Am J Cancer Res 2023;13(3):886-899
www.ajcr.us /ISSN:2156-6976/ajcr0148651

Original Article

Mitochondrial phosphoenolpyruvate carboxykinase inhibits kidney renal clear cell carcinoma malignant progression, leading to cell energy metabolism imbalance

Yeda Chen[1], Zhu Wang[1], Qiong Deng[1], Yuting Chen[2], Hui Liang[1]

[1]*Department of Urology, Affiliated Longhua People's Hospital, Southern Medical University (Longhua People's Hospital), Shenzhen 518109, Guangdong, China;* [2]*Key Laboratory of Environmental Medicine, School of Public Health, Guangdong Medical University, Dongguan 518109, Guangdong, China*

Received December 20, 2022; Accepted February 17, 2023; Epub March 15, 2023; Published March 30, 2023

Abstract: Mitochondrial phosphoenolpyruvate carboxykinase (PCK2) is a key gluconeogenesis enzyme. Its differential expression is related to kidney renal clear cell carcinoma (KIRC) malignancy, possibly by influencing energy metabolism. Therefore, it is possible that PKC2 plays a significant part in the emergence and progression of KIRC. To systematically and comprehensively identify the significance of PCK2 in KIRC, we further studied PCK2 in terms of its relationship to clinical features and various clinical subgroups' prognoses. Moreover, we verified the effect of PCK2 and KIRC cells using experimental methods. PCR and western blotting analyses confirmed PCK2 expression in KIRC cell lines and tissues. As a cell model, we constructed cells that overexpress PCK2. Proliferation was detected by EdU experiments. Scratch tests and transwell assays were used, respectively, to analyze cell migration and invasion. Mass spectrometry detected energy metabolite expression in KIRC cells. The findings revealed that KIRC patients with lower levels of PCK2 expression exhibited shorter progression-free intervals, shorter disease-specific survival, and shorter overall survival. The experimental results showed that compared with 293t, PCK2 was downregulated in three KIRC lines (OSRC-2, 786-0, and A498). Relative to surrounding tissues, PCK2 was downregulated in KIRC. PCK2 overexpression inhibited KIRC cell proliferation, migration, and invasion and upregulated energy metabolite expression. Mass spectrometry revealed that thiamine pyrophosphate, cyclic AMP, beta-D-fructose 6-phosphate, lactate, flavin mononucleotide, NAD, NADP, and D-glucose 6-phosphate were upregulated. PCK2 has the potential to serve as both a diagnostic and prognostic molecular biomarker for KIRC, as well as an independent prognostic risk factor for KIRC. It is hoped that PCK2 will emerge as a therapeutic target for KIRC.

Keywords: Mitochondrial phosphoenolpyruvate carboxykinase, kidney renal clear cell carcinoma, proliferation, migration, invasion, energy metabolism

Introduction

Kidney renal clear cell carcinoma (KIRC) is one of the most common urinary system tumors worldwide [1, 2]. The number of KIRC cases is increasing yearly, with an annual incidence of 4% [3]. KIRC often does not cause obvious clinical symptoms in its early stages. Once symptoms appear, it is often in the advanced stage. However, due to the lack of specific tumor markers for KIRC, its early diagnosis till depends on imaging methods such as B-ultrasound, CT, and MRI [4-6]. Surgical resection is still the main treatment for KIRC [7]. Although resection of malignant tumors can be effective, outcomes are still unsatisfactory [8]. At present, the specific mechanism of the occurrence and development of KIRC is still unclear. Tumor markers are closely related to the occurrence and development of tumors. Finding key mechanisms underlying the malignant progression of tumors and identifying effective and broad-spectrum targets are urgent problems to be solved to improve clinical outcomes.

The pathogenesis of KIRC is extremely complex [9, 10]. The biological behavior of KIRC mainly

involves proliferation, migration, and invasion of tumor cells [11]. In the process of cell metabolism, abnormal energy metabolism is also one of the important manifestations of tumors [12, 13]. In our study, we found that the expression of mitochondrial phosphoenolpyruvate carboxykinase (PCK2) was decreased in KIRC. Abnormal expression may affect the biological behavior of KIRC cells and the process of cellular energy metabolism. PCK2 is a key rate-limiting enzyme in the gluconeogenesis pathway, which is involved in the synthesis of glycerol, amino acids, and nucleotides, as well as in the TCA cycle [14]. PCK2 is a pivotal molecule connecting TCA cycle, glycolysis, and gluconeogenesis, which dynamically regulates intracellular anabolism to provide sufficient energy and metabolic intermediates. PCK2 can supply energy and substances to cells by promoting carbon flow and gluconeogenesis in the TCA cycle and can also promote the Warburg effect by glycolysis. Therefore, PCK2 may affect the occurrence of tumors by regulating energy metabolism. PCK2 has different effects in different tumors, and it has an anti-cancer effect against melanoma and liver cancer [15, 16]. Moreover, PCK2 can promote lung cancer and breast cancer [17]. We were unable to ascertain the mechanism of how PCK2 affects KIRC or find studies on PCK2's influence on the energy metabolism of KIRC through a literature investigation, so our aim was to evaluate the role of PCK2 in proliferation, migration, and invasion of KIRC and its influence on energy metabolites. We hope to provide a theoretical basis of cell biology and energy metabolomics to reveal the mechanisms of KIRC.

Materials and methods

Prognosis and statistical study of survival

Kaplan-Meier plots (progression-free intervals [PFI], disease-specific survival [DSS], and overall survival [OS]) were used for conducting research investigating the link between PCK2 expression and cancer survival. Furthermore, in a diverse group of clinical subgroups associated with KIRC, we evaluated the connection between PCK2 expression and prognosis (as assessed by PFI, DSS, and OS). In order to do statistical analysis and data visualization, respectively, both the survival package and the survminer program were used. The hypothesis was tested with the use of Cox regression, and $P < 0.05$ was considered statistically significant.

Analysis of PCK2 expression in relation to KIRC clinical characteristics

The box plot and table both show the levels of PCK2 expression in KIRC patients with distinct clinical features. RNA-seq and clinical data were acquired from the TCGA database in level 3 HTSeq-fragments per kilobase per million (FPKM) format, then translated to transcripts per million reads (TPM) format, and finally analyzed after log2 conversion. After differentiating the two sets of data with the use of the Wilcoxon rank sum test, and $P < 0.05$ was considered statistically significant.

Clinical specimens

All clinical specimens of kidney cancer and adjacent tissues were sourced from Longhua District People's Hospital of Shenzhen. They were obtained through surgical resection, stored, and transported in liquid nitrogen and stored in a freezer at -80°C.

Reagents

RPMI-1640 medium was purchased from Gibco Thermo Fisher Scientific (USA). Fetal bovine serum (FBS) was purchased from Biological Industries (Beit HaEmek, Israel). The primary antibody to PCK2 (#6924) was purchased from Cell Signaling Technology. The secondary antibody (catalog no. bs-0295G-HRP) was purchased from Bioss Antibodies. Anti-GADPH (#AFF389-50) was purchased from Affinity Biosciences.

Cell culture and transfection

Human KIRC cell lines (OSRC-2, 786-O and A498) were obtained from the cell bank of the Chinese Academy of Sciences (Shanghai). They were cultured in RPMI-1640 medium supplemented with 10% FBS and incubated at 37°C and 5% CO_2. The lentivirus vector was purchased from Shanghai Gene Pharmaceutical Co., Ltd. (Shanghai, China). The lentivirus was used to construct stable transfected cell lines, and then, cells were harvested for PCR and western blotting experiments.

Reverse transcription quantitative PCR (RT-qPCR)

Total RNA was extracted using a kit (Fastgene, Inc; Catalog no. 220010). The extracted RNA was reverse transcribed into cDNA using the Prime kit with DNA Eraser (Takara Bio Inc.). The sequences of primers used were: PCK2 (forward): 5'-GAGCAACGGCTTCTGTCATTT-3'; PCK2 (reverse): 5'-TGCTTGACTCGTACCATGTCC-3'; GAPDH (forward): 5'-AAGAAGGTGGTGAAGCAGG-3'; GAPDH (reverse): 5'-GTCAAAGGTGGAGGAGTGG-3'.

