

# Expression of pyruvate kinase M2 in human bladder cancer and its correlation with clinical parameters and prognosis

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**Background:** Pyruvate kinase M2 (PKM2) is a key regulator of the Warburg effect and has critical functions in glycolysis, contributing to the Warburg effect, tumor growth, angiogenesis, cell division, metastasis, and apoptosis. The high expression of PKM2 in various solid tumors renders it a potential biomarker of tumorigenesis and tumor invasion, but the expression and role of PKM2 in bladder cancer have not been studied extensively.

**Patients and methods:** Western blot and immunohistochemistry (IHC) were used to measure the expression of PKM2, and quantitative real-time polymerase chain reaction (PCR) was performed to determine PKM2 mRNA levels. The relationships between PKM2 expression and clinicopathological parameters and prognosis were analyzed using the Kaplan–Meier plots and a Cox proportional hazards regression model.

**Results:** Compared with paired adjacent normal bladder tissues, PKM2 mRNA and protein levels were found to be higher in urothelial carcinoma of the bladder (UCB) samples by real-time PCR and Western blot. By IHC, high expression of PKM2 was seen in 117 of 215 UCBs (54.4%) and in eight of 90 adjacent normal bladder tissues (8.9%). The expression of PKM2 was significantly associated with grade, stage, and lymph node status ( $P < 0.001$ ). In the univariate survival analysis, a significant association between PKM2 expression and shorter patient survival was observed ( $P < 0.001$ ). In different subsets of UCB patients, we found that PKM2 expression was a prognostic factor in patients with G2 ( $P = 0.009$ ), G3 ( $P < 0.001$ ), pTa/pTis ( $P = 0.006$ ), pT1, pT2–4, and pN– disease ( $P < 0.001$ ). Importantly, PKM2 expression ( $P = 0.003$ ), with tumor histological grade ( $P < 0.001$ ), pT ( $P < 0.001$ ), and pN status ( $P = 0.005$ ), was a significant independent prognostic parameter in the multivariate analysis.

**Conclusion:** PKM2 protein and mRNA are upregulated in UCBs and may serve as molecular markers for a poor prognosis in patients with UCB.

**Keywords:** bladder, urothelial carcinoma, PKM2, prognosis, IHC

## Introduction

Bladder cancer (BCa) is one of the most common malignancies of the urinary tract, of which urothelial carcinoma of the bladder (UCB) is the most frequent histological subtype. Over the past several decades, the incidence of BCa has increased; the incidence of BCa in males and females in developing countries was 16.9/100,000 and 3.7/100,000, respectively, in 2012.<sup>1</sup> Well-recognized tools for predicting the prognosis include clinical staging and pathological grading, while it is difficult to explain why patients with BCa of the same stage and grade have different prognoses. There are few molecular markers that predict the prognosis of BCa.

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Increased conversion of glucose to lactate is a key feature of many cancer cells, which promotes rapid growth. The type L and R pyruvate kinase isoenzymes are encoded by the same gene but are under the control of different promoters.<sup>2–8</sup> The type M1 and M2 pyruvate kinase isoenzymes are different splice products from the same mRNA transcript, resulting in a difference of 21 amino acids.<sup>9,10</sup> Pyruvate kinase M2 (PKM2) has been proposed to be a candidate oncogene that is upregulated in several cancers, including renal cell carcinoma (RCC),<sup>11,12</sup> ovarian cancer,<sup>13,14</sup> breast cancer,<sup>15,16</sup> and lung cancer.<sup>17</sup>

In this study, quantitative real-time polymerase chain reaction (PCR), Western blot, and immunohistochemistry (IHC) were used to detect the expression of PKM2 in tumor and adjacent tissues. In addition, the relationship between the expression of PKM2 and clinicopathological parameters was analyzed. Kaplan–Meier plots and a Cox proportional hazards regression model were used for the univariate and multivariate survival analyses.

## Patients and methods

### Patients and samples

For the qRT-PCR and Western blot analysis, 10 paired fresh UCB tissues and normal bladder tissues adjacent to the tumor (defined as >2.0 cm from the tumor edge) were collected from consecutive patients with UCB who underwent surgery in the Department of Urology, The Second Xiangya Hospital, Central South University (Changsha, People's Republic of China) from January 2012 to November 2012. In addition, 215 formalin-fixed, paraffin-embedded UCB tissues, diagnosed by the Departments of Pathology and Urology, The Second Xiangya Hospital, Central South University (Changsha, People's Republic of China), were retrieved. We selected cases according to the typical pathological diagnosis of UCB, without chemotherapy or radiotherapy prior to surgery.

