6-phosphofructo-2-kinase as therapeutic targets in cancer based on an integrated pan-cancer study

Haimei Qin
The affiliated hospital of Youjiang medical university for nationalities

Junli Wang
The affiliated hospital of Youjiang medical for nationalities

Biyun Liao
the affiliated hospital of Youjiang medical for nationalities

Zhonglin Liu
Southern Medical University Nanfang Hospital

Feng Shi
the affiliated hospital of Youjiang medical for nationalities

Rong Wang (rongwang50@163.com)
the affiliated hospital of Youjiang medical university for nationalities https://orcid.org/0000-0002-0225-1191

Primary research

Keywords: 6-phosphofructo-2-kinase, Immune subtype, Tumor microenvironment, Drug sensitivity, Pan-cancer

DOI: https://doi.org/10.21203/rs.3.rs-47885/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License
Abstract

**Background:** Warburg effect confirm that tumor cells prefer to use glycolysis to perform metabolism glucose metabolism. PFKFB enzymes involve the activity of 6-phosphofructo-1-kinase in the metabolism of glycolysis. But their roles in immunity and the regulation of tumor microenvironment has not been fully understood.

**Methods:** We performed a comprehensive analysis of PFKFB in with immune subtypes, tumour microenvironment, and drug sensitivity from data of pan-cancer. Data of 33 cancer subtypes including RNA-Seq, clinical phenotype, stemness scores of mRNA (RNAss) and DNA-methylation (DNAss), and immune subtypes was downloaded from TCGA. NCI-60 data and RNA-seq data were acquired from the CellMiner. Perl language and R language with packages were used to deal with data.

**Results:** Expression of PFKFB family emerges different heterogeneity in different cancer. PFKFB1, PFKFB3 and PFKFB4 predicted poor prognosis of patients with multiple cancers. All members are associated with immune response. PFKFB3 and PFKFB4 involved tumor microenvironment of pan-cancer. Importantly, our study found that PFKFB genes, especially PFKFB2 and PFKFB4 may contribute to drug resistance in cancer cells.

**Conclusions:** PFKFB family member possess special characters in each cancer based on gene expression and their correlation with immune infiltrates, tumor microenvironment. PFKFB2 and PFKFB4 may act as therapeutic targets in cancer.

Background

Hypoxia has been acknowledged as an important hallmarks of tumors which act as a significant role in growth, invasion and metastasis of tumor cells [1, 2]. Changes in glycolysis rate are applied to adapt hypoxia[3]. The glycolysis capacity of tumor cells is 20–30 times than that of normal cells, which provides a lot of energy and intermediates for tumor metabolism. Activation from glycolysis genes ameliorating or compensating the deficit of oxygen is critical in tolerance of hypoxia [4]. Hypoxia-inducible factor 1 (HIF-1), a key molecular, can induces multiple gene transcriptions that make tumor cells tolerant to hypoxia. Evidences have found that over-expression of HIF-1α or HIF-2α stimulate glycolysis through activating part or all members of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatases (PFKFB) [5].

In the consumption of glycolysis, tumor cells like glycolysis to produce lactic acid more than mitochondrial oxidative phosphorylation even in environment of hyperoxia and we called this action well-known Warburg effect [6]. Fructose-2,6-bisphosphate (F-2,6-BP) is the allosteric activator of 6-phosphofructo-1-kinase. Enzyme PFKFB can maintain the levels of fructose-2,6-bisphosphate to control the activity of 6-phosphofructo-1-kinase in the metabolism of glycolysis. In this enzyme, variable isoform (PFKFB1-PFKFB4) which express many cells and tissues was encoded by four genes. PFKFB enzyme importantly involve the progress of the Warburg effect [7]. Elevated expression of PFKFB influences the
proliferation of tumor cells [8]. Low mRNA Expression of PFKFB4 and higher PFKFB3 mRNA was detected in MKN45 and NUGC3 gastric cancer cell lines. In gastric cancer cell lines and pancreatic cancer cell lines, hypoxia increased the expression level of PFKFB3, PFKFB4, VEGF and Glut1 and are associated with enhanced expression level of HIF-1α protein. These results indicate that PFKFB4 and PFKFB3 genes respond to hypoxia through the approach of HIF-dependent mechanism [9]. In breast cancer cells, phosphorylation of PFKFB3 on Ser461 is followed through function of mRNA transcription on the PFKFB3 promoter which caused by the ERK /RSK pathway [10]. Currently, there is so little research using cell lines about the PFKFB genes in cancer. Additionally, no study was performed to explore this gene family for understanding their roles in different cancers.