Western blotting

Protein samples were lysed at 4°C for 10 min with RIPA lysis buffer. Protein concentration was measured using the Coomassie brilliant blue method. The samples were mixed with sample buffer for 10 min at 95°C. The protein sample was loaded on a 10% SDS gel and electrophoresed for 2 h. Then, the separated protein was transferred to nitrocellulose membrane and blocked with 5% skim milk for 2 h at 24°C. Next, the film was cut according to the molecular mass of the target protein. Subsequently, the membrane was incubated with the primary antibody at 4°C overnight. The next day, the secondary antibody (1:10,000) was incubated at 24°C for 2 h. A scanning instrument (Amersham ImageQuant 800) was used to obtain protein bands. ImageJ was used for grayscale analysis.

Cell EdU assay

Cell proliferation was detected using an EdU cell proliferation detection kit (Ribobio, Guangzhou, China). Cells were incubated with 50 μM EdU for 2 h before fixation, permeabilization, and EdU staining. The nuclei were stained with Hoechst at a concentration of 1 μg/mL for 30 min. The proportion of cells doped with EdU was observed with a fluorescence microscope.

Scratch experiment

The scratch experiment was performed with a culture insert tool. After the cells were digested, they were inoculated into the insert in the middle of the 24-well plate. After the cells filled the insert area, the insert could be removed with tweezers to produce a scratch with a width of 500 μm. Photos were taken over 24 h, picture data were collected, and the experimental results were analyzed.

Transwell migration and invasion experiment

Cells were digested with trypsin and resuspended in 500 μL RPMI-1640 medium, and the cell density was measured using a cell counter. To test invasion, the transwell membrane was coated with matrigel glue. A total of 2×10^4 cells were placed in the upper cavity of the transwell chamber (micropore diameter: 8 μm), and 750 μL RPMI-1640 medium supplemented with 10% FBS was added to the lower cavity, and then the cells were incubated. After 24 h, cells in the upper cavity that have not migrated or invaded were wiped off with cotton swabs, and the chamber was dried at 24°C. Subsequently, the cells were fixed with 4% paraformaldehyde at 24°C for 10 min and stained with crystal violet for 10 min. After the filter membrane had dried, we observed the number of fine migrating and invading cells under a microscope.

Statistical analysis

All data were analyzed using SPSS 23.0 (IBM Corp). The two groups were compared using a t-test. One-way analysis of variance and Bonferroni's test were used to evaluate the differences among multiple groups, and $P < 0.05$ was considered statistically significant.

Results

Using PCK2 as a prognostic factor in KIRC

Significant correlations exist between the PCK2 expression level and the prognostic indicators of KIRC. A worse prognosis was related with low levels of PCK2 expression in patients with KIRC, as measured by OS, DSS, and PFI, according to Cox regression (**Figure 1**).

We analyzed the association relationship between PCK2 and survival (OS, DSS, and PFI) in KIRC subgroups with varying clinical features. In the majority of clinical groups, including subgroups of "age > 60", males, females, M0, N0, T1, T2, "race: white", "histologic grade: G2", "pathological stage: stage I", "serum calcium: low", "hemoglobin: low", "laterality: left", and "laterality: right", reduced PCK2 expression was linked with a worse OS (**Figure 2**).

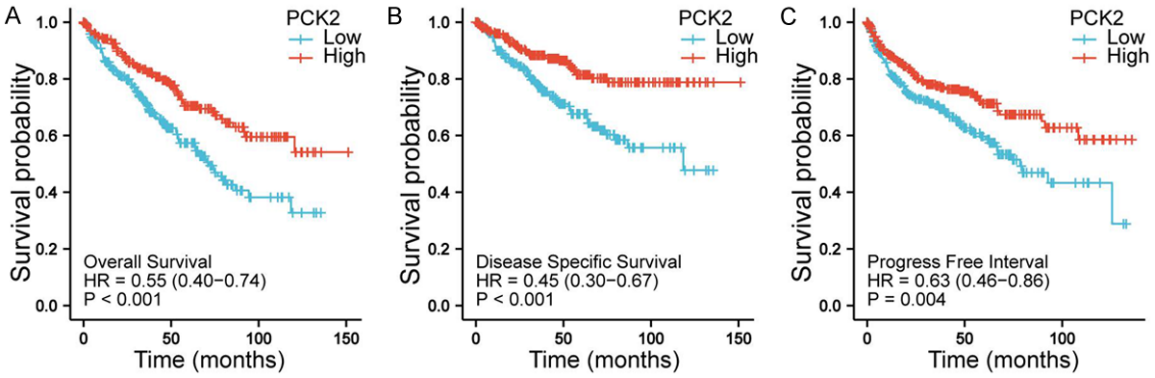

Figure 1. Correlations between PCK2 expression and the prognosis for KIRC. A. Overall survival of PCK2 in KIRC. B. Disease-specific survival of PCK2 in KIRC. C. Progression-free intervals of PCK2 in KIRC.

