Elevating *PKM2* Expression Indicates a Biomarker of Poor Prognosis in Patients With Liver Cancer

Chaoxiang Lv

Northeast Normal University

Qiqi Zhang

Northeast Normal University

Yuanguo Li

Chinese Academy of Agricultural Sciences Changchun Veterinary Research Institute

Entao Li

Chinese Academy of Agricultural Sciences Changchun Veterinary Research Institute

Fangxu Li

Shandong Normal University

Tiecheng Wang (✉ q3504517@126.com)

Chinese Academy of Agricultural Sciences Changchun Veterinary Research Institute <https://orcid.org/0000-0002-0971-5569>

Dandan Zhu

Sichuan Agricultural University

Research

Keywords: Liver cancer, PKM2, Warburg effect, cancer metabolism

Posted Date: June 9th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-593582/v1>

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Abstract

M2 isoform of pyruvate kinase (*PKM2*) plays an important role in reprogramming of cell metabolism which is a hall-marker of tumorigenesis. *PKM2* expression altering is closely related to cancer metabolism and tumor growth. In the present study, we analyzed the role of *PKM2* expression in liver cancer in order to clarify its potential application value in the diagnosis and prognosis of liver cancer patients. In cancerous liver tissues, the *PKM2* expression was significantly higher than normal tissues. High *PKM2* expressing was related to patient's age, gender, histological type, grade, stage, T classification and poor survival. Patients with Higher *PKM2* expression had a shorter OS ($P = 0.0013$) and RFS ($P = 0.027$). ROC and Multivariate Cox analysis showed that high *PKM2* expression was a risk factor for patients' poor prognosis. GSEA identified mitotic spindle, PI3K/Akt/mTOR signaling, notch signaling, apoptosis, G2M checkpoint and Wnt/ β -Catenin signaling were enriched with high *PKM2* expression phenotype. These findings suggested *PKM2* expression has potential as a predictive biomarker for the diagnosis and prognosis of patients with liver cancer.

Introduction

Liver cancer, a clinically common malignant tumor with limited efficacy and poor prognosis, stills a main cause of global cancer-related deaths ^[1]. Despite tremendous progress in the diagnosis and treatment of liver cancer, the overall prognosis of patients is still unsatisfactory ^[2,3]. Therefore, it is extremely valuable and urgent to find more specific and reliable prognostic evaluation indicators of liver cancer.

Metabolic abnormalities are an important hall-marker for tumorigenesis. Pyruvate-kinase (*PKM*) is the enzyme that catalyzes the final step in glycolysis, generating pyruvate and ATP from phosphoenolpyruvate and ADP ^[4]. This process consumes glucose, causing pyruvate to be converted to lactate ^[5]. In mammals, pyruvate kinase has four isoforms, including *PKL*, *PKR*, *PKM1* and *PKM2* ^[6]. The *PKL* and *PKR* show tissue-specific expression in the liver tissue and red-blood cells, respectively ^[7]. They have different first exons and are defined by tissue-specific promoters ^[8]. The *PKM* consists of 12 exons, in which exons 9 and 10 are alternately spliced in mutually exclusive ways to produce *PKM1* and *PKM2* isoforms, respectively ^[9]. *PKM2* is mainly expressed in a range of cancer cells, as well as in fetal and undifferentiated adult tissues, whereas *PKM1* is predominantly expressed in terminally differentiated tissues ^[10]. Previous researches have shown that tissue-specific *PKM1/L/R* expression gradually diminishes and is replaced by *PKM2* expression during tumorigenesis ^[11].

As a key regulator of aerobic glycolysis, the expression of *PKM2* is essential for metabolic regulation ^[12]. In recent years, accumulating studies have shown that *PKM2*, in addition to its involvement in metabolic regulation, can also directly participate in gene transcription as a transcription coactivator and protein kinase, regulating the proliferation and apoptosis of undifferentiated cells ^[13–15]. *PKM2* has also been found to have a potential prognostic role in the treatment of several cancers, including lung cancer ^[16], cervical cancer ^[17] and endometrial carcinoma ^[18–19]. This means that *PKM2* may be used as a predictor in clinical diagnosis and prognosis evaluation of cancer patients.

In the present study, we investigated the relationship between the expression pattern of *PKM2* in cancerous and non-cancerous liver tissues, as well as the association between high *PKM2* expression and histological grade, stage, T/N/M classification, patient age, gender and survival status. The prognostic significance of *PKM2*

expression in liver cancer was also analyzed to clarify its potential application value in the treatment and prognosis of liver cancer patients.

