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Analysis of PKM2 Expression and Prognostic Significance in Liver Cancer Based on TCGA Database

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Abstract

Liver cancer is a malignant and invasive tumor with poor prognosis. Recent studies have shown that the expression of PKM2 is of great significance to cancer. The purpose of this study was to clarify the relationship between the expression of PKM2 and liver cancer and its effect on the prognosis of liver cancer. We downloaded the data of patients with liver cancer and their RNA-seq expression results from The Cancer Genome Atlas database and analyzed the expression of PKM2. We found the expression of PKM2 in liver cancer was significantly higher than that in the control group. There were differences in PKM2 expression among different histological types, histological grades, stages, T classification, vital status, age, gender, and residual tumors. ROC showed the modest diagnostic value of PKM2. According to Kaplan-Meier curve and subgroup analysis, PKM2 is associated with poor overall survival of liver cancer, especially in subgroup of stage I/II (P =0.0044) and histological G1/G2 (P = 0.00013), T1 (P = 0.041), N1 (P = 0.00068), male (P <0.0001), elder (P = 0.0021) and young (P = 0.0086). Univariate and multivariate analysis showed that high PKM2 expression is an independent prognostic factor of liver cancer. In conclusions, high PKM2 expression is an independent prognostic factor of liver cancer and may be a biomarker to evaluate the prognosis of liver cancer.

Keywords: Liver cancer; Biomarkers; PKM2; Prognosis; Diagnosis.

Abbreviations: ADP: Adenosine Diphosphate; AUC: Area Under The Curve; HCC: Hepatocellular Carcinoma; LIHC: Liver Hepatocellular Carcinoma Mixed, Hepatocholangiocarcinoma (Mixed); PEP: Phosphoenolpyruvate; PK: Pyruvate Kinase; PKM2: Pyruvate Kinase Type M2; ROC: Receiver-Operating Characteristic Curve

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Introduction

Liver cancer is a malignant and aggressive tumor which has different histological characteristics and a poor prognosis. As one of the few tumors with a steady increase in morbidity and mortality, liver cancer is the second leading cause of cancer-related deaths in humans in developing countries and the sixth leading cause in developed countries [1,2]. For patients with early-stage liver cancer, surgery, locally destructive treatment, and liver transplantation offer therapeutic potential. The improved of morphological heterogeneity of liver cancer has also promoted the development of targeted therapy [22]. However, these still cannot solve all problems. The treatment of liver cancer is still a serious social and medical problem. Therefore, identifying specific markers for evaluating liver cancer progression has important clinical significance.

Abnormal glucose metabolism is the key to the occurrence and development of cancer cells. Aerobic glycolysis is the main method of cancer cell metabolism [4,5]. Pyruvate kinase (PK) catalyzes the last physiologically irreversible step in glycolysis, the conversion of phosphoenolpyruvate (PEP) to pyruvate by transferring PEP to ADP [6]. In mammals, PK has four protein kinase subtypes encoded by two genes. PKLR gene encodes PKL or PKR subtype [4,7,8]. PKM1 and PKM2 are derived from PKM gene by mutually exclusive splicing of exon 9 and exon 10, respectively. By preserving exon 10, PKM2 has important unique properties in cell metabolic reprogramming and is regulated by complex allosterization, which has become the main competition between proliferative cells and cancer cells [4,9]. In most cancer cells, the expression of PKM2 is increased, suggesting that PKM2 may be an attractive target for cancer therapy [7]. It is worth noting that studies have confirmed that the expression level of PKM2 varies in different types of cancer[10]. However, whether PKM2 is highly expressed in liver cancer and whether it can become a specific marker of liver cancer remains to be further studied.

In this study, we evaluated the expression of PKM2 in liver cancer, analyzed the relationship between PKM2 expression and clinical features, and discussed the potential prognostic significance of PKM2 in patients with liver cancer.