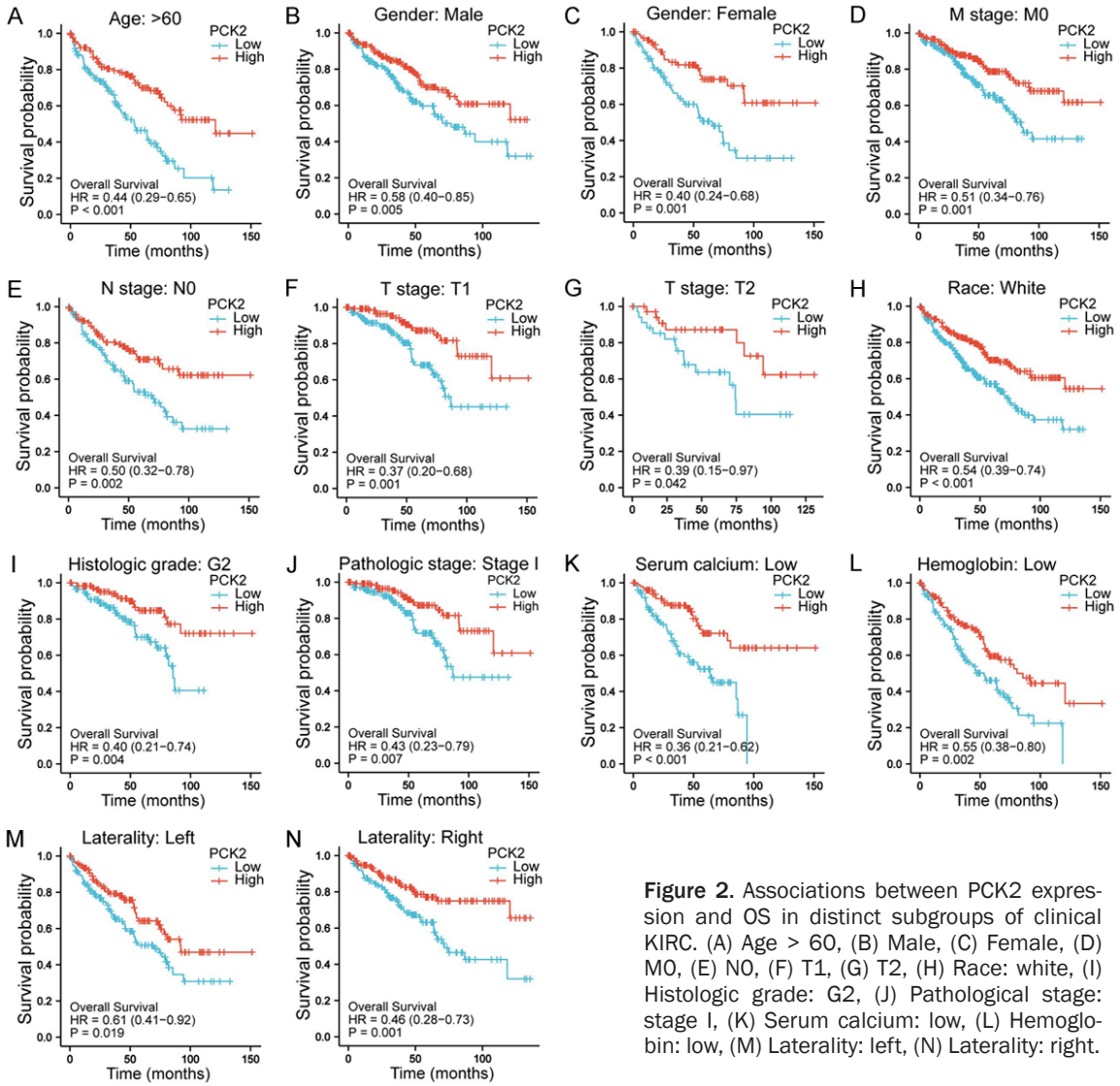

Figure 2. Associations between PCK2 expression and OS in distinct subgroups of clinical KIRC. (A) Age > 60, (B) Male, (C) Female, (D) M0, (E) N0, (F) T1, (G) T2, (H) Race: white, (I) Histologic grade: G2, (J) Pathological stage: stage I, (K) Serum calcium: low, (L) Hemoglobin: low, (M) Laterality: left, (N) Laterality: right.

In the majority of clinical groups, low expression of PCK2 was related with a worse DSS, including the subgroup of "age > 60", males, females, M0, N0, T1, "race: white", "pathologic

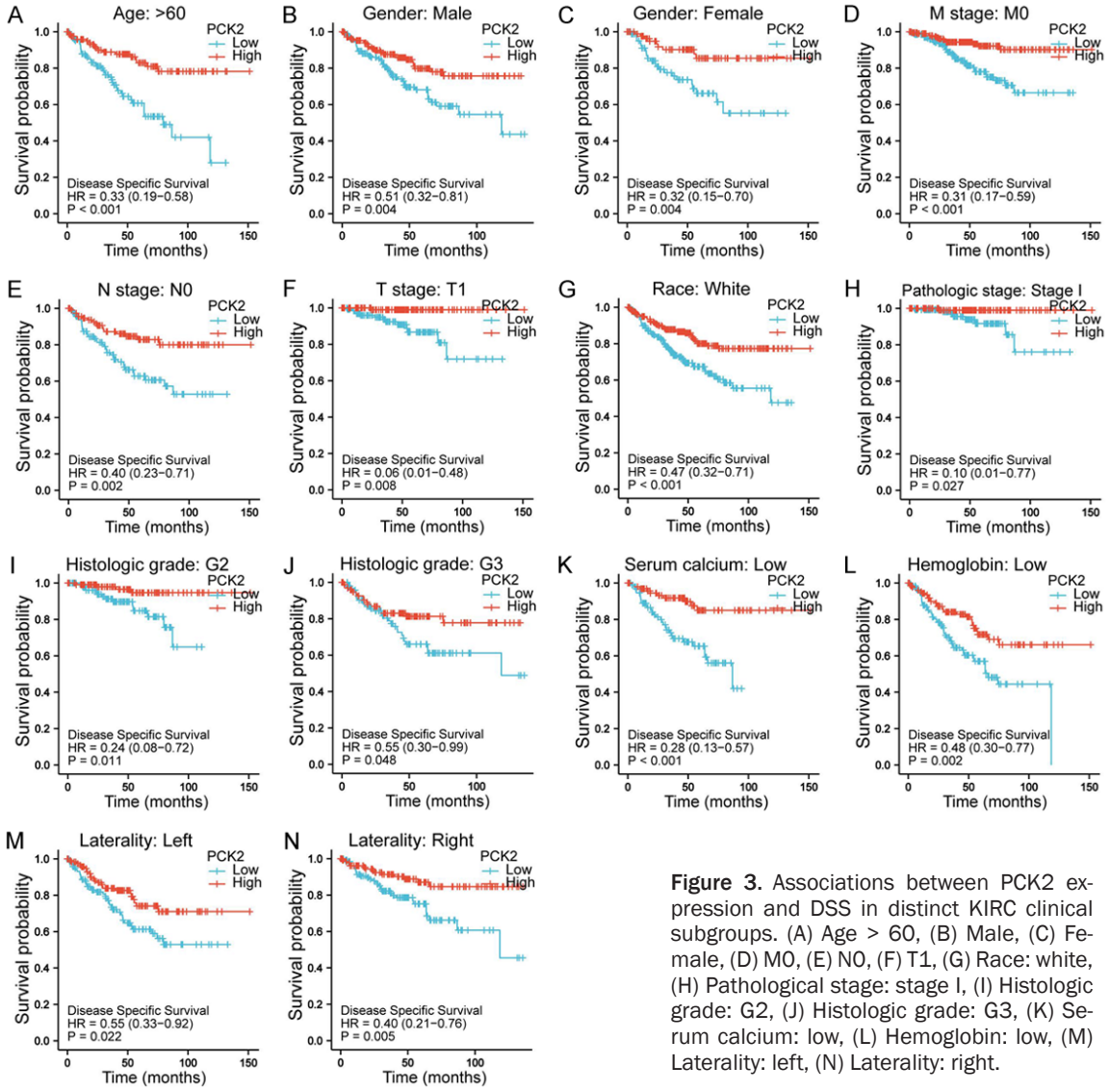

Figure 3. Associations between PCK2 expression and DSS in distinct KIRC clinical subgroups. (A) Age > 60, (B) Male, (C) Female, (D) M0, (E) N0, (F) T1, (G) Race: white, (H) Pathological stage: stage I, (I) Histologic grade: G2, (J) Histologic grade: G3, (K) Serum calcium: low, (L) Hemoglobin: low, (M) Laterality: left, (N) Laterality: right.

stage: stage I", "histologic grade: G2", "histologic grade: G3", "serum calcium: low", "hemoglobin: low", "laterality: left", and "laterality: right" (**Figure 3**).

In the majority of clinical groups, including subgroups of "age > 60", males, M0, T1, "race: white", "pathologic stage: stage I", "histologic grade: G2", "serum calcium: low", "Hemoglobin: low" and "laterality: right" (**Figure 4**), low PCK2 expression was associated with a poorer PFI.

PCK2 is associated with different clinical characteristics of KIRC

PCK2 was divided into high and low groups according to the median expression of PCK2. We discovered a significant difference between PCK2 expression and the clinical features of KIRC (T stage, pathologic stage, N stage, gender, histologic grade, OS event, DSS event, and PFI event) (**Table 1**).

mRNA and protein expression level of PCK2 gene in human renal cell carcinoma cell lines and tissues

RT-qPCR analysis showed that the relative expression of PCK2 in three KIRC cell lines (OSRC-2, 786-O, and A498) was decreased compared with that in the 293t cell line (**Figure 5A**). RT-qPCR and western blotting were, respectively, used to detect the mRNA and protein expression levels in cancer tissues and

