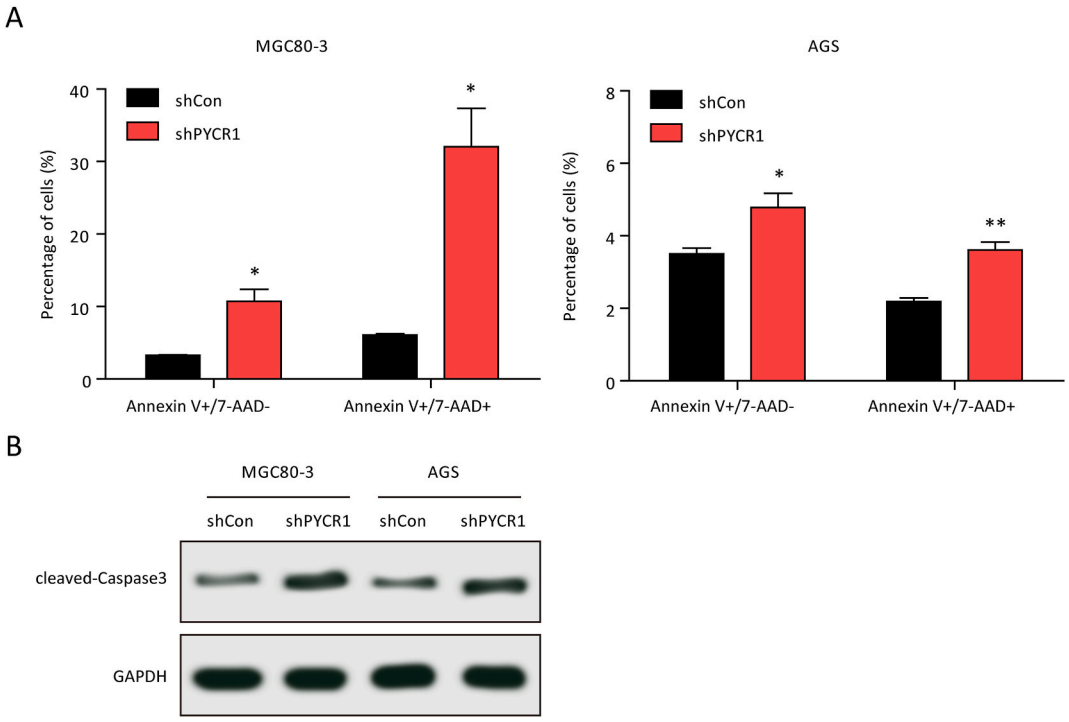


**Fig. 5.** PYCR1 depletion stimulates the apoptotic potential of GC cells A. In MGC80-3 cells, annexin V and 7-AAD double staining was performed to assess apoptosis after shPYCR1 or shCON transduction. B. Western blot examination of cleaved caspase 3 in AGS and MGC80-3 cells was conducted after shPYCR1 or shCON transduction, with GAPDH serving as a loading control.

### *3.3. Depletion of PYCR1 diminishes proliferation and enhances apoptosis in GC cells*

Western blot analysis implied that PYCR1 was expressed in AGS, MGC80-3, and SGC-7901 cells, but not in MKN28 cells (Fig. 3A). To delve into the biological roles of PYCR1 in GC cells, we used shRNAs to deplete PYCR1 expression in AGS and MGC80-3 cells. In both cell lines, PYCR1 protein levels were markedly diminished after transduction with shPYCR1 relative to shCon (Fig. 3B). Subsequently, we employed these shRNAs to understand the relationship between proliferative capacity and PYCR1 expression. The viability of MGC80-3 and AGS cells, which were transduced with shCon and shPYCR1, was evaluated through the MTT assay. The experimental data affirmed markedly restricted viability in both cell lines upon transduction of shPYCR1, as compared to shCon (both $p < 0.001$, Fig. 4). Apoptosis assessment in MGC80-3 and AGS cells following PYCR1 silencing was conducted utilizing double staining with annexin V and 7-aminoactinomycin D (7-AAD). The data unveiled an appreciably higher proportion of apoptotic MGC80-3 cells upon PYCR1 depletion relative to control cells (annexin V+/7-AAD–: 10.72 ± 1.68% *vs.* 3.27 ± 0.09%, $p < 0.05$; annexin V+/7-AAD+: 32.07 ± 5.27% *vs.* 6.08 ± 0.17%, $p < 0.05$; Fig. 5A). Similarly, the apoptotic AGS cell proportion was notably higher in the