Methods

Data mining and collection

We downloaded the clinical data and RNA-seq expression values of with liver cancer patients from TCGA database in R(version3.5.1) [11,12]. The RNA-seq by expectation-maximization expression values were used in the statistical analysis.

Statistical analysis

The data were analyzed by R software (version 3.6.1). The boxplots generated by ggplot2 package in R software (version 3.6.1) was used to describe the difference of mRNA expression among discrete variable groups. χ-2 and Fisher's exact tests were used to analyze the relationship between clinical features and PKM2 expression. The receiver-operating characteristic curve (ROC) was drawn by pROC software package to evaluate the diagnostic ability. According to the optimal cutoff value of vital status determined by the ROC, the patients were divided into two groups: high PKM2 expression group and low PKM2 expression group [13]. The differences of overall survival rate and relapse-free survival rate between low expression group and high expression group were compared by Kaplan-Meier curve, and the P value was calculated by log-rank test [14,15]. Univariate Cox analysis was used to screen related variables, and multivariate Cox analysis was used to evaluate the effect of PKM2 expression on overall survival and relapse-free survival. $P < 0.05$ was statistically significant.

Results

The characteristics of the patient

The gene expression and clinical data of patients with liver cancer were downloaded from the tumor genome map database, with a total of 373 people. Detailed clinical features such as disease classification, TNM stage, residual tumor, survival status, etc., are shown in Table 1.

Table 1: Correlation between the clinicopathologic variables and PKM2 Mrna expression in liver cancer.

Clinical characteristics	Variable	N	(%)	PKM2 mRNA expression					
				high	n (%)	low	n (%)	χ2	*P*
age	<55	117	31.37	41	35.34	76	29.69	0.9 372	0.33 3
	>=55	255	68.36	75	64.66	180	70.31		
gender	Female	121	32.44	49	42.24	72	28.02	6.7 454	0.00 94
	Male	252	67.56	67	57.76	185	71.98		
histological _type	Fibrolamellar Carcinoma	3	0.8	2	1.72	1	0.39	11 822 9	0.00 27
	Hepatocellular Carcinoma (Mixed)	363	97.32	108	93.1	255	99.22		
	Hepatocho langiocarcinoma (Mixed)	7	1.88	6	5.17	1	0.39		

histologic_g rade	G1	55	14.75	10	8.7	45	17.79	12 504 5	0.00 58
	G2	178	47.72	49	42.61	129	50.99		
	G3	123	32.98	52	45.22	71	28.06		
	G4	12	3.22	4	3.48	8	3.16		
stage	I	172	46.11	32	30.19	140	57.61	26 858 6	<0.0 001
	II	87	23.32	30	28.3	57	23.46		
	III	85	22.79	41	38.68	44	18.11		
	IV	5	1.34	3	2.83	2	0.82		
T_classification	T1	182	48.79	36	31.03	146	57.25	26 903 1	<0.0 001
	T2	95	25.47	35	30.17	60	23.53		
	T3	80	21.45	37	31.9	43	16.86		
	T4	13	3.49	8	6.9	5	1.96		
	TX	1	0.27	0	0	1	0.39		
N_classification	N0	253	67.83	77	66.96	176	68.48	3.6 797	0.19 02
	N1	4	1.07	3	2.61	1	0.39		
	NX	115	30.83	35	30.43	80	31.13		
M_classification	M0	267	71.58	84	72.41	183	71.21	0.8 187	0.66 53
	M1	4	1.072	2	1.72	2	0.78		
	MX	102	27.35	30	25.86	72	28.02		
radiation_therapy	NO	340	91.15	105	96.33	235	98.33	0.5 879	0.44 32
	YES	8	2.14	4	3.67	4	1.67		
residual_tumor	R0	326	87.4	93	81.58	233	92.46	11 512 7	0.00 7
	R1	17	4.56	8	7.02	9	3.57		
	R2	1	0.27	0	0	1	0.4		
	RX	22	5.9	13	11.4	9	3.57		
vital_status	DECEASE D	130	34.85	56	48.28	74	28.79	12 516 2	0.00 04
	LIVING	243	65.15	60	51.72	183	71.21		

