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Original Article

Expression of PGAM1 in renal clear cell carcinoma and its clinical significance

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Abstract: Objective: This study is aimed to evaluate the expression of phosphoglycerate mutase 1 (PGAM1) in normal kidney and clear cell renal cell carcinoma (CCRCC), also to evaluate the correlation between PGAM1 expression and clinicopathological features in CCRCC. Methods: PGAM1 expression was detected in 80 cases of normal kidney and 192 cases of CCRCC by immunohistochemistry (IHC). Meanwhile, PGAM1 expression measured in 8 cases of CCRCC and matched normal kidney tissues by Western blot. Then, the correlation between PGAM1 expression and clinicalpathological features was analyzed in CCRCC. Results: IHC results exhibited that the high-expression rate of PGAM1 in CCRCC tissues was 45.8%, which was significantly higher than those in normal kidney tissues (32.5%, P=0.044). Meanwhile, PGAM1 expression in CCRCC was significantly greater compared with those in normal kidney by Western blot. Moreover, PGAM1 expression was significantly associated with age, tumor size and T stage in CCRCC. Conclusion: PGAM1 is highly expressed in CCRCC and correlated with clinicalpathological features, which may contribute to tumor formation and progression.

Keywords: Clear cell renal cell carcinoma, PGAM1, clinicalpathological features, IHC, molecular marker

Introduction

Clear cell renal cell carcinoma (CCRCC) is the most common histological subtype of renal cell carcinoma (RCC), accounting for 75%-85% of cases [1, 2]. CCRCC is difficult to be accurately diagnosed in its early stages and is not sensitive to conventional chemotherapy and radiotherapy [3, 4]. Thus, CCRCC patients have poorer prognosis. The 5-year survival rate of renal cancer is about 55%, but the 5-year survival rate of metastatic renal cell carcinoma is only about 10% [5]. The 5-year survival rate of CCRCC is approximately 5%-15% [6]. Therefore, it is valuable to identify novel potential markers for the early diagnosis and prognostic evaluation in CCRCC.

Phosphoglycerate mutase 1 (PGAM1) is an important enzyme that catalyzes the conversion of 3-phosphoglycerate (3-PG) into 2-phosphoglycerate (2-PG) in glycolysis [7]. Studies have demonstrated that PGAM1 is highly expressed in different types of human cancers, such as hepatocellular carcinoma, oral squamous cell carcinoma, non-small cell lung cancer and colorectal cancer [8-11]. Meanwhile, studies have also found that PGAM1 is involved in tumor growth [12, 13]. Therefore, these studies suggest PGAM1 may be a potential target for cancer therapy. However, the correlation between PGAM1 and CCRCC remains unknown. Here, we evaluate the expression of PGAM1 in CCRCC tissues and analyze its correlation with clinicalpathological features.

Materials and methods

Patients

All samples were obtained from Women and Children's Hospital of Foshan and Nanfang hospital during the year of 2011-2014. 192 cases of CCRCC tissues without lymph node metasta-

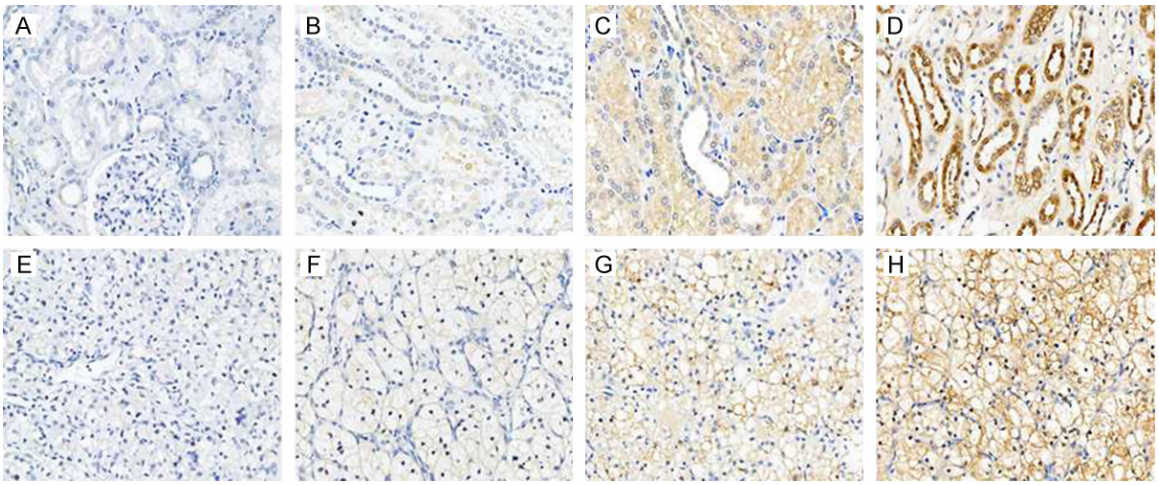

Figure 1. The expression of PGAM1 in normal kidney and CCRCC tissues (40×). A. Negative expression in Normal kidney tissue; B. Weak expression in Normal kidney tissue; C. Moderate expression in Normal kidney tissue; D. Strong expression in Normal kidney tissue; E. Negative expression in CCRCC tissue; F. Weak expression in CCRCC tissue; G. Moderate expression in CCRCC tissue; H. Strong expression in CCRCC tissue.

