Pathology & Oncology Research (2020) 26:853–860
https://doi.org/10.1007/s12253-019-00630-3

ORIGINAL ARTICLE

Overexpression of Pyruvate Kinase M2 in Tumor Tissues Is Associated with Poor Prognosis in Patients with Hepatocellular Carcinoma

Rui Zhao[1,2] · Lei Li[3] · Jinbo Yang[1] · Qinfeng Niu[4] · Han Wang[1] · Xiaodong Qin[1,5] · Ning Zhu[1] · Anchen Shi[6]

Received: 14 March 2018 / Accepted: 4 March 2019 / Published online: 9 March 2019

Abstract

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors, with a high degree of malignancy and a poor prognosis. The aim of this study was to investigate the relationship between expression of pyruvate kinase M2 (PKM2) and prognosis in patients with HCC. The expression levels of PKM2 and PKM1 in 86 cases of HCC were detected by immunohistochemistry. An H score was used to evaluate the expression of PKM, and all patients were further divided into PKM high-expression and PKM low-expression groups. The relationship between PKM2 expression and the clinicopathological parameters and prognosis of patients were subsequently analyzed. Our data suggested that the expression level of PKM2 was significantly higher in HCC tissues than in adjacent tissues and the negatively expression of PKM1 in HCC tissues. Kaplan-Meier analysis revealed that PKM2 expression was strongly associated with survival in HCC patients ($P = 0.001$). The patients in the PKM2 high-expression group had significantly shorter survival times than the patients in the PKM2 low-expression group (hazard ratio for death, 2.358; 95% confidence interval [1.156, 4.812]; $P = 0.018$). In conclusion, these data indicate that PKM2 expression in HCC tissue samples can be used as a prognostic factor for patients with HCC and that high PKM2 expression is correlated with a poor prognosis in HCC patients.

Keywords Pyruvate kinase M2 · Hepatocellular carcinoma · Prognosis · Overall survival rate · Reoccurrence

Introduction

Human hepatocellular carcinoma (HCC), also called malignant hepatoma, is the most common type of liver cancer. HCC is one of the most common aggressive tumors and has a relatively high mortality rate compared to different malignant tumors [1]. Due to a lack an early diagnostic biomarkers or other disease-specific symptoms, most HCC patients are diagnosed at the advanced or later stages, which contributes to the high mortality rate of HCC [2]. Generally, after confirmation of HCC, the overall 5-year survival rate for HCC patients, which is commonly below 18%, is extremely low [1]. HCC typically occurs in individuals who have a chronic liver disease such as viral hepatitis and cirrhosis [3]. Specifically, chronic infections with the hepatitis B virus (HBV) and hepatitis C virus (HCV) can promote the development of hepatocellular carcinoma since the chronic viral infection can repeatedly cause the immune system to attack the liver cells infected by the virus [4, 5]. It is likely that the constant cycle of immune system-mediated damage followed by liver repair leads to mistakes that promote carcinogenesis [4]. Moreover, for HBV, integration of the viral genome into the genome of the infected cell can directly induce HCC [4].

Currently, various treatments have been developed for HCC, such as liver transplantation, surgical resection, transcatheter arterial chemoembolization, radiofrequency ablation, intra-arterial iodine-131–lipiodol administration and a

✉ Jinbo Yang
zhaor@lzu.edu.cn

1 School of Life Sciences, Lanzhou University, Lanzhou 730000, China

2 Department of Hepatology, Lanzhou University Second Hospital, Lanzhou 730030, China

3 Department of Interventional Radiology, Lanzhou University First Hospital, Lanzhou 730030, China

4 Xi'an Medical College Baoji Affiliated Hospital, Baoguang Branch, Baoji 721006, China

5 Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Lanzhou 730046, China

6 School of Basic Medical Sciences, Lanzhou University, Lanzhou 730000, China

receptor tyrosine kinase inhibitor (sorafenib). Although the best prognosis for long-term survival occurs with surgical resection, which involves removing a tumor along with the surrounding liver tissue while preserving enough of the liver remnant for normal body functions, only 10–15% of patients qualify for surgical resection due to extensive disease or poor liver function [6]. Moreover, even though there have been great improvements to traditional treatments, such as successful surgical resection and the use of antiviral drugs in the setting of hepatitis-induced liver cirrhosis, the risk of HCC recurrence is still extremely high and is up to 70% of cases by 5 years [7]. Thus, it is extremely necessary to identify novel and efficient biomarkers for early diagnosis or as therapeutic targets of HCC. In this study, we investigated the correlation between the cytoplasmic level of pyruvate kinase M2 (PKM2) and the prognosis for HCC patients. The expression of PKM2 in 86 cases of HCC was detected by immunohistochemistry. The relationship between PKM2 expression and the clinicopathological parameters and patient prognosis were subsequently analyzed. Our data suggested that PKM2 expression in HCC tissue samples could be used as a prognostic factor for patients with HCC and that high PKM2 expression was correlated with poor prognosis in HCC patients.

