Type Iγ phosphatidylinositol phosphate kinase promotes tumor growth by facilitating Warburg effect in colorectal cancer

Wei Peng, Wei Huang, Xiaoxiao Ge, Liqiong Xue, Wei Zhao, Junli Xue *

Department of Oncology, Shanghai East Hospital, Tongji University School of Medicine, Shanghai 200123, China

A R T I C L E   I N F O
Article history:
Received 26 March 2019
Received in revised form 7 May 2019
Accepted 7 May 2019
Available online 16 May 2019

Keywords:
Colorectal cancer
Warburg effect
Phosphatidylinositol kinase
PIPKIγ
Tumor growth

A B S T R A C T

Background: Emerging evidence suggests that metabolic alterations are a hallmark of cancer cells and contribute to tumor initiation and development. Cancer cells primarily utilize aerobic glycolysis (the Warburg effect) to produce energy and support anabolic growth. The type Iγ phosphatidylinositol phosphate kinase (PIPKIγ) is profoundly implicated in tumorigenesis, however, little is known about its role in reprogrammed energy metabolism.

Methods: Loss- and gain-of-function studies were applied to determine the oncogenic roles of PIPKIγ in colorectal cancer. Transcriptome analysis, real-time qPCR, immunohistochemical staining, Western blotting, and metabolic analysis were carried out to uncover the cellular mechanism of PIPKIγ.

Findings: In this study, we showed that PIPKIγ was frequently upregulated in colorectal cancer and predicted a poor prognosis. Genetic silencing of pan-PIPKIγ suppressed cell proliferation and aerobic glycolysis of colorectal cancer. In contrast, the opposite effects were observed by overexpression of PIPKIγ. Importantly, PIPKIγ-induced proliferative effect was largely glycolysis-dependent. Mechanistically, PIPKIγ facilitated activation of PI3K/Akt/mTOR signaling pathways to upregulate c-Myc and HIF1α levels, which regulate expression of glycolytic enzymes to enhance glycolysis. Moreover, pharmacological inhibition by PIPKIγ activity with the specific inhibitor UNC3230 significantly inhibited colorectal cancer glycolysis and tumor growth.

Interpretation: Our findings reveal a new regulatory role of PIPKIγ in Warburg effect and provide a key contributor in colorectal cancer metabolism with potential therapeutic potentials.

Fund: National Key Research and Development Program of China, Outstanding Clinical Discipline Project of Shanghai Pudong, Natural Science Foundation of China, and Science and Technology Commission of Shanghai Municipality.

© 2019 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Colorectal cancer (CRC) is the third most commonly diagnosed cancer and ranks second in terms of mortality worldwide. Over 1.8 million new colorectal cancer cases and 881,000 deaths are estimated to occur in 2018, accounting for about 10% cancer cases and deaths [1]. Because increased early detection and application of colonoscopy with polypectomy, the clinical outcome of CRC patients has significantly improved during the past decades in many countries [2]. Surgical resection is the primary treatment option for CRC, but even with complete resection the tumor will be eventually recurrent and developed to metastatic disease in many of these patients [3]. Therefore, the long-term survival outlook of CRC is still poor and highlights the development of more effective therapies for this life-threatening disease.

The type I phosphatidylinositol 4-phosphate 5-kinases (PIPKIs) are a family of enzymes that catalyze ATP-dependent phosphorylation of phosphatidylinositol 4-phosphate to generate phosphatidylinositol 4,5-bisphosphate [PI(4,5)P2]. PI(4,5)P2 is subsequently converted into phosphatidylinositol (3,4,5)-trisphosphate [PI(3,4,5)P3] by PI3K. PI(4,5)P2 and PI(3,4,5)P3 are involved in a variety of cellular processes, such as vesicular trafficking, focal adhesion assembly, actin polymerization, endocytosis and agonist-induced calcium signaling [4–6]. PIPKIs comprise a family encoded by three genes that give rise to PIP kinase type Iα (PIPKIα), PIPKIβ and PIPKIγ [7]. In mammalian cells, the different isoforms of PIPKI share very conserved kinase domain but have a high level of sequence divergence at the C-terminus, which allows for their distinct localization and function via...
Research in context

Evidence before this study

Reprogramming metabolism is emerged as a hallmark of cancer. Warburg effect, also known as aerobic glycolysis, can support uncontrolled proliferation of cancer cells by providing abundant cellular buildings. Increased glycolysis contributes to nearly all aspects of the malignant characters of cancer cells.

Added value of this study

This study showed that PIPKιγ is profoundly implicated in colorectal cancer cell proliferation and aerobic glycolysis. PIPKιγ enhances Warburg effect by upregulation of c-Myc and HIF1α levels via activation of PI3K/Akt/mTOR signaling pathways. Pharmacological inhibition of PIPKιγ significantly suppressed tumor growth in vivo.

Implications of all the available evidence

This finding suggests that PIPKιγ is a critical glycolysis modulator and provide a potential target for anti-tumor therapy for colorectal cancer.