High expression of PKM2 in liver cancer

We measured the difference in the expression of PKM2 mRNA between patients with liver cancer patients and normal controls, and expressed this result with boxplots. As shown in figure 1, the expression of PKM2 in patients with liver cancer patients is significantly higher than that in normal subjects (P=1.8e-08). There were also differences in PKM2 expression among different histological types (P=0.0025), histological grade (P=0.00013), stage (P <0.0035), T classification (P=9.6e-05), vital status (P=0.001), age (P=0.041), gender (P=0.0022) and residual tumor (P<0.0066). The expression of PKM2 increased gradually with the progress of disease stage and T classification. However, there was no significant correlation between the expression of PKM2 and N classification (P=0.32), M classification (P=0.65), and whether received radiotherapy (P=0.33).

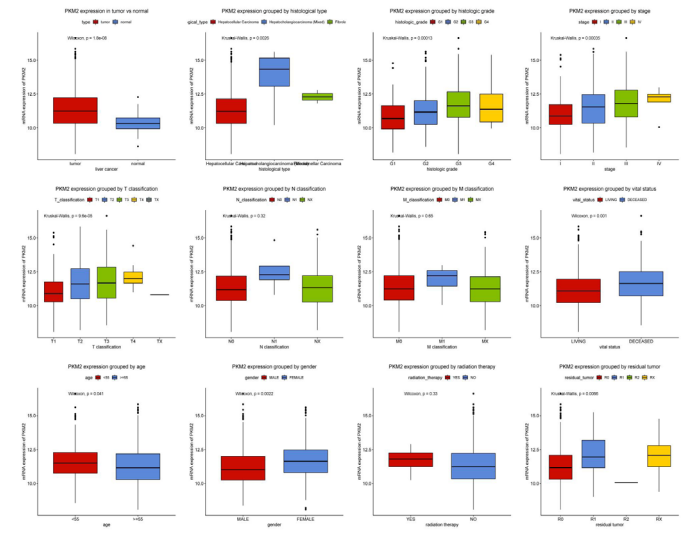

Figure 1: The different PKM2 expressions in the boxplot. The expression of PKM2 is grouped by type, histologic type, histologic grade, stage, T classification, N classification, M classification, and vital status, age, gender, radiation therapy, residual tumor.

Diagnostic capability of PKM2

As shown in the ROC in figure 2, and the area under the curve (AUC) is 0.745, indicating that PKM2 has moderate diagnostic ability. Subsequently, similar results were shown in the subgroup analysis of different stages. (AUC: 0.699 for stage I, 0.757 for stage II, 0.786 for stage III, 0.860 for stage IV).

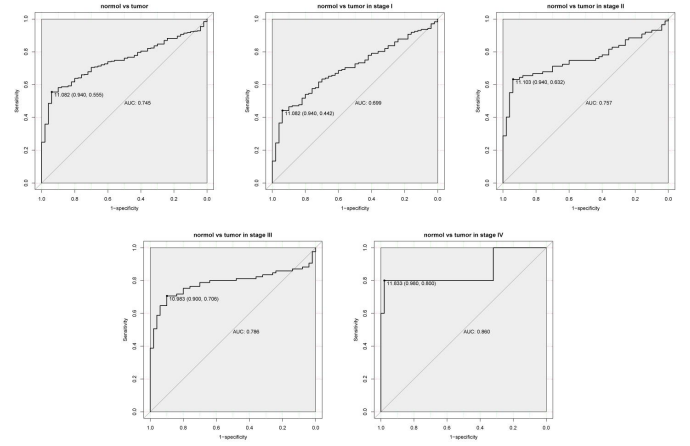

Figure 2: The ROC curve of PKM2 in LIHC cohort. Nontumor sample and tumor sample. Nontumor sample and tumor sample of stage I. Nontumor sample and tumor sample of stage II. Nontumor sample and tumor sample of stage III. Nontumor sample and tumor sample of stage IV.