Materials and Methods

Patients and Ethics Statements

All liver cancer tissues and corresponding normal adjacent samples were collected from 86 patients who had undergone routine surgery at the second hospital affiliated with Lanzhou University from January 2011 to December 2012. HCC was confirmed in all patients by pathological examination. This study was approved by the Ethical Committee of Lanzhou University, and written informed consent was obtained from every individual involved. The study methodologies conformed to the standards set by the Declaration of Helsinki. The clinicopathological features and demographic data of the 86 HCC cases are summarized in Table 1.

Liver Histology

Hematoxylin-ensin (H&E) staining was used for liver pathological evaluation. H&E staining kit was purchased by Jiancheng Bioengineering Institute (Nanjing, China). The kit was used according to the corresponding manufacturers' instruction. The sections were counterstained with hematoxylin (Sigma-Aldrich) and visualized with an Olympus CX22 microscope.

Table 1 Clinicopathological characteristics of patients with HCC

Characteristic	No. of patients	%
Age (years)		
Median	50	
Range	38–70	
Gender		
Female	29	33
Male	57	67
Clinical symptoms		
Negative	22	26
Positive	64	74
HBsAg		
Negative	31	36
Positive	55	64
HBV-DNA		
$\leq 1 \times 10^4$	52	60
$>1 \times 10^4$	34	40
ALT (U/L)		
≤40	35	41
>40	51	59
TBIL (mmol/l)		
≤25.8	48	56
>25.8	38	44
AFP		
≤20	35	41
21–400	19	22
>400	32	37
Tumor size (cm)		
≤5	38	44
>5	48	56
Liver cirrhosis		
No	12	14
Yes	74	86
Microvascular invasion		
Absent	54	63
Present	32	37
Cancer embolus		
No	58	67
Yes	28	33
Tumor encapsulation		
Complete	59	69
None	27	31
Lymphatic metastasis		
No	72	84
Yes	14	16
Distant metastasis		
No	76	88
Yes	10	12
Tumor differentiation		
I + II	79	92
III + IV	7	8

Immunohistochemistry Analysis

The expression levels of PKM2 and PKM1 in the HCC tissues and adjacent healthy tissues were detected by immunohistochemistry. Briefly, paraffin sections were subsequently deparaffinized with xylene and rehydrated in decreasing concentrations of ethanol. Antigen retrieval was performed by heating the sections in an antigen retrieval buffer (pH 6.0) in a microwave cooker at 90 °C for 45 min. After antigen retrieval, endogenous peroxidase activity was blocked by adding 3% H_2O_2 for at least 10 min, and the endogenous biotin enzyme in the tissues was blocked with normal goat serum (Sigma-Aldrich, St. Louis, MO, USA) for 30 min at room temperature (RT). Then, the sections were incubated with primary antibody forPKM2 (1:800 dilution) (Cat#3198, Cell Signaling Technology, Beverly, MA, USA) and PK #15821, Proteintech, Wuhan, Hubei, China) overnight at 4 °C. After PKM2 and PKM1 antibodies incubation, the sections were washed with PBS and incubated with biotin-labeled anti-rabbit antibody (1:100 dilution) (Sigma-Aldrich, St. Louis, MO, USA) at 37 °C for 60 min and washed three times with PBS. Horseradish peroxidase-labeled streptavidin was added to the sections after the PBS washes and incubated at 37 °C for 30 min. The specific reaction between the PMK2 or PKM1 antibody and their targets were visualized with the DAB kit (Sigma-Aldrich). The sections were counterstained with hematoxylin (Sigma-Aldrich) and visualized with an Olympus CX22 microscope.