2. Materials and methods

2.1. Cell culture and reagent

Human colorectal cancer cell lines LOVO, Caco-2, SW620, and SW480 were purchased from the Institute of Biochemistry and Cell Biology, Chinese Academy of Science (Shanghai, China); LS174T, HCT116, COL0205, and the normal colonic epithelial cell NCM460 were derived from the American Tissue Culture Collection (ATCC, Manassas, VA, USA). All cells were cultured in DMEM (Dulbecco’s modified Eagle’s medium, Gibco) or RPMI 1640 medium (Gibco, USA) medium supplemented with 10% fetal bovine serum (FBS, Gibco, USA), and the antibiotics penicillin (500 units/mL) and streptomycin (200 μg/mL). Cells were maintained at a humidified incubator with 5% CO2 atmosphere. 2-Deoxy-d-glucose (2-DG, D8375) and galactose (G0750) were purchased from Sigma (Shanghai, China). The specific inhibitor of PIPKιγ (UNC3230) was purchased from Tocris Bioscience (#5271/10, USA).

2.2. Generation of stable PIPKιγ knockdown cells

Lentiviral shRNA negative control and shRNA oligonucleotides targeting human PIPKιγ listed below were designed and synthesized by GenePharma (Shanghai, China). The sequences for the PIPKιγ shRNA were: sh-1, 5′-TGATGAACTCCTGTCGTCTTTTTTC-3′; sh-2, 5′-GGCTGCTCTGACGGAGAAGTTICATCCAGAGATAGAAACTTTCCAGGACCAGGCTTTTTTC-3′; sh-3, 5′-TTGTCCTCGACGGAGAAGTTICATCCAGAGATAGAAACTTTCCAGGACCAGGCTTTTTTC-3′; and sh-Ctrl, 5′-TTGTCCTCGACGGAGAAGTTICATCCAGAGATAGAAACTTTCCAGGACCAGGCTTTTTTC-3′. The lentivirus LV2 (pGLVU6/Puro) plasmids were transfected into human embryonic kidney 293 T cells using Lipofectamine 2000 (Invitrogen, USA) according to the manufacturer’s manual. Colorectal cancer cells were then infected with lentivirus medium from the packaging cells 48 h after transfection in the presence of 8 μg/mL polybrene. After infection overnight, virus-containing medium was replaced by normal culture medium. The stable clones were selected with 2 μg/mL puromycin. Expression levels of PIPKιγ were confirmed by Western blotting analysis.

2.3. Transfection

For overexpression of PIPKιγ, the whole sequence of PIPKιγ, j2, mPIPKeepιγ, j2–1 and mPIPKeepιγ, j2–2 was synthesized by GenePharma (Shanghai, China) and then subcloned to the pcDNA3.1 plasmid. Six si- lent mutations were introduced in the sh-PIPKeepιγ target sequence in order to make it resistant to corresponding shRNA. The siRNA sequences for PIP4K2C were: si-1 sense, 5′-CCAGUAAUUAUCAGAUCGTTT-3′; si-1 antisense, 5′-GGATGAACTCCGATTGTATTTC-3′; and sh-Ctrl, 5′-GATGGAACTCCGATTGTATTTC-3′. The lenti-virius LV2 (pGLVU6/Puro) plasmids were transfected into human embryonic kidney 293 T cells using Lipofectamine 2000 (Invitrogen, USA) according to the manufacturer’s manual. Colorectal cancer cells were then infected with lentivirus medium from the packaging cells 48 h after transfection in the presence of 8 μg/mL polybrene. After infection overnight, virus-containing medium was replaced by normal culture medium. The stable clones were selected with 2 μg/mL puromycin. Expression levels of PIPKιγ were confirmed by Western blotting analysis.

2.4. Gene expression microarrays

For RNA preparation, total RNA from sh-CTRL and sh-PIPKeepιγ cells were extracted according to standard protocol. RNA was processed and profiled on whole human Genome Microarray (4 × 44 K, Agilent) as recommended by the manufacturer. Background subtraction and normalization of probe set intensities were performed using Robust Multiaarray Analysis (RMA). The microarray data is available at GEO database GSE130761. Gene set enrichment analysis (GSEA) was performed on the Broad Institute Platform and statistical significance (false discovery rate, FDR) was set at 0.25.
2.5. Real-time quantitative PCR

Total RNA was extracted from indicated cells using Qiagen RNeasy kits (Qiagen, Valencia, CA). RNA quality and quantity were determined using Nanodrop™ spectrophotometer (Nanodrop products, Wilmington, CA). Next, 1 μg of total RNA was reverse transcribed using a First Strand cDNA Synthesis Kit (ThermoFisher Scientific) to synthesize complementary DNA (cDNA). Subsequently, the cDNA product was subjected to PCR amplification on a PCR thermocycler (Applied Biosystems) to analyze the expression of mRNA; β-actin was used as an internal control. The PCR primers sequences used in this study are shown in supplemental Table 1. Relative quantification was performed using the comparative 2^ΔΔCt method.