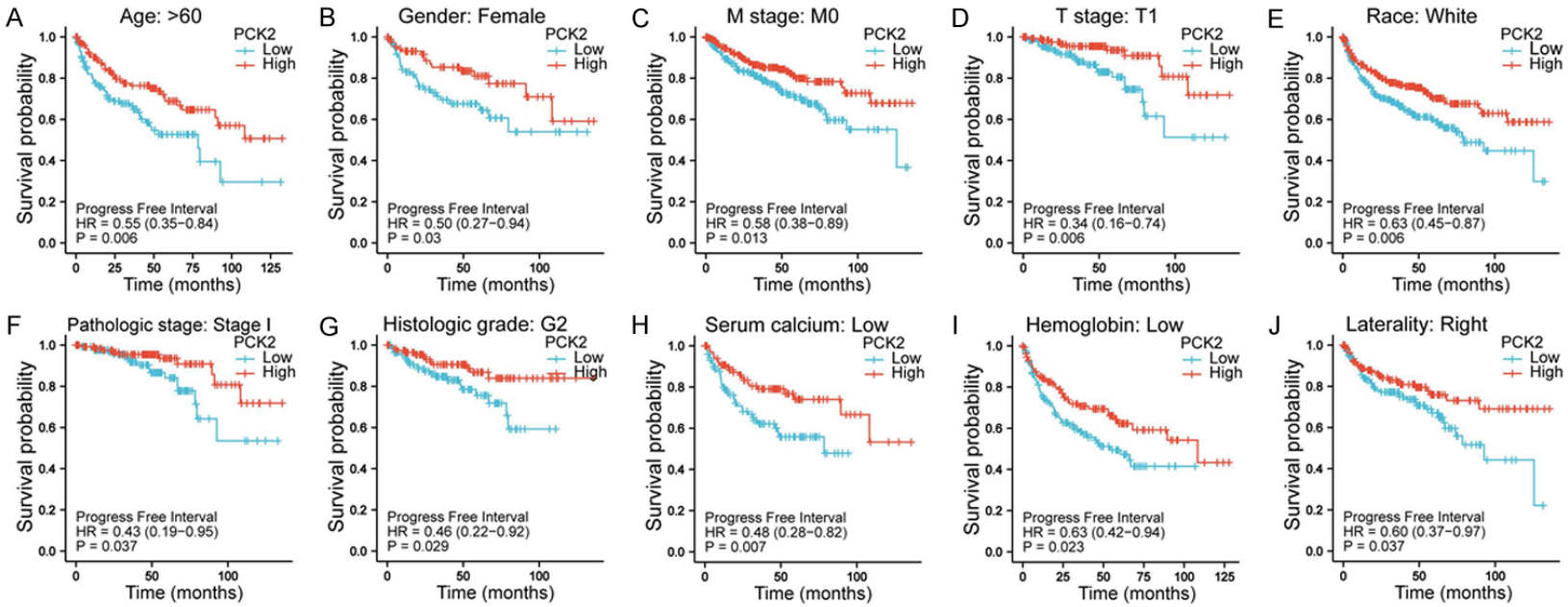

Figure 4. Associations between PCK2 expression and PFI in diverse KIRC subgroups. (A) Age > 60, (B) Male, (C) M0, (D) T1, (E) Race: white, (F) Pathological stage: stage I, (G) Histologic grade: G2, (H) Serum calcium: low, (I) Hemoglobin: low, (J) Laterality: right.

Table 1. Clinical characteristics of KIRC patients

Characteristic	Low expression of PCK2	High expression of PCK2	*P*
n	269	270	
T stage, n (%)			0.005
T1	120 (22.3%)	158 (29.3%)	
T2	46 (8.5%)	25 (4.6%)	
T3	97 (18%)	82 (15.2%)	
T4	6 (1.1%)	5 (0.9%)	
Pathologic stage, n (%)			0.002
Stage I	114 (21.3%)	158 (29.5%)	
Stage II	38 (7.1%)	21 (3.9%)	
Stage III	70 (13.1%)	53 (9.9%)	
Stage IV	45 (8.4%)	37 (6.9%)	
N stage, n (%)			0.034
N0	122 (47.5%)	119 (46.3%)	
N1	13 (5.1%)	3 (1.2%)	
Gender, n (%)			0.016
Female	79 (14.7%)	107 (19.9%)	
Male	190 (35.3%)	163 (30.2%)	
Histologic grade, n (%)			0.043
G1	4 (0.8%)	10 (1.9%)	
G2	111 (20.9%)	124 (23.4%)	
G3	102 (19.2%)	105 (19.8%)	
G4	47 (8.9%)	28 (5.3%)	
OS event, n (%)			< 0.001
Alive	164 (30.4%)	202 (37.5%)	
Dead	105 (19.5%)	68 (12.6%)	
DSS event, n (%)			< 0.001
Alive	192 (36.4%)	228 (43.2%)	
Dead	71 (13.4%)	37 (7%)	
PFI event, n (%)			0.022
Alive	176 (32.7%)	202 (37.5%)	
Dead	93 (17.3%)	68 (12.6%)	

adjacent tissues of six patients. Compared with adjacent normal tissues, the mRNA and protein expression levels of PCK2 in cancer tissues were lower (**Figure 5B-D**).

Efficiency verification of overexpression of PCK2

To evaluate the role of PCK2 in KIRC cells, we overexpressed PCK2 in three cell lines, namely OSRC-2, 786-O, and A498 and detected the expression levels of mRNA and protein by qRT-PCR and western blotting. Compared with the cells in the control group, the expression levels of mRNA and protein in OSRC-2, 786-O, and A498 cell lines increased (**Figure 5E-G**).

Overexpression of PCK2 inhibits KIRC proliferation

The activity of cells was evaluated through EdU experiments. Cells overexpressing PCK2 showed decreased activity in OSRC-2, 786-O, and A498 cell lines, indicating that overexpression of PCK2 can inhibit cell proliferation compared with that of control cells (**Figure 5H**, **5I**).

Overexpression of PCK2 inhibits KIRC migration

A scratch test was used to detect the migration ability of cells. The results were as follows (**Figure 6A**, **6B**): In OSRC-2, 786-O, and A498 cell lines, 24 h post-scratch, compared with that in the control group, the migration ability of cells in the PCK2-overexpressing group was weakened, indicating that PCK2 overexpression could inhibit the migration of cells. A transwell assay was used to detect the migration ability of cells. After 24 h culture in the transwell chamber, crystal violet was used to stain cells passing through micropores. Results were as follows (**Figure 6C**, **6D**): Compared with the control group, the number of cells passing through micropores in the overexpression PCK2 group decreased, indicating that PCK2 overexpression can inhibit cell migration.

PCK2 overexpression inhibits KIRC invasion

A transwell assay was used to detect the invasion ability of cells. In OSRC-2, 786-O, and A498 cell lines, after 24 h culture in transwell chamber, the cells passing through micropores were stained with crystal violet. The results show that (**Figure 6E**, **6F**) compared with that in the control group, the number of cells passing through micropores in the PCK2 overexpression group decreased, indicating that overexpression of PCK2 could inhibit the invasion of cells.