Patient Grouping

All sections were reviewed by two independent pathologists who were blinded to the patient information. For the quantifications of the immunohistochemistry results, five fields of each part of the tissue were randomly selected under a light microscope. The total numbers of positive cells and tumor cells were determined, and the color intensity was recorded. A semi-quantitative method was used to evaluate each section as previously described [8]. Quantification of the IHC data for each patient was conducted as previously described [9]. Briefly, 3 fields were randomly selected from each section for counting the percentage of PKM positively stained cells. The percentage of positive cells (P) was assigned a score from 0 to 3. A score of 0 indicated no positive cells. A score of 1 indicated a cell positivity rate of less than 20%. A score of 2 indicated a cell positivity rate higher than 20% but less than 75%. A score of 3 indicated a cell positivity rate higher than 75%. The staining score of each positive cell was further assigned as 0 (no stain), 1 (yellow stain), 2 (tan stain) and 3 (dark tan stain). The sum of the multiplication of the PKM-positive percentage and staining score was used to classify the final grading of the PKM-positive level for each section. Samples with final scores less than 4 were defined as PKM low expression, while samples with final scores equal to or greater than 4 were defined as PKM high expression.

Statistical Analysis

Statistical analyses were performed using SPSS 13.0 (SPSS Inc.). The expression differences between the proteins were analyzed with chi-squared test. Correlation analyses were performed using Spearman's rank correlation. The Kaplan-Meier estimate was used for the univariate analysis of prognosis. The data comparison among the different groups was performed using the log rank test. The Cox proportional hazards model was used for the multivariate analysis of prognosis. A P value less than 0.05 was considered statistically significant.

Results

PKM2 and PKM1 Expressions in Different HCC Samples

PKM2 is an isoenzyme of the glycolytic enzyme pyruvate kinase, which is expressed in cells with a high rate of nucleic acid synthesis, such as normal proliferating cells, embryonic cells, and especially tumor cells [10–12]. In the HCC samples collected in our study, the positivity rate of PKM2 expression for all 86 HCC cases was 100% and the positivity rate of PKM1 expression for all 86 HCC cases was 0%, suggesting the specificity of the used PKM2 antibody at tissue level. A representative immunohistochemistry images of PKM2 and PKM1 expressions of each group were shown as Fig. 1.

Association between the PKM2 Expression Level and Clinicopathological Features in HCC Patients

Based on the statistical analysis of PKM2 expression in the cytoplasm, all patients involved were divided into the PKM2-high group and PKM2-low group. The association between the PKM2 expression levels and other factors was analyzed to characterize the relationships. Based on our results, high PKM2 expression was correlated with the female sex, an HBV-DNA copy number $> 1 \times 10^4$, an ALT level > 40 U/L, a TBIL >25.8 mmol/l, an AFP level from 21 to 400, a tumor size >5 cm and a cancer embolus ($P < 0.05$) (Table 2).

Survival Analysis for HCC Patients with Different PKM2 Expression Levels

After surgery, all involved patients underwent follow-up for information related to the patient recovery, HCC recurrence and patient survival time. Based on the data from all patients, the median survival time was 24 months. The 1-year, 2-year and 3-year overall survival rates were 71.9%, 43% and