In our study, we download the data of pan-cancer form The Cancer Genome Atlas Program (TCGA). Next, we analyzed their expression profile and the association of expression, overall survival of cancers, tumor microenvironment and drug sensitivity. activity. We look forward to provide novel knowledge in therapeutic targets of cancers using PFKFB genes from the aspect of tumor microenvironment, tumor immunology and drug sensitivity.

**Methods**

**Obtaining of TCGA pan-cancer data**

UCSC Xena (https://xena.ucsc.edu/) is an open website which can get data from The Cancer Genome Atlas (TCGA) [11]. Therefore, we used the UCSC Xena to obtain data of 33 cancer subtypes (Table 1) including RNA-Seq, clinical phenotype, stemness scores of mRNA (RNAss) and DNA-methylation (DNAss), and immune subtypes. We removed the cancer that number of normal tissue samples less than five. Hence, gene expression of tumors and adjacent normal in the rest cancer types were analyzed. Patients with survival information were employed in the investigation of PFKFB gene family expression and overall survival in cancers.
Table 1  
Pan-cancer from The Cancer Genome Atlas (TCGA)

| Abbreviation | Full name                                           | Abbreviation | Full name                                           |
|--------------|-----------------------------------------------------|--------------|-----------------------------------------------------|
| ACC          | Adrenocortical carcinoma                            | LUSC         | Lung squamous cell carcinoma                        |
| BLCA         | Bladder Urothelial Carcinoma                        | MESO         | Mesothelioma                                        |
| BRCA         | Breast invasive carcinoma                           | OV           | Ovarian serous cystadenocarcinoma                   |
| CESC         | Cervical squamous cell carcinoma and endocervical adenocarcinoma | PAAD         | Pancreatic adenocarcinoma                           |
| CHOL         | Cholangiocarcinoma                                  | PCPG         | Pheochromocytoma and Paraganglioma                  |
| COAD         | Colon adenocarcinoma                                | PRAD         | Prostate adenocarcinoma                             |
| DLBC         | Lymphoid Neoplasm Diffuse Large B-cell Lymphoma     | READ         | Rectum adenocarcinoma                               |
| ESCA         | Esophageal carcinoma                                | SARC         | Sarcoma                                             |
| GBM          | Glioblastoma multiforme                             | SKCM         | Skin Cutaneous Melanoma                             |
| HNSC         | Head and Neck squamous cell carcinoma               | STAD         | Stomach adenocarcinoma                              |
| KICH         | Kidney Chromophobe                                  | TGCT         | Testicular Germ Cell Tumors                         |
| KIRC         | Kidney renal clear cell carcinoma                   | THCA         | Thyroid carcinoma                                   |
| KIRP         | Kidney renal papillary cell carcinoma               | THYM         | Thymoma                                             |
| LAML         | Acute Myeloid Leukemia                              | UCEC         | Uterine Corpus Endometrial Carcinoma                |
| LGG          | Brain Lower Grade Glioma                            | UCS          | Uterine Carinosarcoma                               |
| LIHC         | Liver hepatocellular carcinoma                      | UVM          | Uveal Melanoma                                      |
| LUAD         | Lung adenocarcinoma                                 |              |                                                     |

**Analysis of tumor microenvironment**

To survey the impact of gene expression in tumor microenvironment, immune score and stromal score were applied to assess the infiltration degree immune cells and stromal cells in pan-cancer. The establishment of evaluation system was based on the TCGA expression data. Estimate score also can reflect the purity of tumor cells. Immune subtypes including C1 (wound healing), C2 (INF-\(\gamma\) dominant), C3 (inflammatory), C4 (lymphocyte depleted), C5 (immunologically quiet), and C6 (TGF\(\beta\) dominant) were
used to observe immune infiltrates. Stemness score (DNA methylation based) and stemness score (RNA based) measured the stemness characteristics of tumor cells.

### Analysis of drug sensitivity

CellMiner (https://discover.nci.nih.gov/cellminer/), which is used to detect and identify potential anticancer drugs, can provide a quick entry for calculating the correlation between genes and drug activity[12]. NCI-60 data and RNA-seq data of same samples was acquired from the “download data sets” in CellMiner. The correlation of gene expression and drug sensitivity was evaluated using Pearson correlation.