Materials And Methods

Data mining and preprocessing from The Cancer Genome Atlas (TCGA) database

The RNAseq data of *PKM2* (gene ID: 5315) and clinical information of liver hepatocellular carcinoma (LIHC) patient were obtained from The Cancer Genome Atlas (TCGA) database. The RNAseq value is estimated as $\log_2(x + 1)$ converted RSEM normalized counts, and these data were processed using R software (version 4.0.1).²⁰

Gene Set Enrichment Analysis

To explore the distribution of predefined genomes and determine the potential mechanism to influence the effect of *PKM2* expression on the prognosis of LIHC patients, we opted for GSEA (version 4.0.3). This analysis was performed through the "h.all.v7.2.symbols.gmt" gene set in the Molecular Signatures database.²¹ Gene-sets with a normal P value <0.05 was regarded as significantly enriched.

Statistical Analysis

R software package was used for statistical analysis. The ggplot2 package in R was performed for visualization. Chi-square test was used to evaluate the correlation between *PKM2* expression and clinical characteristics of liver cancer. ROC analysis was used to evaluate the diagnostic significance of *PKM2* expression. Kaplan-meier survival curve was used to analyze the correlation between *PKM2* expression and patients OS and RFS. Univariate and multivariate Cox regression analysis were used to verify the correlation between *PKM2* expression and survival rate and other clinical features. Statistical significance was defined as $P < 0.05$.

Results

The patients' clinical characteristics and expression pattern of *PKM2* in human liver cancer

We obtained RNAseq of *PKM2* expression and clinical information of 377 liver cancer patients from the TCGA database, including the patient age and gender; as well as histological type, histological grade, pathologic stage, T/N/M classification, radiation therapy, survival status and relapse (Table 1). Subsequently, we found that the expression of *PKM2* in tumors was significantly higher than normal tissues ($P < 0.0001$, Fig. 1). Furthermore, *PKM2* expression was also closely related to histological grade ($P = 0.00011$), histological stage ($P = 0.00024$), T classification ($P < 0.0001$), age ($P = 0.0336$), gender ($P = 0.0136$) and survival status ($P = 0.00261$).

Diagnostic significance of *PKM2* expression and relationship with clinical characteristics in human liver cancer

To evaluate the role of *PKM2* expression in diagnosis, ROC analysis was performed. We found that *PKM2* expression had well diagnostic value (AUC = 0.744; Fig. 2A). In addition, we also analyzed the diagnostic value of *PKM2* expression in different stages of the liver cancer, including AUC value of 0.698 in the first stage, 0.768 in the second stage, 0.786 in the third stage and 0.860 in the fourth stage (Fig. 2B-E). Subsequently, we analyzed the association between *PKM2* expression and clinical features of liver cancer by divided patients into two groups (high or low *PKM2* expression) according to median expression cutoff (Table 2). We found that high *PKM2*

expression was related to patient age ($P = 0.05$), gender ($P = 0.002$), histological type ($P = 0.041$), histological grade ($P = 0.003$), pathologic stage ($P = 0.001$), T classification ($P = 0.000$) and poor survival ($p = 0.020$).

Correlation of PKM2 Expression with OS in liver cancer patient

We previously demonstrated the value of *PKM2* expression in the diagnosis of liver cancer. To further evaluate the correlation of *PKM2* expression with patient OS, Kaplan-Meier curves was performed. We found that high *PKM2* expression was able to shorten patient OS ($P = 0.0013$; Fig. 3). Subsequently, subgroup analysis also showed that high *PKM2* expression significantly affected the OS for patients with grade G1/G2 ($P = 0.025$), grade G3/G4 ($P = 0.047$) stage I/II ($P = 0.0082$), T3 ($P = 0.045$), N0 ($P = 0.0097$), M0 ($P = 0.03$), and M1/MX cancers ($p = 0.012$). Univariate and multivariate Cox analyses indicated that *PKM2* was a risk factor for poor OS (hazard ratio HR = 1.47, 95% confidence interval CI: 1.01–2.14, $P = 0.047$, Table 3). Interestingly, we found the high *PKM2* expression was a risk factor for poor OS in male patients (hazard ratio HR = 1.47, 95% confidence interval CI: 1.01–2.14, $P = 0.047$, Table 4), but not in female ($P = 0.91$; Table S1).