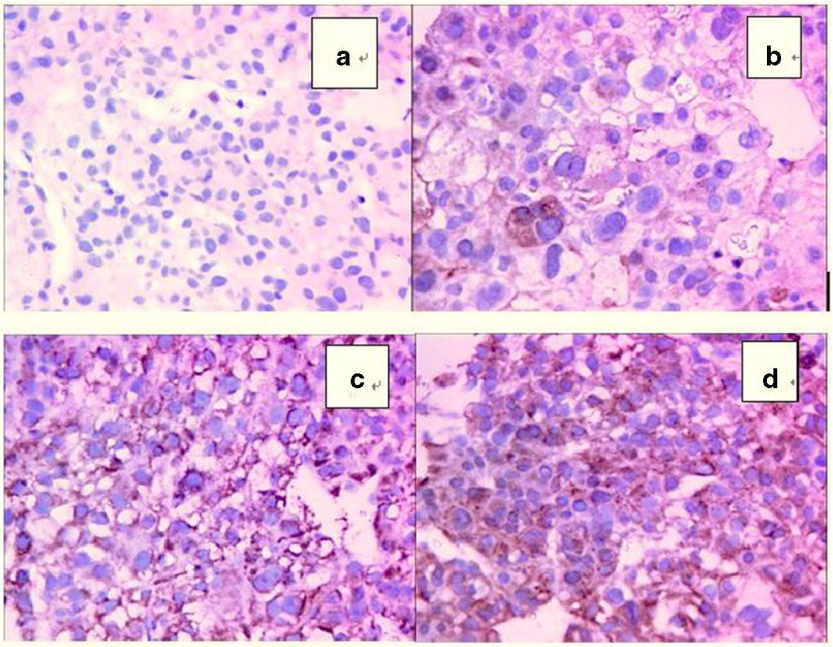

Fig. 1 HE-staining in normal liver tissue, and expressions of PKM2 and PKM1 in normal liver tissue and tissue samples obtained from HCC patients. **a**: HE-staining of normal liver tissue. **b**: The expressions of PKM2 in normal liver tissue and tissue samples obtained from HCC patients. a) PKM2 was negatively expressed in normal liver tissue; b) PKM2 was mainly expressed in the cytoplasm at a low level in highly differentiated HCC tissue; c) Moderate expression of PKM2 in the cytoplasm in moderately differentiated HCC tissue; d) High expression of PKM2 in the cytoplasm in poorly differentiated HCC tissue. **c**: The expressions of PKM1 in normal liver tissue and tissue samples obtained from HCC patients. a) PKM1 was positively expressed in cytoplasm and partial cell membranes of normal liver tissue; b) PKM1 was negatively expressed in highly differentiated HCC tissue; c) PKM1 was negatively expressed in moderately differentiated HCC tissue; d) PKM1 was negatively expressed in poorly differentiated HCC tissue. The sections were photographed at 400× magnification

29.1%, respectively. Based on the Kaplan-Meier survival analysis, the overall survival rates of the PKM2-high group were significantly lower than those of the PKM2-low group ($P = 0.001$) (Fig. 2). For the reoccurrence rate, the PKM2-high group also showed a significantly high rate of reoccurrence relative to the PKM2-low group (Fig. 3). On the one hand, the univariate analysis suggested that the PKM2 expression level, sex, clinical symptoms and tumor differentiation were associated with prognosis of HCC (Table 3). On the other hand, the multivariate analysis suggested that the clinical symptoms, tumor differentiation, and the PKM2 expression level were associated with HCC prognosis (Table 3). Taken together, these data suggested that high PKM2 expression in patients with HCC was the main factor affecting the prognosis of HCC.

Discussion

A common feature of cancer cells is dysregulation of metabolic pathways to meet the unique biological requirements of cancer cells [13]. Otto Warburg made his landmark observation of cancer cell metabolism 80 years ago when he discovered that cancer cells consumed more glucose and produced a large amount of lactate even in a well-oxygenized environment, a process known as aerobic glycolysis or the Warburg effect [13]. In contrast to normal cells, which maximize ATP production by mitochondrial oxidative phosphorylation of glucose, cancer cells generate much less ATP from glucose by aerobic glycolysis [13].

Pyruvate kinase catalyzes the last procedure of glycolysis, dephosphorylation of phosphoenolpyruvate to pyruvate, and is responsible for ATP production within the glycolytic sequence [12]. However, unlike mitochondrial respiration, energy regeneration by pyruvate kinase is independent of the oxygen supply and allows survival of tissues, such as in solid tumors, under hypoxic conditions [12]. There are two isozymes that are encoded by the PKM gene: PKM1 and PKM2. PKM1 and PKM2 are different splicing products of the M-gene with exon 9 and exon 10 for PKM1 and PKM2, respectively [11, 14]. PKM1 and PKM2 differ by 23 amino acids within a 56-amino acid stretch (aa 378–434) at their carboxy terminus [11, 14].

Generally, PKM1 is predominantly expressed in tissue that is strongly dependent on a high rate of energy regeneration, such as muscle or the brain [15, 16]. However, PKM2 is preferentially overexpressed in tumors cells [17]. Based on our current knowledge, PKM2 overexpression in tumor cells is generally accompanied by overexpression of oncoprotein c-Myc [18, 19]. c-Myc activates the transcription of heterogeneous nuclear ribonucleoproteins (hnRNPs) I, A1 and A2,