### Statistics processing

The Perl Programming Language and R software with packages was used to integrate data. Wilcox test were used to analyzed the differences between tumor samples and corresponding normal samples in pan-cancer. The results of comparison were displayed by boxplots in various cancers. The association of gene expression and overall survival in patients of pan-cancer were estimated by cox proportional hazard regression models.

The association between gene expression and stemness scores, stromal score, immune score, estimate score, and drug sensitivity was detected using Spearman correlation. Kruskal-Wallis was used to analyze the relation of gene expression and clinical outcomes, immune components. P < 0.05 was consider as statistic differences. All graphs were drawn using R software with packages.

### Results

#### Expression of PFKFB gene family in pan-cancer

We examined the expression of four members of the PFKFB family in all 33 kinds of cancers in pan-cancer data. As shown in (Fig. 1) and (Fig. 2), the phenomenon of tumor heterogeneity in expression of PFKFB members was revealed. Some members including PFKFB2, PFKFB3, PFKFB4 host higher expression and the relatively lower PFKFB1 level in the pan-cancer. PFKFB1, PFKFB2, PFKFB3 present higher expression or low expression in tumor tissues. No significant differences also been found in several cancers. But PFKFB4 expression is different in tumor samples comparing normal samples. In terms of the internal expression relationship of PFKFB family member, we found the negatively maximal correlation in PFKFB3 and PFKFB4 (r = 0.37, P < 0.001); positively maximal correlation in PFKFB3 and PFKFB1 (r = -0.22, P < 0.001).

**Figure 1** Expression of PFKFB member in rumor tissues and normal tissues. **A.** Boxplot of PFKFB members in pan-cancer. **B.** Heatmap of PFKFB members in tumor tissues and normal tissues that the number of normal samples more than 5. **C.** Correlation of expression levels among the members in PFKFB gene family.
**Association between PFKFB expression and overall survival in pan-cancer**

In order to recognize the predictive ability of PFKFB member in tumourigenesis of pan-cancer, we used cox proportional hazard regression models to identify their roles in overall survival of pan-cancer. Forest plots in (Fig. 3) shown the significant relationship with the P value less than 0.05. Finally, we found that expression of PFKFB member was associated with overall survival in patients with cancers. Different expression manifested different direction of association. PFKFB1 predicted poor prognosis of patients with COAD, KIRC and THCA. PFKFB3 predicted poor prognosis of patients with ACC, CESC and LIHC. PFKFB4 predicted poor prognosis of patients with CESC, GBM, KICH, LIHC, LUSC, PCPG, SARC and THCA.

**PFKFB genes involved immune response and tumor microenvironment of cancer**

In the study about the function of PFKFB in T cell activation, the authors reported that inhibition of the PFKFB3 kinase activity can decrease the activation in T cells and suppress T cell dependent immunity. The results indicate that small molecule antagonists of PFKFB3 may be the effective in inhibiting T cell immunity. However, it is unknown that whether PFKFB genes associated with immune response in tumor microenvironment. Therefore, we investigated the correlation between PFKFB genes and immune infiltration in cancers. Immune infiltration correlated with the expression level PFKFB family members in pan-cancer (Fig. 4). PFKFB2 expression is higher in the C1 and C5. PFKFB3 expression is higher in C5 and C6 and PFKFB4 expression is higher in C1, C2 and C5. We also analyzed the association of expression levels of PFKFB genes and the infiltration stromal cells in tumors indicated using stromal scores. Different degree from association of PFKFB genes and stromal score in cancers was found, (Fig. 5). The deep correlation between PFKFB genes and stromal score mainly occurred in ACC, COAD, DCBC, KICH, LAML, LGG, LIHC, MESO, PCPG, PRAD, TGCT, THCA and UCS. PFKFB3 showed significantly positive correlation with stromal score in KICH, PCPG and PRAD. PFKFB4 showed significantly positive correlation with stromal score in LAML and significantly negative correlation with stromal score in UCS.

**Figure 4** Association of PFKFB gene family expression with immune infiltration in pan-cancer. ***, P < 0.001**

**Figure 5** Correlation matrix plots between PFKFB gene family expression and stromal scores in cancers. The size of the dots represents the size of the correlation value.

