Original Article

The ratio of PKM1/PKM2 is the key factor affecting the glucose metabolism and biological function of colorectal cancer cells

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Background: Despite evidence suggesting a significant role of pyruvate kinase muscle isozyme (PKM) in cancer development, its particular function in colorectal cancer (CRC) remains unclear. This study aimed to elucidate the specific role and mechanism of PKM and its isoforms, PKM1 and PKM2, in the progression of CRC.

Methods: We analyzed PKM, PKM1, and PKM2 expression in CRC tissues and their correlation with clinicopathological features. Plasmids were constructed to modulate these isoforms' expression in CRC cells. Cellular behavior changes, including glucose metabolism alterations, were assessed using the Seahorse Energy Meter, and the Cell Counting Kit-8 (CCK8) assay to determine the inhibitory concentration of 5-fluorouracil (5-FU) on different CRC cell groups.

Results: Our results showed significant PKM overexpression in CRC tissues, which was correlated with negative prognostic factors such as advanced T stages and lymph node metastasis. A lower *PKM1/PKM2* ratio was associated with these adverse outcomes. Functionally, *PKM1* overexpression decreased cell migration and invasion, increasing 5-FU sensitivity. Conversely, *PKM2* overexpression promoted malignant traits and reduced 5-FU sensitivity. Intriguingly, the introduction of glycolysis inhibitors attenuated the impact of *PKM* on the biological functions of CRC cells, suggesting a glycolysis-dependent mechanism.

Conclusions: This study establishes the *PKM1/PKM2* ratio as crucial in CRC progression and 5-FU response. PKM1 overexpression reduces CRC malignancy and increases 5-FU sensitivity, while *PKM2* does the opposite. Notably, glycolysis inhibitors lessen *PKM*'s impact on CRC cells, highlighting a glycolysis-dependent mechanism. These insights suggest targeting *PKM* isoforms and glycolysis pathways as a promising CRC therapeutic strategy, potentially enhancing treatment efficacy.

Keywords: Pyruvate kinase; chemotherapy sensitivity; clinicopathological features; glucose metabolism

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Introduction

Colorectal cancer (CRC) is leading a sustained increase in the burden of healthcare in China (1). In 2020, China recorded over 555,000 new CRC cases and more than 286,000 deaths, constituting over 75% of the CRC cases in East Asia (2). The steadily rising incidence of CRC poses a serious threat to human health (3). Recently, the search for novel biomarkers and more effective targeted therapies has emerged as a key area of research. Tumor cells primarily derive their energy from aerobic glycolysis, a process that directly initiates nutrient uptake (4). Most tumors are characterized by significantly increased glucose uptake and

accompanying metabolic alterations (5). Numerous genes and pathways, including the upregulation of oncogenes *RAS* and *Src*, mutations in the tumor suppressor gene *P53*, and alterations in the *PI3K/AKT/mTOR* pathway, are implicated in the regulation of glucose metabolism in tumors. Such changes result in metabolic reprogramming of cancer cells (6), particularly in enhancing glucose uptake and glycolysis (7). Molecules that regulate the reprogramming of tumor glycometabolism are increasingly recognized as promising targets for cancer diagnosis and therapy.

Serving as a pivotal enzyme in the process of aerobic glycolysis, pyruvate kinase muscle isozyme (PKM) plays a key role as a rate-limiting factor in glycolysis, significantly influencing glucose metabolism. PKM exists in two isoforms: PKM1 and PKM2. A decrease in *PKM1* expression coupled with an increase in *PKM2* can lead to the Warburg effect, driving tumor proliferation in cancer cells (8-10). Nonetheless, the specific impacts of *PKM1* and *PKM2* on tumors remain widely debated (11). A recent study (12) suggests that shifts in the PKM1/PKM2 ratio may reprogram glucose metabolism, steering it towards either aerobic glycolysis or oxidative phosphorylation.

In our prior research (13), we established that the oncogene tripartite motif containing 29 (TRIM29) modulates glucose metabolism by the ubiquitin-mediated degradation of *PKM1/2*, predominantly targeting *PKM1*, thereby facilitating cancer progression. This study aims to investigate the expression patterns of *PKM1* and *PKM2* in clinical specimens and to substantiate their impact on cellular biological functions and drug responsiveness via cellular assays. We present this article in accordance with the MDAR reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-154/rc).

Highlight box

Key findings

- This study investigated pyruvate kinase muscle isozyme (PKM) and its isoforms *PKM1/PKM2* in colorectal cancer (CRC).
- PKM was found to be overexpressed in CRC, linked to poor prognosis.
- Lower *PKM1/PKM2* ratio correlates with advanced CRC stages and lymph node metastasis.
- *PKM1* overexpression reduces CRC cell migration/invasion and increases 5-fluorouracil (5-FU) sensitivity.
- *PKM2* overexpression enhances CRC malignancy and reduces 5-FU sensitivity.

What is Known and what is new?

- *PKM* has a significant role in cancer, with *PKM1/PKM2* implicated in various cancers. *PKM* was found to be overexpressed in CRC, with *PKM1/PKM2* ratio critical for progression and treatment response.
- *PKM1* inhibits, *PKM2* promotes CRC malignancy, affects 5-FU effectiveness.

What is the implication, and what should change now?

- *PKM1/PKM2* ratio is critical for CRC glucose metabolism and cell functions.
- This study opens avenues for CRC treatments targeting *PKM1/PKM2*, improving 5-FU efficacy.
- Future research should explore interventions altering these isozymes for better CRC treatment outcomes.

Methods

Bioinformatics analysis

We screened genes and found oncogenes related to glucose metabolism in CRC using our team's previous gene chip data (14) (Gene Expression Omnibus access number: GSE104836) and the Gene Expression Profiling Interactive Analysis (GEPIA) database information (http://gepia.cancer-pku.cn/).

Human CRC tissue samples

Paraffin specimens from 50 CRC patients were collected from The Fourth Hospital of Hebei Medical University (Hebei, China) between 2018 and 2019 for immunohistochemical (IHC) tests. Clinicopathological features, such as tumor invasion and N stage, were simultaneously summarized. In addition, we purchased a complementary DNA (cDNA) chip (cDNA-HColA060CS02) from Shanghai Outdo Biotech Co. Ltd. (Shanghai, China) for quantitative polymerase chain reaction (q-PCR) to determine the expression levels of *PKM*, *PKM1*, and *PKM2* genes in CRC tissue, as well as their correlation with TNM staging (tumor size, lymph node involvement, and metastasis). The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Board of The Fourth Hospital of Hebei Medical University (No. 2020KY151) and individual consent for this retrospective analysis was waived.