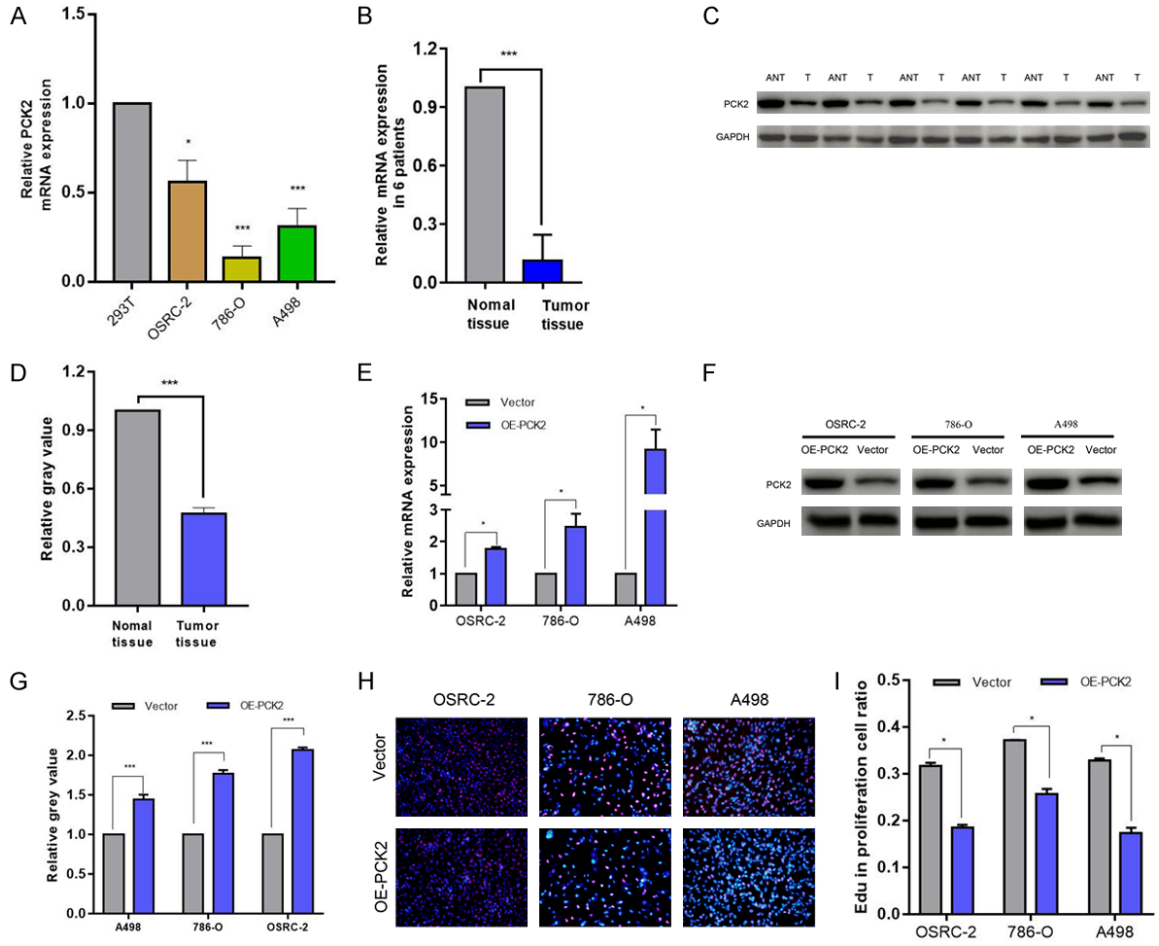

Figure 5. mRNA and protein expression levels of PCK2. PCK2 inhibits growth of KIRC cells. (A) Relative mRNA expression levels of PCK2 in KIRC cell lines. (B) Relative mRNA expression levels of PCK2 in KIRC primary tumor tissues and normal tissues. (C) PCK2 protein expression in KIRC adjacent non-neoplastic tissues (ANT) and tumor tissues (T). (D) Quantification of protein expression levels in (C). (E) Relative mRNA expression levels of PCK2-overexpressing KIRC cell lines. (F) Protein expression levels in PCK2-overexpressing KIRC cell lines in KIRC cell lines. (G) Quantification of protein expression levels in (B). (H) Cell viability detected using the EdU assay. (I) Quantification of proliferating cell ratio. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Overexpression of PCK2 leads to abnormal expression of energy metabolites

A498 cells overexpressing PCK2 were analyzed using mass spectrometry. The results of spectrum quantification of various energy metabolites were as follows: **Table 2** (Quantitative analysis of energy metabolites by HPLC-MS/MS) shows that the expression of eight energy metabolites was upregulated, namely thiamine pyrophosphate, cyclic AMP, beta-D-fructose 6-phosphate, lactate, flavin mononucleotide, NAD, NADP, and D-glucose 6-phosphate (**Figure 7**).

Discussion

We investigated the roles of PCK2 in KIRC. We found that the expression of PCK2 could significantly inhibit the proliferation, migration, and invasion of KIRC. We detected the energy metabolites in a A498 cell line overexpressing PCK2 and found that the expression of eight energy metabolites increased, namely thiamine pyrophosphate, cyclic AMP, beta-D-fructose 6-phosphate, lactate, flavin mononucleotide, NAD, NADP, and D-glucose 6-phosphate. Thus, targeting the PCK2 protein may be a useful strategy to inhibit KIRC progression.

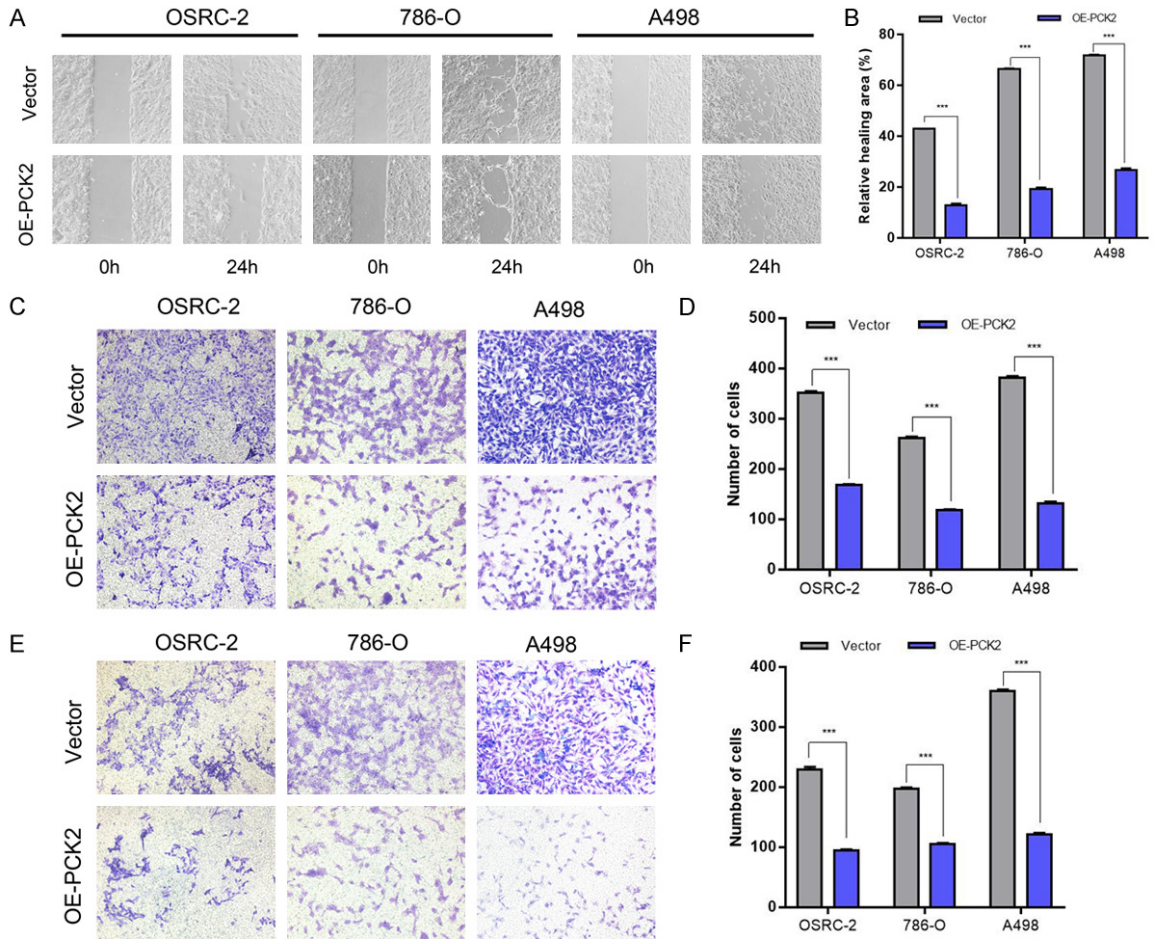

Figure 6. PCK2 inhibits migration and invasion of KIRC cells. (A) Representative micrographs of the wound healing assay at 0 and 24 h using KIRC cells. (B) Quantification of the results shown in (A). (C) Migration of KIRC cells evaluated using a transwell assay. (D) Quantification of the results shown in (C). (E) Invasion ability of KIRC cells evaluated using a transwell assay. (F) Quantification of the results shown in (E).