**Association of PFKFB genes with tumor stemness and chemotherapy sensitivity**
Acquisition of stem cell-like characteristics maintain the growth and proliferation of cancer cells. Two methods including RNA stemness score from mRNA expression (RNAss) and DNA stemness from DNA methylation pattern (DNAss) can be used to assess tumor stemness. Members of PFKFB gene family have different levels of association with RNAss and DNAss (Fig. 6A, 6B). PFKFB3 was negatively associated with RNAss and DNAss (P < 0.001). PFKFB4 was positively correlated with RNAss and DNAss (P < 0.001). Results suggest that PFKFB3 and PFKFB4 carrying RNAss and DNAss may identify stemness in cancers. Next, we investigated the relationship of PFKFB gene expression and NCI-60 cell with sensitivity of chemotherapy drugs. We observed that levels of PFKFB2 expression showed great heterogeneity in different drugs. Except Afatinib and Kahalide f, increased expression of PFKFB2 was related to increased drug resistance of many chemotherapy drugs (r > 0.4 and P < 0.001). PFKFB4 was associated with increased drug resistance of Vemurafenib (r > 0.4 and P < 0.001) (Fig. 7). Expression PFKFB2 have opposite associations with in different drugs.

**Figure 6** Association of PFKFB gene expression and tumor stemness. **A.** Correlation matrix based on RNAss **B.** Correlation matrix based on DNAss

**Figure 7** The association between PFKFB expression and drug sensitivity

**Analysis of PFKFB gene family in breast cancer**

The study of PFKFB gene family in cancer is still restricted. In breast cancer cells, it has been reported that PFKFB3 is involved the proliferation, migration, invasion and angiogenesis in breast cancer and glucose metabolism in HER2 + cells [13, 14]. We explored the PFKFB gene family in the breast cancer according to TCGA data. All members were significantly differential expression in breast cancer tissues and adjacent normal tissues (P < 0.001). The association between PFKFB gene expression and immune subtypes in breast cancer was analyzed using the same method in pan-cancer. Expression of all members were associated with immune infiltrate types (P < 0.001), (Fig. 8). We further investigated the correlation of PFKFB gene expression and stromal score. As shown in (Fig. 9), these members were correlated with stromal scores of breast cancer (P < 0.001). PFKFB genes were related to immune score (P < 0.001) and tumor purity (Estimate score), (P < 0.001).

**Figure 8** Association between PFKFB gene expression and immune infiltration in breast cancer

**Figure 9** Correlation between PFKFB gene expression and RNAss, DNAss, stromal score, immune score, and Estimate Score.

**Discussion**

In the development of cancer, the metabolism ability of cancer cells is abnormal. The exploitation of modulating metabolism of cancer cells may be a potentially therapeutic approach[15–18]. Development of different therapeutic approaches need to selectively base on glucose consumption. Importantly, high glycolytic activity is regarded as a metabolic hallmark of cancer. So, inhibition of glycolysis promotes death of cell and has gradually been a novel strategy[19–21]. At present, most studies focus on an
inhibitor named 2-DG which can be phosphorylated by hexokinase. The use of 2-DG can help adriamycin and paclitaxel to elevate reacting in human osteosarcoma-bearing mice and of small cell lung cancer[22]. In fact, complete break of glycolysis will lead to severe adverse effects. Therefore, screening the targets from the glycolysis mainly rely on the genes encoding enzymes which control the rate of metabolism. In the survival micro-environments of tumor cells under hypoxia, PFKFB genes are activated by hypoxia and the important nodes in signal for the change of glycolytic phenotype. PFKFB isoenzymes possess the feature of controllability and targeting point. Inhibitors of PFKFBs could be employed to cancer treatment. Aspirin can improve the sorafenib resistance in HCC by the PFKFB3 inhibition[23, 24].

