PYCR1 Promotes Proliferation, Invasion and Drug-Resistance of Lung Cancer Cells by Regulating STAT3-Mediated PI3K/AKT and NF- κ B Signaling Pathway

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EDITORIAL NOTE:

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Abstract

Background: Aberrant expression of PYCR1 has been proved to be one of the most pivotal regulators of tumor progress and metastasis. However, the detailed role of PYCR1 in promotion of NSCLC progress is not investigated thoroughly. The present study was aimed to thoroughly investigate the effect of PYCR1 in the growth of NSCLC and the underlying mechanisms, so that provide valuable theoretical basis for efficient treatment of NSCLC.

Methods: The expressions of PYCR1 and its target genes and downstream signals were respectively determined by Western-blot assay and RT-qPCR experiment. Cell growth of NSCLC cells was investigated using the CCK-8 kit while the proliferation assay was performed with the help of EDU staining. The NSCLC-bearing mice mode was established to evaluate the effect of PYCR1 on the progress of NSCLC and the underlying mechanisms *in vivo*.

Results: It was firstly demonstrated that the PYCR1 was overexpressed in lung cancer tissues and cells and overexpression of PYCR1 contributed to significantly enhanced proliferation, invasion, and drug-resistance of cancer cells and progress of NSCLC tissues. Further studies revealed that up-regulation of PYCR1 level resulted to the elevation of bioactivity of STAT3. Additionally, the positive correlation between the expression of PYCR1 and STAT3 indicated that the PYCR1 promotes the growth of NSCLC through targeting regulation the STAT3 levels. Furthermore, activation of STAT3 by PYCR1 was proved to be able of awaking the PI3K/AKT and NF- κ B signaling pathway, which was extremely important to the metabolism, proliferation, cell survival, and growth of many cancer cell types.

Conclusion: In conclusion, our study demonstrated that PYCR1 was specifically overexpressed in lung cancer and closely related to rapid progress and drug-resistance thorough regulating the STAT3-mediated PI3K/AKT and NF- κ B signaling pathway. It may provide a valuable strategy for treatment of malignant lung cancer.

Background

As one of the most malignant tumors, lung cancer was generally characterized with fast growth rate, high morbidity, and terrible mortality [1, 2]. According to the statistics, the morbidity and mortality of lung cancer ranks first among men [3]. The non-small cell lung cancer (NSCLC) represents one of the most aggressive type of lung cancer, which accounts for 85% all mortality cases globally [4, 5]. Treatment of NSCLC are still seriously blocked by the rapid metastasis, fast progress, and insufficient therapy approaches [6]. Additionally, majority of patients with lung cancer will develop multi-drug resistance in the later stage, resulting in insensitive to chemotherapy and high mortality [7, 8].

Pyrroline-5-carboxylate reductase 1 (PYCR1), an enzyme involved in cell metabolism, has been shown to be up-regulated in many cancer types [9–12]. Previous study has proved that knockdown of PYCR1 significantly inhibited the proliferation, colony, and invasion of prostate cancer cells [9]. In addition, over expression of PYCR1 was detected in breast cancer and down-regulation of PYCR1 levels markedly

decrease the proliferation ability of breast cancer cells, while obviously enhanced the apoptosis rate of cancer cells [10]. Importantly, high level of PYCR1 was also detectable in NSCLC and it was high expression of PYCR1 closely related to the progression of NSCLC [11]. However, the aberrant expression of PYCR1 in NSCLC and its role and mechanisms in regulating progress of lung cancer are still need to be thoroughly investigated, so as to provide a new theoretical basis for the diagnosis and treatment of lung cancer.

The signal transducer and activator of transcription protein (STAT), belongs to the family of cytoplasmic transcription factors, has been demonstrated played significant role in the signal transduction through multiple mechanisms [13]. Increasing evidences revealed that the STAT3 was involved in the development and progression of many cancer types through participation in a series of tumorigenic processes such as cell proliferation and survival [14]. Moreover, aberrant expression and constitutive activation of STAT3 have been detected in a variety of tumors such as the gastric cancer, breast cancer, prostate tumor, and NSCLC [13–15]. Additionally, knockdown of STAT3 was proved to be facilitate to enhance the chemotherapy efficacy of malignant cancer by inhibition cell proliferation, invasion, and inhibition of drug-resistance [16].