Each patient was staged according to the 2009 The Union for International Cancer Control staging system.<sup>18</sup> In brief, the 215 patients included 145 males and 70 females, with a median age of 62 years (range: 20–88). The median follow-up time for overall survival (OS) was 62 months. Ninety-nine patients underwent radical cystectomy and 116 underwent transurethral resection of the bladder tumor. The clinicopathological features of the 215 patients are summarized in Table 1. Written informed consent was obtained from the patients for use of their tissue specimens and medical data, and the study protocol was approved by the Institutional Research Ethics Committee of Second Xiangya Hospital, Central South University.

**Table 1** Correlation between PKM2 expression and clinicopathological characteristics of UCB patients

Characteristics	PKM2 protein			P-value <sup>a</sup>
	Total cases	Low	High	
Age (years)				0.273
≤65 <sup>b</sup>	113	56	57	
>65	102	42	60	
Gender				0.465
Male	145	69	76	
Female	70	29	41	
Histological grade				<0.001
G1	71	55	16	
G2	62	29	33	
G3	82	14	68	
pT classification				<0.001
pTa/pTis	61	42	19	
pT1	61	33	28	
pT2–4	93	23	70	
pN classification				<0.001
pN <sup>-</sup>	169	95	74	
pN <sup>+</sup>	46	3	43	
Tumor size (cm)				0.336
≤3 <sup>c</sup>	119	58	61	
>3	96	40	56	
Tumor multiplicity				0.132
Unifocal	110	56	54	
Multifocal	105	42	63	

**Notes:** <sup>a</sup>Chi-square test. <sup>b</sup>Median age. <sup>c</sup>Mean size.

**Abbreviations:** PKM2, pyruvate kinase M2; UCB, urothelial carcinoma of the bladder.

### real-time PCR analysis

Total RNA was isolated using TRizol reagent (Thermo Fisher Scientific, Waltham, MA, USA). RNA was reverse-transcribed to cDNA using the RevertAid™ H Minus First Strand cDNA Synthesis Kit (Thermo Fisher Scientific) according to the manufacturer's instructions. The qRT-PCR was performed using SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA) in a total reaction volume of 25 µL on an ABI PRISM 7900HT Sequence Detection System (Applied Biosystems) as follows: denaturation for 5 minutes at 95°C; 40 cycles of denaturation for 20 seconds at 94°C, annealing for 20 seconds at 61°C, extension for 20 seconds at 72°C; and a final extension for 5 minutes at 72°C. The specificity of the amplified products was determined by melting curve analysis. The relative expression levels of mRNA were calculated by the 2<sup>-ΔΔCt</sup> method and expressed as relative quantification values, normalized to β-actin. Experiments were repeated in triplicate. The primers used in this study were as follows: PKM2 forward (5'-ATTATTTGAGGAACTCCGCCGCT-3') and reverse (5'-ATTCCGGGTCACAGCAATGATGG-3); β-actin forward (5'-CATTAAGGAGAAGCTGTGCT-3') and reverse (5'-GTTGAAGGTAGTTTCGTGGA-3').

## Western blot

Tissue proteins were lysed in radioimmunoprecipitation assay buffer, and the total protein concentration was determined by the Bradford method: 20  $\mu$ g of protein was loaded onto 8% denaturing Sodium dodecyl sulfate polyacrylamide gel electrophoresis for electrophoresis. The blots were probed with the primary antibodies overnight at 4°C, followed by incubation with the appropriate secondary antibodies at room temperature for 1 hour. PKM2 rabbit monoclonal anti-PKM2 (1:1,000; Cell Signaling Technology, Danvers, MA, USA) and goat anti-rabbit (1:1,000; Santa Cruz Biotechnology, Dallas, TX, CA) antibodies were used to detect PKM2 protein.

## Immunohistochemical staining

Deparaffinized and rehydrated tissue sections were pre-treated with 3% peroxidase in methanol for 10 minutes at room temperature. Antigen retrieval was performed by boiling the sections in citrate buffer (pH 6.0) for 30 minutes. After preincubation with the blocking buffer (10% normal goat serum in PBS) for 1 hour at room temperature, the sections were incubated with PKM2 (diluted 1:200) (Cat:4053; Cell Signaling Technology) overnight at 4°C, followed by incubation with the secondary antibody (KIT-5010, MaxVision™) for 1 hour. PKM2 signals were visualized by 3,3'-diaminobenzidine staining.