Relationship between expression of PKM2 and clinical features

We analyzed the relationship between the expression of PKM2 and the clinical features of liver cancer. As shown in Table 1, we found that the high expression of PKM2 was closely related to gender (P=0.0094), histological type (P=0.0027), histological grade (P=0.0058), stages (P<0.01), T classification (P<0.01), residual tumor (P=0.007), vital status (P=0.0004) and overall survival (P=0.0003).

PKM2 expression is correlated with poor prognosis in liver cancer patients

To evaluate the effect of PKM2 expression on the prognosis of patients with liver cancer, we used Kaplan-Meier survival curve combined with log-rank test to evaluate the relationship between PKM2 expression and overall survival and relapse freesurvival. As shown in figure 3, there was a significant difference in overall survival between patients with PKM2 high expression and patients with low PKM2 expression (P<0.0001). Patients with high expression of PKM2 had poorer overall survival, but there was no significant difference in relapse-free survival (*P*=0.49).

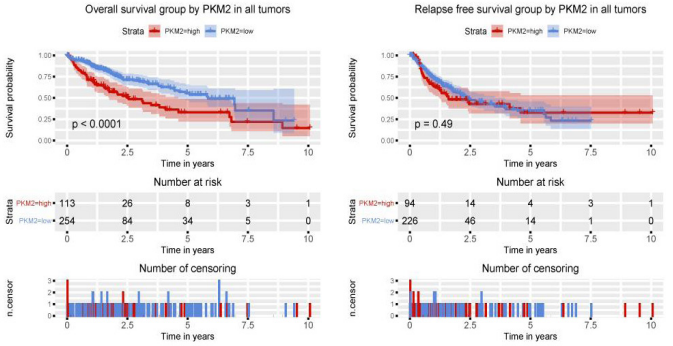

Figure 3: Kaplan–Meier curves produced survival analysis of PKM2 expression in terms of overall survival and Relapse free survival.

Subgroup analysis of the prognostic value of PKM2 expression in term of overall survival

To further explore the prognostic value of PKM2 in term of overall survival, we conducted a subgroup analysis. The results showed that patients with high PKM2 expression had poor overall survival in subgroup of stage I/II (P=0.0044) and histological G1/G2 (P=0.00013), T1 (P=0.041), N1 (P=0.00068), male (P<0.0001), elder (P=0.0021) and young (P=0.0086).

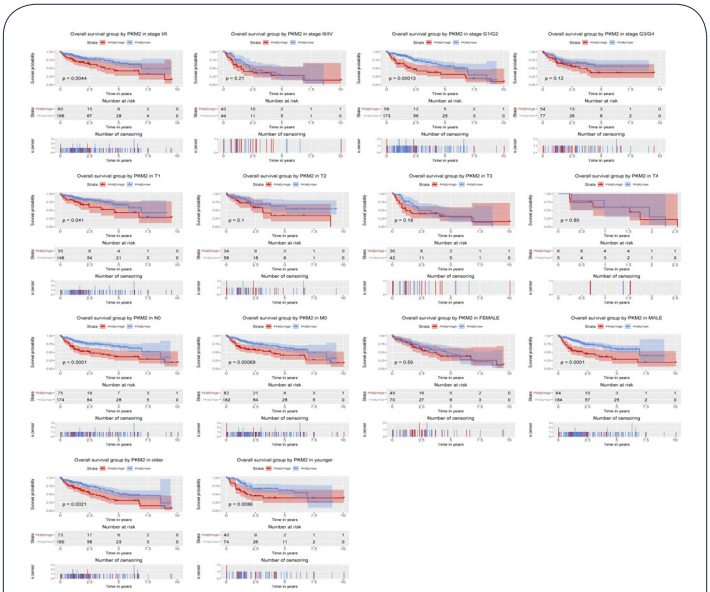

Figure 4: Survival analysis of PKM2 expression in terms of overall survival. Kaplan–Meier curves produced survival analysis of clinical stage (I/II and III/IV) and subgroup analysis of histological grade (G1/G2 and G3/G4), T classification (T1, T2, T3, and T4), N0, M0, female and male, and older and younger.