The present study was aim to explore the effect of PYCR1 on proliferation, invasion and drug resistance of lung cancer cells and the underlying mechanisms. Additionally, the mutual relation between the PYCR1 and STAT3 was further investigated.

Materials And Methods

Chemicals and reagents

The cell counting kit-8 (CCK-8) was purchased from Dojindo Molecular Technologies (Gaithersburg, MD). 5-Fluorouracil was obtained from Aladdin (Shanghai, China). Primary antibodies and horseradish peroxidase (HRP)-conjugated secondary antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The complete RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 µg/ml streptomycin was purchased from Hyclone (GE Healthcare Life Sciences, Logan, UT, USA). The EdU kit was obtained from Guangzhou RiboBio Co., Ltd. (Guangzhou, China) and the Hoechst 33342 was purchased from Molecular Probes (Eugene, OR, USA).

Cell culture and animals

The human lung adenocarcinoma cell lines H520, H1299, H1975, and A549 cells were achieved from the Chinese Academy of Medical Sciences (Shanghai, China) while the normal cell line BEAS-2B was obtained from the American Type Culture Collection (ATCC, Rockville). All the cells were cultured in the RPMI-1640 medium as mentioned above under a 5% CO₂ atmosphere at 37°C. The male nude mice with weight of 20 ± 2g and average of 4~5 weeks were obtained from BK Lab Animal Ltd. (Shanghai, China).

Cell transfection

For cell experiments, A549 cells were transfected respectively in advance with various siRNAs (GenePharma, Shanghai, China) including the Ctrl-siRNA, si-PYCR1, p-PYCR1, and STAT3. The cell transfections were performed using the Lipofectamine 2000 (Invitrogen, NY, USA) with the help of plasmid vectors pcDNA3.1. Finally, the transfection efficiencies were evaluated after 48 h of the transfection using the Western-blot assay.

Cell proliferation and invasion

Cells were seeded in 96-well plates at the density of 5×10^3 cells/well. After an overnight of incubation, the old medium was replaced with fresh cell culture medium and continue to culture for 24 h. Then 50 mM EdU was added and co-incubated with cells for 2 h followed by fixation with 4% formaldehyde for 15 min. After that, the cells were incubated with 1X Apollo reaction cocktail and stained with Hoechst 33342 for 30 min. Finally, the results were visualized by the fluorescence microscope (Carl Zeiss).

To investigate the invasion ability, cells were seeded on the upper chamber of trans-well at the density of 1×10^5 cells/well. The lower compartment was filled with 00 mL of complete medium containing 20% FBS. Then the cells were allowed to incubate for 24 h. After that, the non-invading cells on the upper chamber were removed while the invaded cells were fixed by 4% paraformaldehyde. For quantitative analysis, the cells were stained with hematoxylin followed by counting cell numbers using Image J software.

CCK-8 assay

5×10^3 cells were seeded in 96-well plates and cultured at 37°C. After treated as indicated, the cells were allowed to grow for 24. Then 10 μ L CCK-8 solution was added into each well of the plates. After incubation for 4 h, the absorbance was determined by microplate reader (BioTek Instruments, Winooski, VT) with the detection wavelength was set at 450 nm.

Western blotting analysis

The radioimmunoprecipitation assay lysis buffer (Cell Signaling Technology, Inc.) was applied to extract the total protein of cell samples. Then the protein concentration was determined using the BCA Protein Assay kit (Pierce Biotechnology, Inc., Rockford, IL, USA). For separation of the protein samples, 10% SDS-PAGE (Sigma-Aldrich) was applied and the gel was allowed to run for 20 min under 80 V followed by 100 min under 120 V. After that, the samples were transferred to a polyvinylidene fluoride membrane (EMD Millipore, Billerica, MA, USA) followed by blocking with 5% w/v non-fat dry milk (Sigma-Aldrich). For protein detection, corresponding primary antibodies were introduced and incubated with protein samples for overnight at 4°C. HRP-labeled secondary antibodies was subsequently added and allowed to incubate with samples for 1 h under room temperature. Finally, an enhanced chemiluminescence kit (KPL, Inc., Gaithersburg, MD, USA) was used to determine the immunobands followed by analysis with ImageJ (version 1.48; National Institutes of Health, Bethesda, MD, USA) software.