Each section was evaluated independently by two pathologists. Each slide was observed in five fields of view, with each field of view magnified 400 times to observe 100 cells. The expression of PKM2 was scored according to staining intensity and extent. The absence of reactivity was considered to be negative. According to the percentage of positively stained area, the sections were given scores of 0 (0%), 1 (1%–25%), 2 (26%–50%), 3 (51%–75%), and 4 points (76%–100%). Also, according to the degree of staining, the samples received a score of 0 (negative: no staining), 1 (weak positive: light yellow), 2 (medium positive: brown), or 3 points (strong positive: tan). Both scores were added to yield the final score (0–7 points). Final scores >3 indicated high expression and those  $\leq$ 3 indicated low expression.<sup>19</sup>

## Statistical analyses

All statistical analyses were performed with SPSS 17.0. The association of PKM2 expression with clinicopathological features of the BCa patients was assessed using the  $\chi^2$  test. Survival was analyzed by the Kaplan–Meier method. Log-rank test was used to compare different survival curves. Univariate and multivariate survival analyses were performed using the Cox proportional hazards regression model.

Differences were considered to be significant if the *P*-value by the two-tailed test was <0.05.

## Results

### Expression of PKM2 mRNA and protein in paired UCB and adjacent tissues

The relative expression of PKM2 mRNA in UCB and adjacent normal tissues was  $5.878 \pm 1.346$  and  $3.683 \pm 0.7561$ , respectively ( $P < 0.001$ ) (Figure 1J). The relative expression of PKM2 protein in BCa tissues was  $1.347 \pm 0.576$ , which was significantly higher than in adjacent normal tissues ( $0.642 \pm 0.255$ ) ( $P < 0.001$ ) (Figures 1I–1K).

### Expression of PKM2 in UCBs by IHC

We further examined the expression of PKM2 protein in 215 paraffin-embedded UCB tissues and 90 matched normal tissues by IHC. PKM2 expression was substantially upregulated in tumor tissues compared with normal tissues. Of all UCB tissues, 54.4% (117/215) showed high expression of PKM2, compared with 8.9% (8/90) of controls. Positivity for PKM2 occurred primarily in the cytoplasm (Figure 1A–H and Table 2).

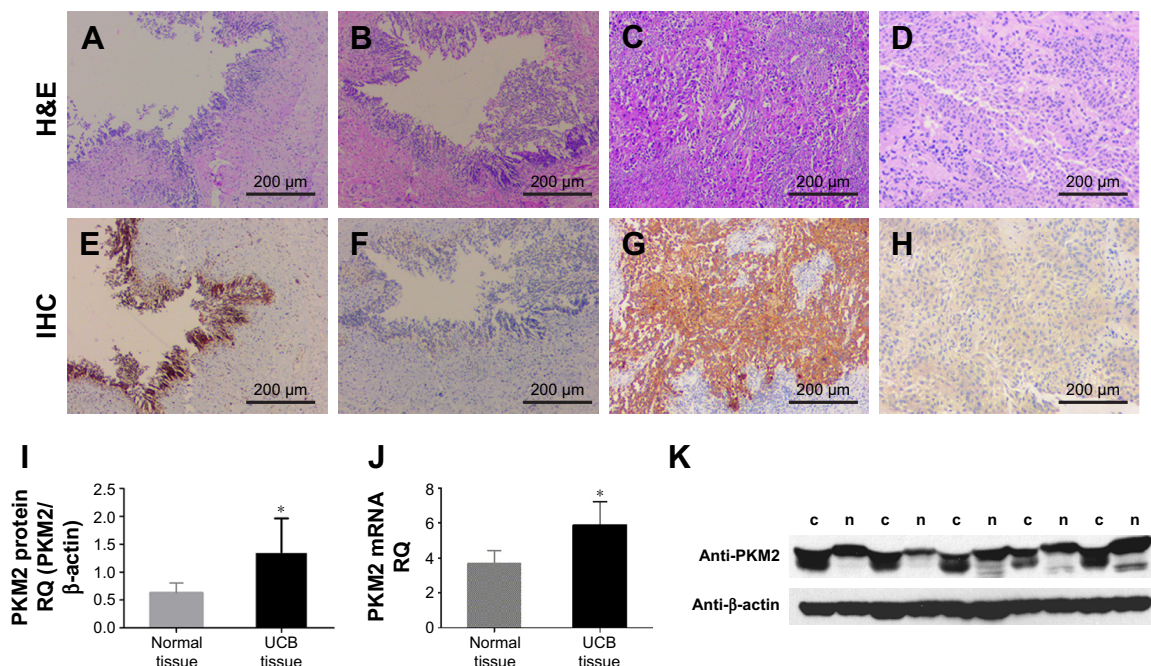
### Correlation between PKM2 expression and clinicopathological features

The relationship between the clinicopathological characteristics of UCB patients and PKM2 levels was analyzed. Only three factors – T classification ( $P < 0.001$ ), histological grade ( $P < 0.001$ ), and N classification ( $P < 0.001$ ) – were significantly associated with high expression of PKM2 (Table 1).