IHC staining

We used IHC staining to analyze the protein expression of

PKM, PKM1, and PKM2 in the 50 collected CRC samples. An antibody against PKM (Proteintech, Wuhan, China) was applied at a dilution of 1:500, an antibody against PKM1 (Proteintech, Wuhan, China) was applied at a dilution of 1:500, and an antibody against PKM2 (Proteintech, Wuhan, China) was applied at a dilution of 1:500. IHC results were assessed independently by at least two pathologists. The positive expression of PKM protein was distinguished by (1+), (2+), and (3+), representing weak positive, medium positive, and strong positive expression, respectively, while unstained was defined as (–).

q-PCR

The cDNA chip was removed from –20 ℃ and placed at room temperature for 1 minute. The following solution was prepared in a centrifuge tube: Power SYBR Green PCR Master Mix 610 μL, forward primer (20 μM) 30.5 μL, reverse primer (20 μM) 30.5 μL, ddH_2O 549 μL. Then mix the above mixture well, and add 20 μL/well into the cDNA array. Seal the cDNA chip with a new sealing film, and then place the pore plate on ice for 15 minutes to fully dissolve the freeze-dried cDNA. After gently shaking, centrifuge at 2,000–6,000 rpm for 1 minute. Place the orifice plate into the PCR instrument and set the program as follows: activation 50 ℃ for 2 minutes, pre-soak 95 ℃ for 10 minutes, denaturation 95 ℃ for 15 seconds, annealing 60 ℃ for 1 minute (40 cycles), melt curve 65 ℃ → 95 ℃, with a temperature rise of 0.5 ℃ every 5 seconds. qPCR assays were performed to quantify PKM1, PKM2, and GAPDH mRNA levels. We use the comparative Ct method (ΔΔCt) to analyze the relative expression of genes. The fold change was evaluated as $2^{-\Delta\Delta Ct}$. The primers of PKM1 were: forward 5'-CCAGCTTCCCGATCAGTGG-3', reverse 5'-AGGAAGTCGGCACCTTTCTG-3'. The primers of PKM2 were: forward 5'-CGAGCCTCAAGTCACTCCACAG-3', reverse 5'-AACATTCATGGCAAAGTTCACCC-3'. The primers of GAPDH were: forward 5'-GCACCGTCAAGGCTGAGAAC-3', reverse 5'-TGGTGAAGACGCCAGTGGA-3'.

Cell culture

The Type Culture Collection of the Chinese Academy of Science (Shanghai, China) purchased the SW480 and HCT116 cell lines. Short tandem repeat (STR) profiling at the time of purchase can authenticate all cell lines. Both of the cell lines were mycoplasma-free cells. SW480 was cultured in Dulbecco's modified Eagle's medium (DMEM) (Thermo, MA, Waltham, USA). HCT116 was cultured in RPMI-1640 medium (Thermo, MA, Waltham, USA). Both mediums contained 1% antibiotics and 10% fetal bovine serum (FBS).

Plasmid transfection

An expression construct was generated by subcloning PCR-amplified full-length human *PKM*, *PKM1*, or *PKM2* cDNA into an Ez-NEG-M98 (Gene Copoeia, Guangzhou, China) to overexpress *PKM*, *PKM1*, or *PKM2* in cells. At the same time, we used an empty vector as a negative control.

Cell migration and Matrigel invasion assays

To assess the migration and invasion capabilities of cancer cells, we used Transwell culture plates (Corning Inc., NY, USA). For the migration assay, 1.5×10^5 cells were seeded into upper chambers without Matrigel, and a chemotactic agent was added below. Cells that migrated to the lower chambers were counted post-incubation using a microscope. For the invasion assay, membranes were precoated with 60 μL of Matrigel. Conditions mirrored the migration assay, and cells that penetrated the Matrigel and reached the lower chambers were counted at experiment's end. Additionally, for wound healing assay, cells in 6-well plates were scratched with a 10 μL pipette tip upon reaching confluency, and scratch closure was imaged at 0, 24, and 48 hours using an Olympus IX71 inverted microscope at 100× magnification. Migration was quantified by measuring the initial scratch width and the remaining gap at each time point, providing a comprehensive analysis of cell motility and invasive behavior. 2-Deoxy-D-glucose (2-DG), as a glycolysis inhibitor, was introduced in this experiment to explore whether PKM, PKM1, and PKM2 affect the migration and invasion of colon cancer cells through the glycolysis pathway.

Cell proliferation assay

Cell Counting Kit-8 (CCK8) assay can evaluate the cell viability. We cultured the cells in 96-well plates with a density of 3,000 cells/well and then performed using the CCK8 assay kit (Promega, Fitchburg, WI, USA) for 72 hours. An enzyme marker can measure the absorbance (A) values at 450 nm of different groups to reflect the cell viability of each group. We repeated this assay three times.

Extracellular acidification rate (ECAR) and oxygen consumption rate (OCR) assays

To detect the ECAR and OCR of CRC cancer cells, the Seahorse XF Glycolysis Stress Test Kit (Agilent Technologies, Beijing, China) and Seahorse XF Cell Mito Stress Test Kit (Agilent Technologies) were used. The experiment utilized the SW480 and HCT116 cell lines. Cells were inoculated with a density of 1.5×10^4 per well into the XF96 healthy plate and were allowed to adhere overnight. The proton static loss in the glycolysis process was taken as the real-time detection value and was incubated in the non-buffered culture medium for 1 hour before detection. When measuring the ECAR value, 10 mM of glucose, 4 μM of oligomycin, and 80 mM of 2-DG were sequentially injected at each designated time point. When calculating the OCR value, inject 4 μM oligomycin, 0.5 μM reversible oxidative phosphorylation inhibitor FCCP (p-trifluoromethoxy carboxyl cyanide phenylhydrazone), and 0.5 μM mitochondrial complex I inhibitor rotenone and mitochondrial complex III inhibitor antimycin A (Rote/AA) in sequence at each designated time point. Digest cells after testing and count the number of cells. ECAR measurements are normalized based on cell count in units of mpH/minute/1,000 cells and OCR in units of pmol/minute. Each sample is tested four times.

The half inhibitory concentration (IC50) of 5-fluorouracil (5-FU) detected with CCK8 assay

The 96-well culture plates had four groups of cells (pc-Control, pc-PKM1, pc-PKM2, pc-PKM), 5×10^3 cells for each well, six wells/group, and negative control was proceeding at the same time. Different concentrations of 5-FU were added to each group (final concentrations were 0, 5, 10, 20, 40, 80, and 120 μg/mL) after cell adhesion. After 72 hours incubation in the incubator, 20 μL CCK8 solution was added into each well. After 2 hours, we measured the absorbance (A) value at 450 nm on the enzyme marker to reflect the viability of cells in each group. Subsequently, we added the 2-DG into the four groups and repeated this experiment. Using Prism 5.0 software, we calculated the IC50 of 5-FU.

Statistical analyses

We used two-tailed Student's *t*-tests to evaluate differences between groups and analyzed correlations with the Chi-squared test. Statistical analyses were conducted using GraphPad Prism (version 5, La Jolla, CA, USA) and SPSS (version 21.0, USA). Differences were considered statistically significant at $P<0.05$. Each experiment was conducted at least three times (*P<0.05, **P<0.01, ***P<0.001, and ****P<0.0001).