RT-qPCR experiments

The total RNA in cells and tumor tissues was extracted using the Trizol reagent (Invitrogen, Carlsbad, CA). After the concentration of RNA was determined, the reverse transcription (RT) reaction was performed by the PrimeScript RT Master Mix (Perfect Real Time; Takara Bio Inc., Tokyo, Japan). Subsequently, the PCR reaction was performed thorough the ABI PRISM 7900 Real-Time system (Applied Biosystems, Foster City, CA). Finally, the $2^{-\Delta\Delta C_t}$ method was applied to determine the levels of various RNA with all RNA molecules were normalized to the GAPDH. The primers were adopted here were displayed as follows: PYCR1 forward, ACACCCACAACAAGGAGAC, and reverse, CTGGAGTGTGGTCATGCAG; PI3K forward, CATCACTTCCTCCTGCTCTAT, and reverse, CAGTTGTTGGCAATCTTCTTC; AKT forward, GGACAACCGCCATCCAGACT, and reverse, GCCAGGGACACCTCCATCTC; NF- κ B forward, GCCGTGGAGTACGACAA, and reverse, CGGTTTCCCATTCTAGTATGT; MMP-1 forward, AAATAGTGGCCCAGTGGTTG, and reverse, CACATCAGGCACTCCACATC; VEGF forward, TGTCTAATGCCCTGGAGCCT, and reverse, TTAAGTCAAGCTGCCTCGCC; P-gp forward, CCCATCATTGCAATAGCAGG, and reverse, GTTCAAAGTCTGCTCCTGA; GAPDH forward TGTGTCCGTCGTGGATCTGA, and reverse, CCTGCTTCACCACCTTCTTGA.

Immunofluorescence staining

Cells were fixed with 4% paraformaldehyde for 10 min and then washed with PBS before incubated with 0.5% Triton-20 (Sigma-Aldrich) for 10 min. After that, the samples were blocked with 4% bovine serum albumin (Sigma-Aldrich) for 1 h under room temperature. Then primary antibodies were added and incubated with samples for overnight at 4°C followed by incubated with fluorochrome-conjugated secondary antibody for 1h at room temperature. The results were analyzed under a confocal laser scanning microscopy (Leica TCS SP5II; Leica Microsystems GmbH, Wetzlar, Germany).

Immunohistochemistry (IHC) assay

The tumor tissues were sectioned for 5- μ m thick and then fixed with 4% paraformaldehyde for 10 min. After they were blocked with 4% bovine serum albumin, primary antibodies was introduced and co-incubated with the samples for overnight at 4°C. To visualize the target proteins, the horse radish peroxidase (HRP)-labeled secondary antibodies was allowed to react with the samples for 1 h. Subsequently, the sections were stained with 3,3'-diaminobenzidine for 10 min and counterstained with 0.5% hematoxylin for 1 min before evaluation under the confocal laser scanning microscopy.

Statistical analysis

Results in the study were presented as means $\pm$ standard error of the mean (SEM) using GraphPad Prism 7 software. Two and one-way analysis of variance (ANOVA) were used for compare between different treatments with p-values < 0.05 considered significant, < 0.01 very significant and < 0.001 highly significant.

Results

Overexpression of PYCR1 was detected in lung cancer tissues and cell lines

As determined by the RT-qPCR and Western-blot assay, the expression levels of PYCR1 in lung cancer tissues was significantly higher than that of the normal adjacent tissues (Figure 1A and B). Such results were further confirmed by the cellular PYCR1 expression. As shown in Figure 1C and 1D, the normal BEAS-2B cells expressed the lowest level of PYCR1. In contrast, obvious higher levels of PYCR1 was detected in the lung cancer cell lines, including the H520, H1299, H1975, and A549 cells. Of great importance, the drug-resistant A549 cells expressed the strongest signal of PYCR1. In this case, the A549 cells were selected as the cell model for further experiments.

Transfection with si-PYCR1 significantly inhibited the proliferation, invasion, and drug resistances of A549 cells

Firstly, the transfection efficiency was determined with results shown in Figure 2A revealed that cells transfection with si-PYCR1 lead to obvious down-regulation of PYCR1 expression in A549 cells. However, negligible changes were determined in the cells transfected with Ctrl-siRNA.