Cancer cells change their original physiological metabolic pathways to maintain their rapid growth and proliferation. This phenomenon is called metabolic reprogramming. Metabolic reprogramming is an important feature of tumor cells [18, 19]. The Warburg effect is a typical example of metabolic reprogramming of tumor cells [20, 21]. Tumors tend to exhibit high glucose utilization and lactic acid secretion rates. In many cases, rapidly proliferating solid tumors exist for long periods in microenvironments with nutrient depletion and hypoxia due to insufficient local blood perfusion. To maintain their rapid proliferation, solid tumors must undergo metabolic reprogramming to adapt to various stress conditions, illustrating their obvious metabolic flexibility [22]. The enhanced expression of eight energy metabolites may be related to the proliferation of KIRC cells.

There are two forms of phosphoenolpyruvate carboxykinase (PEPCK) in human tissues, namely cytoplasmic phosphoenolpyruvate carboxykinase (PCK1) and mitochondrial phosphoenolpyruvate carboxykinase (PCK2) [23, 24]. PCK1 is only expressed in the liver, kidney, and small intestine, while PCK2 is widely expressed [25, 26]. To date, much research has focused on the function of PCK1 [27-29]. However, little is known about the function and role of PCK2, particularly its role in transcriptional and translational regulation and tumor metabolic reprogramming. In recent years, PCK2 has been found to be involved in the remodeling of glucose and lipid metabolism in the tumor microenvironment and plays an important role in the metabolic reprogramming of tumor cells [30]. PCK2 is located in the mitochondria where it plays an important metabolic regulation role in

Table 2. Quantitative analysis of energy metabolites by HPLC-MS/MS

Metabolite name	Transitions	Retention time (min)	Fold change	*P*-value
Thiamine pyrophosphate (TPP)	423.0/302.0	9.549925243	2.693178715	0.001981404
Cyclic AMP	328.0/134.0	2.294758473	2.924216719	0.004964789
Beta-D-fructose 6-phosphate	259.1/97.0	8.036902428	5.105632925	0.005336338
Lactate	89.2/43.0	2.113546702	1.896659418	0.006579511
Flavin mononucleotide (FMN)	455.1/97.0	5.706043288	2.160335993	0.008713741
NAD	662.1/540.0	6.99460364	2.478152627	0.028995718
NADP	742.1/620.0	10.86071924	1.497427754	0.038684385
D-Glucose 6-phosphate	259.0/97.0	9.018184023	5.346450978	0.048094643
Dihydroxyacetone phosphate	169.1/97.0	6.931829805	1.745534718	0.051943188
NADPH	744.1/408.0	10.8607685	1.702771414	0.067866805
NADH	664.1/408.0	7.006379973	2.018240665	0.078314045
Guanosine 5'-diphosphate (GDP)	442.0/79.0	9.864741325	2.062502692	0.097215741
GMP	362.1/79.1	8.515646024	2.385321703	0.117013604
L-Malic acid	133.0/115.1	5.858238732	1.988735194	0.142919883
Adenosine monophosphate	346.1/79.0	6.840498086	1.919485291	0.153371388
Succinate	117.1/73.0	5.398342339	1.72723084	0.234620109
ADP	426.0/79.0	8.216146246	1.616641747	0.246789257
Citrate	191.1/87.1	8.691795111	14.33340046	0.320494498
Isocitrate	191.0/73.0	8.611581905	21.32596191	0.35978066
cis-Aconitate	173.1/85.1	7.891126679	119.8010097	0.363937489
Oxaloacetate	131.1/87.1	5.207381672	0.892087627	0.3681783
alpha-Ketoglutarate	145.2/101.1	4.563744159	1.163344106	0.393019673
Acetyl coenzyme A (Acetyl-CoA)	808.2/408.1	6.764476947	1.444415485	0.537365161
3-Phospho-D-glycerate	185.0/97.0	8.6781922	0.56419499	0.655424008
Phosphoenolpyruvate	167.1/79.0	8.376516446	0.584543509	0.671825314
D-Fructose 1, 6-bisphosphate	339.1/97.0	11.75677073	1.155564746	0.711815852
Guanosine 5'-triphosphate (GTP)	522.0/159.1	11.03649425	1.202520004	0.803775018
Adenosine 5'-triphosphate (ATP)	506.0/159.0	9.44134118	0.864031228	0.832133115

gluconeogenesis, the TCA cycle, and glycolysis. PCK2 is a key rate-limiting enzyme in the gluconeogenesis pathway, which catalyzes the conversion of oxaloacetate (OAA) and GTP into phosphoenolpyruvate (PEP), GDP, and CO_2 [31]. Furthermore, PCK2 controls the cataplerosis of non-oxidized amino acid carbon skeletons in the TCA cycle. That is, it oxidizes the carbon skeletons of amino acids such as glutamic acid and alanine flowing into the TCA cycle, to prevent the accumulation of TCA cycle intermediate products in mitochondria [32]. Gluconeogenesis can be regarded as a simple but incomplete reverse reaction of glycolysis, and they are coordinated and regulated in cells, so the effect of PCK2 on gluconeogenesis is accompanied by the influence of the glycolytic pathway.

PCK2, located at the hub of glycolysis, gluconeogenesis, and the TCA cycle, dynamically regulates intracellular anabolism to provide sufficient energy and metabolic intermediates. Metabolic reprogramming provides an important growth advantage to cancer cells, enabling them to rely on limited nutrients in the tumor microenvironment to grow [33]. The activity of PCK2 not only depends on the level of mtGTP produced by succinate-CoA ligase GDP-forming subunit beta (SUCLG2) in the TCA cycle [32, 34] but is also subject to rapid transcription regulation, thus affecting tumor proliferation and growth.

High-precision quantitative proteomic analysis of hepatocellular carcinoma and its adjacent tissues showed that PCK2 expression was significantly downregulated in hepatocellular carcinoma [35]. Leithner et al. [36] found that PCK2 expression and activity in NSCLC cells and tissues such as A549, H23, and H1299 increased, and PCK2 activity in lung cancer tis-