2.6. Immunohistochemical analysis

The colorectal cancer tissue microarray used in this study was purchased from Zhuoli Biotech (#COC1504, http://www.zhuoliotech.com, Shanghai, China). For immunohistochemical analysis, paraffin-embedded tissue sections were deparaffinized in xylene and rehydrated through descending concentrations of ethanol. Then antigen retrieval was performed by boiling in 10 mM citrate buffer (pH 6.0) for 10 min, followed by treatment with 3% hydrogen peroxide to block endogenous peroxidase. After washing with 1 × PBS for three times, the sections were incubated with a primary antibody overnight at 4 °C. The antibodies used for immunohistochemistry were listed as follows: PIPKγ (1:200, Proteintech, 27.640-1-AP), PCNA (1:5000, Cell Signaling Technology, #2983), p-S6K (1:1000, Cell Signaling Technology, #2971), mTOR (1:1000, Cell Signaling Technology, #2920), Akt (1:1000, Cell Signaling Technology, #4685), p-mTOR (1:200, Abcam, ab109192), p-Akt (1:200, Cell Signaling Technology, #4060), Proteins embedded tissue sections were deparaffinized in xylene and rehydrated through descending concentrations of ethanol. Then antigen retrieval was performed by boiling in 10 mM citrate buffer (pH 6.0) for 10 min, followed by treatment with 3% hydrogen peroxide to block endogenous peroxidase. After washing with 1 × PBS for three times, the sections were incubated with a primary antibody overnight at 4 °C. The antibodies used for immunohistochemistry were listed as follows: PIPKγ (1:200, Proteintech, 27.640-1-AP), PCNA (1:5000, Cell Signaling Technology, #2983), p-S6K (1:1000, Cell Signaling Technology, #2971), mTOR (1:1000, Cell Signaling Technology, #2920), Akt (1:1000, Cell Signaling Technology, #4685), p-mTOR (1:200, Abcam, ab109192), p-Akt (1:200, Cell Signaling Technology, #4060), Proteins (1:300, Abcam, ab113642), c-Myc (1:500, Abcam, ab32072), GLUT1 (1:200, Proteintech, 21,829-1-AP), LDHA (1:1000, Cell Signaling Technology, 19,987-1-AP), and PDK1 (1:200, Proteintech, 10,026-1-AP). The next day, the HRP-conjugated secondary antibody was added for 45 min at room temperature. The immunoreactivity was developed with 3,3′-diaminobenzidine (DAB). Finally, the sections were counterstained with hematoxylin and scoring was evaluated by two investigators blinded to the clinical information.

2.7. Western blot analysis

Cell lystates were separated using SDS-PAGE and then electrophoretically transferred onto PVDF membranes. After blocking with 5% defatted milk for 1 h at room temperature, the PVDF membranes were incubated with primary antibodies overnight at 4 °C, followed by incubation with HRP-conjugated secondary antibodies at room temperature for 45 min. β-actin antibody was used as loading control. Immunoblots were developed using the Pierce™ Western ECL Blotting substrate (Thermofisher Scientific, Waltham, MA) and ChemiDoc Touch image system (Bio-Rad). The antibodies used were listed as follows: PIPKγ (1:2000, Abcam, ab109192), p-Akt (1:2000, Cell Signaling Technology, #4060), Akt (1:1000, Cell Signaling Technology, #4685), p-mTOR (1:1000, Cell Signaling Technology, #2971), mTOR (1:1000, Cell Signaling Technology, #2983), p-S6K (1:1000, Cell Signaling Technology, #9204), S6K (1:1000, Cell Signaling Technology, #9202), HIF1α (1:1000, Abcam, ab113642), c-Myc (1:1000, Abcam, ab32072), and β-actin (1:1000, Abcam, ab8227).

2.8. Measurement of glucose and lactate

Colorectal cancer cells with indicated genetic manipulations were cultured at normal condition for 2 days and culture medium was collected. Lactate production was measured using a commercial Lactate Assay Kit (Sigma) according to the manufacturer’s protocol. For glucose uptake assay, the culture medium was replaced with phenol-red free DMEM with 10% FBS in continuous culture for 2 days. Glucose levels in the culture medium were measured using a Colorimetric Glucose Assay Kit (BioVision) as recommended by the manufacturer. All values were normalized on the basis of the total protein level (BCA protein assay, Thermo Fisher Scientific, USA).

2.9. Measurement of extracellular acidification rate (ECAR) and oxygen consumption ratio (OCR)

In vitro real-time ECAR and OCR was monitored with the Seahorse XF96 Flux Analyzer (Seahorse Bioscience) in accordance to the manufacturer’s instructions. In brief, colorectal cancer cells were seeded at a density of 2–3 × 10^4 per well. For ECAR assay, colorectal cancer cells were pre-incubated with unbuffered media for 1 h, followed by a sequential injection of 10 mM glucose, 1 mM oligomycin and 80 mM 2-DG to detect ECAR. For measurement of mitochondrial respiration, OCR was assessed by sequential injection of 1 mM oligomycin, carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone (FCCP, Sigma-Aldrich, C2920) and 2 mM antimycin A and rotenone (Sigma-Aldrich). The final output data were normalized by cell number or total protein level as demonstrated by BCA assay.

2.10. Cell proliferation and colony formation assay

Cell counting kit-8 (CCK-8, Dojindo Molecular Technologies, Japan) assay was performed to measure cell proliferation. Briefly, 2 × 10^3 cells were seeded and cultured in 96-well plates for 6 days. CCK-8 assay was carried out everyday according to the manufacturer’s protocol. All experiments were performed independently in triplicate. Absorbance was recorded at 450 nm using a microplate reader. For colony formation assay, colorectal cancer cells were seeded in 6-well plate at a density of 1 × 10^4 cells per well. After continuous culture for 12–14 days, colonies formed were stained with 0.1% crystal violet. Each experiment was performed in triplicate and repeated twice.