As shown in Figure 2B, silencing the PYCR1 expression led to an obvious down-regulated proliferation ability of A549 cells. Further investigation revealed that knockdown of PYCR1 resulted in dramatically decreased expression of vascular endothelial growth factor (VEGF) (Figure 2C), the primary genes regulating the proliferation of cells. Then the invasion ability of A549 cells was evaluated after various treatments. Results displayed that the invasion rate of A549 cells was markedly inhibited after transfection with si-PYCR1 by decreasing the levels of matrix metalloproteinase-1 (MMP-1), the main genes regulating cell invasion (Figure 2D and 2E). Whether silencing of PYCR1 could alleviate the drug-resistance of malignant lung cancer cells was subsequently determined. As shown in Figure 2F, down-regulation of PYCR1 levels significantly enhanced the chemotherapy effect of 5-Fu, a well-known anti-cancer agent. Additionally, further investigation exhibited that the expression of P-gp, the most important drug-resistant related protein, was dramatically down-regulated in A549 cells after transfection with si-PYCR1.

Overexpression of PYCR1 dramatically enhanced the proliferation, invasion, and drug resistances of A549 cells

Determination of the transfection efficiency revealed that the significantly higher expression of PYCR1 was detected in the A549 cells transfection with p-PYCR1 while not the Vector (Figure 3A). Further studies exhibited that high levels of PYCR1 was quite helpful in the proliferation of A549 cells through up-regulation of the VEGF expression (Figure 3B and 3C). Moreover, increase of PYCR1 expression also facilitated the invasion of A549 cells by elevation of the MMP-1 levels (Figure 3D and 3E). In contrast to the above results, transfection of A549 cells with p-PYCR1 significantly increased the IC₅₀ value of 5-FU and up-regulated the expression of P-glycoprotein (P-gp).

PYCR1 enhanced cell progress thorough targeting the STAT3

As shown in Figure 4A, the binding site between the STAT3 and PYCR1 was predicated by the ChIPBase. Subsequently, the binding of PYCR1 with STAT3 was further confirmed using the CoIP experiments. The results clearly illustrated that similar expression trend was obtained between the PYCR1 and STAT3. For further confirmation, the Western-blot assay and RT-qPCR assay was conducted to determine the levels of STAT3 after treating cells with p-PYCR1. Interestingly, transfection the lung cancer cells with p-PYCR1 dramatically increased the level of STAT3 (Figure 4B). Spearman's analysis further revealed that the expression of STAT3 was liner correlation to the PYCR1 expression. Taking these results together, it could be concluded that the STAT3 was the targeting gene of PYCR1.

PYCR1 promoted the proliferation, invasion, and drug resistances of A549 cells thorough regulating the STAT3 expression

As shown in Figure 5A, proliferation assay revealed that transfection of A549 cells with STAT3 significantly enhanced the proliferation ability. However, the proliferation rate of A549 cells was signally down-regulated by transfection with si-PYCR1. Further investigation displayed that transfection of cells with STAT3 led to obvious increase of VEGF expression while treated with si-PYCR1 resulted in contrary results (Figure 5B). Invasion assay showed that the STAT3 treated A549 cells have the strongest invasion rate by increase of MMP-1 expression (Figure 5C and 5D). Interestingly, such favorable effect could be signally discounted by additional transfection with si-PYCR1, suggested that the si-PYCR1 was against the expression of STAT3. IC₅₀ values in Figure 5E exhibited that high expression of STAT3 contributed to an adverse effect on the chemotherapy efficacy of 5-Fluorouracil (5-Fu), which was mainly because that overexpression of STAT3 significantly increase the P-gp level in A549 cells (Figure 5F). Finally, qualitative analysis of various proteins in A549 cells after various treatment was determined. The results shown in Figure 5G exhibited the similar trend to above with transfection with STAT3 significantly enhanced the expression of VEGF, MMP-1, and P-gp.

PYCR1 promoted the activation of STAT3-mediated PI3K/AKT and NF-κB Signaling Pathway

As shown in Figure 6A, overexpression of STAT3 led to high expression of PI3K, p-PI3K, AKT, and p-AKT, suggested that high level of STAT3 contributed to the activation of PI3K/AKT signaling pathway. Additionally, transfection with STAT3 also resulted in up-regulation of NF-κB p65 and p-p65, indicated that the NF-κB signaling pathway could also be activated by STAT3. For confirmation, RT-qPCR experiments were performed. As demonstrated in Figure 6B-6D, the levels of p-P13K/PI3K, p-AKT/AKT, and p-p65/p65 were signally elevated after transfection with STAT3. Moreover, the immunofluorescence analysis obtained the similar results as above with the stat3 treated cells achieved the highest levels of p65 and AKT (Figure 6E).