High PKM2 is an independent risk factor of liver cancer patients' overall survival

To select the potential variables associated with overall survival, we conducted a univariate analysis of key variables including PKM2 expression, clinical stage, TNM classification, residual tumors, etc. Multivariate analysis with the cox proportional hazard model showed that T classification (HR=1.790, 95%CI: 1.412-2.270, P<0.001), residual tumor (HR=1.341, 95%CI: 1.043-1.723, P<0.022) and PKM2 expression (HR=1.594, 95%CI: 1.106-2.297, P<0.012) were independent risk factors affecting the overall survival of liver cancer patients (Figure 5).

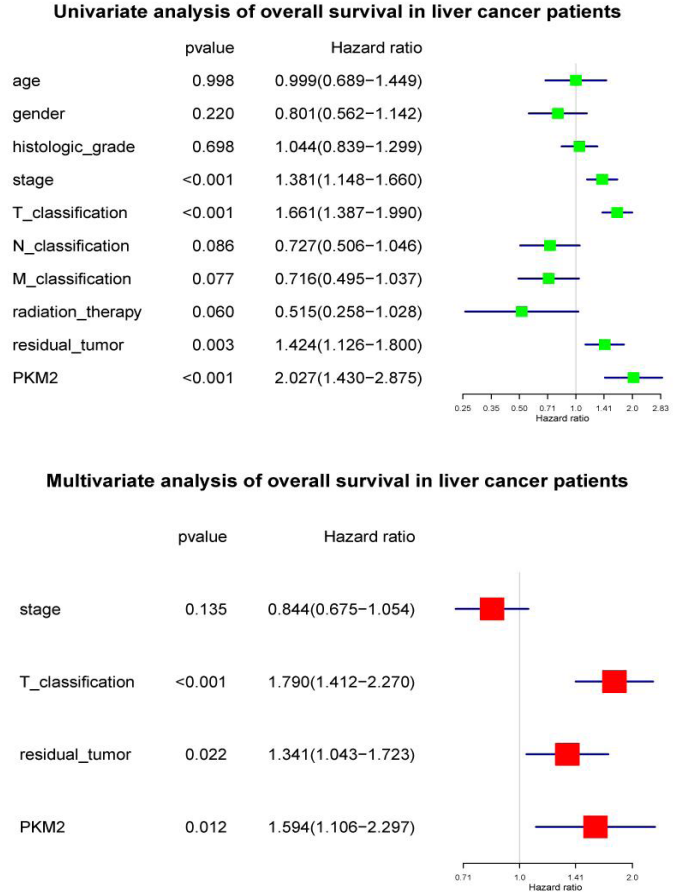

Figure 5: Univariate analysis and Multivariate analysis of overall survival in liver cancer patients. Statistically significant, *P* <0.05.

Subgroup analysis of the prognostic value of PKM2 expression in term of relapse free survival

Although the high expression of PKM2 had no significant effect on the relapse-free survival of liver cancer patients, we conducted a subgroup analysis to mining its prognostic value in specific population. The results showed no significant in subgroup such as stage, histological grade, TNM classification, gender, and age.