Table 2 Association between PKM2 expression and the clinicopathological features

Clinicopathological indexes		PKM2		
		Low	High	*p*
Age (year)	≤50	19	24	0.829
	>50	20	23	
Sex	Female	7	22	0.004
	Male	33	24	
Clinical symptoms	Negative	15	7	0.026
	Positive	26	38	
HBsAg	Negative	11	20	0.346
	Positive	26	29	
HBV-DNA	$\leq 1 \times 10^4$	30	22	0.014
	$>1 \times 10^4$	11	23	
ALT (U/L)	≤40	23	12	0.003
	>40	18	33	
TBIL(mmol/l)	≤25.8	29	19	0.000
	>25.8	7	31	
AFP	≤20	26	10	0.000
	21–400	7	12	
	>400	4	27	
Tumor size (cm)	≤5	22	16	0.006
	>5	15	33	
Liver cirrhosis	No	4	8	0.551
	Yes	34	40	
Microvascular invasion	Absence	30	25	0.024
	Present	11	20	
Cancer embolus	No	32	26	0.004
	Yes	7	21	
Tumor encapsulation	Complete	29	30	0.170
	None	9	18	
Lymphatic metastasis	No	35	37	0.656
	Yes	5	9	
Distant metastasis	No	34	42	0.097
	Yes	8	2	
Tumor differentiation	I + II	38	41	0.204
	III + IV	1	6	

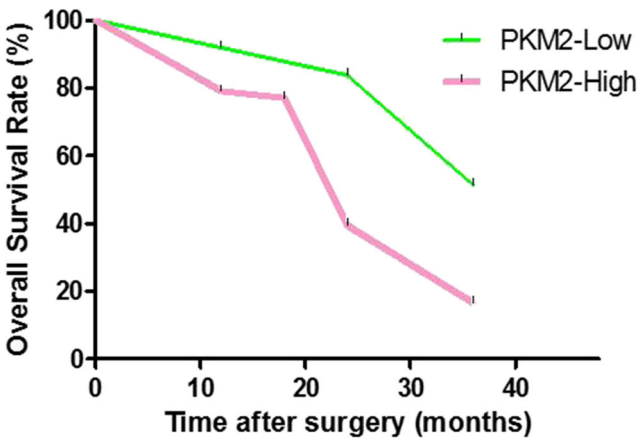

Fig. 2 The relationship between the PKM2 expression level and the overall survival rate of HCC patients

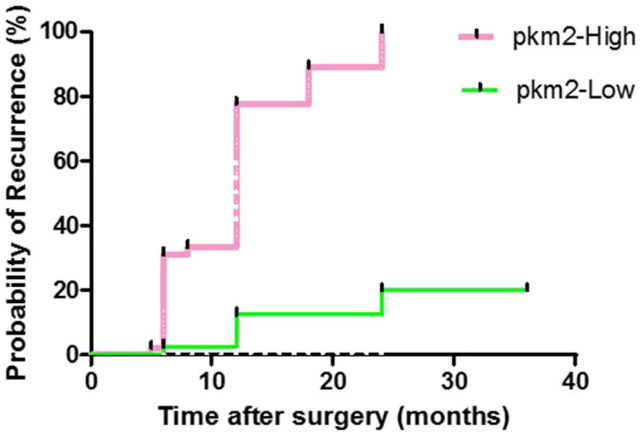

Fig. 3 The relationship between the PKM2 expression level and the recurrence rate of HCC after surgery

which bind and repress exon 9-encoding RNA sequences [18, 19]. Therefore, c-Myc promotes the preferable splicing of PKM2 mRNA, which allows synchronous expression of the PKM2 isoform [18, 19].

On the one hand, PKM1 forms a stable, constitutively active tetramer that has high pyruvate kinase activity [20]. On the other hand, the PKM2 conformation is affected by numerous allosteric effectors and posttranslational modifications and switches between dimeric and tetrameric forms in tumor cells [20]. Reversion from PKM1 to PKM2 is an indispensable stage that shifts glucose metabolism toward aerobic glycolysis, a metabolic phenotype that is amenable to providing favorable energetics, biosynthetic intermediates and redox power for rapidly dividing tumor cells [21, 22]. Although a bi-functional role for PKM2 has been proposed, the PKM2 level in circulation or tissue has been shown to act as a biological marker for diagnosis and prognosis of several types of cancer [23–25].