### Relationship between clinicopathological features, PKM2 expression, and UCB patient survival: univariate survival analysis

To determine the clinical significance of PKM2 expression in UCB patients, survival curves were created, and we found that overexpression of PKM2 correlated with a poor prognosis in the UCB patients in this study. Both the OS and disease-free survival (DFS) rates of UCB patients with low expression of PKM2 were higher than those of high expressors (Figures 2 and 3; both  $P < 0.001$ ). The univariate Cox proportional hazards regression model demonstrated that patients with high PKM2 expression had a greater risk of death than those with low expression (hazard ratio [HR]=0.132, 95% confidence interval [CI]: 0.073–0.239,  $P < 0.001$ ) (Table 2).

Furthermore, PKM2 expression was demonstrated to be a prognostic factor for OS in UCB patients with G2 ( $P = 0.009$ ),



**Figure 1** Expression of PKM2 in UCBs and adjacent normal bladder tissues. **Notes:** (A–D) Representative PKM2 and H&E stains are shown. Positivity for PKM2 was observed primarily in the cytoplasm. (A and B) Adjacent normal bladder tissues observed by H&E staining (×40). (C and D) Bladder cancer tissues observed by H&E staining (×40). (E) High and (F) low expression of PKM2 in adjacent normal bladder tissues observed by IHC (×40). (G) High and (H) low expression of PKM2 in bladder cancer tissues observed by IHC (×40). (I and K) Relative expression of PKM2 protein detected by Western blot in 10 UCB cases. Expression levels were normalized to β-actin. (J) Relative expression of PKM2 mRNA detected by real-time-PCR in 10 UCB cases. \**P*<0.001. **Abbreviations:** PKM2, pyruvate kinase M2; UCB, urothelial carcinoma of the bladder; H&E, hematoxylin and eosin; IHC, immunohistochemistry; PCR, polymerase chain reaction; RQ, relative quantification; c, bladder cancer tissues; n, normal bladder tissues.

**Table 2** Univariate analysis of different prognostic factors in 215 patients with UCB

Characteristics	Total cases	HR (95% CI)	P-value
Age (years)			0.802
≤65	113	1	
>65	102	0.948 (0.626–1.436)	
Gender			0.06
Male	145	1	
Female	70	0.559 (0.366–0.854)	
Histological grade			<0.001
G1	71	1	
G2	62	0.032 (0.010–0.101)	
G3	82	0.348 (0.243–0.607)	
pT classification			<0.001
pTa/pTis	61	1	
pT1	61	0.032 (0.010–0.103)	
pT2–4	93	0.240 (0.141–0.409)	
pN classification			<0.001
pN <sup>-</sup>	169	1	
pN <sup>+</sup>	46	0.130 (0.083–0.203)	
Tumor size (cm)			0.155
≤3	119	1	
>3	96	0.741 (0.489–1.122)	
Tumor multiplicity			0.095
Unifocal	110	1	
Multifocal	105	0.937 (0.619–1.417)	
PKM2 expression			<0.001
Low	98	1	
High	117	0.132 (0.073–0.239)	

**Abbreviations:** UCB, urothelial carcinoma of the bladder; HR, hazard ratio; CI, confidence interval; PKM2, pyruvate kinase M2.