Effect of PKM2 expression on RFS among liver cancer patients

Next, we used Kaplan-Meier curves to explore the correlation of *PKM2* expression with patient RFS (Fig. 4). Survival analysis showed that high *PKM2* expression was associated with poor RFS ($P = 0.027$). Additionally, subgroup analysis also showed that high *PKM2* expression significantly affected the RFS for patients with N0/N1 ($P = 0.039$), and M0/M1 ($P = 0.004$). However, contrary to expectations, we did not observe that high *PKM2* expression is a risk factor for RFS by Univariate Cox analyses (Table S2). Unexpectedly, we found that high *PKM2* expression is not a risk factor for poor RFS in both male and female patients with LIHC (Table S2, 3).

High PKM2 expression-related signaling pathway

Identifying the activation of signaling pathways would facilitate a better understanding of molecular interactions, reactions and relationships, as well as disease process.²² To determine the signaling pathways activated in LIHC, we used GSEA to analyze the high and low *PKM2* expression datasets. The results showed that mitotic spindle, PI3K/Akt/mTOR signaling, notch signaling, apoptosis, G2M checkpoint and Wnt/ β -Catenin signaling were enriched to the high *PKM2* expression phenotype (Table 5, Fig. 5).

Discussion

In the current study, we investigated the role of tumor *PKM2* expression as a predictor in cancerous and healthy liver tissues. This observation clearly shows that the *PKM2* level of tumors is significantly higher than healthy tissues, and elevating expression of *PKM2* is associated with histological grade, stage and vital status. In addition, univariate and multivariate COX analysis indicated that high *PKM2* expression was a predictor for decreased OS in the diagnosis and treatment of liver cancer patients.

Pyruvate Kinase Isotype M2 (*PKM2*) was a key enzyme involved in Warburg effect, and its activity was important for tumor metabolism and growth regulation.²³ In most cancer cells, high *PKM2* expression was able to promote cancer cell proliferation and increase the degree of tumor malignancy.²⁴ In addition, the necrosis and renewal of tumor cells can cause *PKM2* to be released into surrounding tissues, peripheral blood, feces of patients with gastrointestinal malignancies, and pleural effusion of patients with chest tumors, which can be used as

biomarkers to detect tumor metabolism and proliferation.^{25, 26} In early tumors, *PKM2* expression showed heterogeneity, but the staining of metastatic tumor tissue was uniform and strong.²⁷ This also indicated that *PKM2* may play an important role in the occurrence and development of tumors. Previous studies had shown that the re-expression of *PKM2* in tumor cells not only has important significance in regulating tumor cell sugar metabolism, but also gives tumor cells the advantage of selective growth.²⁸ In this study, our results show that *PKM2* expression also gradually increased as histologic grade increased from G1 to G4, as histologic stage increased from I to IV, and as T classification increased from T1 to T4. These results suggested that *PKM2* expression was closely related with staging and grading of cancer, indicating that it can be used as a prognostic monitoring indicator in LIHC.

The phenotype of gene expression and genetic characteristics in tumors are related to signal activation occurrence and development of tumor. It had been reported that knockout of *PKM2* in HCC cells inhibit cell proliferation and induce apoptosis in vivo and in vitro.²⁹ In addition, *PKM2* knockout was able to be used as a chemotherapeutic sensitizer of docetaxel in non-small cell lung cancer cells, resulting in cell viability inhibition, G2/M cell arrest and increased apoptosis.³⁰ Moreover, *PKM2* promotes cell migration and inhibits autophagy by mediating PI3K/AKT activation, and promotes the malignant development of gastric cancer.³¹ In fact, our results indicate that *PKM2* expression is related to liver cancer progression and malignant tumors, and the implicit mechanism may be linked to mitotic spindle, PI3K/Akt/mTOR signaling, notch signaling, apoptosis, G2M checkpoint and Wnt/ β -Catenin signaling as GSEA identified. These findings, combined with previous reports, greatly enrich our comprehension and understanding of the physiological role of *PKM2* in tumor development and deterioration.

Recent studies have shown that the *PKM2* expression was also a potential histopathological marker for the differential diagnosis of malignant and precancerous endometrial lesions, and high *PKM2* expression in endometrial cancer is conducive to poor prognosis.³²⁻³⁴ C Papadaki *et al.* also indicated that *PKM2* as a biomarker for chemosensitivity to front-line platinum-based chemotherapy in patients with metastatic non-small-cell lung cancer.³⁵ In this study, we explored the correlation between high *PKM2* expression and clinical diagnosis. In addition, high *PKM2* expression reduces OS in patients. Interestingly, the high *PKM2* expression was significantly correlated with OS in male patients ($P = 0.00011$), but not in female ($P = 0.91$; Fig. 3). Additionally, although not significant at the 5% level, high *PKM2* expression is a risk factor for RFS in liver cancer male patients ($P = 0.071$; Fig. 4). Univariate and multivariate Cox analysis suggested that high *PKM2* was an independent risk factor for poor OS in male patients, not in female patients with LIHC (Table 4 and S1). Thus, we speculate that *PKM2* may be more suitable for male patients as a potential biomarker for diagnosis and prognosis in liver cancer.