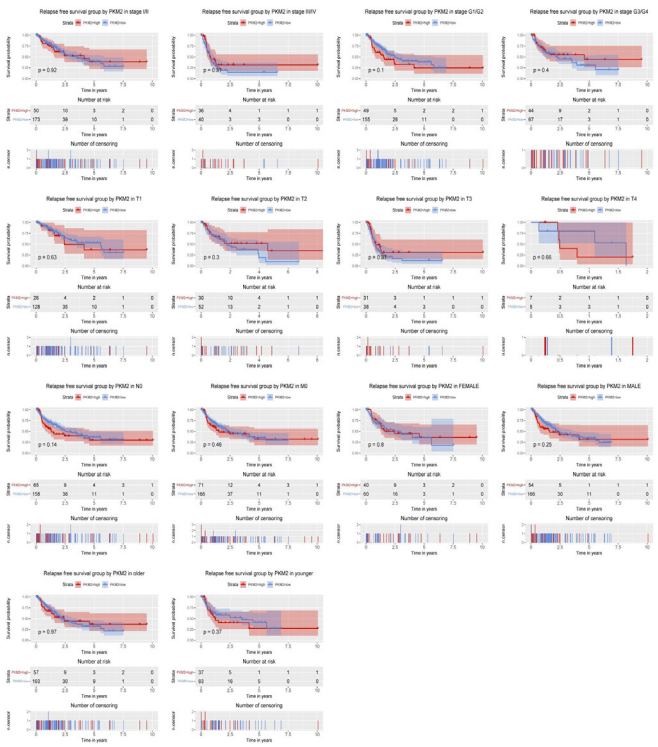

Figure 6: Survival analysis of PKM2 expression in terms of relapse-free survival. Kaplan–Meier curves produced survival analysis of clinical stage (I/II and III/IV) and subgroup analysis of histological grade (G1/G2 and G3/G4), T classification (T1, T2, T3, and T4), N0, M0, female and male, older and younger.

High PKM2 is not an independent risk factor of liver cancer patients' relapse free survival

To select the potential variables associated with overall survival, we conducted a univariate analysis of key variables including PKM2 expression, clinical stage, TNM classification, residual tumors, etc. Further multivariate cox analysis showed that T classification (HR=1.635, 95%CI: 1.256-2.126, P<0.001) and residual tumors (HR=1.334, 95%CI: 1.053-1.691, P=0.017) were independent factors affecting relapse-free survival (Figure 7).

Discussion

In this study, we confirmed the role of PKM2 in liver cancer. PKM2 is highly expressed in patients with liver cancer, which is closely related to gender, histological type, histologic grade, stage, T classification and residual tumor. At the same time, we proved the relationship between the high expression of PKM2 and the prognosis of liver cancer, we consider that PKM2 can be used as a biomarker to evaluate the prognosis of liver cancer.

Aerobic glycolysis is a common feature of tumor tissue. As a rate-limiting enzyme that catalyzes the last step of glycolysis, PKM2 has attracted much attention in tumor research in recent years. In recent years, a large number of studies have shown that PKM2 is preferentially expressed in malignant tumors and plays an important role in cancer cell proliferation and tumor growth [16]. Inhibiting the activity of PKM2, destroying the stability of PKM2 structure can reduce the glycolysis activity of PKM2, which inhibit the development and formation of tumor [17]. The results of this study show that the expression of PKM2 in patients with liver cancer is significantly higher than that in the normal group,