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

Results

The screening and validation of PKM

According to our previous study (15), we identified numerous differentially expressed genes in CRC tissues compared to para-cancer tissues: 1,606 upregulated and 1,615 downregulated mRNAs (*Figure 1A*). Pathway enrichment analysis revealed that glucose metabolism was crucial for the onset and progression of CRC (*Figure 1B*). We integrated three datasets: critical enzymes in glucose metabolism, genes overexpressed in our previous gene chip, and genes overexpressed in the GEPIA database. PKM was identified as a common mRNA (*Figure 1C*). *PKM*, a key enzyme in glycolysis, significantly regulates this metabolic pathway. GEPIA database indicated that *PKM* expression in CRC tissue was markedly higher than in para-cancer tissues (*Figure 1D*). Using IHC on 50 pairs of CRC and para-cancer tissues, we observed PKM protein in the cytoplasm and nucleus of CRC cells. We categorized PKM protein expression into four grades based on IHC staining intensity: PKM (–), PKM (1+), PKM (2+), and PKM (3+) (*Figure 1E*). Among the 50 pairs of CRC tissues and para-cancer tissues, there were 40 cases of high expression of PKM protein, PKM (2+), and PKM (3+) in CRC tissues (40/50, 80%), while only 19 cases of high expression of PKM protein in para-cancer tissues (19/50, 38%) (*Figure 1F*). Then, we assessed the relationship between PKM protein expression and clinicopathologic parameters, such as T stage and lymph node metastasis. Of those with high PKM protein expression, 65% (26/40) were T3 + T4 and 55% (22/40) had lymph node metastasis, whereas those with low PKM protein expression had 30% (3/10) T3 + T4 and 40% (4/10) with lymph node metastasis. Elevated PKM protein expression in CRC tissue was associated with advanced T stage and increased lymph node metastasis (*Figure 1G,1H*), suggesting PKM's potential role in CRC progression.

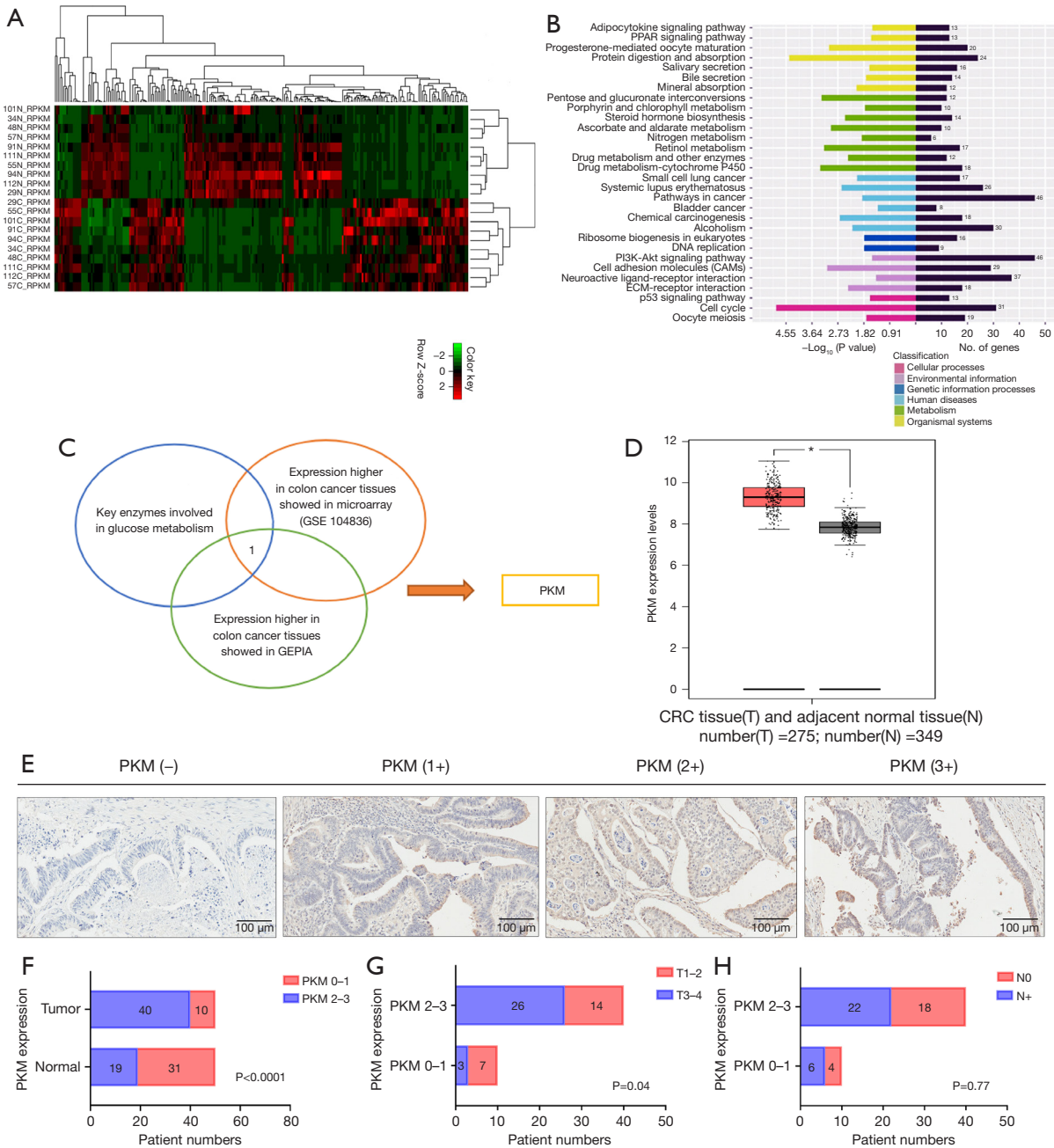

Figure 1 The expression of PKM in CRC tissues and its relationship with clinicopathologic parameters. (A) There were 1,606 upregulated mRNAs in CRC tissues compared with para-cancer tissues. (B) The pathway enrichment analysis of these differential mRNAs. (C) The screening process of PKM. (D) The expression level of PKM in CRC tissues and para-cancer tissues in public database (GEPIA). (E) Representative IHC staining of PKM in CRC tissues (200×). (F) Protein expression of PKM in 50 pairs of CRC tissues and para-cancer tissues by IHC test. (G) The correlation between PKM protein expression and T stage. (H) The correlation between PKM protein expression and lymph node metastasis. * P<0.05. RPKM, reads per kilo base of transcript per million mapped reads; ECM, extracellular matrix; GSE, Gene Set Enrichment; GEPIA, Gene Expression Profiling Interactive Analysis; PKM, pyruvate kinase muscle isozyme; CRC, colorectal cancer; IHC, immunohistochemical.