Previous studies have suggested an important role for PKM2 in tumorigenesis of hepatocellular carcinoma [26–29]. An in vitro study has suggested that PKM2 knockdown by shRNA inhibits tumor progression of HCC by inducing apoptosis and cell cycle arrest [30]. Data gained from HCC patients suggests that overexpression of PKM2 is correlated with a high TNM stage and level of vascular invasion of HCC [27]. Patients with HCC who are positive for PKM2 expression and negative for TRIM35 expression have shorter overall survival and time-to-recurrence rates than patients who are negative for PKM2 and positive for TRIM35 [27]. A clinical data analysis indicated that increased PKM2 expression in HCC was correlated with vascular invasion and intrahepatic metastasis. Moreover, high PKM2 level was strongly correlated with AFP, multiplicity, TNM stage and tumour differentiation in cirrhosis HCC [31]. These studies suggested high PKM2 expression played an important role in poor prognostic of HCC. In our study, by dividing the patients into a PKM2-high group and PKM2-low group, high PKM2 expression was correlated with various clinical

Table 3 Cox regression analysis between PKM2 and clinicopathological features

Factors	Patient no	3-year overall survival	Univariate analysis HR (95% CI) *P* value	Multivariate analysis HR (95% CI) *P* value
Age (year)			0.966 (0.563–1.658)	
≤50	42	25	P 0.901	
>50	44	37		
Gender			0.495 (0.291–0.841)	
Female	29	20	P 0.01	
Male	57	41		
Clinical symptoms			0.417 (0.189–0.922)	2.398 (1.084–5.304)
Negative	22	17	P 0.031	P 0.031
Positive	64	45		
HBsAg			0.831(0.48–1.44)	
Negative	28	17	P 0.509	
Positive	58	42		
HBV-DNA			1.028 (0.575–1.84)	
≤500	21	15	P 0.925	
>500	65	34		
ALT(U/L)			1.189 (0.698–2.024)	
≤40	34	29	P 0.524	
>40	52	32		
TBIL(mmol/l)			0.904 (0.521–1.566)	
≤25.8	48	46	P 0.904	
>25.8	38	18		
AFP			1.344 (0.792–2.28)	
≤20	36	29	P 0.274	
21–400	16	14		
>400	34	20		
Tumor size (cm)			0.999 (0.589–1.695)	
≤5	38	32	P 0.997	
>5	48	30		
Liver cirrhosis			1.081 (0.463–2.524)	
No	12	6	P 0.858	
Yes	74	55		
Microvascular invasion			1.295 (0.755–2.221)	
Absent	54	42	P 0.348	
Present	32	20		
Cancer embolus			0.673 (0.39–1.16)	
No	58	46	p 0.154	
Yes	28	16		
Tumor encapsulation			0.915 (0.51–1.639)	
Complete	58	44	P 0.764	
None	28	18		
Lymphatic metastasis			0.753 (0.368–1.538)	
No	72	55	P 0.436	
Yes	14	7		
Distant metastasis			0.49 (0.119–2.013)	
No	76	61	0.323	
Yes	10	1		
Tumor differentiation			0.508 (0.262–0.985)	1.969 (1.015–3.82)
I + II	79	58	P 0.045	P 0.045
III + IV	7	3		
PKM2 staining			2.705 (1.495–4.896)	2.436 (1.324–4.689)
Low	42	38	P 0.001	P 0.011
High	44	24		

features, such as the HBV-DNA copy number, ALT level, TBIL, AFP level, tumor size and cancer embolus. The survival analysis of the two groups of patients suggested that the overall survival rate of the PKM2-high group was significantly lower than that of the PKM2-low groups. Moreover, the PKM2-high group showed a significantly higher rate of reoccurrence than the PKM2-low group.

Conclusion

In this study, by investigating the expression of pyruvate kinase M2 (PKM2) expression and its relationship with prognosis in 86 HCC patients, it was demonstrated that the expression level of PKM2 was significantly higher in HCC tissues than in adjacent healthy tissues. The Kaplan-Meier analysis

revealed that patients with high expression of PKM2 had significantly shorter survival times than patients with low expression of PKM2. Therefore, we concluded that PKM2 expression in the HCC tissue samples could be used as a prognostic factor for patients with HCC and that high PKM2 expression correlated with a poor prognosis of HCC patients.

Funding This study was supported by the Gansu Province Natural Science Youth fund (No. 1506RJYA225).

Compliance with Ethical Standards

Disclosure Nothing to disclose.

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

Ethical Approval Cairo University Ethical Committee. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Human/Animal Rights Statement This article contain all liver cancer tissues and corresponding normal adjacent samples were collected from 86 patients who had undergone routine surgery at the second hospital affiliated with Lanzhou University from January 2011 to December 2012.