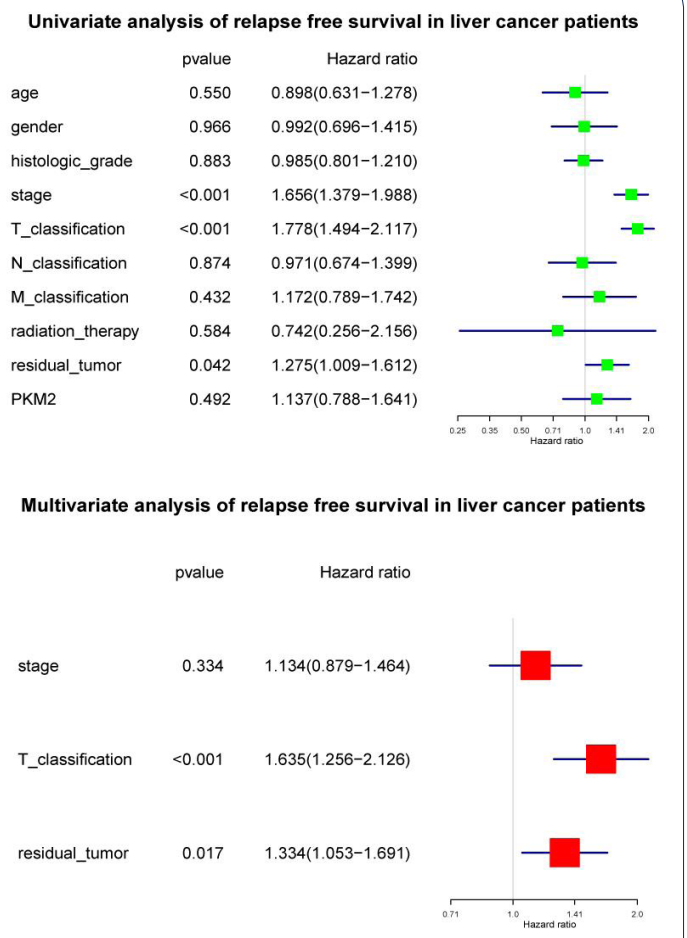

Figure 7: Univariate analysis and Multivariate analysis of relapse-free survival in liver cancer patients. Statistically significant, P <0.05.

which fully illustrates the role of PKM2 in the proliferation of liver cancer cells, which is also consistent with the results of other tumor studies. In addition, the expression of PKM2 is different in different histological types of liver cancer, and the expression of PKM2 is the highest in cholangiocarcinoma, suggesting that it may be related to the occurrence of liver cancer. At the same time, with the development of disease stage and T classification, the expression of PKM2 gradually increased, indicating that PKM2 is closely related to the progression of liver cancer, and the increase of PKM2 activity can make cancer cells produce more energy for utilization. The expression of PKM2 is higher in patients less than 55 years old, which may be related to the regulation of cancer metabolism. Interestingly, the expression of PKM2 is higher in male patients, and the relationship between its expression and gender can be further explored.

Studies have shown that PKM2 can be used as a biomarker of renal and testicular cancer and has a particular diagnostic value [18-21]. According to the results of ROC curve of PKM2, PKM2 also shows reasonable diagnostic ability for liver cancer, and is expected to be combined with other markers in the diagnosis of liver cancer in the future.

PKM2 plays an important role in the prognosis of patients with liver cancer. According to the results of the study, we found that patients with high expression of PKM2 had a lower overall survival rate. It is especially obvious in stage I / II and G1 /G2, which provide corresponding help for the targeted therapy of liver cancer in the future. The high expression of PKM2 has a significant effect on the overall survival rate of male patients, but not in female patients. The complex interaction of PKM2 in genitourinary and endocrine system needs to be further studied. At the same time, PKM2 is an independent risk factor affecting the prognosis of liver cancer, which provides evidence for PKM2 to become a biomarker of liver cancer.

This study confirmed the close relationship between the expression of PKM2 and liver cancer, and explored the important role of PKM2 in the prognosis. It provides a new idea for the diagnosis and prognosis of liver cancer. However, the role of PKM2 and related mechanism is complex, more in-depth research is needed in the future.

Conclusion

PKM2 is highly expressed in patients with liver cancer and is related to a variety of clinical features. High PKM2 expression is an independent risk factor affecting the prognosis of liver cancer, and can be used as a biomarker to evaluate the prognosis of patients with liver cancer.

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Conflicts of interest: All authors have completed the ICMJE uniform disclosure form. The authors have no conflicts of interest to declare.