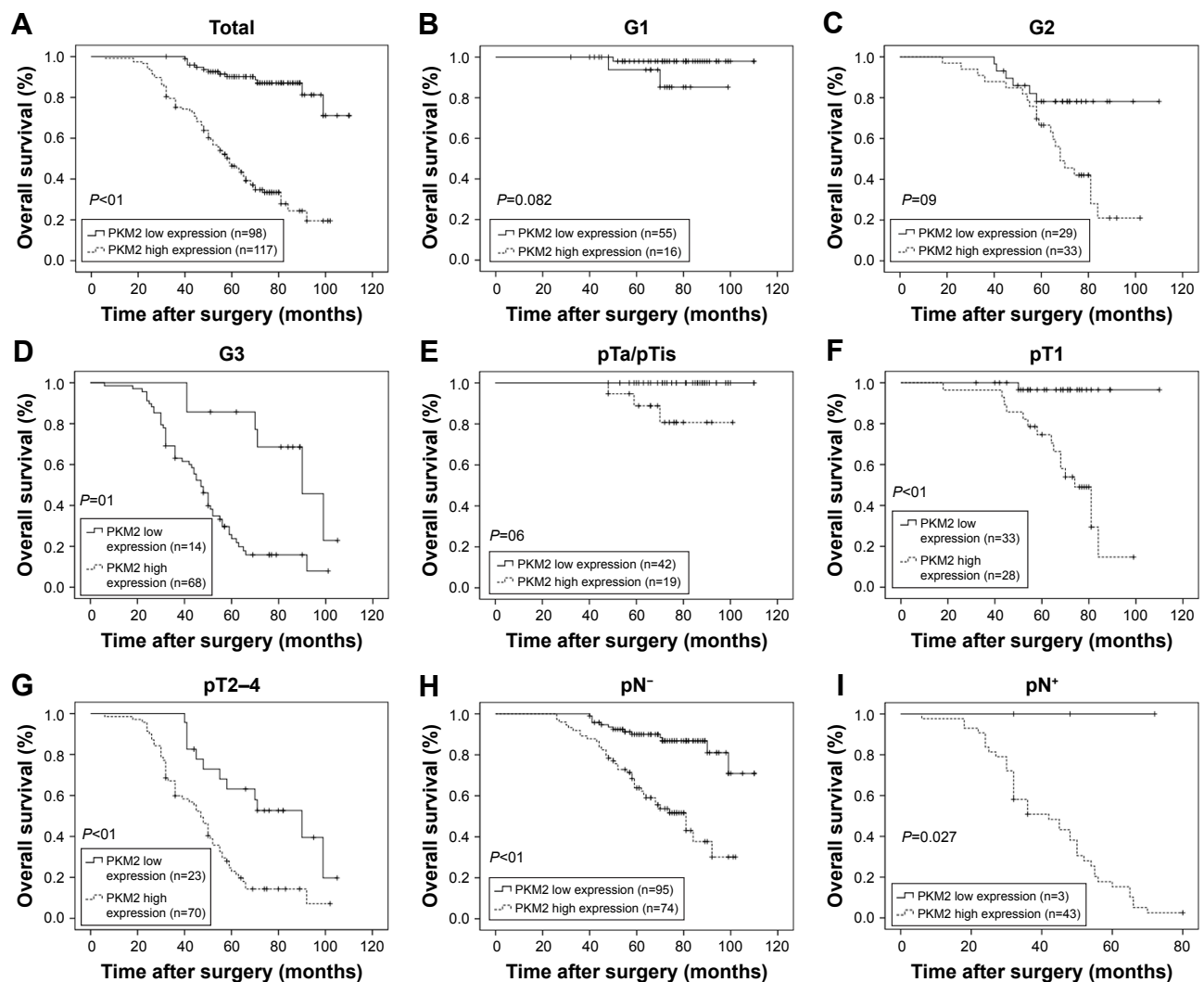
G3 (*P*=0.001), pTa/pTis (*P*=0.006), pT1 (*P*<0.001), pT2–4 (*P*<0.001), N<sup>-</sup> (*P*<0.001), and pN<sup>+</sup> disease (*P*<0.027) (Figure 2). The univariate analysis indicated that OS was related to histological grade (*P*<0.001), T classification (*P*<0.001), N classification (*P*<0.001), and expression of PKM2 (*P*<0.001).

PKM2 expression was also confirmed to be a prognostic factor for DFS in UCB patients with G1 (*P*<0.001), G2 (*P*=0.006), G3 (*P*<0.001), pTa/pTis (*P*<0.001), pT1 (*P*<0.001), pT2–4 (*P*<0.001), and pN<sup>-</sup> disease (*P*<0.001) (Figure 3).

### Multivariate analysis using Cox proportional hazards regression model for independent prognostic factors

In the multivariate analysis, when controlling for the effects of standard clinicopathological parameters, the expression of PKM2 was found to be an independent prognostic factor of OS (HR=3.197, 95% CI: 1.682–6.076, *P*<0.001) (Table 3). Moreover, we also confirmed histological grade (HR=2.446, 95% CI: 1.671–3.579, *P*<0.001), T classification (HR=2.867, 95% CI: 1.901–4.323, *P*<0.001), and N classification (HR=2.013, 95% CI: 1.236–3.279, *P*=0.005) to be independent predictors of OS in patients with UCB (Table 3).