Expression of PKM1 and PKM2 protein in CRC and their correlation with clinicopathological parameters

We further analyzed PKM1 and PKM2 expression, the two isoforms of PKM, in 50 paraffin-embedded specimens. *Figure 2A*,*2B* display the varied expression levels of PKM1 and PKM2 in CRC tissues. Further analysis revealed 11 cases (11/18, 61.1%) of T3 + T4 and 12 cases (12/18, 66.7%) of lymph node-positive in CRC with high PKM1 protein expression, while there were 18 cases (18/32, 56.3%) of T3 + T4 and 16 cases (16/32, 50.0%) of lymph node-positive in CRC with low PKM1 protein expression (*Figure 2C*,*2D*). Likewise, there were 18 cases (18/27, 66.7%) of T3 + T4 and 18 cases (18/27, 66.7%) of lymph node-positive in CRC with high PKM2 protein expression, while there were 11 cases (11/23, 47.8%) of T3 + T4 and 10 cases (10/23, 43.5%) of lymph node-positive in CRC with low PKM2 protein expression (*Figure 2E*,*2F*). Overall, these findings suggest that the expression levels of PKM1 and PKM2 proteins are not significantly correlated with the T and N stages of CRC, respectively. We postulated that the PKM1/PKM2 ratio, rather than their individual expression levels, correlates with clinicopathological parameters. We categorized the PKM1/PKM2 ratio into two groups: PKM1/PKM2 <0.5 and PKM1/PKM2 ≥0.5. In the PKM1/PKM2 ≥0.5 group, 14 cases (42.4%) were T3 + T4 and 15 cases (45.5%) had lymph node involvement. Conversely, in the PKM1/PKM2 <0.5 group, 15 cases (88.2%) were T3 + T4 and 13 cases (76.5%) had lymph node involvement. A significant statistical difference was observed between the two groups (*Figure 2G*,*2H*).

Expression of PKM1 and PKM2 in gene chips, and the correlation between PKM1/PKM2 ratio and TNM staging

The gene chip analyzed 30 CRC samples and their corresponding para-cancer tissues. The quantitative reverse transcription polymerase chain reaction (qRT-PCR) results indicated a significantly higher expression of PKM1 in CRC tissues compared to para-cancer tissues (*Figure 3A*), with PKM2 following a similar trend (*Figure 3B*). Interestingly, a more pronounced difference was observed in the PKM1/PKM2 ratio between CRC and para-cancer tissues. The PKM1/PKM2 ratio in CRC tissues was notably higher than in para-cancer tissues, showing a more pronounced difference than either PKM1 or PKM2 independently (*Figure 3C*). Additionally, the area under the curve (AUC) analysis suggested that with a cutoff value of 1 for the PKM1/PKM2 ratio, it could reliably predict T and TNM stages in CRC patients. However, its predictive accuracy for N staging was marginally reduced (*Figure 3D-3F*). We categorized the PKM1/PKM2 ratio into two groups: PKM1/PKM2 <1 and PKM1/PKM2 ≥1. Findings indicated that a PKM1/PKM2 ratio of <1 correlated with more adverse T and TNM stages (*Figure 3G*,*3H*).

Effects of PKM, PKM1, and PKM2 on migration and invasion in vitro

For a functional study of PKM, PKM1, and PKM2, we introduced their respective plasmids into SW480 and HCT116 cells. Concurrently, a negative control group was established to eliminate any interference. qRT-PCR analysis revealed a notable overexpression of PKM post-transfection with the PKM plasmid in HCT116 cells (Figure S1A). Likewise, PKM1 and PKM2 displayed significant overexpression after their respective plasmid transfections in HCT116 cells (Figure S1B,S1C). Similarly, in SW480 cells, we achieved successful overexpression of PKM, PKM1, and PKM2 (Figure S1D-S1F). We employed Transwell assays to assess cell migration and invasion capacities. Results indicated a significant decrease in migrating cell counts in both SW480 and HCT116 cells post-PKM1 plasmid transfection.

Conversely, the number of migrating cells significantly increased after transfection with the PKM2 plasmid. This trend was negated when cells were treated with 2-DG, a glycolytic inhibitor (*Figure 4A*,*4B*). For the invasion assays, the upper chamber membranes were precoated with 60 µL of Matrigel to simulate the extracellular matrix. Findings from the invasion tests mirrored those of the migration assays, demonstrating consistency in the effects of PKM2 overexpression and 2-DG treatment (*Figure 4C*,*4D*). In wound healing assay performed on the SW480 and HCT116 cell lines, we observed variations in tumor migration capacity: the cells transfected with pc-PKM1 demonstrated a reduced ability to migrate, while those transfected with pc-PKM2 showed an increased migratory capacity (Figure S2). In summary, our findings indicate that PKM1 acts as a tumor suppressor gene, while PKM2 functions as an oncogene, modulating glycolysis.

The effect of PKM, PKM1 and PKM2 on glucose metabolism

To further examine PKM1 and PKM2's influence on glucose metabolism in CRC cells, we assessed the ECAR