Ethical statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

References

1. Li L, Wang H. Heterogeneity of liver cancer and personalized therapy. Cancer Lett. 2016; 379: 191-197.
2. Liu CY, Chen KF, Chen PJ. Treatment of Liver Cancer. Cold Spring HarbPerspect Med. 2015; 5: a021535.
3. Sia D, Villanueva A, Friedman SL, Llovet JM. Liver Cancer Cell of Origin,Molecular Class, and Effects on Patient Prognosis. Gastroenterology. 2017; 152: 745-761.
4. Zhou Y, Liu K, Liu Y, Tan L. MicroRNA-34a inhibit hepatocellular carcinoma progression by repressing hexokinase-1. Journal of cellular biochemistry. 2018.
5. Liu M, Wang Y, Ruan Y, Bai C,Qiu L et al. PKM2 promotes reductive glutamine metabolism. Cancer Biol Med. 2018;15: 389-399.
6. Dayton TL, Jacks T, Vander Heiden MG. PKM2, cancer metabolism, and the road ahead. EMBO Rep. 2016; 17: 1721-1730.
7. Dong G, Mao Q, Xia W, XU Y, WANG J, et al. PKM2 and cancer: The function of PKM2 beyond glycolysis. Oncol Lett. 2016; 11: 1980-1986.
8. Zhang Z, Deng X, Liu Y, Liu Y, Sun L, Chen F. PKM2, function and expression and regulation. Cell Biosci. 2019; 9: 52.
9. Wong N, Ojo D, Yan J, Tang D. PKM2 contributes to cancer metabolism. Cancer Lett. 2015; 356: 184-191.

10. Azoitei N, Becher A, Steinestel K, Rouhi A, Diepold K, et al. PKM2 promotes tumor angiogenesis by regulating HIF-1alpha through NF-kappaB activation. Mol Cancer. 2016; 15: 3.

11. Samur MK. RTCGAToolbox: a new tool for exporting TCGA Firehose data. PLoS One. 2014; 9: e106397.

12. Team RC. R: A language and environment for statistical computing. MSOR connections. 2014; 1.

13. Robin X, Turck N, Hainard A, Tiberti N, Lisacek F, et al. pROC: an open-source package for R and S+ to analyze and compare ROC curves. BMC Bioinformatics. 2011; 12: 77.

14. Therneau TM. A Package for Survival Analysis in S. 1999.

15. Lin H, Zelterman D. Modeling Survival Data: Extending the Cox Model. Technometrics. 2002; 44: 85-86.

16. Zhou Z, Li M, Zhang L, Zhao H, Şahin O, et al. Oncogenic Kinase-Induced PKM2 Tyrosine 105 Phosphorylation Converts Nononcogenic PKM2 to a Tumor Promoter and Induces Cancer Stem-like Cells. Cancer Res. 2018;78: 2248-2261.

17. Lv L, Li D, Zhao D, Lin R, Chu Y, et al. Acetylation targets the M2 isoform of pyruvate kinase for degradation through chaperone-mediated autophagy and promotes tumor growth. Mol Cell. 2011; 42: 719-730.

18. Weinberger R, Appel B, Stein A, Metz Y, Neheman A, et al. The pyruvate kinase isoenzyme M2 (Tu M2-PK) as a tumour marker for renal cell carcinoma. Eur J Cancer Care (Engl). 2007;16: 333-337.

19. Wechsel HW, Petri E, Bichler KH, Feil G. Marker for renal cell carcinoma (RCC): the dimeric form of pyruvate kinase type M2 (Tu M2-PK). Anticancer Research. 1999; 19: 2583-2590.

20. Pottek T, Muller M, Blum T, Hartmann M. Tu-M2-PK in the blood of testicular and cubital veins in men with testicular cancer. Anticancer Research. 2000; 20: 5029-5033.

21. Eigenbrodt E, Basenau D, Holthusen S, Mazurek S, Fischer G. Quantification of tumor type M2 pyruvate kinase (Tu M2-PK) in human carcinomas. Anticancer Research. 1997; 17: 3153-3156.