To our best knowledge, we first performed an analysis of PFKFB gene family based on systemic pan-cancer. The expression levels of PFKFB gene emerged heterogeneity in different tumors. PFKFB2, PFKFB3, PFKFB4 expression is higher and the relatively lower PFKFB1 level in the pan-caner. The negatively maximal correlation in PFKFB3 and PFKFB4 was found. Expression of PFKFB3 was associated with the poor prognosis of patients with ACC, CESC and LIHC. Additionally, PFKFB4 predicted poor prognosis of patients with CESC, GBM, KICH, LIHC, LUSC, PCPG, SARC and THCA. The Warburg effect is also associated with the activities of many immune cells. We found that PFKFB2 expression is higher in the wound healing and immunologically quiet. PFKFB3 expression was associated with immunologically quiet and TGFβ dominant and PFKFB4 expression is higher in wound healing, INF-γ dominant and immunologically quiet. TGF-β1 upregulates mRNA and protein expression of PFKFB3 resulting in increased concentration of fructose 2,6-bisphosphate. These members impact different immune types. PFKFB3 and PFKFB4 was associated with RNAss and DNAss. PFKFB3 and PFK1 expression can distinguish induced pluripotent stem cells and cancer stem cells. PFKFB2 was related to sensibility of many chemotherapy drugs. Expression of all members were associated with immune infiltrate types. These members were correlated with stromal scores, immune score and tumor purity. Currently, no study was reported in the genes family from many aspects. PFKFB2 expression is necessary not only for steady-state F2,6BP levels, but also glycolytic activity and proliferation of pancreatic adenocarcinoma cells[25]. PFKFB2 also is the target of some miRNAs which participates the development of cancers. PFKFB2 act as the target of miR-182 to modulate GB-associated stromal cells-induced glycolysis in glioma cells[26]. The expression of PFKFB2 was inhibited by a mechanism that miR-613 could directly bind to the 3'UTR of PFKFB2. This way regulated glycolysis metabolism and cell growth[27]. As an important regulator of glycolysis, studies have suggested that PFKFB3 is associated with many characters of cancer, including carcinogenesis, cancer cell proliferation, vessel aggressiveness, drug resistance and tumor microenvironment[28]. HIF-1α can activated HRE-D in the promoter region of PFKFB4 bladder cancer cells[29]. PFKFB4 expression facilitated the glycolysis pathway and promotes stemness of breast cancer[30]. In our study, we also found that PFKFB4 expression was associated with stemness of breast cancer. The role of each member as tumor suppressor or promoter in specific cancer types needs to be confirmed by experiments.

**Conclusions**
Importantly, PFKFB2 and PFKFB4 were also associated with increased drug sensitivity of a few drugs. Increased expression of PFKFB2 was related to increased drug resistance of many chemotherapy drugs. PFKFB4 was associated with increased drug resistance of Vemurafenib. These data suggest that PFKFB may involve in sensitivity or resistance of drug treatment and can be act as therapeutic targets to reply drug induced resistance.

Abbreviations
TCGA: The Cancer Genome Atlas; TME: Tumour microenvironment.

Declarations

Authors’ contributions
Conceptualization, RW; Methodology, JL W; Writing-original draft, HM Q; writing-review & editing, BY L, ZL L, F S; Supervision, R W. All authors read and approved the final manuscript.

Funding
This study was supported by National Natural Science Foundation of China (No. 81560461), 139 medical high-level talent training plan and thousands of young and middle-aged backbone teacher cultivation plan of Guangxi, high-level talent training plan of Youjiang medical university for nationalities and the affiliated hospital of Youjiang medical university for nationalities.

Availability of data and materials
The data that support the findings of this study are available in UCSC Xena (https://xena.ucsc.edu/) and CellMiner (https://discover.nci.nih.gov/cellminer/).

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

References
1. Bhandari V, Hoey C, Liu LY, Lalonde E, Ray J, Livingstone J, Lesurf R, Shiah YJ, Vujcic T, Huang X et al: Molecular landmarks of tumor hypoxia across cancer types. Nature genetics 2019, 51(2):308-318.
2. Wigerup C, Påhlman S, Bexell D: Therapeutic targeting of hypoxia and hypoxia-inducible factors in cancer. *Pharmacology & therapeutics* 2016, 164:152-169.

3. Lee P, Chandel NS, Simon MC: Cellular adaptation to hypoxia through hypoxia inducible factors and beyond. *Nature reviews Molecular cell biology* 2020, 21(5):268-283.

4. He Y, Kim H, Ryu T, Kang Y, Kim JA, Kim BH, Lee JH, Kang K, Lu Q, Kim K: δ-Catenin overexpression promotes angiogenic potential of CWR22Rv-1 prostate cancer cells via HIF-1α and VEGF. *FEBS letters* 2013, 587(2):193-199.