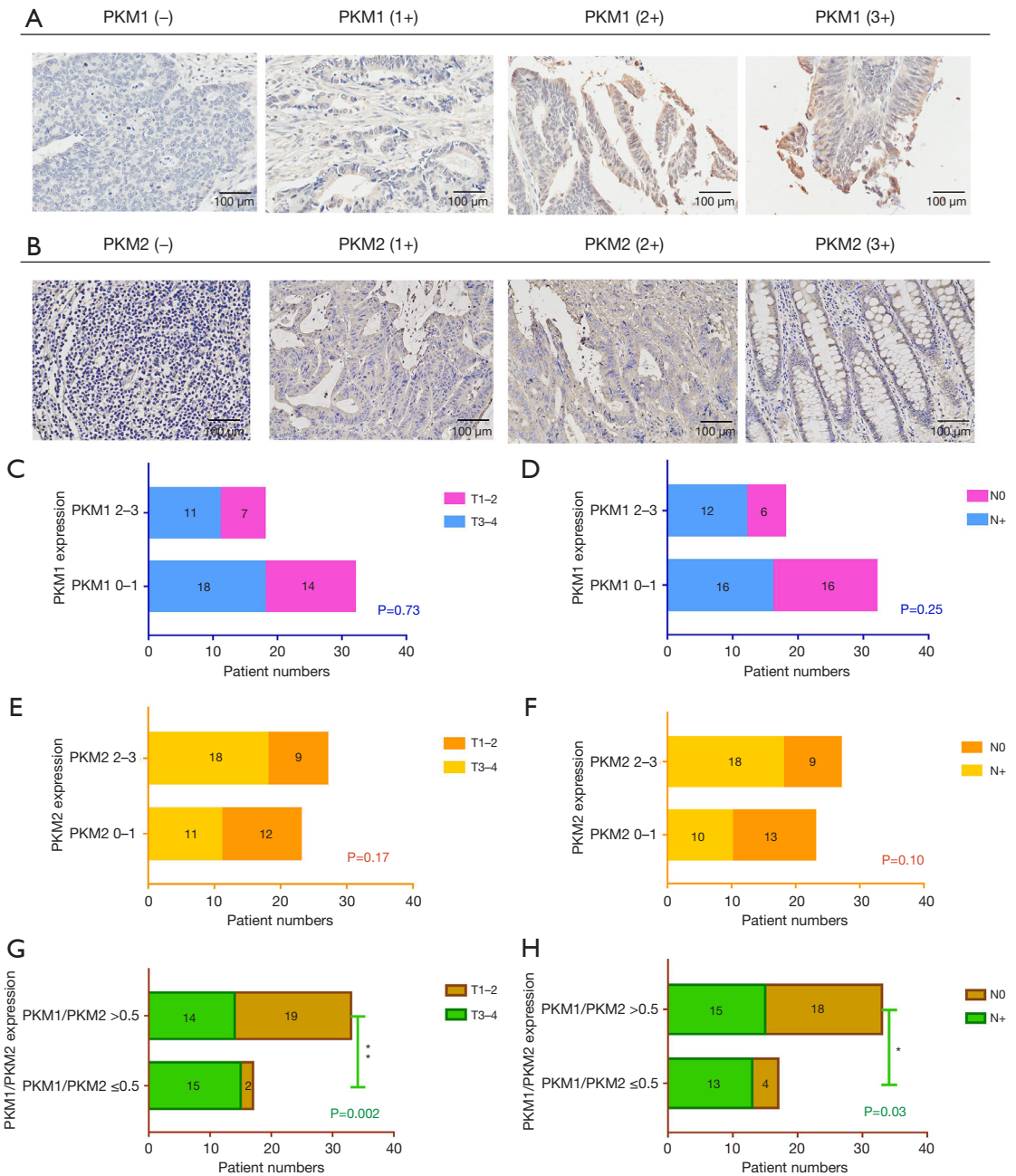

Figure 2 Expression of PKM1 and PKM2 protein in CRC and their correlations with clinicopathological parameters. (A,B) IHC staining was employed to analyze the protein expression levels of PKM, PKM1, and PKM2 in 50 collected CRC samples (200×). (C) There was no significant correlation between PKM1 protein expression and T staging. (D) There was no significant correlation between PKM1 protein expression and lymph node metastasis. (E) There was no significant correlation between PKM2 protein expression and T staging. (F) There was no significant correlation between PKM2 protein expression and lymph node metastasis. (G) There was a significant correlation between the ratio of PKM1/PKM2 and T staging. (H) There was a significant correlation between the ratio of PKM1/PKM2 and lymph node metastasis. *, P<0.05; **, P<0.01. PKM, pyruvate kinase muscle isozyme; CRC, colorectal cancer; IHC, immunohistochemical.

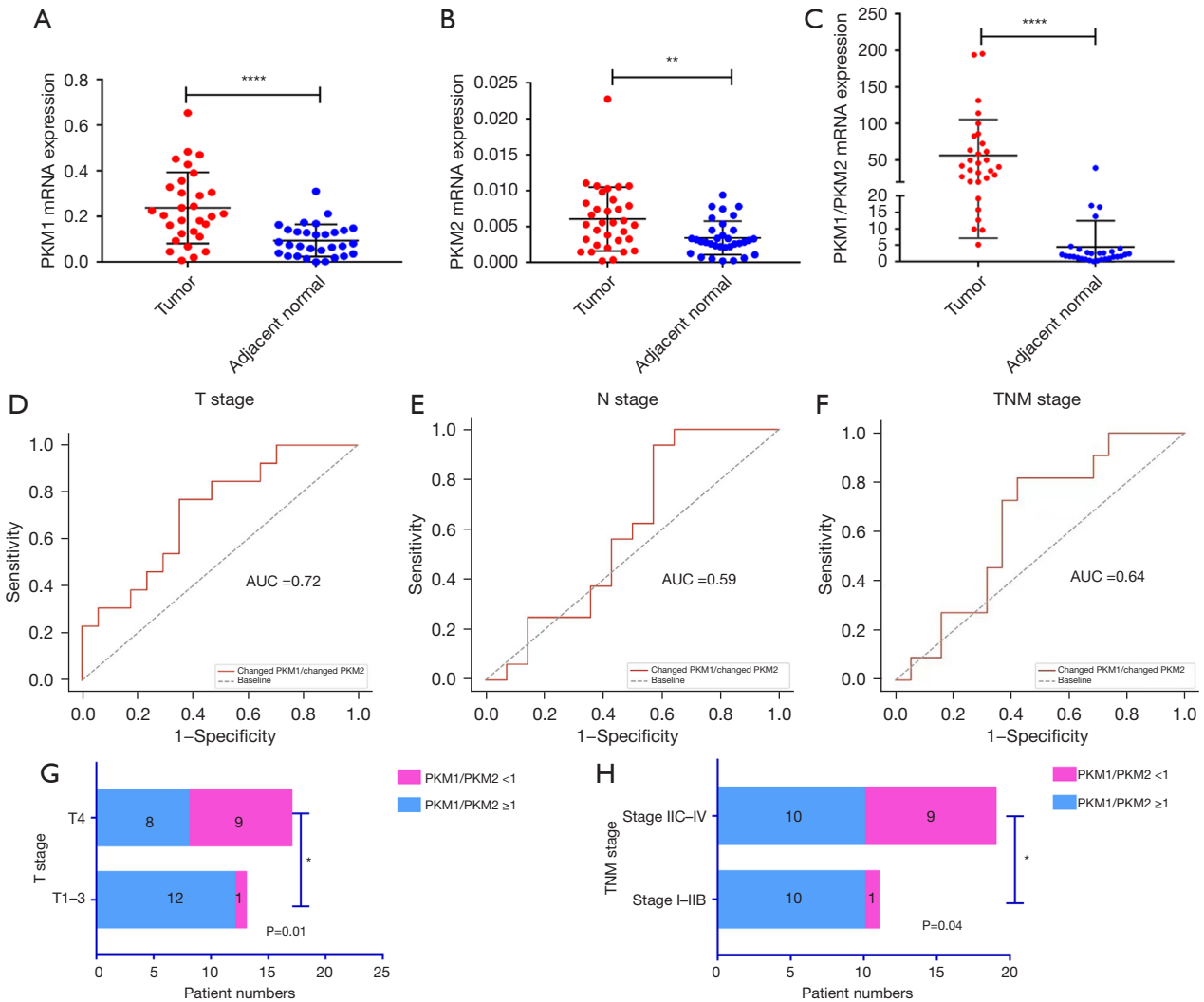

Figure 3 PKM1/PKM2 <1 of mRNA expression was associated with worse T staging and TNM staging. (A) The PKM1 mRNA in CRC tissues was significantly higher than that in para-cancer tissues. (B) The PKM2 mRNA in CRC tissues was significantly higher than that in para-cancer tissues. (C) The ratio of PKM1/PKM2 mRNA in CRC tissues was significantly higher than that in para-cancer tissues. (D,F) The ratio of PKM1/PKM2 (the ratio was 1) can effectively predict T stage and TNM stage in CRC patients. (E) The ratio's predictive value for N staging was slightly lower. (G,H) PKM1/PKM2 <1 was associated with worse T staging and TNM staging. *, P<0.05; **, P<0.01; ****, P<0.0001. PKM, pyruvate kinase muscle isozyme; AUC, area under the curve; CRC, colorectal cancer; TNM staging, tumor size, lymph node involvement, and metastasis.