PYCR1 promoted lung tumor growth thorough the activation of STAT3-mediated PI3K/AKT and NF-κB Signaling Pathway

Tumor images in Figure 7A and quantitative analysis in Figure 7B showed that the PYCR1 treated mice exhibited the largest tumor volumes while contrary result was obtained by si-PYCR1 transfection. Immunohistochemical analysis revealed that treatment of PYCR1 led to high expression of VEGF, MMP-1, and P-gp in tumor tissues (Figure 7C), indicated that the PYCR1 promoted lung cancer growth thorough enhancing cancer cells proliferation, invasion, and drug-resistance. Quantitative analysis by RT-qPCR displayed that overexpression of PYCR1 contributed to high expression of p-P13K/PI3K (Figure 7D), p-AKT/AKT (Figure 7E), and p-p65/p65 (Figure 7F) in lung cancer tissues. Such results could be further confirmed by qualitative evaluation via Western-blot assay (Figure 7G).

Discussion

Lung cancer represents one of the most commonly diagnosed cancer with rapid progress, high metastatic, and high mortality [1–3]. Despite the development of a wide range of novel therapeutic methods, the treatment efficacy of lung cancer remain dissatisfactory with and overall 5-year survival rate was only 10% around [2, 4]. It have been demonstrated that a wide array of proteins were involved in the tumorigenesis, progress, metastasis, and drug-resistance of lung cancer [17–19]. Among those molecules, PYCR1 represents one of the most seductive proteins because overexpression of PYCR1 was suggested to be the most related factor that contributed to poor outcomes of patients with malignant cancer [11–13]. In the present study, we demonstrated that the levels of PYCR1 was significantly higher in lung cancer tissues and cells than of the tumor adjacent normal tissues and normal cells.

It has been previously demonstrated that aberrant activation of PYCR1 was detected in a wide array of cancer types, including colorectal cancer [20], prostate cancer [21], and breast cancer [22]. Additionally, the aberrant expression of PYCR1 significantly influenced the tumor size, grade, subtypes, and even that survival of patient with malignant cancer [23]. Recent studies revealed that high level of PYCR1 was facilitated to tumor cells proliferation and depletion of PYCR1 signally suppressed the competence of tumor growth and invasion [23, 24]. Our study demonstrated that high expression of PYCR1 was exactly quite helpful in the proliferation and invasion of lung cancer cells thorough increase the VEGF and MMP-1 levels, respectively. Additionally, up-regulation of PYCR1 in lung cancer cells was closely related to drug-resistance by dramatically increased the level of P-gp expression. In contrast, silencing the PYCR1 expression in lung cancer cells resulted in signally down-regulated ability of proliferation, invasion and drug-resistance of cancer cells.

As a transcription factor, STAT3 has been detected with overexpression and/or hyperactivation in multiple cancer types [25]. Thorough transcription of anti-apoptotic and pro-proliferative genes, STAT3 played significantly role in the enhancement of cell proliferation, invasion, and drug-resistance of cancer cells [25]. Additionally, it was also demonstrated that STAT3 was able of direct interaction with mitochondrial DNA to facilitate the Ras-dependent malignant transformation and cancer progression [25–27]. Of great importance, the aberrant expression of STAT3 was suggested to be one of the most important factor that associated with poor overall survival rates in patients with malignant cancer [27]. Our study revealed that elevation of STAT3 expression significantly enhanced the proliferation ability of lung cancer cells. More

importantly, it was demonstrated that the STAT3 could be activated by PYCR1 and the level of STAT3 in lung cancer was almost linear correlation to the expression of PYCR1. It was exhibited that up-regulation of PYCR1 in cells resulted in significant increase of STAT3 expression. In contrast, decrease of PYCR1 level by treating cells with si-PYCR1 dramatically down-regulated the STAT3 expression, which finally led to insufficient proliferation, invasion and more sensitivity to chemotherapy.