In conclusion, we provided a reliable and comprehensive analysis of *PKM2* expression patterns, diagnosis value and prognosis significance. It has possible to be a valuable potential prognostic biomarker and therapeutic target for liver cancer. It will give us a comprehensive understanding of the biological functions of *PKM2* and provide new reference value through its regulated network of signaling pathway. This will help formulate a better supervision, diagnosis and treatment strategies for malignant tumor in clinical applications.

Declarations

Acknowledgements

This work was supported by Sichuan Social Science Planning Project (SC18B080). It also wanted to thank Pro. Chaofeng Lv from Southwestern University of Finance and Economics for the providing assistance in the early stage of manuscript writing.

Authors' contributions

Chaoxiang Lv and Qiqi Zhang collected and analyzed the data. Yuanguo Li and Fangxu Li performed the data curation. Tian Qin performed visualization of data-set. Dandan Zhu, Tiecheng Wang and Chaoxiang Lv manuscript writing and revision. All authors read and approved the final manuscript.

Conflicts of Interest

The authors declared that there were no conflicts of interest regarding the publication of this manuscript.

Data availability statement

The original data-set used in this manuscript have been deposited in the TCGA database (<https://cancergenome.nih.gov/>).

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Tables

Table 1 Patient clinical characteristic in the present study

Parameters	Variables	Numbers (%)
Gender	Male	255 (67.64)
	Female	122 (32.36)
Histological type	Fibrolamellar carcinoma	3 (0.81)
	Hepatocellular carcinoma	367 (97.35)
	Hepatochoangiocarcinoma(mixed)	7 (1.84)
Histologic grade	NA	5(1.33)
	G1	55 (14.59)
	G2	180 (47.75)
	G3	124 (32.89)
	G4	13 (3.45)
Pathologic stage	NA	22(5.83)
	I	175 (46.42)
	II	88 (23.34)
	III	86 (22.81)
	IV	6 (1.59)
M classification	M0	272 (72.15)
	M1	4 (1.06)
	MX	101(26.79)
N classification	NA	1(0.27)
	N0	257 (68.17)
	N1	4 (2.26)
	NX	115(30.5)
T classification	NA	2(0.53)
	T1	185 (49.07)
	T2	95 (25.20)
	T3	81 (21.49)
	T4	13 (4.45)
	TX	1(0.27)
Radiation therapy	NA	23(6.10)
	NO	345 (91.52)
	Yes	9 (2.39)

Vital status	Dead	132 (35.01)
	Survival	245 (64.99)
Relapse	NA	27(7.16)
	NO	235(62.33)
	YES	115(30.51)
<i>PKM2</i>	NA	7(1.86)
	High	185(49.07)
	Low	185(49.07)

NA: not available

Table 2 Associations between the clinicopathologic variables and *PKM2* expression.

Parameters	Variables	Numbers	PKM2				χ^2	P-value
			high	Prop %	low	Prop %		
age	≥55	257	144	64.29	113	71.98	3.837	0.050
	<55	120	80	35.71	40	28.02		
Genger	Male	255	138	61.61	117	76.47	9.175	0.002
	Female	122	86	38.39	36	23.53		
Histological type	Fibrolamellar carcinoma	3	3	1.59	0	0	6.401	0.041
	Hepatocellular carcinoma	360	180	95.24	180	99.45		
	Hepatocholangiocarcinoma (mixed)	7	6	3.17	1	0.55		
Histologic grade	G1	55	19	10.22	36	20.11	13.882	0.003
	G2	176	84	45.16	92	51.40		
	G3	122	77	41.40	45	25.14		
	G4	12	6	3.22	6	3.35		
Pathologic stage	I	175	84	41.18	91	62.33	15.840	0.001
	II	87	58	28.43	29	19.86		
	III	86	61	29.90	25	17.12		
	IV	2	1	0.49	1	0.69		
M classification	M0	272	161	72.85	111	73.03	0.001	0.970
	M1	101	60	27.15	41	26.97		
N classification	N0	257	113	68.90	144	69.23	0.829	0.363
	N1	115	51	31.10	64	30.77		
T classification	T1	185	90	40.54	95	63.33	23.660	0.000
	T2	93	63	28.38	30	20.00		
	T3	81	56	25.22	25	16.67		
	T4	13	13	5.86	0	0		
Radiation therapy	NO	338	170	96.59	168	98.25	0.940	0.332
	Yes	9	6	3.41	3	1.75		
Vital status	Dead	132	89	39.73	43	28.1	5.402	0.020
	Survival	245	135	60.27	110	71.9		

Table 3 Univariate and Multivariate analysis of over survival in patients with liver cancer.