and OCR in HCT116 cells across three groups: pc-PKM1, pc-PKM2, and a control vector group, utilizing the hippocampal energy meter. PKM1 overexpression led to a significant decrease in ECAR and an increase in OCR relative to the control group. Conversely, PKM2 overexpression resulted in a significant rise in ECAR and a reduction in OCR when compared to the control group (*Figure 5A,5B*). The same experiment was replicated in SW480 cells, yielding consistent results (*Figure 5C,5D*). Additionally, we categorized SW480 and HCT116 cells into four groups: pc-PKM, pc-PKM1, pc-PKM2, and a control vector group. Overexpression of PKM exhibited minimal impact on ECAR and OCR (*Figure 5E,5F*). The data suggest the PKM1/PKM2 ratio change, rather than total PKM alterations, primarily drives the shift between oxidative phosphorylation and glycolysis in cancer cell.

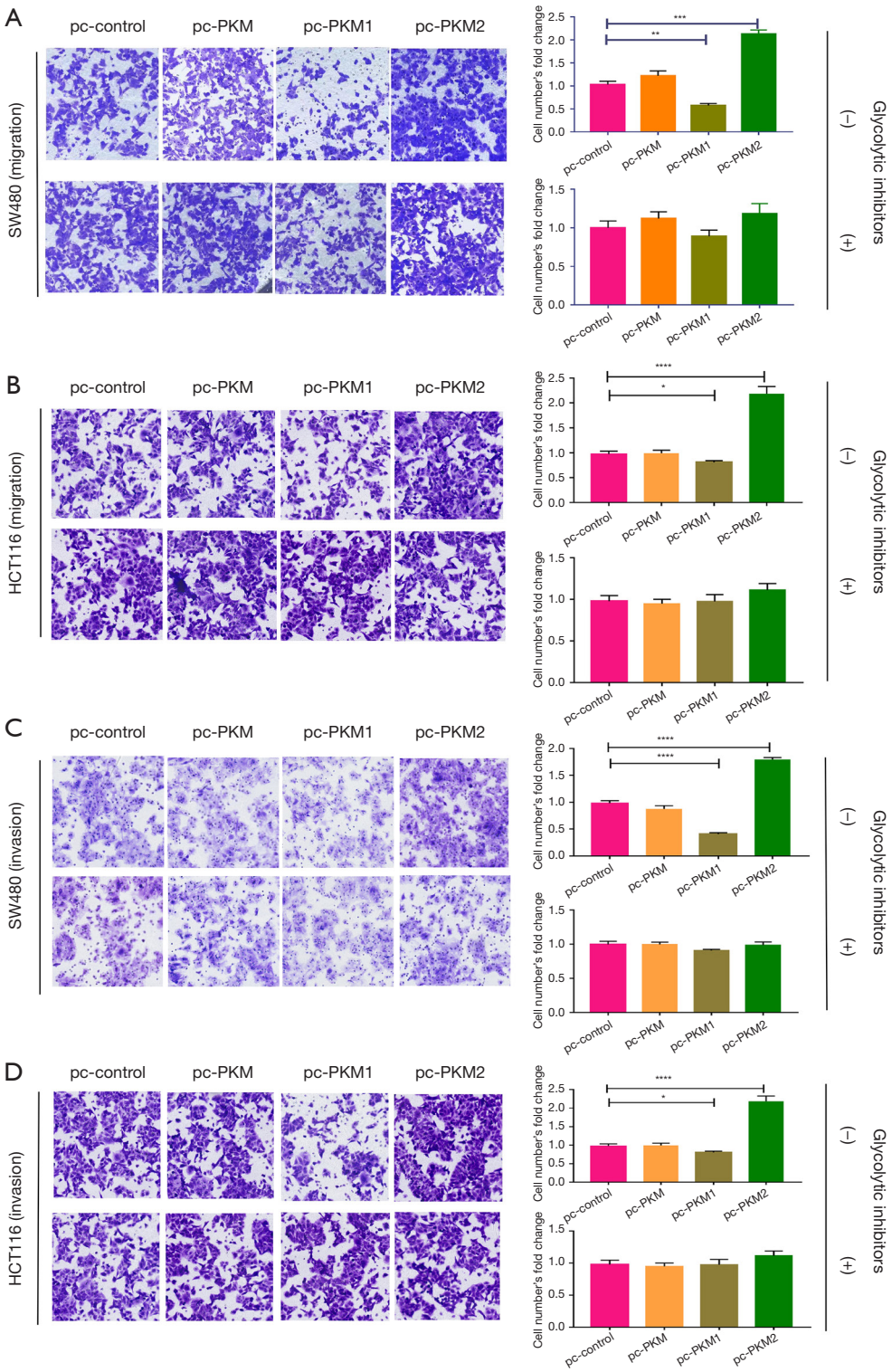

Figure 4 PKM1 and PKM2 play the role of tumor suppressor genes and oncogenes through glycolysis. (A) Transwell migration assays of SW480. The error bars represent the standard deviation. (B) Transwell migration assays of HCT116. (C) Transwell invasion assays of SW480. (D) Transwell invasion assays of HCT116. Migrated and invaded cells were stained with 0.1% crystal violet to facilitate counting. All images were captured using an Olympus IX71 inverted microscope at 100× magnification. *, P<0.05; **, P<0.01; ***, P<0.001; ****, P<0.0001. PKM, pyruvate kinase muscle isozyme.