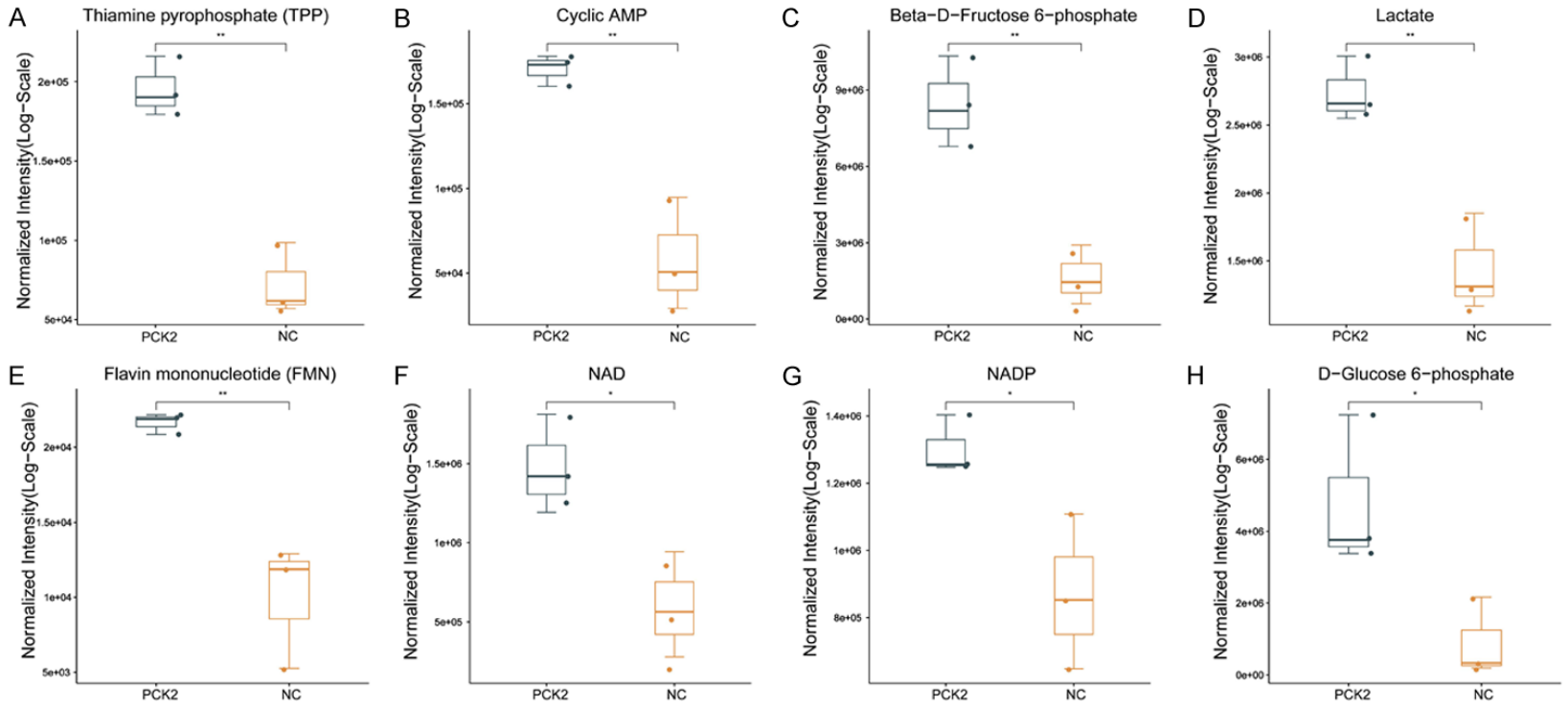

Figure 7. Quantitative assessment of energy metabolites using mass spectrometry. (A) Thiamine pyrophosphate, (B) Cyclic AMP, (C) Beta-D-Fructose 6-phosphate, (D) Lactate, (E) Flavin mononucleotide, (F) NAD, (G) NADP, and (H) D-Glucose 6-phosphate. $^{*}P < 0.05$, $^{**}P < 0.01$.

sues was three-fold higher than that in normal lung tissues. In a nude mouse model of xenotransplantation of lung cancer cells, silencing PCK2 reduced tumor growth and average tumor weight compared with that in the control group in vivo [37]. In addition, exogenous overexpression of PCK2 in lung cancer cells can increase the migration ability of cells, and knocking down endogenous PCK2 can cause the opposite result; however, there is no obvious significant change in cell proliferation ability, suggesting that PCK2 can promote metastasis in lung cancer. Similarly, PCK2 expression is upregulated in colon cancer, gastric cancer, and pancreatic neuroendocrine tumors [38-40]. PCK2 expression and function are variable, even contrary, and it can promote and inhibit cancer. PCK2 participates in various anabolic and metabolic reprogramming processes in tumor cells, promotes the occurrence and development of tumors in various ways, and enables tumor cells to adapt to the microenvironment of local hypoxia, hunger, and limited nutrients to grow and survive. Intracellular energy metabolism is a complex regulatory network. However, there have been no related reports on energy metabolism in KIRC. Whether the energy metabolism regulatory network plays an important role in the occurrence and development of KIRC requires further exploration. In-depth molecular mechanism research may provide new insights into KIRC treatment.

There were some shortcomings in this study. First, only the A498 cell line was used to detect energy metabolites, and detection in more cell lines should provide stronger evidence. Second, how energy metabolism mediates the occurrence of PCK2 and tumors needs to be further explored.

Conclusions

This study shows that overexpression of PCK2 can inhibit the malignant progression of KIRC, and upregulate the expression of eight energy metabolites. Therefore, PCK2 has the potential to be a novel and valuable oncotarget protein for human kidney cancer.

Acknowledgements

This work was supported by the Shenzhen Science and Technology Program (Basic Research Project; No. JCYJ20210324125010029), the Longhua Science and Technology Innovation Fund (No. LHKJCXJCYJ202002), the Medical Key Discipline of Longhua District (No. MKD202007090201), the National Natural Science Foundation of China (No. 82103876), China Postdoctoral Science Foundation (No. 2022M713281). We are thankful to all patients for providing samples.

Disclosure of conflict of interest

None.

Address correspondence to: Hui Liang, Department of Urology, Affiliated Longhua People's Hospital, Southern Medical University (Longhua People's Hospital), Shenzhen 518109, Guangdong, China. E-mail: lianghui8689@smu.edu.cn; Yuting Chen, Key Laboratory of Environmental Medicine, School of Public Health, Guangdong Medical University, Dongguan 518109, Guangdong, China. E-mail: yutingchen@gdmu.edu.cn

References

[1] Fang Y, Ji W and Yan C. Research progress of PI3K/PTEN/AKT signaling pathway associated with renal cell carcinoma. Dis Markers 2022; 2022: 1195875.

[2] Burgess KE and DeRegis CJ. Urologic oncology. Vet Clin North Am Small Anim Pract 2019; 49: 311-323.

[3] Siegel RL, Miller KD, Fuchs HE and Jemal A. Cancer statistics, 2021. CA Cancer J Clin 2021; 71: 7-33.

[4] Kuusk T, Grivas N, de Bruijn R and Bex A. The current management of renal cell carcinoma. Minerva Med 2017; 108: 357-369.

[5] Tyson MD and Chang SS. Optimal surveillance strategies after surgery for renal cell carcinoma. J Natl Compr Canc Netw 2017; 15: 835-840.

[6] Sankineni S, Brown A, Cieciera M, Choyke PL and Turkbey B. Imaging of renal cell carcinoma. Urol Oncol 2016; 34: 147-155.

[7] Krokidis ME, Kitrou P, Spiliopoulos S, Karnabatidis D and Katsanos K. Image-guided minimally invasive treatment for small renal cell carcinoma. Insights Imaging 2018; 9: 385-390.

[8] Van Poppel H, Da Pozzo L, Albrecht W, Matveev V, Bono A, Borkowski A, Colombel M, Klotz L, Skinner E, Keane T, Marreaud S, Collette S and Sylvester R. A prospective, randomised EORTC intergroup phase 3 study comparing the oncologic outcome of elective nephron-sparing surgery and radical nephrectomy for low-stage