**Figure 2** Kaplan-Meier survival analysis of PKM2 expression in patients with UCB (log-rank test).

**Notes:** Patients with high expression of PKM2 were significantly associated with poorer overall survival ( $P<0.001$ ). **(A)** Total, the probability of survival of all patients with UCB: low expression (solid line),  $n=98$ ; high expression (dashed line),  $n=117$ . **(B)** G1, the probability of survival of G1 patients with UCB: low expression (solid line),  $n=55$ ; high expression (dashed line),  $n=16$ . **(C)** G2, the probability of survival of G2 patients with UCB: low expression (solid line),  $n=29$ ; high expression (dashed line),  $n=33$ . **(D)** G3, the probability of survival of G3 patients with UCB: low expression (solid line),  $n=14$ ; high expression (dashed line),  $n=68$ . **(E)** pTa/pTis, probability of survival of pTa/pTis patients with UCB: low expression (solid line),  $n=42$ ; high expression (dashed line),  $n=19$ . **(F)** pT1, the probability of survival of pT1 patients with UCB: low expression (solid line),  $n=33$ ; high expression (dashed line),  $n=28$ . **(G)** pT2-4, probability of survival of pT2-4 patients with UCB: low expression (solid line),  $n=23$ ; high expression (dashed line),  $n=70$ . **(H)** pN<sup>-</sup>, the probability of survival of pN<sup>-</sup> patients with UCB: low expression (solid line),  $n=95$ ; high expression (dashed line),  $n=74$ . **(I)** pN<sup>+</sup>, probability of survival of pN<sup>+</sup> patients with UCB: low expression (solid line),  $n=3$ ; high expression (dashed line),  $n=43$ .

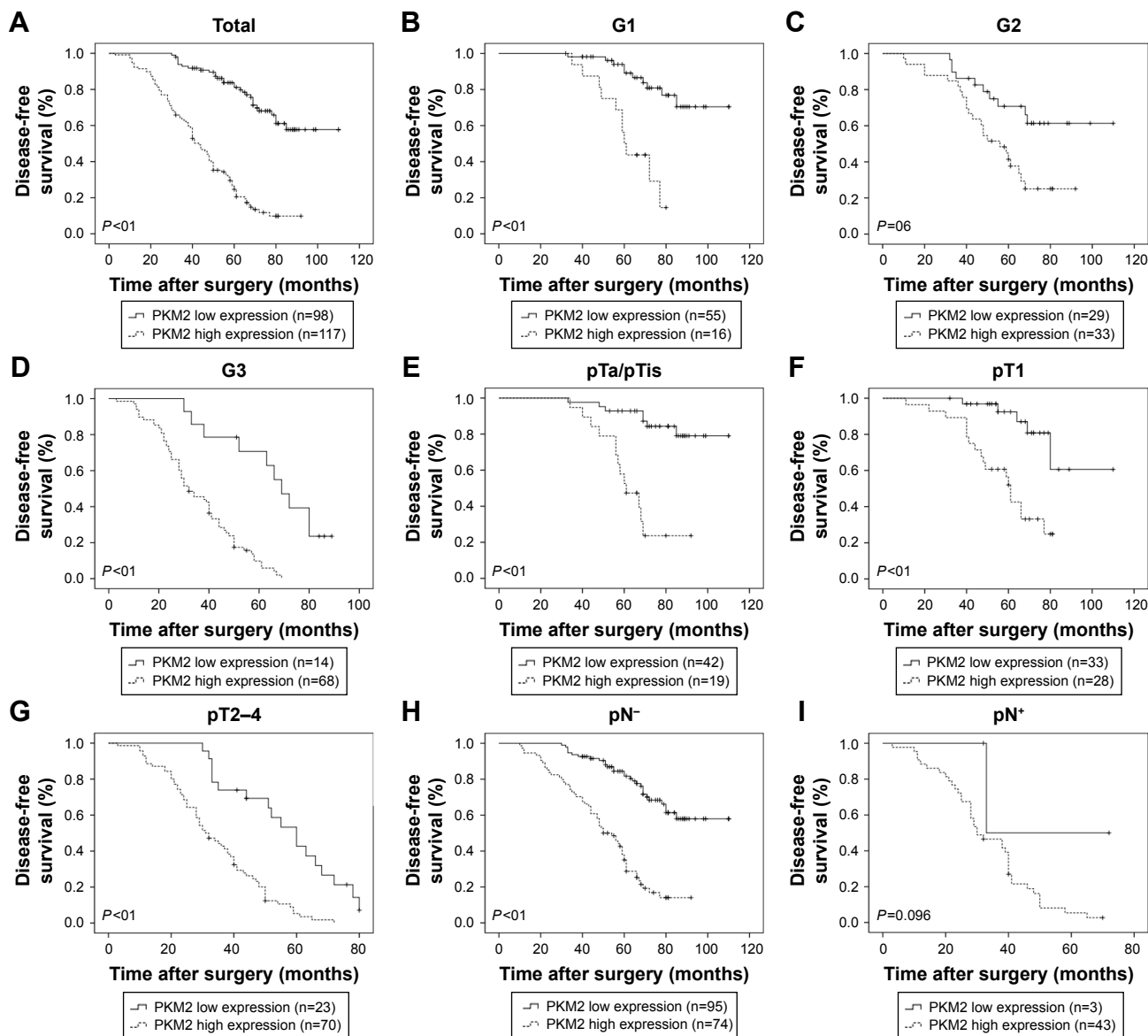
**Abbreviations:** PKM2, pyruvate kinase M2; UCB, urothelial carcinoma of the bladder.