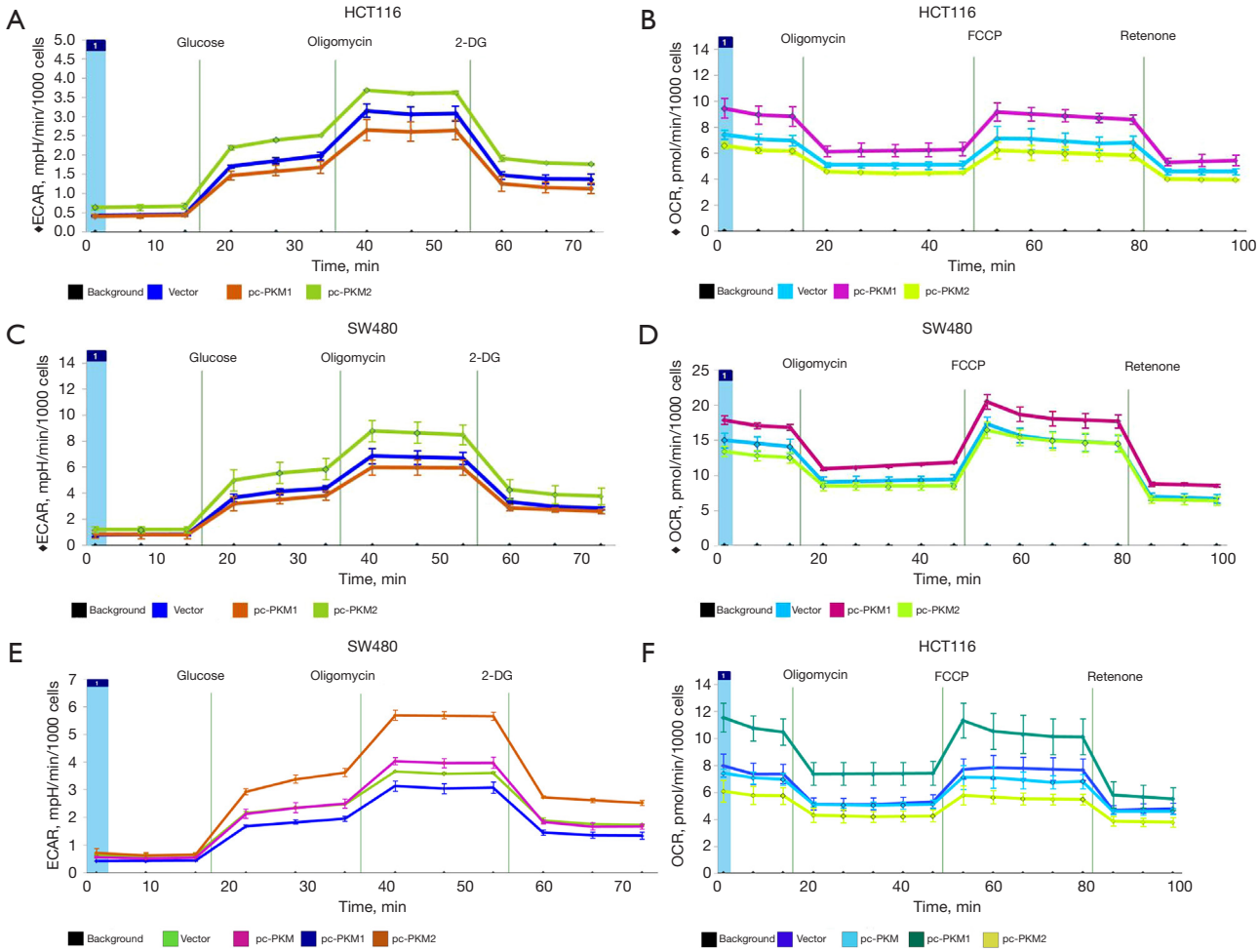

Figure 5 The effect of PKM, PKM1 and PKM2 on glucose metabolism. (A,B) In HCT116 cells, the overexpression of PKM1 decreased ECAR and increased OCR, the overexpression of PKM2 increased ECAR and decreased OCR compared with the control group. (C,D) The above experiment was repeated in SW480 cells. (E,F) There was no significant difference in ECAR and OCR between the PKM overexpression group and the control group. ECAR, extracellular acidification rate; PKM, pyruvate kinase muscle isozyme; FCCP, p-trifluoromethoxy carboxyl cyanide phenylhydrazone; 2-DG, 2-deoxy-D-glucose; OCR, oxygen consumption rate.

The changes of PKM1 and PKM2 can affect 5-FU drug sensitivity

SW480 cell lines transfected with pc-PKM, pc-PKM1, pc-PKM2, and pc-Control were subjected to CCK8 assays to evaluate the influence of PKM, PKM1, and PKM2 on proliferation. There was no significant difference in growth rate between the pc-Control and pc-PKM groups. Elevated *PKM1* expression compared to the control group significantly reduced cell proliferation. Conversely, elevated *PKM2* expression markedly enhanced cell proliferation. This trend was nullified upon adding 2-DG to the cells (*Figure 6A*). Cells overexpressing PKM showed no significant change in IC50 compared to the control. *PKM1*-overexpressing cells were more sensitive to 5-FU with a significantly decreased IC50, whereas *PKM2*-overexpressing cells exhibited reduced sensitivity to 5-FU and a notably increased IC50 value. This trend was negated upon the addition of 2-DG (*Figure 6B*).

Discussion

As we all know, glucose is the primary energy source for most cells. Glucose is transported into cells via glucose transporters (GLUTs) on the cell membrane and is subsequently metabolized. Under adequate

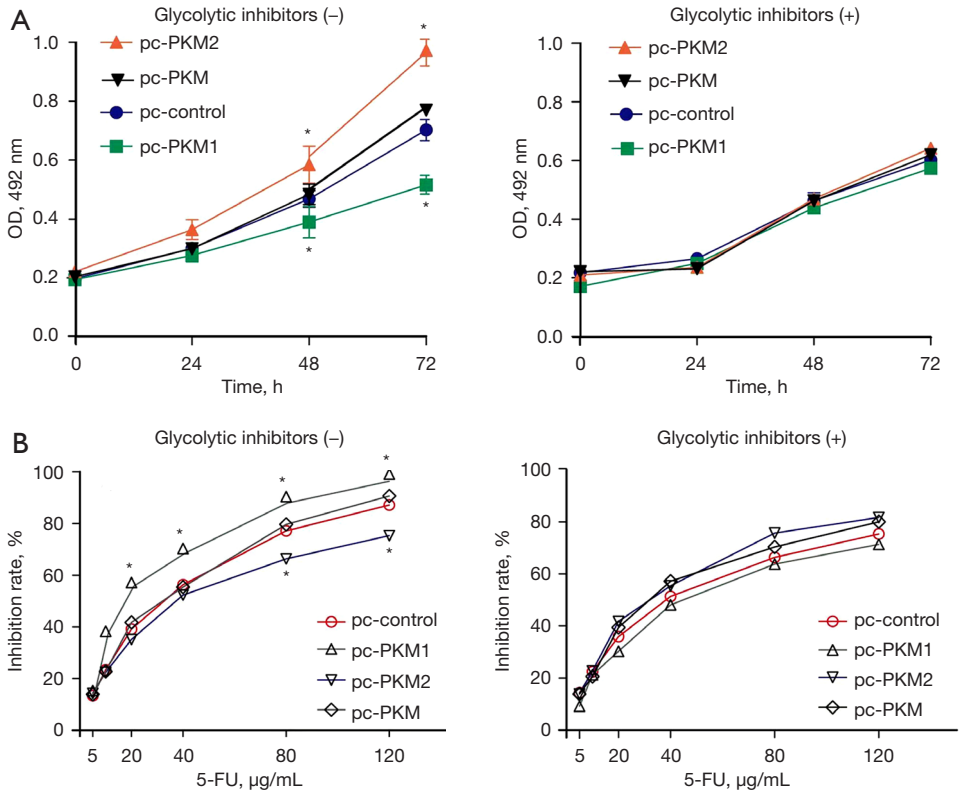

Figure 6 The effect of PKM1 and PKM2 on 5-FU drug sensitivity. (A) CCK8 assay without or with glycolytic inhibitors. Each point indicates the mean of spectrometric absorbance OD492 ± standard deviation of three independent experiments. (B) IC50 assay without or with glycolytic inhibitors in different groups. *, P<0.05. OD, optical density; PKM, pyruvate kinase muscle isozyme; 5-FU, 5-fluorouracil; CCK8, Cell Counting Kit-8; IC50, half inhibitory concentration.