Informed Consent Informed consent was obtained from all individual participants included in the study.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References

1. Altekruse SF, McGlynn KA, Dickie LA, Kleiner DE (2012) Hepatocellular carcinoma confirmation, treatment, and survival in surveillance, epidemiology, and end results registries, 1992-2008. Hepatology 55:476–482. https://doi.org/10.1002/hep.24710
2. Li H, Huang W, Luo R (2015) The microRNA-325 inhibits hepatocellular carcinoma progression by targeting high mobility group box 1. Diagn Pathol 10:117. https://doi.org/10.1186/s13000-015-0323-z
3. Zeng QA, Qiu J, Zou R, Li Y, Li S, Li B, Huang P, Hong J, Zheng Y, Lao X, Yuan Y (2012) Clinical features and outcome of multiple primary malignancies involving hepatocellular carcinoma: a long-term follow-up study. BMC Cancer 12:148. https://doi.org/10.1186/1471-2407-12-148
4. Chen CJ, Yang HI, Su J, Jen CL, You SL, Lu SN, Huang GT, Iloeje UH, REVEAL-HBV Study Group (2006) Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. Jama 295:65–73. https://doi.org/10.1001/jama.295.1.65
5. Bosch FX, Ribes J, Diaz M, Cleries R (2004) Primary liver cancer: worldwide incidence and trends. Gastroenterology 127:S5–S16
6. Ang SF, Ng ES, Li H, Ong YH, Choo SP, Ngeow J, Toh HC, Lim KH, Yap HY, Tan CK, Ooi LL, Cheow PC, Chung AY, Chow PK, Foo KF, Tan MH (2015) The Singapore liver cancer recurrence (SLICER) score for relapse prediction in patients with surgically resected hepatocellular carcinoma. PLoS ONE 10:e0118658. https://doi.org/10.1371/journal.pone.0128058
7. Llovet JM, Burroughs A, Bruix J (2003) Hepatocellular carcinoma. Lancet 362:1907–1917. https://doi.org/10.1016/S0140-6736(03)14964-1
8. Yu G, Yu W, Jin G, Xu D, Chen Y, Xia T, Yu A, Fang W, Zhang X, Li Z, Xie K (2015) PKM2 regulates neural invasion of and predicts poor prognosis for human hilar cholangiocarcinoma. Mol Cancer 14:193. https://doi.org/10.1186/s12943-015-0462-6
9. Yamada S, Fuchs BC, Fujii T, Shimoyama Y, Sugimoto H, Nomoto S, Takeda S, Tanabe KK, Kodera Y, Nakao A (2013) Epithelial-to-mesenchymal transition predicts prognosis of pancreatic cancer. Surgery 154:946–954. https://doi.org/10.1016/j.surg.2013.05.004
10. MacDonald MJ, Chang CM (1985) Pancreatic islets contain the M2 isoenzyme of pyruvate kinase. Its phosphorylation has no effect on enzyme activity. Mol Cell Biochem 68:115–120
11. Dombrauckas JD, Santarsiero BD, Mesecar AD (2005) Structural basis for tumor pyruvate kinase M2 allosteric regulation and catalysis. Biochemistry 44:9417–9429. https://doi.org/10.1021/bi0474923
12. Vaupel P, Harrison L (2004) Tumor hypoxia: causative factors, compensatory mechanisms, and cellular response. Oncologist 9:4–9. https://doi.org/10.1634/theoncologist.9-90005-4
13. Wong N, De Melo J, Tang D (2013) PKM2, a central point of regulation in cancer metabolism. Int J Cell Biol 2013:242513:1–11. https://doi.org/10.1155/2013/242513
14. Noguchi T, Inoue H, Tanaka T (1986) The M1- and M2-type isozymes of rat pyruvate kinase are produced from the same gene by alternative RNA splicing. J Biol Chem 261:13807–13812
15. Eigenbrodt E, Reinacher M, Scheefers-Borchel U, Scheefers H, Friis R (1992) Double role for pyruvate kinase type M2 in the expansion of phosphometabolite pools found in tumor cells. Crit Rev Oncog 3:91–115
16. Mazurek S, Boschek CB, Hugo F, Eigenbrodt E (2005) Pyruvate kinase type M2 and its role in tumor growth and spreading. Semin Cancer Biol 15:300–308. https://doi.org/10.1016/j.semcancer.2005.04.009
17. Steinberg P, Klingelhoffer A, Schafer A, Wust G, Weisse G, Oesch F, Eigenbrodt E (1999) Expression of pyruvate kinase M2 in preneoplastic hepatic foci of N-nitrosomorpholine-treated rats. Virchows Arch 434:213–220. https://doi.org/10.1007/s004280050330
18. David CJ, Chen M, Assanah M, Canoll P, Manley JL (2010) HnRNP proteins controlled by c-Myc deregulate pyruvate kinase mRNA splicing in cancer. Nature 63:364–368. https://doi.org/10.1038/nature08697
19. Dong G, Mao Q, Xia W, Xu Y, Wang J, Xu L, Jiang F (2016) PKM2 and cancer: the function of PKM2 beyond glycolysis. Oncol Lett 11:1980–1986. https://doi.org/10.1038/nature08697
20. Li Z, Yang P, Li Z (2014) The multifaceted regulation and functions of PKM2 in tumor progression. Biochim Biophys Acta 1846:285–296. https://doi.org/10.1016/j.bbcan.2014.07.008
21. Christofk HR, Vander Heiden MG, Harris MH, Ramanathan A, Gerszten RE, Wei R, Fleming MD, Schreiber SL, Cantley LC (2008) The M2 splice isoform of pyruvate kinase is important for cancer metabolism and tumour growth. Nature 452:230–233. https://doi.org/10.1038/nature06734
22. Tamada M, Suematsu M, Saya H (2012) Pyruvate kinase M2: multiple faces for conferring benefits on cancer cells. Clin Cancer Res 18:5554–5561. https://doi.org/10.1158/1078-0432.CCR-12-0859
23. Schulze G (2000) The tumor marker tumor M2-PK: an application in the diagnosis of gastrointestinal cancer. Anticancer Res 20:4961–4964