## Discussion

PKM2, an isoform of pyruvate kinase, is abundantly expressed in rapidly proliferating tissues, such as embryonic tissues and tumors, and in differentiated tissues.<sup>20</sup> The metabolism of many tumor cells is characterized by elevated glucose uptake but a lower rate of oxidative phosphorylation. This persistence of high lactate production by tumors in the presence of oxygen, known as aerobic glycolysis, was first noted by Warburg >75 years ago.<sup>21</sup> Compared with normal cells, tumor cells give priority to anaerobic oxidation, even under sufficient oxygen, resulting in greater lactic acid production, which is mediated by PKM2 in many tumor cells. PKM2

is necessary for the shift in cellular metabolism to aerobic glycolysis, which promotes tumorigenesis, cell division, apoptosis, and metastasis.<sup>22,23</sup> Several types of tumor cells have been shown to express the embryonic M2 isoform of pyruvate kinase exclusively.<sup>24,25</sup>

Overexpression of PKM2 is closely associated with many human cancers, such as RCC,<sup>11,26</sup> ovarian cancer,<sup>13,14</sup> breast cancer,<sup>15</sup> and lung cancer.<sup>17</sup> Bluemlein et al found that PKM2 is highly expressed in BCa by mass spectrometry.<sup>27</sup> In the current study, the expression of PKM2 was examined in BCa tissues. Collectively, these findings suggest a potential oncogenic role of PKM2 in BCa.



**Figure 3** Kaplan–Meier disease-free survival analysis of PKM2 expression in patients with UCB (log-rank test).

**Note:** Patients with high expression of PKM2 were significantly associated with poorer disease-free survival ( $P < 0.001$ ). **(A)** Total, the probability of survival of all patients with UCB: low expression (solid line),  $n = 98$ ; high expression (dashed line),  $n = 117$ . **(B)** G1, the probability of survival of G1 patients with UCB: low expression (solid line),  $n = 55$ ; high expression (dashed line),  $n = 16$ . **(C)** G2, the probability of survival of G2 patients with UCB: low expression (solid line),  $n = 29$ ; high expression (dashed line),  $n = 33$ . **(D)** G3, the probability of survival of G3 patients with UCB: low expression (solid line),  $n = 14$ ; high expression (dashed line),  $n = 68$ . **(E)** pTa/pTis, probability of survival of pTa/pTis patients with UCB: low expression (solid line),  $n = 42$ ; high expression (dashed line),  $n = 19$ . **(F)** pT1, the probability of survival of pT1 patients with UCB: low expression (solid line),  $n = 33$ ; high expression (dashed line),  $n = 28$ . **(G)** pT2–4, probability of survival of pT2–4 patients with UCB: low expression (solid line),  $n = 23$ ; high expression (dashed line),  $n = 70$ . **(H)** pN<sup>-</sup>, the probability of survival of pN<sup>-</sup> patients with UCB: low expression (solid line),  $n = 95$ ; high expression (dashed line),  $n = 74$ . **(I)** pN<sup>+</sup>, probability of survival of pN<sup>+</sup> patients with UCB: low expression (solid line),  $n = 3$ ; high expression (dashed line),  $n = 43$ .

**Abbreviations:** PKM2, pyruvate kinase M2; UCB, urothelial carcinoma of the bladder.

**Table 3** Multivariate analysis of overall patient survival (Cox regression model)

Characteristics	HR	95% CI	P-value
PKM2 expression (low vs high)	3.197	1.682–6.076	<0.001
Histological grade (G1 vs G2 vs G3)	2.446	1.671–3.579	<0.001
pT classification (pTa/pTis vs pT1 vs pT2–4)	2.867	1.901–4.323	<0.001
pN classification (pN <sup>-</sup> vs pN <sup>+</sup> )	2.013	1.236–3.279	0.005
Tumor size (cm) ( $\leq 3$ vs $> 3$ )	1.235	0.728–1.692	0.331

**Abbreviations:** HR, hazard ratio; CI, confidence interval; PKM2, pyruvate kinase M2.

Previous studies have demonstrated that PKM2 is a critical oncogene in various human cancers. Papadaki et al analyzed the expression of PKM2 in tumor samples from 148 and 157 non-small-cell lung cancer (NSCLC) patients treated with front-line platinum-based chemotherapy in the training and validation sets, respectively. In addition, 85 NSCLC patients treated with non-platinum-containing regimens were included as a control group. In the validation and training sets, high PKM2 mRNA levels were associated with decreased

progression-free survival (PFS), OS, and disease control rate (all  $P < 0.05$ ) compared with patients with low PKM2 levels. No correlation was found between PKM2 mRNA levels and PFS or OS in the control group. Multivariate analysis revealed that high PKM2 mRNA expression was an independent predictive factor of a poor outcome.<sup>28</sup>