2.11. Tumor xenograft experiment

Pathogen-free female athymic nude mice (5 weeks old, 18–20 g weigh) used in this study were managed at SPF Laboratory Animal Center in full accordance with the guidelines by the U.S. National Institutes of Health Guide for the Care and Use of Laboratory Animals. Tumor cells (SW480, 1 × 10^6) were injected subcutaneously into the right scapular region of nude. The tumor size was monitored every week, and the volume was calculated with the following formula: Volume = (width^2 × length)/2. At the termination of the experiment, mice were sacrificed, and tumors were harvested, weighed, and fixed in formalin and embedded in paraffin or directly stored at −80 °C. For the pharmacological inhibition assay, mice were randomly divided into two groups when bore visible tumors (200 mm^3); in the test group, mice were intraperitoneally injected with 5 mg/kg UNC3230 three times a week for three weeks; in the control group, mice were treated with saline containing 0.01% DMSO. All the animal studies were approved by the Animal Care and Use Committee of Shanghai East Hospital, Tongji University School of Medicine.

2.12. Statistical analysis

Results were presented in the form of means ± standard deviation (SD). Group difference was assessed using one-way ANOVA (SPSS 23.0) or the Student t-test (two-tailed). A two-sided p-value of <0.05 was considered statistically significant. *p < 0.05, **p < 0.01 and ***p < 0.001.

3. Results

3.1. PIPKγ is highly expressed in colorectal cancer and predicts a poor prognosis

To determine the expression profile of PIPKγ in colorectal cancer, real-time qPCR and Western blotting analysis were performed in
colorectal cancer cell lines and the normal colonic epithelial cell NCM460. As shown in Fig. 1A and B, PIPKιγ mRNA and protein level were frequently overexpressed in colorectal cancer cell lines compared with the normal epithelial cell. Then, immunohistochemical analysis of a tissue microarray containing 75 matched tumor and non-tumor tissues were carried out to comprehensively characterize PIPKιγ expression in colorectal cancer tissues. The result showed that PIPKιγ was more highly expressed in colorectal cancer tissues compared to corresponding non-tumor tissues (Fig. 1C). Moreover, Kaplan-Meier curves showed that high PIPKιγ level was correlated with a reduced overall survival in colorectal cancer patients (Fig. 1D). Similar prognostic value of PIPKιγ was also conformed in colon adenocarcinoma (COAD) and rectal adenocarcinoma (READ) patients derived from the Cancer Genome Atlas (TCGA) cohort (Fig. 1E). Collectively, these data above suggested that PIPKιγ may act as oncogene in colorectal cancer tumorigenesis and progression.

3.2. PIPKιγ promotes tumor growth in colorectal cancer

Next, COAD samples derived from TCGA cohort was stratified into 2 groups (high versus low) based on median PIPKιγ value. Gene set enrichment analysis (GSEA) revealed that PIPKιγ was profoundly implicated in several oncogenic pathways, such as KEGG_Pathway in cancer, KEGG_Colorectal cancer, and BENPORATH_Proliferation (Fig. 2A). To
confirm the oncogenic roles of PIPKγ1 in colorectal cancer, loss-of-function studies were performed in two cell lines, SW480 and LOVO, which had higher intrinsic PIPKγ1 protein level. As shown in Fig. 2B, two specific shRNA targeting pan-PIPKγ1 led to significant reduction in PIPKγ1 protein. By CCK-8 assay (Fig. 2C) and colony formation assay (Fig. 2D), we found that silencing of PIPKγ1 significantly inhibited the cell proliferation of colorectal cancer cells. To confirm the specific role of PIPKγ1 in colorectal cancer, PIPKγ1 knockdown was rescued by expressing a PIP5K1C mRNA made resistant to the shRNA by six silent mutations (mPIPKγ1) and coding for a wild-type PIPKγ1_i2 protein. Western blotting analysis demonstrated that re-expression of mPIPKγ1_i2 completely restored the protein level of PIPKγ1 (Fig. 2B). As expected, mPIPKγ1_i2 largely blocked the growth-inhibiting effect induced by PIPKγ1 knockdown (Fig. 2C, D). Furthermore, gain-of-function studies revealed that PIPKγ1_i2 overexpression dramatically enhanced colorectal cancer cell proliferation in vitro (Supplementary Fig. 1). Using the subcutaneous xenograft model, we demonstrated that silencing of pan-PIPKγ1 in SW480 cells remarkably suppressed its
tumor-forming capacity, which can be restored by re-expression of mPIPKIγ2 (Fig. 2E). Consistent with the tumor-promoting role of PIPKγ, PCNA staining revealed that PIPKγ knockdown decreased tumor cell proliferation in vivo (Fig. 2F). Taken together, our results strongly suggested that in colorectal cells, PIPKγ plausibly participates in the regulation of tumor growth.

3.3. PIPKγ regulates glycolysis in colorectal cancer

To elucidate the mechanism by which PIPKγ promotes tumor growth, an Agilent gene expression microarray was used to determine the transcriptional changes after PIPKγ knockdown. Gene set enrichment analysis showed that the gene sets related to glycolysis, PI3K/
Akt/mTOR, mTORC1 signaling, hypoxia, Myc targets V1, and Myc targets V2 negatively correlated with PIPKIγ downregulation in SW480 cells (Fig. 3A). The top-scoring genes altered in the six gene sets included many glycolysis-related genes, such as SLC16A3, SLC2A1, PFKP, LDHA, and TKTL1, indicating that loss of PIPKIγ contributes to weakened glycolysis (Fig. 3B). To interrogate the potential regulatory role of PIPKIγ in tumor glucose metabolism, several experiments were performed to fully characterize metabolic alterations after PIPKIγ knockdown. Firstly, real-time qPCR analysis of glycolytic genes showed that SLC2A1, HK2, PFKL, PKM2, LDHA, and PDK1 were significantly downregulated by silencing of PIPKIγ (Fig. 4A and B); IHC analysis showed that PIPKIγ knockdown led to remarkable reduction in GLUT1, LDHA, and PDK1 protein expression in tumor tissues (Supplementary Fig. 2). Secondly, measurement of glucose and lactate in the cell culture medium demonstrated that PIPKIγ knockdown led to pronounced drop in glucose uptake and lactate production (Fig. 4C and D). Finally, the Seahorse XF96...