Phosphatidylinositol-3-kinase (PI3K)-protein kinase B (AKT) pathway has been recommended as one of the key intracellular signal transduction pathway that contributed to promotion of metabolism, proliferation, cell survival, and invasion of cancer cells [28–30]. Previous studies demonstrated that the PI3K/Akt pathway was directly related to multiple functions of cancer cells, such as the quiescence, proliferation, and cancer progress [28, 29]. In the present study, high level of STAT3, resulted from transfection of cells or treating tumor-bearing mice with PYCR1, contributed to high expression of PI3K, p-PI3K, AKT, and p-AKT. Therefore, it could be concluded that aberrant expression of PYCR1 in lung cancer cells or tissues was facilitated to the activation of PI3K/AKT signaling pathway. Additionally, PI3K and AKT can regulate the activation of the NF- κ B pathway through the phosphorylation of I κ B α [31]. In this case, blocking of the PI3K/AKT pathway and NF- κ B was supposed to be a favourable option for cancer treatment [32]. Our further experiments revealed that high expression of STAT3 also contributed to the up-regulation of NF- κ B p65 and p-p65, suggested that NF- κ B signaling pathway was activated after treating by PYCR1. It was further demonstrated that the levels of p-P13K/PI3K, p-AKT/AKT, and p-p65/p65 could be signally elevated by transfection with PYCR1. Taking these together, our study confirmed that the PYCR1 promoted the activation of STAT3-mediated PI3K/AKT and NF- κ B signaling pathway.

Conclusion

In conclusion, the present study confirmed that high levels of PYCR1 was detectable in A549 cells and overexpression of PYCR1 contributed to promotion of cancer cell proliferation, invasion, and drug-resistance. Importantly, our studies further revealed that PYCR1 promoted progress of NSCLS thorough targeting the STAT3-mediated PI3K/AKT and NF- κ B signaling pathway. The findings suggested that inhibition PYCR1 and targeting PI3K/AKT and NF- κ B signaling pathway may be a valuable strategy for treatment of NSCLS.

Abbreviations

NSCLC, Non-small cell lung cancer; PYCR1, Pyrroline-5-carboxylate reductase 1; STAT3, Signal transducer and activator of transcription 3; FBS, fetal bovine serum; VEGF, Vascular endothelial growth factor; MMP-1, Matrix metalloproteinase; P-gp, P-glycoprotein; 5-FU, 5-Fluorouracil; PI3K/AKT, Phosphatidylinositol-3-kinase (PI3K)-protein kinase B (AKT), IHC, Immunohistochemistry; HRP, horse radish peroxidase.

Declarations

Authors' contributions

Zhanwu Yu, Gebang Wang, and Hongxu Liu were responsible for the guarantor of integrity of the entire study and preparation, editing, and review of the manuscript. Chenlei Zhang, Yu Liu, and Wei Chen were respectively involved in the definition of intellectual content and literature research. Wei Chen and Haoyou Wang responsible for the data acquisition and analysis. Hongxu Liu revised the manuscript.

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Availability of data and materials

The data are available from the authors upon request.

Ethics approval and consent to participate

The animal experiments in the present study were all performed in accordance with guidelines evaluated and approved by the ethics committee of Cancer Hospital of China Medical University.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

Figure 1

PYCR1 expression levels in lung tumors and cells, and respectively compared with that in tumor adjacent normal tissues and normal cell lines. (A) Expression of PYCR1 in lung cancers and para-carcinoma tissues determined by the RT-qPCR experiments. (B) Qualitative and semi-quantitative analysis of the PYCR1 expression levels in lung tumor and adjacent normal tissues by Western-blot assay. (C) Cellular expression of PYCR1 analyzed in various lung cancer cell lines and compared with the normal cells. (D) Qualitative and semi-quantitative analysis of PYCR1 expression in lung cancer cells and normal cells via the Western-blot assay. All experiments were performed repeatedly three times. * $P < 0.05$, ** $P < 0.01$, significantly higher than the tumor adjacent normal tissues or BEAS-2B cells.