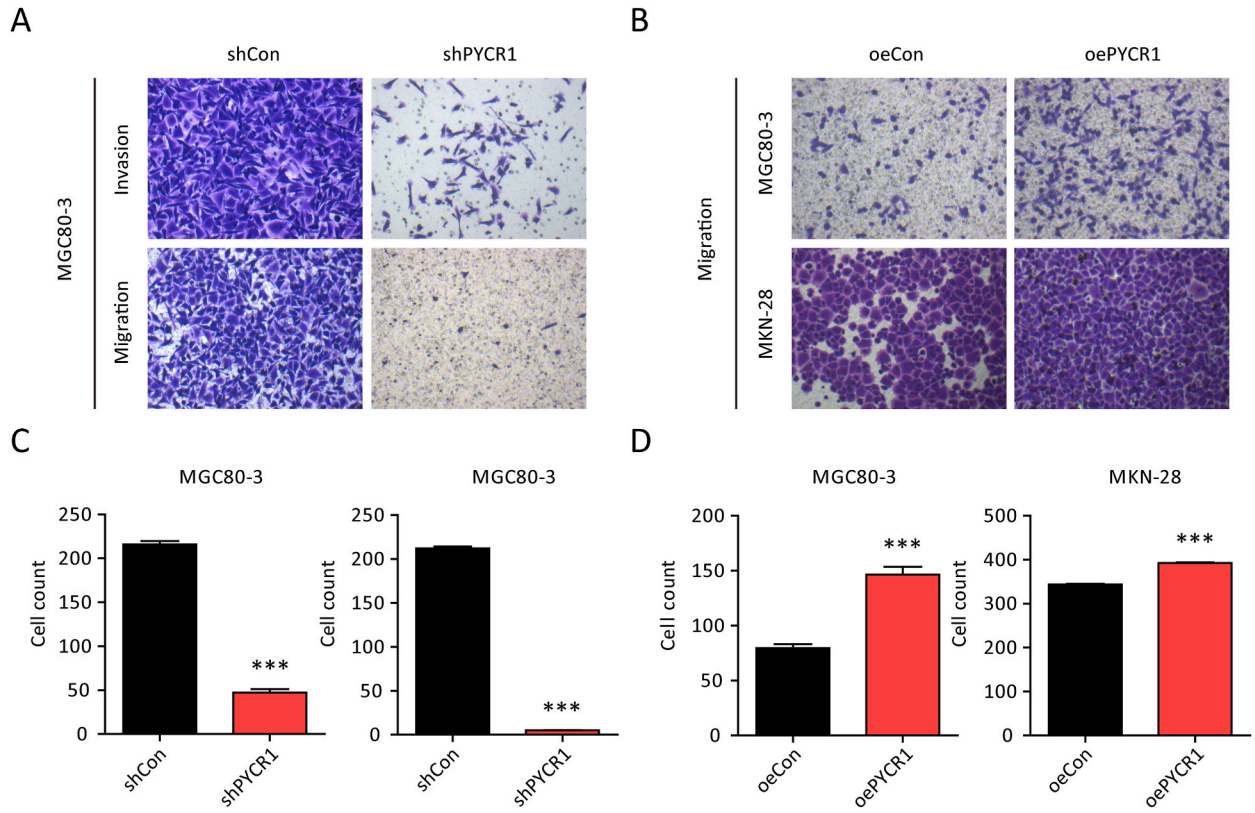


**Fig. 6.** Effect of PYCR1 depletion and overexpression on migration of GC cells A., D. Transwell migration and invasion assays were performed on MGC80-3 cells following transduction with either shPYCR1 or shCon. B., E. Transwell migration assays were conducted on MGC80-3 and MKN28 cells following transduction with either oePYCR1 or oeCon. C. Western blot analysis of PYCR1 expression in MGC-803 and MKN28 cells following transduction with oePYCR1 or oeCon. Protein level quantitation was normalized to GAPDH and β-actin.

PYCR1–depleted group compared with the control (annexin V+/7-AAD-: 4.78 ± 0.39% *vs.* 3.50 ± 0.16%, $p < 0.05$; annexin V+/7-AAD+: 3.61 ± 0.22% *vs.* 2.19 ± 0.11%, $p < 0.01$; Fig. 5A). As depicted in Fig. 5B, the cleaved caspase 3, an apoptotic protein, was augmented in both MGC80-3 and AGS cells after PYCR1 depletion.