Fig. 4. Glycolytic changes induced by PIPKIγ. (A) Summary of the glycolytic transporters, enzymes, and metabolites. (B–E) The impact of PIPKIγ on the expression of glycolytic enzymes (B), glucose uptake (C), lactate production (D), and extracellular acidification ratio (ECAR) (E) of SW480 and LOVO cells. *P < .05 and **P < .01.
Fig. 5. PIPKIγ knockdown leads to inhibition of the PI3K/Akt/mTOR/c-Myc-HIF1α signaling pathway. (A) Western blot analysis for changes of PI3K/Akt/mTOR signaling pathway and c-Myc and HIF1α levels in sh-Ctrl, sh-PIPKIγ, and sh-PIPKIγ+mPIPKIγ_i2 cell lysates against indicated antibodies. (B) Cell lysates were harvested from ov-Ctrl and ov-PIPKIγ_i2 cells in the presence or absence of LY294002 or Rapamycin. Then proteins in PI3K/Akt/mTOR signaling pathway and c-Myc and HIF1α levels were analyzed by Western blotting. (C-F) The impact of PIPKIγ_i2 overexpression on the glucose uptake (C), lactate production (D), ECAR (E), and colony formation ability (F) of SW480 and LOVO cells in the presence of LY294002 or Rapamycin. *P < .05; **P < .01; ***P < .001.
Flux Analyser revealed that PIPKγ knockdown decreased extracellular acidification rate (ECAR) with minimal implications to oxygen consumption ratio (OCR), suggesting that PIPKγ mainly induces significant alterations to glycolysis but not TCA cycle (Fig. 4E and Supplementary Fig. 3). In line with the function of PIPKγ in the regulation of cell proliferation, the decreased glycolytic metabolism induced by silencing of PIPKγ can be completely restored by mIPPKγ_i2. Moreover, overexpression of PIPKγ significantly promoted the glycolytic activity of colorectal cancer cells as demonstrated by elevated glycolytic genes, increased glucose uptake and lactate production, and upregulated ECAR (Supplementary Fig. 4). Thus, these results strongly supported that PIPKγ is involved in the Warburg effect of colorectal cancer cells.

3.4. Loss of PIPKγ causes inactivation of the PI3K/Akt/mTOR/c-Myc-HIF1α signaling pathway

As described above, our results suggested that PI3K/AKT/mTOR signaling, hypoxia, and Myc targets are regulated by PIPKγ (Fig. 3A). HIF1α and c-Myc are two critical transcription factors implicated in the Warburg effect by regulating expression of glycolytic enzymes [23,25]. Interestingly, both of them can be regulated by the PI3K/Akt/mTOR pathway. Therefore, we hypothesized that PIPKγ may regulate glycolysis by activation of the PI3K/Akt/mTOR/c-Myc-HIF1α signaling pathway. To test this hypothesis, we firstly examined PI3K/Akt/mTOR activity upon PIPKγ knockdown. Western blotting analysis showed that silencing of PIPKγ reduced the phosphorylation levels of Akt, mTOR and its major target S6K, which can be rescued by mIPPKγ_i2 (Fig. 5A and Supplementary Fig. 5A). In PIPKγ_i2-overexpressing colorectal cancer cells, PI3K/Akt/mTOR activity was markedly increased compared to the control cells (Fig. 5B and Supplementary Fig. 5B). Of note, PIPKγ-induced elevation of c-Myc and HIF1α was largely compromised by addition of PI3K inhibitor (LY294002) or mTOR inhibitor (Rapamycin) (Fig. 5B and Supplementary Fig. 5B). Consistently, inhibition of PI3K/Akt/mTOR signaling also blocked the glucose utilization (Fig. 5C), lactate secretion (Fig. 5D), ECAR (Fig. 5E), and survival advantage (Fig. 5F) induced by PIPKγ. Collectively, these results suggested that PIPKγ may activate PI3K/Akt/mTOR signaling pathway, which further increases c-Myc and HIF1α level to promote aerobic glycolysis in colorectal cancer cells.

3.5. PIPKγ-mediated growth advantage is glycolysis-dependent

The Warburg effect is emerged as a key contributor to tumor initiation and progression, and blocking the Warburg effect greatly inhibited tumorigenesis. Therefore, we investigated whether the Warburg effect is an important mechanism contributing to PIPKγ-mediated oncogenic roles in colorectal cancer. To test this hypothesis, SW480 and LOVO cells were cultured in medium containing 5 mM 2-Deoxy-D-glucose (2-DG), which competitively inhibits the production of glucose-6-phosphate from glucose at the phosphoglucoisomerase level. In concordant with previous report, 2-DG clearly inhibited the anchorage-independent growth of colorectal cancer cells (Fig. 6A). Notably, 2-DG also abolished the suppressive effect of PIPKγ knockdown on anchorage-independent growth of SW480 and LOVO cells (Fig. 6A). To further confirm our observation, we replaced glucose in the culture medium with galactose, which has a much lower rate than glucose entry into glycolysis. As a result, growth disadvantage induced by PIPKγ knockdown was largely abolished by galactose (Fig. 6B). Taken together, these results strongly suggest that PIPKγ-mediated Warburg effect promotes colorectal cancer tumorigenesis.