24. Wong N, Yan J, Ojo D, De Melo J, Cutz JC, Tang D (2014) Changes in PKM2 associate with prostate cancer progression. Cancer Investig 32:330–338. https://doi.org/10.3109/07357907.2014.919306
25. Hardt PD, Ngoumou BK, Rupp J, Schnell-Kretschmer H, Kloer HU (2000) Tumor M2-pyruvate kinase: a promising tumor marker in the diagnosis of gastro-intestinal cancer. Anticancer Res 20:4965–4968
26. Fan F, Wu H, Liu Z, Hou X, Chen W, Wang A, Lu Y (2016) Nuclear PKM2 expression, an independent risk factor for ER after curative resection of hepatocellular carcinoma. Biomed Pharmacother 84:1858–1864. https://doi.org/10.1016/j.biopha.2016.10.108
27. Chen Z, Lu X, Wang Z, Jin G, Wang Q, Chen D, Chen T, Li J, Fan J, Cong W, Gao Q, He X (2015) Co-expression of PKM2 and TRIM35 predicts survival and recurrence in hepatocellular carcinoma. Oncotarget 6:2538–2548. https://doi.org/10.18632/oncotarget.2991
28. Xu Q, Liu X, Zheng X, Yao Y, Liu Q (2014) PKM2 regulates Gli1 expression in hepatocellular carcinoma. Oncol Lett 8:1973–1979. https://doi.org/10.3892/ol.2014.2441
29. Fan FT, Shen CS, Tao L, Tian C, Liu ZG, Zhu ZJ, Liu YP, Pei CS, Wu HY, Zhang L, Wang AY, Zheng SZ, Huang SL, Lu Y (2014) PKM2 regulates hepatocellular carcinoma cell epithelial-mesenchymal transition and migration upon EGFR activation. Asian Pac J Cancer Prev 15:1961–1970
30. Liu WR, Tian MX, Yang LX, Lin YL, Jin L, Ding ZB, Shen YH, Peng YF, Gao DM, Zhou J, Qiu SJ, Dai Z, He R, Fan J, Shi YH (2015) PKM2 promotes metastasis by recruiting myeloid-derived suppressor cells and indicates poor prognosis for hepatocellular carcinoma. Oncotarget 6:846–861. https://doi.org/10.18632/oncotarget.2749
31. Liu Y, Wu H, Mei Y, Ding X, Yang X, Li C, Deng M, Gong J (2017) Clinicopathological and prognostic significance of PKM2 protein expression in cirrhotic hepatocellular carcinoma and non-cirrhotic hepatocellular carcinoma. Sci Rep 7:15294. https://doi.org/10.1038/s41598-017-14813-y