### 3.4. PYCR1 depletion and overexpression yield conflicting results on metastasis

This investigation proceeded to examine the function of PYCR1 in metastasis *via* Transwell assays (Fig. 6A and C). In addition to establishing a cellular model with knockdown of PYCR1, we also established a stable overexpression model of PYCR1 in MGC80-3 and MKN28 cells (Fig. 6B). The statistics data revealed migration and invasion of MGC80-3 cells was curtailed upon shPYCR1 transduction compared with shCon (5.1 ± 0.2 *vs.* 212.2 ± 2.0 and 47.3 ± 4.0 *vs.* 215.9 ± 3.8, respectively; both $p < 0.001$, Fig. 6D). Moreover, in both cell lines, PYCR1 overexpression fostered the migration compared to oeCon (146.5 ± 7.1 *vs.* 79.7 ± 3.4, and 392.8 ± 1.1 *vs.* 344.1 ± 1.0; both $p < 0.001$, Fig. 6D).

### 3.5. PYCR1 modulates GC apoptosis and metastasis via the PI3K/AKT signaling

The effects of PYCR1 on the levels and activation status of proteins involved in apoptosis and metastasis–related signaling pathways were evaluated by Western blot and qRT-PCR. In MGC80-3 cells, PYCR1 depletion led to a decrease in the extent of PI3K and AKT phosphorylation, while ectopic expression of PYCR1 facilitated p-PI3K and p-AKT (Fig. 7A). We further assayed downstream metastasis-related proteins of the PI3K/AKT pathway, and observed an elevation in E-cadherin and a reduction in Snail upon PYCR1 knockdown (Fig. 7B). Conversely, diminished E-cadherin levels and increased Snail levels were observed in MGC80-3 cells following PYCR1 overexpression.

## 4. Discussion

Cancer cells alter their metabolism to increase survival and promote proliferation and metastasis [24,25]. Proline biosynthesis can promote protein production, which is required for proliferation, and the silencing of PYCR1 is sufficient to impair proliferation in some

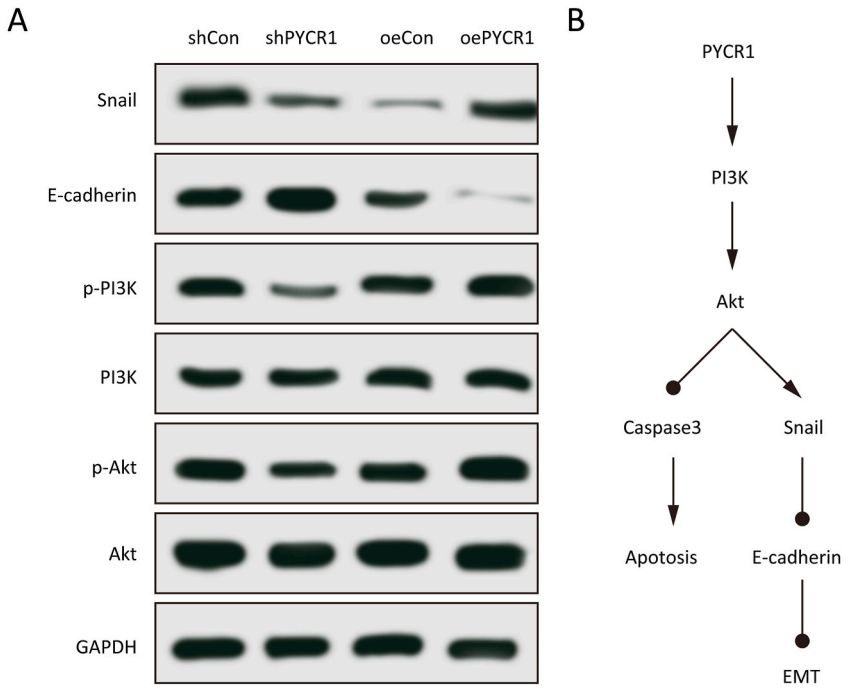


**Fig. 7.** PYCR1 regulates GC cell apoptosis and metastasis *via* the PI3K/AKT signaling A. Western blot analysis of control and transduced cells, as normalized to GAPDH. B. Possible molecular mechanisms by which PYCR1 may regulate the malignant phenotypes of GC cells.

cancers [26]. In addition, high expression of PYCR1 is significantly connected to the unfavorable prognosis among breast cancer patients [27]. This finding implies that PYCR1 serves as a potential oncogene; however, its mechanism of action in GC remains largely unexplored.