3.6. Pharmacological inhibition of PIPKγ suppresses tumor growth

To test the therapeutic value of targeting PIPKγ in colorectal cancer, we treated colorectal cancer cells with a selective PIPKγ inhibitor, UNC3230. As a result, UNC3230 significantly inhibited the glycolytic...
phenotypes of SW480 and LOVO cells as revealed by reduced glucose uptake, lactate release, and ECAR (Fig. 7A and B). In xenograft tumors formed by SW480 cells, blocking PIPKIγ activity with UNC3230 significantly reduced tumor burden, glucose level, and lactate level in the subcutaneous model (Fig. 7C and D). In addition, immunohistochemical analysis of the xenograft tissues showed that c-Myc and HIF1α immunoreactivity were markedly downregulated by UNC3230 treatment. Similar observations were also found in the glucose transporter (GLUT1), LDHA, and PDK1 (Fig. 7E). Notably, UNC3230 can also efficiently inhibit PIP4K2C activity. We therefore performed a loss-of-function study of PIP4K2C in SW480 and LOVO cells. The results showed that knockdown of PIP4K2C showed no significant influence on CRC cell proliferation and glycolysis, indicating that the inhibitory role of UNC3230 was largely mediated by PIPKIγ (Supplementary Fig. 6). Collectively, these data above clearly showed that PIPKIγ activity is responsible for its oncogenic role in glycolysis and tumor growth.

4. Discussion

Enhanced Warburg effect is a distinctive hallmark of cancer cells and often correlates oncogenic phenotypes and poor prognosis in cancer patients [26,27]. This metabolic character provides sufficient cellular buildings and energetic needs for cancer cells to promote proliferation and avoid apoptosis [20]. Interestingly, many human malignancies including CRC exhibit an increased glycolytic activity. Thus, revealing the critical contributor in the Warburg effect is of paramount importance to identify new therapeutic targets for colorectal cancer. In the present study, we identified PIPKIγ as a key regulator of Warburg effect in colorectal cancer and uncovered its underlying molecular mechanism. Through in vitro and in vivo studies, PIPKIγ was demonstrated to be a promising molecular target for CRC treatment.

PIPKIγ is a major phosphoinositide-generating enzyme that controls polyphosphoinositide metabolism. Increased expression of PIPKIγ is
frequently noticed in human cancer cell lines and primary tumors. In pancreatic cancer, we previously showed that PIPKγ is upregulated in all cancer cell lines detected and pY639-PIP-Kγ exhibits remarkably strong staining in tumor tissues indicative of a pathogenic role for PIPKγ during malignant transformation [17]. Moreover, pY639-PIP-Kγ is also markedly elevated in invasive breast ductal carcinoma and correlates elevated histological grade, suggesting the important implications of PIPKγ in tumor progression [28]. Using a tissue microarray containing 438 breast carcinomas tissues, Sun et al. showed a significant inverse correlation between strong PIPKγ expression and overall patient survival [29]. Consistently, we found that PIPKγ is commonly overexpressed in human colorectal cancer cell lines and tumor tissues. Analysis of a CRC tissue microarray and TCGA cohorts with clinical follow-ups showed that elevated PIPKγ expression level positively correlated with reduced overall survival rate, indicating that PIPKγ might act as a new prognostic factor or biomarker for colorectal cancer.

Through generation of P(1,4,5)P2, PIPKγ is critically important in a variety of biological processes, such as focal adhesion assembly [6,30], ciliogenesis [31], centriole duplication [32], and leukocyte recruitment [33]. Notably, PIPKγ is also widely implicated in many oncogenic phenotypes, such as cell proliferation [13,34], migration [35], invasion [12,28], and the epithelial to mesenchymal transition process [14]. The dysregulated expression pattern of PIPKγ prompted us to investigate its neoplastic activities in colorectal cancer. By both gain- and loss-of-function studies, we confirmed the growth-promoting effect of PIPKγ in colorectal cancer cells. Through whole transcriptomic gene expression analysis, the altered glucose metabolism induced by PIPKγ was revealed. During tumor growth, hypoxia and metabolic stress will be occurred in most solid tumors. To survive under this harsh microenvironment, cancer cells exhibit a metabolic shift from oxidative phosphorylation to glycolysis, which supports growth advantage by providing anabolic precursors and minimizing the reactive oxygen species in the mitochondria [19,36]. Our functional study showed that PIPKγ can enhance the Warburg effect in colorectal cancer cells as demonstrated by glucose uptake, lactate production, extracellular acidification ratio, and expression of glycolytic enzymes. Importantly, blocking glycolysis by 2-DG or galactose largely compromised the growth-promoting effect of PIPKγ. However, PIPKγ had no significant impact on mitochondrial respiration as revealed by oxygen consumption ratio, suggesting its preferential roles in regulating aerobic glycolysis. In vitro, PIPKγ knockdown led to 40–50% reduction in tumor glycolysis, suggesting that other oncogenic inputs involved in colorectal cancer cell glycolysis. In vivo, PIPKγ knockdown resulted in approximately 50% reduction in tumor growth. Previously, we have demonstrated that PIPKγ can regulate PD-LI expression by activating NF-κB, suggesting that PIPKγ might exhibit a role in the immune microenvironment [9] and the anti-tumor activity for targeting PIPKγ might be enhanced in immune-competent models. Therefore, further works are warranted regarding the therapeutic value of targeting PIPKγ in colorectal cancer.