Figure 2

Silencing the PYCR1 expression significantly inhibited the proliferation, invasion, and drug resistances of A549 cells. (A) Transfection efficacy of ctrl-siRNA and si-PYCR1 determined by Wester-blot assay. (B) Proliferation of A549 cells after transfected with ctrl-siRNA and si-PYCR1 determined using the immunocytochemical analysis. (C) Investigation of the VEGF level in the ctrl-siRNA and si-PYCR1 transfected A549 cells. (D) Invasion rate of A549 cells after respectively treated by ctrl-siRNA and si-PYCR1. (E) MMP-1 levels in the ctrl-siRNA and si-PYCR1 transfected A549 cells. (F) IC₅₀ values of 5-Fu on A549 cells treated by ctrl-siRNA or si-PYCR1, and compared with the untreated cells. (G) P-gp expression in the ctrl-siRNA and si-PYCR1 transfected A549 cells. All experiments were performed repeatedly three times. **P<0.01, significantly higher than the control group.

Figure 3

Overexpression of PYCR1 significantly promoted the proliferation, invasion, and drug resistances of A549 cells. (A) Transfection efficacy of vector and p-PYCR1 determined by Western-blot assay. (B) Proliferation of A549 cells after transfected with vector and p-PYCR1 determined using the immunocytochemical analysis. (C) Investigation of the VEGF level in the vector and p-PYCR1 transfected A549 cells. (D) Invasion rate of A549 cells after respectively treated by vector and p-PYCR1. (E) MMP-1 levels in the vector and p-PYCR1 transfected A549 cells. (F) IC50 values of 5-Fu on A549 cells treated by vector and p-PYCR1, and compared with the untreated cells. (G) P-gp expression in the vector and p-PYCR1 transfected A549 cells. All experiments were performed repeatedly three times. **P<0.01, significantly higher than the control group.

Figure 4

Overexpression of PYCR1 significantly up-regulated the level of STAT3 in lung cancer cells. (A) The binding site between the STAT3 and PYCR1 was predicated by the ChIPBase and further confirmed using the CoIP experiments. (B) The levels of STAT3 after treating cells with p-PYCR1 was detected by Western-blot assay. (C) Spearman's analysis of the expression correlation between the STAT3 and PYCR1.

* $P < 0.05$, # $P < 0.05$, significantly higher than the control group.

Figure 5

PYCR1 promoted the proliferation, invasion, and drug resistances of A549 cells thorough regulating the STAT3 expression. (A) Proliferation of A549 cells after transfected with various genes. (B) Determination of VEGF levels in various genes transfected cells by RT-qPCR. (C) Invasion rated of of A549 cells after transfected with various genes. (D) Determination of MMP-1 levels in various genes transfected cells by RT-qPCR. (E) IC50 values of A549 cells after transfected with various genes. (F) Determination of P-gp levels in various genes transfected cells by RT-qPCR. (G) Qualitative analysis of VEGF, MMP-1, and P-gp levels in various genes transfected cells by Western-blot assay. All experiments were performed repeatedly three times. * $P < 0.05$, ** $P < 0.01$, significantly higher than the control group.

Figure 6

PYCR1 promoted the activation of STAT3-mediated PI3K/AKT and NF-κB Signaling Pathway. (A) Qualitative analysis of the PI3K/AKT and NF-κB signaling pathway related genes in A549 cells after various treatment by Western-blot assay. Quantitative investigation of the levels of p-P13K/PI3K (B), p-AKT/AKT (C), and p-p65/p65 (D) in various genes treated A549 cells by RT-qPCR experiments. (E) Immunofluorescence analysis of p65 and AKT in A549 cells after various treatment. All experiments were performed repeatedly three times. *P<0.05, **P<0.01, significantly higher than the control group.

Figure 7

PYCR1 promoted lung tumor growth thorough the activation of STAT3-mediated PI3K/AKT and NF-κB Signaling Pathway. (A) Tumor images of lung cancer after treated by various strategies. (B) Tumor weights of lung cancer after various treatments. (C) Immunohistochemical analysis of VEGF, MMP-1, and P-gp levels in tumor tissues post transfection with different genes. Quantitative investigation of the levels of p-P13K/PI3K (D), p-AKT/AKT (E), and p-p65/p65 (F) in various genes treated tumor tissues by RT-qPCR experiments. (G) Qualitative analysis of the PI3K/AKT and NF-κB signaling pathway related genes in tumor tissues after various treatment by Western-blot assay. * $P < 0.05$, ** $P < 0.01$, significantly higher than the control group.

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