The findings of this investigation demonstrated pronounced upregulation of PYCR1 in GC tissues in contrast to noncancerous tissues, accompanied by a noteworthy correlation between PYCR1 expression in GC tissues and shorter OS. Upon depletion of PYCR1, GC cell lines displayed diminished proliferative capacity, facilitated apoptotic potential, and curbed migratory ability, whereas contrasting effects were observed upon PYCR1 overexpression. These findings propose the potential role of PYCR1 as a pivotal driver gene in GC. Furthermore, PYCR1 may serve as an independent prognostic biomarker for individuals diagnosed with GC.

The dysregulation of the PI3K/AKT signaling is implicated in multiple cellular processes, such as proliferation, tumorigenesis, invasion, and migration [28]. The enhanced activation of PI3K and AKT, responsible for substrate phosphorylation, is frequently observed in a wide array of cancer types [29], indicating its potential significance in GC. Our results uncover that PYCR1 actively activates and modulates the PI3K/AKT pathway in GC cell lines. In addition, downstream apoptosis– and metastasis–related molecules under the influence of PI3K/AKT signaling were identified. In GC cells, it was substantiated that PYCR1 depletion resulted in an increase in E-cadherin level and a decline in Snail level. Conversely, ectopically expressed PYCR1 diminished E-cadherin levels and raised Snail levels. These findings indicate that PYCR1 might facilitate metastatic events *via* the induction of Snail-mediated epithelial-mesenchymal transition (EMT). Moreover, caspase 3 activation was heightened in GC cells in the presence of PYCR1 depletion. Collectively, it can inferred that PYCR1 is implicated in the modulation of apoptotic and metastatic capacities of GC cells primarily through orchestrating the PI3K/AKT signaling.

There are some limitations to our study. First, we did not determine whether PYCR1-mediated proline metabolism plays an important role in GC. In addition, it has been shown that epigenetic modification of PYCR1 plays an important role in the occurrence and development of GC [30]. Therefore, whether epigenetic modification of PYCR1 is related to the activation of PI3K/AKT pathway remains to be further studied.

## 5. Conclusion

In conclusion, this study provides evidence supporting the potential oncogenic role of PYCR1 in GC. PYCR1 sheds light on the underlying mechanistic actions governing the growth of GC through its involvement in the stimulation of proliferation and metastasis and suppression of apoptosis *via* the PI3K/AKT signaling. Moreover, these findings emphasize the significance of PYCR1 as a promising molecular target for the diagnosis and targeted therapeutic interventions of GC.

## Data availability statement

Data associated with the study has not been deposited into a publicly available repository and data will be made available on request.

## CRediT authorship contribution statement

**Huijie Xiao:** Writing – original draft, Funding acquisition. **Jiannan Huang:** Writing – original draft. **Haitao Wu:** Investigation. **YuYing Li:** Software, Conceptualization. **Yizhuo Wang:** Project administration.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e26883.