The PI3K/Akt signaling pathways are often activated in human cancers [37,38]. These pathways are initiated by the generation of PI(3,4,5)P3 by PI3K-mediated phosphorylation of PI(4,5)P2. Previous, Thapa et al. clearly demonstrated the mechanism by which PIPKγ couples with PI3K to activate PI3K/Akt signaling [34]. Of note, PI3K/Akt signaling and its downstream mTORC1 complex are central regulators of glycolysis [18]. Akt can enhance glucose transporter activity and promote glycolysis by activation of hexokinase and phosphofructokinase. Indeed, PIPKγ knockdown markedly inhibited the activation of PI3K/Akt/mTORC1 signaling. Inhibition of PI3K using LY294002 or mTORC1 by rapamycin blocked enhanced glycolysis and growth advantage induced by PIPKγ, suggesting that PI3K/Akt/mTORC1 signaling is responsible for PIPKγ-mediated functions. It is well known that the Warburg effect can be regulated by several transcriptional factors [39], especially HIF1α and c-Myc [40,41]. HIF1α can increase expression of glycolytic enzymes such as LDHA, as well as PKD limit entry of pyruvate in TCA cycle by inhibiting the activity of pyruvate dehydrogenase [25]. Increased c-Myc can activate numerous genes involved in glycolysis and lactate production [23]. PIPKγ knockdown suppressed, while over-expression increased HIF1α and c-Myc levels. Inhibition of PI3K/Akt/mTORC1 signaling downregulated PIPKγ-induced HIF1α and c-Myc levels indicative of the role of PI3K/Akt/mTORC1/HIF1α-c-Myc axis in the PIPKγ-mediated glycolysis. Although the HIF1α and c-Myc levels are regulated by PI3K/Akt/mTORC1 signaling in colorectal cancer cells, we cannot fully exclude other inputs influenced by PIPKγ in the contribution of increased HIF1α and c-Myc.

In conclusion, for the first time, we identified PIPKγ as a novel regulator for aerobic glycolysis in human colorectal cancer cells. Our current results not only provide insight into the oncogenic roles of PIPKγ in colorectal cancer, but also the molecular mechanisms by which PIPKγ regulates aerobic glycolysis. However, further investigations are warranted concerning the roles of PIPKγ in reprogrammed metabolism, including glutamine metabolism and fatty acid metabolism. Given pharmacological inhibition of PIPKγ activity significantly suppressed tumor growth, our findings may provide alternative strategies for the treatment of colorectal cancer.

Funding sources

This work was supported by the National Key Research and Development Program of China (No. 2017YFC1308900); The Outstanding Clinical Discipline Project of Shanghai Pu dong (PWYgy2018-02); the grant from the Natural Science Foundation of China (81502510); and the grant from Science and Technology Commission of Shanghai Municipal (17411968800).

Conflicts of interest statement

The authors declare no conflicts of interest.

Authors’ contributions

Junli Xue and Wei Peng conceived the study plan. Wei Peng, Wei Huang, and Xiaohao Ge performed the experiments, analyzed the data and finished the manuscript writing. Liqiong Xue and Wei Zhao contributed to the in vivo experiments. Junli Xue supervised this study and edited the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ebiom.2019.05.015.

References

[1] Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68(6):394–424.
[2] Atkin W, Wooldrage K, Brenner A, Martin J, Shah U, Perera S, et al. Adenoma surveillance and colorectal cancer incidence: a retrospective, multicentre, cohort study. Lancet Oncol 2017;18(6):823–34.
[3] Angenete E. The importance of surgery in colorectal cancer treatment. Lancet Oncol 2019;20(1):6–7.
[4] Schill NJ, Anderson RA. Two novel phosphatidylinositol-4-phosphate 5-kinase type I gamma splice variants expressed in human cells display distinct cellular targeting. Biochem J 2009;422(3):473–82.
[5] Ling K, Doughman RL, Iyer VV, Firestone AJ, Baiistro SE, Mosher DF, et al. Tyrosine phosphorylation of type I gamma phosphatidylinositol phosphate kinase by Src regulates an integrin-Talin switch. J Cell Biol 2003;163(6):1339–49.
[6] Ling K, Doughman RL, Firestone AJ, Bunce MW, Anderson RA. Type I gamma phosphatidylinositol phosphate kinase targets and regulates focal adhesions. Nature 2002;420(6911):89–93.
[7] Heck JN, Mellman DL, Ling K, Sun Y, Waggoner MP, Schill NJ, et al. A conspicuous connection: structure defines function for the phosphatidylinositol-phosphate kinase family. Curr Rev Biochem Mol Biol 2007;42(1):15–39.
[8] Barlow CA, Laishram RS, Anderson RA. Nuclear phosphoinositidases: a signaling enigma wrapped in a compartmental conundrum. Trends Cell Biol 2010;20(1):25–35.
[9] Xue J, Chen C, Qi M, Huang Y, Wang L, Gao Y, et al. Type I gamma phosphatidylinositol phosphate kinase regulates PD-L1 expression by activating NF-kappaB. Oncotarget 2017;8(26):42414–27.