5. Wolf A, Agnihotri S, Micallef J, Mukherjee J, Sabha N, Cairns R, Hawkins C, Guha A: Hexokinase 2 is a key mediator of aerobic glycolysis and promotes tumor growth in human glioblastoma multiforme. *The Journal of experimental medicine* 2011, 208(2):313-326.

6. Bartrons R, Caro J: Hypoxia, glucose metabolism and the Warburg's effect. *Journal of bioenergetics and biomembranes* 2007, 39(3):223-229.

7. Yi M, Ban Y, Tan Y, Xiong W, Li G, Xiang B: 6-Phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 and 4: A pair of valves for fine-tuning of glucose metabolism in human cancer. *Molecular metabolism* 2019, 20:1-13.

8. Minchenko OH, Tsuchihara K, Minchenko DO, Bikfalvi A, Esumi H: Mechanisms of regulation of PFKFB expression in pancreatic and gastric cancer cells. *World journal of gastroenterology* 2014, 20(38):13705-13717.

9. Bobarykina AY, Minchenko DO, Opentanova IL, Moenner M, Caro J, Esumi H, Minchenko OH: Hypoxic regulation of PFKFB-3 and PFKFB-4 gene expression in gastric and pancreatic cancer cell lines and expression of PFKFB genes in gastric cancers. *Acta biochimica Polonica* 2006, 53(4):789-799.

10. Mondal S, Roy D, Sarkar Bhattacharya S, Jin L, Jung D, Zhang S, Kalogera E, Staub J, Wang Y, Xuyang W et al: Therapeutic targeting of PFKFB3 with a novel glycolytic inhibitor PFK158 promotes lipophagy and chemosensitivity in gynecologic cancers. *International journal of cancer* 2019, 144(1):178-189.

11. Goldman MJ, Craft B, Hastie M, Repečka K, McDade F, Kamath A, Banerjee A, Luo Y, Rogers D, Brooks AN et al: Visualizing and interpreting cancer genomics data via the Xena platform. *Nature biotechnology* 2020, 38(6):675-678.

12. Reinhold WC, Sunshine M, Liu H, Varma S, Kohn KW, Morris J, Doroshow J, Pommier Y: CellMiner: a web-based suite of genomic and pharmacologic tools to explore transcript and drug patterns in the NCI-60 cell line set. *Cancer research* 2012, 72(14):3499-3511.

13. Peng F, Li Q, Sun JY, Luo Y, Chen M, Bao Y: PFKFB3 is involved in breast cancer proliferation, migration, invasion and angiogenesis. *International journal of oncology* 2018, 52(3):945-954.

14. O'Neal J, Clem A, Reynolds L, Dougherty S, Imbert-Fernandez Y, Telang S, Chesney J, Clem BF: Inhibition of 6-phosphofructo-2-kinase (PFKFB3) suppresses glucose metabolism and the growth of HER2+ breast cancer. *Breast cancer research and treatment* 2016, 160(1):29-40.