oxygen conditions, cells predominantly utilize oxidative phosphorylation for energy production. Pyruvate enters the mitochondria and is decarboxylated to produce acetyl CoA, which then participates in the tricarboxylic acid (TCA) cycle to release energy. Under hypoxic conditions, cells derive energy from glycolysis. In the cytoplasm, pyruvate is reduced to lactate by the enzyme lactate dehydrogenase (LDH), aiding cellular adaptation in hypoxic conditions. Tumor cells undergo metabolic reprogramming during cancer development to adapt to changes in nutrition, inadequate blood supply, and the hypoxic microenvironment of primary lesions. Even in oxygen-rich conditions, tumor cells primarily derive energy from glycolysis rather than oxidative phosphorylation. This metabolic shift is recognized as a hallmark of tumor cells and contributes to tumor growth (16). The specific glucose metabolism pattern in tumor cells is termed the Warburg effect or aerobic glycolysis (17,18).

Since the 1980s, the widespread application of fluoro-2-deoxy-D-glucose (FDG) positron emission tomography (PET) in clinical practice has led researchers to discover an interesting phenomenon: most tumors exhibit abnormal glucose metabolism. A more pronounced glucose absorption is associated with stronger malignant biological behavior of the cancer. Numerous studies have demonstrated that metformin, a classic antidiabetic drug, possesses anticancer properties, playing a significant role in tumor prevention and treatment (19-23). The malignant transformation of adenomas is a crucial step in the onset and progression of colon cancer. It is reported that aerobic glycolysis emerges as a unique feature during the CRC phase of the adenoma-carcinoma sequence (24). Researchers have investigated the metabolic alterations in CRC and identified significant differences in metabolite phenotypes between CRC tissue and normal tissue, encompassing glucose metabolism. Some critical molecules in the glucose metabolism pathway are closely associated with the prognosis of cancer patients (25). Concurrently, reprogramming of glycolytic metabolism

can promote the invasion and metastasis of CRC (26). Therefore, we are further convinced that reprogramming glucose metabolism is crucial to the onset and progression of tumors. In our previous study (13), we also preliminarily confirmed the critical role of metabolic abnormalities in CRC. Based on the aforementioned observations, this study aims to reprogram glucose metabolism to explore its role in the malignant behavior of CRC.

Tumor suppressor genes and oncogenes can regulate the reprogramming of glucose metabolism in cancer cells. It is well-known that *KRAS* and *BRAF* genes significantly impact the malignant behavior of CRC. Reports indicate that mutations *KRAS* G13D or *BRAF* V600E directly affect glycolysis (27). *KRAS* contributes to reprogramming glucose metabolism in cancer cells by enhancing glucose uptake and inducing the expression of key glycolytic enzymes (28). Researchers found that *c-MYC* can drive glycolysis by upregulating genes within the glycolytic pathway across various cancer types (29). Mutations in *p53* also promote the reprogramming of glucose metabolism (30). *PTEN* prevents the reprogramming of glucose metabolism by reducing the rate of glycolysis (31). Additionally, research on crucial enzymes related to glucose metabolism reprogramming in tumor cells is garnering increased attention. Some studies indicate that the reduction of *PKM1* expression, coupled with the re-expression of *PKM2* leading to tumor proliferation, generally triggers the Warburg effect in cancer cells (8-10). In this study, we observed that *PKM2* overexpression significantly enhanced the migration and invasion ability of CRC cells, while *PKM1* overexpression substantially inhibited these abilities in colon cancer cells. This discovery aligns with the previous research findings of Shuyu Yu's team (32). We further confirmed the modulation of CRC cells' biological functions through the glycolytic pathway by *PKM1* and *PKM2*. Simultaneously, we discovered that the *PKM1/PKM2* ratio, rather than the total PKM, is a crucial factor affecting glucose metabolism and the biological function of CRC cells.

Acquired drug resistance presents a common challenge in cancer treatment. Compared to oxidative phosphorylation, aerobic glycolysis in tumor cells consumes more glucose by up-regulating glucose transporters, particularly in chemotherapy-resistant tumor cells (33). Lactic acid, a glycolysis metabolite, plays a crucial role in the biological process of drug resistance (34). Reports suggest that multi-kinase inhibitor anti-angiogenic drugs [tyrosine kinase inhibitors (TKIs)] can down-regulate glycolysis in breast and lung tumor models to overcome TKI resistance (35). Sun *et al.* discovered that Shenmai injection can augment the anti-tumor efficacy of cisplatin via glucose metabolism reprogramming (36). Additionally, reprogramming of glucose metabolism can influence the tumor's immune microenvironment. During this process, cancer cells consume substantial glucose and produce lactate, leading to a low energy and oxygen state in the tumor microenvironment (TME) that facilitates immune evasion by cancer cells (37). Dong *et al.* also posited that targeting glucose metabolism reprogramming could be a strategy to combat drug resistance (38). There are limited studies on the sensitivity of metabolic reprogramming to chemotherapy drugs in CRC. In this study, we discovered that modulating the expression of PKM1 and PKM2 in tumor cells can regulate the sensitivity of CRC cells to 5-FU drugs. This discovery aids in further enhancing the treatment sensitivity of patients with CRC.

Despite the absence of clinical interventions for abnormal tumor metabolism, investigating the regulatory network of *PKM* ratio in glucose metabolism of colon cancer may illuminate new treatment methodologies and targets. We anticipate that as research deepens, critical molecules in glucose metabolism will be paired with corresponding inhibitors or activators. Ultimately, for the betterment of patient outcomes, successful clinical transformation is imperative in the future.

Conclusions

Our study identifies the *PKM1/PKM2* ratio as a crucial factor in CRC progression and response to 5-FU treatment. We demonstrate that *PKM2* overexpression enhances CRC malignancy, while *PKM1* acts oppositely. These findings align with the known Warburg effect in cancer metabolism, suggesting new therapeutic approaches targeting glucose metabolism in CRC. Our results also point to a potential strategy in overcoming drug resistance in CRC by modulating metabolic pathways. Further research into these mechanisms may offer novel targets for CRC therapy, aiming to improve patient outcomes.

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Footnote

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-154/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Board of The Fourth Hospital of Hebei Medical University (No. 2020KY151) and individual consent for this retrospective analysis was waived.

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