Lim et al analyzed PKM2 expression in gastric cancer using gene expression microarrays and tissue microarrays. Their results revealed that PKM2 expression was significantly increased in primary gastric cancers compared with adjacent normal tissues from the same patients. Furthermore, PKM2 expression was associated with shorter OS ( $P < 0.05$ ).<sup>29</sup> Chao et al evaluated PKM2 expression in ovarian cancer, reporting that patients with higher PKM2 levels correlated significantly with malignancy groups ( $P < 0.001$ ) and pathogenesis models ( $P < 0.001$ ) and had poor PFS ( $P = 0.01$ ) compared with patients exhibiting lower PKM2 levels.<sup>30</sup>

In this study, we collected 10 UCB tissues. The expression of PKM2 in UCB tissues was detected by Western blot, qRT-PCR, and IHC. The results showed that the protein and mRNA levels of PKM2 in UCBs were significantly higher than in the control group. By analyzing the relationship between PKM2 protein and mRNA expression and clinicopathological features, we found that the expression of PKM2 correlated positively with the clinical stage and tumor grade of UCB patients. Furthermore, patients with high PKM2 expression had a poor prognosis. The results demonstrate that PKM2 is a potentially valuable biomarker that predicts recurrence and survival after surgical resection and that upregulation of PKM2 may provide a selective advantage in UCB tumorigenesis.

Obtaining sufficient energy is essential for cell survival. Cells grow primarily by glycolysis and oxidative phosphorylation, the latter of which is more efficient – in normal tissues, ~90% of ATP is derived from oxidative phosphorylation vs 10% from glycolysis.<sup>31</sup> In the presence of oxygen, most mature differentiated cells mainly metabolize glucose into carbon dioxide by oxidation of glycolytic pyruvate in the mitochondrial tricarboxylic acid cycle, maximizing energy production with little lactic acid. Only in the absence of oxygen is pyruvate converted into lactic acid by lactate dehydrogenase. However, regardless of whether they are under aerobic or anaerobic conditions, tumor cells consume large amounts of glucose and produce significant amounts of lactic acid.

PKM2 can directly or indirectly regulate the proliferation, apoptosis, and metastasis of tumor cells.<sup>22</sup> PKM2 regulates tumor angiogenesis through two mechanisms: 1) enhancement of endothelial cell proliferation, migration, and cell–extracellular matrix adhesion;<sup>8,32</sup> and 2) a PKM2/NF- $\kappa$ B/miR-148a/152 feedback loop.<sup>33</sup> With regard to apoptosis,

PKM2 phosphorylates Bcl2 to prevent the binding of Cul3-based E3 ligase to Bcl2, leading to its degradation and the inhibition of apoptosis.<sup>34</sup> Concerning tumor metastasis, PKM2 is highly associated with STS-induced epithelial–mesenchymal transition, which is related to the migration and invasion of cancer cells.<sup>35</sup>

It has been reported that the concentration of blood PKM2 is associated with the sensitivity of lung cancer to chemotherapy drugs.<sup>36</sup> The serum level of PKM2 in RCC patients was higher than in the healthy control group, and the serum level of PKM2 correlated with stage and metastasis status in RCC patients. Serum PKM2 levels decreased to normal after radical nephrectomy, whereas in cases of recurrence or metastasis, they rose again, indicating that serum PKM2 level is a biomarker for surveillance, recurrence, and metastasis in RCC.<sup>26,37</sup> Therefore, PKM2 measurement is a useful tool for the diagnosis of cancer and may be used as a diagnostic marker for tumors and a marker of drug efficacy and tumor recurrence in RCC. We determined PKM2 protein and mRNA levels in BCa tissues and found that this marker was associated with TNM staging and pathological grading. Thus, PKM2 may serve as a biomarker for BCa and contribute to its progressive features and poor prognosis.

Overall, this study has several limitations, including its retrospective design and the small cohort of cases from a single center. Furthermore, because of the complicated mechanism of UCB, more studies are needed to elucidate the specific mechanism of PKM2 in UCB.

## Conclusion

The expression of PKM2 protein and mRNA is increased significantly in UCB and correlates positively with histological grade and TNM staging. PKM2 may be involved in BCa growth and metastasis and may serve as a tumor marker for the degree of malignancy of BCa.

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## Disclosure

The authors report no conflicts of interest in this work.

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