## References

[1] F. Bray, et al., Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, CA Cancer J Clin 68 (6) (2018) 394–424.
[2] F. Lordick, et al., Unmet needs and challenges in gastric cancer: the way forward, Cancer Treat Rev. 40 (6) (2014) 692–700.
[3] A.D. Ricci, A. Rizzo, G. Brandi, DNA damage response alterations in gastric cancer: knocking down a new wall, Future Oncol. 17 (8) (2021) 865–868.
[4] M. Santoni, et al., The impact of gender on the efficacy of immune checkpoint inhibitors in cancer patients: the MOUSEION-01 study, Crit. Rev. Oncol. Hematol. 170 (2022) 103596.
[5] A. Rizzo, et al., Third- and later-line treatment in advanced or metastatic gastric cancer: a systematic review and meta-analysis, Future Oncol. 16 (2) (2020) 4409–4418.
[6] A.D. Ricci, et al., Novel HER2-directed treatments in advanced gastric carcinoma: AnotHER paradigm shift? Cancers 13 (7) (2021).
[7] A.F. Okines, et al., Epirubicin, oxaliplatin, and capecitabine with or without panitumumab for advanced esophagogastric cancer: dose-finding study for the prospective multicenter, randomized, phase II/III REAL-3 trial, J. Clin. Oncol. 28 (25) (2010) 3945–3950.
[8] T. Doi, et al., Multicenter phase II study of everolimus in patients with previously treated metastatic gastric cancer, J. Clin. Oncol. 28 (11) (2010) 1904–1910.
[9] L. Marano, et al., c-Met targeting in advanced gastric cancer: an open challenge, Cancer Lett. 365 (1) (2015) 30–36.
[10] E. Adams, L. Frank, Metabolism of proline and the hydroxyprolines, Annu. Rev. Biochem. 49 (1980) 1005–1061.
[11] J. De Ingeniis, et al., Functional specialization in proline biosynthesis of melanoma, PLoS One 7 (9) (2012) e45190.
[12] C. Chen, M.B. Dickman, Proline suppresses apoptosis in the fungal pathogen Colletotrichum trifolii, Proc. Natl. Acad. Sci. U.S.A. 102 (9) (2005) 3459–3464.
[13] S.P. Donald, et al., Proline oxidase, encoded by p53-induced gene-6, catalyzes the generation of proline-dependent reactive oxygen species, Cancer Res. 61 (5) (2001) 1810–1815.
[14] S.A. Maxwell, G.E. Davis, Differential gene expression in p53-mediated apoptosis-resistant vs. apoptosis-sensitive tumor cell lines, Proc. Natl. Acad. Sci. U.S.A. 97 (24) (2000) 13009–13014.
[15] K. Polyak, et al., A model for p53-induced apoptosis, Nature 389 (6648) (1997) 300–305.
[16] G.C. Yeh, S.C. Harris, J.M. Phang, Pyrroline-5-carboxylate reductase in human erythrocytes, J. Clin. Invest. 67 (4) (1981) 1042–1046.
[17] R. Possemato, et al., Functional genomics reveal that the serine synthesis pathway is essential in breast cancer, Nature 476 (7360) (2011) 346–350.
[18] T. Ernst, et al., Decrease and gain of gene expression are equally discriminatory markers for prostate carcinoma: a gene expression analysis on total and microdissected prostate tissue, Am. J. Pathol. 160 (6) (2002) 2169–2180.
[19] H. Okabe, et al., Genome-wide analysis of gene expression in human hepatocellular carcinomas using cDNA microarray: identification of genes involved in viral carcinogenesis and tumor progression, Cancer Res. 61 (5) (2001) 2129–2137.
[20] O. Greengard, A. Herzfeld, The undifferentiated enzymic composition of human fetal lung and pulmonary tumors, Cancer Res. 37 (3) (1977) 884–891.
[21] A. Herzfeld, O. Greengard, W.V. McDermott, Enzyme pathology of the liver in patients with and without nonhepatic cancer, Cancer 45 (9) (1980) 2383–2388.
[22] S. Xiao, et al., Pyrroline-5-carboxylate reductase 1 (PYCR1) upregulation contributes to gastric cancer progression and indicates poor survival outcome, Ann. Transl. Med. 8 (15) (2020) 937.
[23] B. Zhang, et al., JMJD8 promotes malignant progression of lung cancer by maintaining EGFR stability and EGFR/PI3K/AKT pathway activation, J. Cancer 12 (4) (2021) 976–987.
[24] I. Elia, et al., Organ-specific cancer metabolism and its potential for therapy, Handb. Exp. Pharmacol. 233 (2016) 321–353.
[25] S.Y. Lunt, S.-M. Fendt, Metabolism – a cornerstone of cancer initiation, progression, immune evasion and treatment response, Curr. Opin. Struct. Biol. 8 (2018) 67–72.
[26] F. Loayza-Puch, et al., Tumour-specific proline vulnerability uncovered by differential ribosome codon reading, Nature 530 (7591) (2016) 490–494.
[27] J. Ding, et al., Human mitochondrial pyrroline-5-carboxylate reductase 1 promotes invasiveness and impacts survival in breast cancers, Carcinogenesis 38 (5) (2017) 519–531.

[28] S. Faes, O. Dormond, PI3K and AKT: unfaithful partners in cancer, Int. J. Mol. Sci. 16 (9) (2015) 21138–21152.
[29] I.A. Mayer, C.L. Arteaga, The PI3K/AKT pathway as a target for cancer treatment, Annu. Rev. Med. 67 (2016) 11–28.
[30] S. Xiao, et al., Epigenetic modification facilitates proline synthase PYCR1 aberrant expression in gastric cancer, Biochim Biophys Acta Gene Regul Mech 1865 (6) (2022) 194829.