	Univariate analysis			Multivariate analysis		
	Hazard Ratio	CI95	P-value	Hazard Ratio	CI95	P-value
Age	1.04	0.72-1.50	0.851			
Gender	0.82	0.58-1.17	0.276			
Histological type	0.99	0.26-3.27	0.987			
Histologic grade	1.10	0.87-1.38	0.443			
Pathologic stage	1.64	1.34-2.00	0.000	1.12	0.45-2.80	0.799
M classification	1.29	1.07-1.55	0.007	1.29	0.99-1.68	0.064
N classification	1.25	1.04-1.50	0.017	1.02	0.78-1.33	0.893
T classification	1.65	1.38-1.97	0.000	1.46	0.61-3.51	0.396
Radiation therapy	0.91	0.29-2.87	0.875			
<i>PKM2</i>	1.773	1.25-2.53	0.002	1.47	1.01-2.14	0.047

Table 4 Univariate and Multivariate analysis of over survival in male patients with liver cancer.

	Univariate analysis			Multivariate analysis		
	Hazard Ratio	CI95	P-value	Hazard Ratio	CI95	P-value
Age	1.27	0.79-2.04	0.321			
Histological type	1.01	0.16-6.04	0.992			
Histologic grade	1.05	0.79-1.41	0.741			
Pathologic stage	1.91	1.47-2.49	0.000	0.97	0.24-3.97.	0.970
M classification	1.26	1.00-1.60	0.054	1.15	0.82-1.60	0.424
N classification	1.31	1.04-1.65	0.022	1.08	0.78-1.49	0.423
T classification	1.90	1.51-2.38	0.000	1.95	0.51-7.48	0.325
Radiation therapy	0.83	0.20-3.40	0.798			
<i>PKM2</i>	2.36	1.51-3.70	0.000	2.01	1.24-3.24	0.004

Table 5 Gene set enrichment analysis in phenotype low among liver cancer.

Name	ES	NES	NOM p-value
HALLMARK_MITOTIC_SPINDLE	0.55	1.91	0.000
HALLMARK_PI3K_AKT_MTOR_SIGNALING	0.45	1.83	0.006
HALLMARK_NOTCH_SIGNALING	0.55	1.83	0.008
HALLMARK_APOPTOSIS	0.38	1.65	0.008
HALLMARK_G2M_CHECKPOINT	0.61	1.83	0.025
HALLMARK_WNT_BETA_CATENIN_SIGNALING	0.48	1.59	0.028

ES: Enrichment score; NES: normalized enrichment score; NOM: nominal;

Figures

Figure 1

PKM2 expression pattern in human liver cancer. The expression of PKM2 was compared in cancerous and normal liver tissues; as well as in groups with different histological grade, histological stage, T classification, N classification, M classification, patient age, patient gender and survival status.

Figure 2

Diagnosis significance of PKM2 expression in liver cancer. (A) ROC curves of PKM2 expression were compared in cancerous liver tissues and normal liver tissues. (B-E) ROC curves of PKM2 expression in different stages of the liver cancer, including I, II, III, and IV.

Figure 3

Correlation of PKM2 expression with OS in liver cancer. Kaplan-Meier curves was used to evaluate relationship between PKM2 expression and OS in all patients, as well as subgroup analysis (Female, Male, G1/G2, G3/G4, stage I/II, stage III/IV, T1-T4, N0, N1/NX, M0 and MX).

Figure 4

Effect of PKM2 expression on RFS among liver cancer patients. Kaplan-Meier curves of RFS of all liver cancer cases were compared, and sub-component analysis of different classifications (Female, Male, G1/G2, G3/G4, stage I/II, stage III/IV, T1-T4, N0, N1/NX, M0 and MX).

Figure 5

Gene-Set Enrichment plots. GSEA results showing differential enrichment of genes related to mitotic spindle, PI3K/Akt/mTOR signaling, notch signaling, apoptosis, G2M checkpoint and Wnt/ β -Catenin signaling in LIHC cases with high PKM2 expression.

Supplementary Files

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