renal cell carcinoma. Eur Urol 2011; 59: 543-552.
[9] Zhang Y, Lai H and Tang B. Abnormal expression and prognosis value of COG complex members in kidney renal clear cell carcinoma (KIRC). Dis Markers 2021; 2021: 4570235.
[10] Yin L, Li W, Wang G, Shi H, Wang K, Yang H and Peng B. NR1B2 suppress kidney renal clear cell carcinoma (KIRC) progression by regulation of LATS 1/2-YAP signaling. J Exp Clin Cancer Res 2019; 38: 343
[11] Li Z, Nong D, Li B, Wang H, Li C, Chen Z, Li X, Huang G, Lin J, Hao N and Li W. Effect of AKT silence on malignant biological behavior of renal cell carcinoma cells. BMC Urol 2022; 22: 129.
[12] Xiong Z, Xiao W, Bao L, Xiong W, Xiao H, Qu Y, Yuan C, Ruan H, Cao Q, Wang K, Song Z, Wang C, Hu W, Ru Z, Tong J, Cheng G, Xu T, Meng X, Shi J, Chen Z, Yang H, Chen K and Zhang X. Tumor cell "slimming" regulates tumor progression through PLCL1/UCP1-mediated lipid browning. Adv Sci (Weinh) 2022; 9: e2202011.
[13] Liu M, Pan Q, Xiao R, Yu Y, Lu W and Wang L. A cluster of metabolism-related genes predict prognosis and progression of clear cell renal cell carcinoma. Sci Rep 2020; 10: 12949.
[14] Zhao J, Li J, Fan TWM and Hou SX. Glycolytic reprogramming through PCK2 regulates tumor initiation of prostate cancer cells. Oncotarget 2017; 8: 83602-83618.
[15] Luo S, Li Y, Ma R, Liu J, Xu P, Zhang H, Tang K, Ma J, Liu N, Zhang Y, Sun Y, Ji T, Liang X, Yin X, Liu Y, Tong W, Niu Y, Wang N, Wang X and Huang B. Downregulation of PCK2 remodels tricarboxylic acid cycle in tumor-repopulating cells of melanoma. Oncogene 2017; 36: 3609-3617.
[16] Liu MX, Jin L, Sun SJ, Liu P, Feng X, Cheng ZL, Liu WR, Guan KL, Shi YH, Yuan HX and Xiong Y. Metabolic reprogramming by PCK1 promotes TCA cataplerosis, oxidative stress and apoptosis in liver cancer cells and suppresses hepatocellular carcinoma. Oncogene 2018; 37: 1637-1653.
[17] Vincent EE, Sergushichev A, Griss T, Gingras MC, Samborska B, Ntimbane T, Coelho PP, Blagih J, Raissi TC, Choiniere L, Bridon G, Loginicheva E, Flynn BR, Thomas EC, Tavare JM, Avizonis D, Pause A, Elder DJ, Artyomov MN and Jones RG. Mitochondrial phosphoenolpyruvate carboxykinase regulates metabolic adaptation and enables glucose-independent tumor growth. Mol Cell 2015; 60: 195-207.
[18] Hanahan D and Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011; 144: 646-674.
[19] DeBerardinis RJ, Lum JJ, Hatzivassiliou G and Thompson CB. The biology of cancer: metabolic reprogramming fuels cell growth and proliferation. Cell Metab 2008; 7: 11-20.
[20] Cassim S, Raymond VA, Dehbidi-Assadzadeh L, Lapierre P and Bilodeau M. Metabolic reprogramming enables hepatocarcinoma cells to efficiently adapt and survive to a nutrient-restricted microenvironment. Cell Cycle 2018; 17: 903-916.
[21] Zhou X, Curbo S, Li F, Krishnan S and Karlsson A. Inhibition of glutamate oxaloacetate transaminase 1 in cancer cell lines results in altered metabolism with increased dependency of glucose. BMC Cancer 2018; 18: 559.
[22] Boroughs LK and DeBerardinis RJ. Metabolic pathways promoting cancer cell survival and growth. Nat Cell Biol 2015; 17: 351-359.
[23] Brearley MC, Daniel Z, Loughna PT, Parr T and Brameld JM. The phosphoenolpyruvate carboxykinase (PEPCK) inhibitor, 3-mercaptopicolinic acid (3-MPA), induces myogenic differentiation in C2C12 cells. Sci Rep 2020; 10: 22177.
[24] Seenappa V, Joshi MB and Satyamoorthy K. Intricate regulation of phosphoenolpyruvate carboxykinase (PEPCK) isoforms in normal physiology and disease. Curr Mol Med 2019; 19: 247-272.
[25] Mendez-Lucas A, Hyrossova P, Novellasdemunt L, Vinals F and Perales JC. Mitochondrial phosphoenolpyruvate carboxykinase (PEPCK-M) is a pro-survival, endoplasmic reticulum (ER) stress response gene involved in tumor cell adaptation to nutrient availability. J Biol Chem 2014; 289: 22090-22102.
[26] Modaressi S, Brechtel K, Christ B and Jungermann K. Human mitochondrial phosphoenolpyruvate carboxykinase 2 gene. Structure, chromosomal localization and tissue-specific expression. Biochem J 1998; 333: 359-366.
[27] Xiang J, Zhang Y, Tuo L, Liu R, Gou D, Liang L, Chen C, Xia J, Tang N and Wang K. Transcriptomic changes associated with PCK1 overexpression in hepatocellular carcinoma cells detected by RNA-seq. Genes Dis 2020; 7: 150-159.
[28] Tuo L, Xiang J, Pan X, Hu J, Tang H, Liang L, Xia J, Hu Y, Zhang W, Huang A, Wang K and Tang N. PCK1 negatively regulates cell cycle progression and hepatoma cell proliferation via the AMPK/p27 (Kip1) axis. J Exp Clin Cancer Res 2019; 38: 50.
[29] Tang Y, Zhang Y, Wang C, Sun Z, Li L, Cheng S and Zhou W. Overexpression of PCK1 gene antagonizes hepatocellular carcinoma through the activation of gluconeogenesis and suppression of glycolysis pathways. Cell Physiol Biochem 2018; 47: 344-355.
[30] Zhao J, Li J, Fan T and Hou SX. Glycolytic reprogramming through PCK2 regulates tumor initi-

ation of prostate cancer cells. Oncotarget 2017; 8: 83602-83618.
[31] Montal ED, Dewi R, Bhalla K, Ou L, Hwang BJ, Ropell AE, Gordon C, Liu W, DeBerardinis RJ, Sudderth J, Twaddel W, Boros LG, Shroyer KR, Duraisamy S, Drapkin R, Powers RS, Rohde JM, Boxer MB, Wong K and Girnun GD. PEPCK coordinates the regulation of central carbon metabolism to promote cancer cell growth. Mol Cell 2015; 60: 571-583.
[32] Wang Z and Dong C. Gluconeogenesis in cancer: function and regulation of PEPCK, FBPase, and G6Pase. Trends Cancer 2019; 5: 30-45.
[33] Montal ED, Bhalla K, Dewi RE, Ruiz CF, Haley JA, Ropell AE, Gordon C, Haley JD and Girnun GD. Inhibition of phosphoenolpyruvate carboxykinase blocks lactate utilization and impairs tumor growth in colorectal cancer. Cancer Metab 2019; 7: 8.
[34] Stark R, Pasquel F, Turcu A, Pongratz RL, Roden M, Cline GW, Shulman GI and Kibbey RG. Phosphoenolpyruvate cycling via mitochondrial phosphoenolpyruvate carboxykinase links anaplerosis and mitochondrial GTP with insulin secretion. J Biol Chem 2009; 284: 26578-26590.
[35] Gao Y, Wang X, Sang Z, Li Z, Liu F, Mao J, Yan D, Zhao Y, Wang H, Li P, Ying X, Zhang X, He K and Wang H. Quantitative proteomics by SWATH-MS reveals sophisticated metabolic reprogramming in hepatocellular carcinoma tissues. Sci Rep 2017; 7: 45913.
[36] Leithner K, Hrzenjak A, Trotzmuller M, Moustafa T, Kofeler HC, Wohlkoenig C, Stacher E, Lindenmann J, Harris AL, Olschewski A and Olschewski H. PCK2 activation mediates an adaptive response to glucose depletion in lung cancer. Oncogene 2015; 34: 1044-1050.
[37] Leithner K, Triebl A, Trotzmuller M, Hinteregger B, Leko P, Wieser BI, Grasmann G, Bertsch AL, Zullig T, Stacher E, Valli A, Prassl R, Olschewski A, Harris AL, Kofeler HC, Olschewski H and Hrzenjak A. The glycerol backbone of phospholipids derives from noncarbohydrate precursors in starved lung cancer cells. Proc Natl Acad Sci U S A 2018; 115: 6225-6230.
[38] Chun SY, Johnson C, Washburn JG, Cruz-Correa MR, Dang DT and Dang LH. Oncogenic KRAS modulates mitochondrial metabolism in human colon cancer cells by inducing HIF-1alpha and HIF-2alpha target genes. Mol Cancer 2010; 9: 293.
[39] Fernández-Coto DL, Gil J, Hernández A, Herrera-Goepfert R, Castro-Romero I, Hernández-Márquez E, Arenas-Linares AS, Calderon-Sosa VT, Sanchez-Aleman MÁ, Mendez-Tenorio A, Encarnación-Guevara S and Ayala G. Quantitative proteomics reveals proteins involved in the progression from non-cancerous lesions to gastric cancer. J Proteomics 2018; 186: 15-27.
[40] Chu PY, Jiang SS, Shan YS, Hung WC, Chen MH, Lin HY, Chen YL, Tsai HJ and Chen LT. Mitochondrial phosphoenolpyruvate carboxykinase (PEPCK-M) regulates the cell metabolism of pancreatic neuroendocrine tumors (pNET) and de-sensitizes pNET to mTOR inhibitors. Oncotarget 2017; 8: 103613-103625.