15. Li X, Wenes M, Romero P, Huang SC, Fendt SM, Ho PC: Navigating metabolic pathways to enhance antitumour immunity and immunotherapy. *Nature reviews Clinical oncology* 2019, 16(7):425-441.
16. Cirotti C, Contadini C, Barilà D: SRC Kinase in Glioblastoma News from an Old Acquaintance. *Cancers* 2020, 12(6).
17. Wang YP, Lei QY: Perspectives of Reprogramming Breast Cancer Metabolism. *Advances in experimental medicine and biology* 2017, 1026:217-232.
18. De Matteis S, Ragusa A, Marisi G, De Domenico S, Casadei Gardini A, Bonafè M, Giudetti AM: Aberrant Metabolism in Hepatocellular Carcinoma Provides Diagnostic and Therapeutic Opportunities. *Oxidative medicine and cellular longevity* 2018, 2018:7512159.
19. Sheng H, Tang W: Glycolysis Inhibitors for Anticancer Therapy: A Review of Recent Patents. *Recent patents on anti-cancer drug discovery* 2016, 11(3):297-308.
20. Xintaropoulou C, Ward C, Wise A, Queckborner S, Turnbull A, Michie CO, Williams ARW, Rye T, Gourley C, Langdon SP: Expression of glycolytic enzymes in ovarian cancers and evaluation of the glycolytic pathway as a strategy for ovarian cancer treatment. *BMC cancer* 2018, 18(1):636.
21. Shi Y, Liu S, Ahmad S, Gao Q: Targeting Key Transporters in Tumor Glycolysis as a Novel Anticancer Strategy. *Current topics in medicinal chemistry* 2018, 18(6):454-466.
22. Pattni BS, Jhaveri A, Dutta I, Baleja JD, Degterev A, Torchilin V: Targeting energy metabolism of cancer cells: Combined administration of NCL-240 and 2-DG. *International journal of pharmaceutics* 2017, 532(1):149-156.
23. Li S, Dai W, Mo W, Li J, Feng J, Wu L, Liu T, Yu Q, Xu S, Wang W et al.: By inhibiting PFKFB3, aspirin overcomes sorafenib resistance in hepatocellular carcinoma. *International journal of cancer* 2017, 141(12):2571-2584.
24. Long Q, Zou X, Song Y, Duan Z, Liu L: PFKFB3/HIF-1α feedback loop modulates sorafenib resistance in hepatocellular carcinoma cells. *Biochemical and biophysical research communications* 2019, 513(3):642-650.
25. Ozcan SC, Sarioglu A, Altunok TH, Akkoc A, Guzel S, Guler S, Imbert-Fernandez Y, Muchut RJ, Iglesias AA, Gurpinar Y et al.: PFKFB2 regulates glycolysis and proliferation in pancreatic cancer cells. *Molecular and cellular biochemistry* 2020, 470(1-2):115-129.
26. He Z, You C, Zhao D: Long non-coding RNA UCA1/miR-182/PFKFB2 axis modulates glioblastoma-associated stromal cells-mediated glycolysis and invasion of glioma cells. *Biochemical and biophysical research communications* 2018, 500(3):569-576.
27. Liu H, Chen K, Wang L, Zeng X, Huang Z, Li M, Dong P, Chen X: miR-613 inhibits Warburg effect in gastric cancer by targeting PFKFB2. *Biochemical and biophysical research communications* 2019, 515(1):37-43.
28. Shi L, Pan H, Liu Z, Xie J, Han W: Roles of PFKFB3 in cancer. *Signal transduction and targeted therapy* 2017, 2:17044.
29. Zhang H, Lu C, Fang M, Yan W, Chen M, Ji Y, He S, Liu T, Chen T, Xiao J: HIF-1α activates hypoxia-induced PFKFB4 expression in human bladder cancer cells. *Biochemical and biophysical research communications* 2016, 476(3):146-152.
30. Gao R, Li D, Xun J, Zhou W, Li J, Wang J, Liu C, Li X, Shen W, Qiao H et al: CD44ICD promotes breast cancer stemness via PFKFB4-mediated glucose metabolism. Theranostics 2018, 8(22):6248-6262.

Figures

Figure 1

Expression of PFKFB member in rumor tissues and normal tissues. A. Boxplot of PFKFB members in pan-cancer. B. Heatmap of PFKFB members in tumor tissues and normal tissues that the number of normal samples more than 5. C. Correlation of expression levels among the members in PFKFB gene family.
Figure 2

Detailed distribution of PFKFB member expression in tumor tissues and normal tissues. ***, P<0.001; ***, P<0.01; *, P<0.05
Figure 3

Association of PFKFB expression with overall survival in different cancers. Cox proportional hazard regression was utilized in the association analysis.
**Figure 4**

Association of PFKFB gene family expression with immune infiltration in pan-cancer. ***, P<0.001

**Figure 5**

Correlation matrix plots between PFKFB gene family expression and stromal scores in cancers. The size of the dots represents the size of the correlation value.
Figure 6

Association of PFKFB gene expression and tumor stemness. A. Correlation matrix based on RNAss. B. Correlation matrix based on DNAss.
Figure 7

The association between PFKFB expression and drug sensitivity
Figure 8

Association between PFKFB gene expression and immune infiltration in breast cancer
Correlation between PFKFB gene expression and RNAss, DNAss, stromal score, immune score, and Estimate Score.

**Figure 9**

| Cancer: BRCA | PFKFB1 | PFKFB2 | PFKFB3 | PFKFB4 |
|-------------|--------|--------|--------|--------|
| Gene expression | ![Graph](image) | ![Graph](image) | ![Graph](image) | ![Graph](image) |

RNAss, DNAss, Stromal Score, Immune Score, ESTIMATE Score