[10] Lee SY, Voronov S, Letinic K, Nainz AC, Di Paolo G, De Camilli P. Regulation of the interaction between PIPKI gamma and Talin by proline-directed protein kinases. J Cell Biol 2005;168(5):789–99.

[11] Legate KR, Takahashi S, Bonakdar N, Fabry B, Boettiger D, Zent R, et al. Integrin adhesion and force coupling are independently regulated by localized PtdIns(4,5)2 synthesis. EMBO J 2011;30(22):4539–53.

[12] Li L, Kolodziej T, Jafari N, Chen J, Zhu H, Rajfur Z, et al. Cdk5-mediated phosphorylation regulates phosphatidylinositol 4-gosphate 5-kinase type I gamma 90 activity and cell invasion. FASEB J 2019;33(1):631–42.

[13] Li H, Xiao N, Wang Y, Wang R, Chen Y, Pan W, et al. Smurf1 regulates lung cancer cell growth and migration through interaction with and ubiquitination of PIPKI gamma. Oncogene 2017;36(41):5698–80.

[14] Thapa N, Tan X, Choi S, Wise T, Anderson RA. PIPK/PIPKigamma and Talin couple phosphoinositide and adhesion signaling to control the epithelial to mesenchymal transition. Oncogene 2017;36(7):899–911.

[15] Schramp M, Thapa N, Heck J, Anderson R. PIPKIgamma regulates beta-catenin transcriptional activity downstream of growth factor receptor signaling. Cancer Res 2011;71(4):1282–91.

[16] Wu Z, Li X, Sunkara M, Spearman H, Morris AJ, Huang C. PIPKIgamma regulates focal adhesion dynamics and colon cancer cell invasion. PLoS One 2011;6(9):e24775.

[17] Chen C, Wang X, Fang J, Xue J, Xiong X, Huang Y, et al. EGF-induced phosphorylation of type I gamma phosphatidylinositol phosphate kinase promotes pancreatic cancer progression. Oncotarget 2017;8(26):42621–37.

[18] Cairns RA, Harris IS, Mak TW. Regulation of cancer cell metabolism. Nat Rev Cancer 2011;11(3):85–95.

[19] Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science 2009;324(5930):1029–33.

[20] Lunt SY, Vander Heiden MG. Aerobic glycolysis: meeting the metabolic requirements of cell proliferation. Annu Rev Dev Biol 2011;27:441–64.

[21] Shukla SK, Purohit V, Mehla K, Gunda V, Chaika NV, Vernucci E, et al. MUC1 and HIF-1 oncoprotein cooperatively regulate PIPKIgamma expression and activity in cancer. J Biol Chem 2015;290(30):18843–50.

[22] Liu P, Cheng H, Roberts TM, Zhao JJ. Targeting the phosphoinositide 3-kinase pathway to control pancreatic cancer development. Oncogene 2017;36(41):5668–79.

[23] Dang CV, Le A, Gao P. MYC-induced cancer cell energy metabolism and therapeutic opportunities. Clin Cancer Res Off J Am Assoc Cancer Res 2009;15(21):6479–83.

[24] Jiang SH, Li J, Dong FY, Yang JY, Liu DJ, Yang XM, et al. Increased serotinin signaling contributes to the Warburg effect in pancreatic tumor cells under metabolic stress and promotes growth of pancreatic tumors in mice. Gastroenterology 2017;153(1):277-91 e19.

[25] Iansante V, Choy PM, Fang SW, Liu Y, Chai JG, Dyson J, et al. PARP14 promotes the Warburg effect in hepatocellular carcinoma by inhibiting JNK1-dependent PKM2 phosphorylation and activation. Nat Commun 2015;6:7882.

[26] Sun Y, Turbin DA, Ling K, Thapa N, Leung S, Huntsman DG, et al. Type I gamma phosphatidylinositol phosphate kinase modulates invasion and proliferation and its expression correlates with poor prognosis in breast cancer. Breast Cancer Res BCR 2010;12(1):R6.

[27] Nader GP, Erazzity EJ, Gundersen G, FAK, Talin and PIPKIgamma regulate endocytosed integrin activation to polarize focal adhesion assembly. Nat Cell Biol 2016;18(5):491–503.

[28] Xu Q, Zhang Y, Wei Q, Huang Y, Hu J, King K. Phosphatidylinositol phosphate kinase PIPKI gamma and phosphatase INPP5E coordinate initiation of ciliogenesis. Nat Commun 2016;7:10777.

[29] Xu Q, Zhang Y, Xiong X, Huang Y, Salisbury JL, Hu J, et al. PIPKIgamma targets to the centrosome and restrains centriole duplication. J Cell Sci 2014;127:1293–305 Pt 6.

[30] Nader GP, Ezratty EJ, Gundersen G, FAK, Talin and PIPKIgamma regulate endocytosed integrin activation to polarize focal adhesion assembly. Nat Cell Biol 2016;18(5):491–503.

[31] Chen C, Wang X, Fang J, Xue J, Xiong X, Huang Y, et al. EGF-stimulated phosphorylation of type I gamma phosphatidylinositol phosphate kinase promotes pancreatic cancer progression. Oncotarget 2017;8(26):42621–37.

[32] Zhang C, Li J, Jiang Y, Wu R, Zhao Y, Hong X, et al. Tumour-associated mutant p53 drives the Warburg effect. Nat Commun 2013;4:2935.

[33] Semenza GL. HIF-1 mediates metabolic responses to intratumoral hypoxia and oncogenic mutations. J Clin Invest 2013;123(9):3664–71.