Table 1. PGAM1 expression in CCRCC tissues and normal tissues

Types	N	PGAM1 expression				*P* values
		Negative	Weak positivity	Moderate positivity	Strong positivity	
CCRCC tissues	192	59	45	71	17	0.017
Normal tissues	80	20	34	20	6	

Table 2. PGAM1 expression in CCRCC tissues and normal tissues

Types	N	Low-expression	High-expression	*P* values
CCRCC tissues	192	104	88	0.044
Normal tissues	80	54	26	

sis and distant metastasis and 80 cases of matched normal kidney tissues were enrolled in this study. All CCRCC patients didn't receive radiotherapy and chemotherapy. CCRCC patients including 60 cases of male and 132 cases of female were aged from 26 to 84 years (mean age, 57 years). Patients' clinicalpathological features including tumor size, tumor location, T stage, tumor location and smoke history were obtained from hospitalized records. Histological diagnosis was confirmed by three pathologists. This study was supported by the Ethnics Committee of Women and Children's Hospital of Foshan and Nanfang hospital.

Immunohistochemical staining

Tissues were fixed with 10% formalin and embedded in paraffin. Tissue sections were cut into 4 μm thickness and dewaxed by xylene. Citrate buffer (pH 6.0) was used for antigen retrieval using autoclave sterilizer method. All sections were incubated in 3% H_2O_2 for 10 min at room temperature to block endogenous peroxidase activity. Then, sections were blocked by fetal bovine serum (FBS) for 15 min, followed by an incubation for 2 hour at room temperature with anti-PGAM1 antibody, (diluted 1:200, Abcam, Cambridge, UK). After rinse in phosphate buffered saline (PBS) for 10min, sections were incubated with secondary biotinylated antibody (Jingqiao, Beijing, China) for 30 min at room temperature. Subsequently, sections were reacted with 3,3'-diaminobenzidine substrate solution (Jingqiao, Beijing, China) and counterstained with hematoxylin. The immunohistochemical staining was evaluated by the percentage and intensity of positive cells. The percentage of positive cells was recorded as 0 (<5%), 1 (6-25%), 2 (26-50%), 3 (51%-75%) and 4 (>75%). The staining intensity of positive cells was record as 0 (negative), 1 (weakly positive), 2 (moderately positive) and 3 (strongly positive). Total score (0-12) was calculated by multiplying the two parameters. When the total score of each section was greater than or equal to 6, it would be considered as high-expression. While

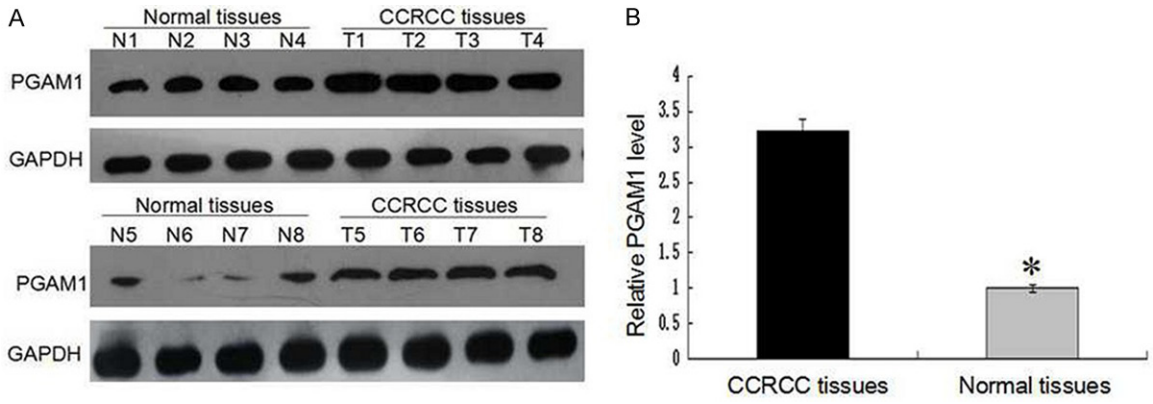

Figure 2. PGAM1 expression in CCRCC and matched normal kidney tissues was quantitatively detected by Western blot. A. Western blot results showed that PGAM1 expression in CCRCC was increased compared with those in normal kidney tissues. B. Statistical analysis of PGAM1 expression in CCRCC and matched normal kidney tissues. *P<0.05

Table 3. Correlation between PGAM1 expression and clinicopathological features

Clinicopathological features	N	Low-expression	High-expression	*P* value
Gender				
Male	60	27	33	0.898
Female	132	77	55	
Age				
>57	92	47	12	0.005
≤57	100	57	43	
Tumor size				
<5 cm	134	80	54	0.027
≥5 cm	58	24	34	
T stage				
T1	112	70	42	0.008
T2-T3	80	34	46	
Tumor location				
Left	102	54	48	0.251
Right	90	50	40	
Smoke history				
Positive	52	24	28	0.195
Negative	140	80	60	

the total score of each section was less than 6, it would be regarded as low-expression. Sections were reviewed separately by two pathologists in a double-blinded manner.

Western blotting

Total protein was extracted from froze fresh tissues by protein lysates and inhibitors. Protein concentration was quantified by bicinchoninic acid (BCA) assay method. A total of 50 mg protein lysates were separated by 10% SDS-PAGE and electrotransferred to polyvinylidene fluoride membranes (ImmobilonP; Millipore, Bedford, MA, USA). Then, membranes was blocked in 5% skimmed milk for 2 h at room temperature. Membranes were immunoblotted with anti-PGAM1 monoclonal antibody (Abcam, Cambridge, UK) and anti-GAPDH monoclonal antibody overnight at 4°C (Abcam, Cambridge, UK), followed by secondary antibodies. Signals were developed by enhanced chemiluminescence (Pierce, Rockford, IL, USA).

Statistical analysis

All data were analyzed by SPSS 19.0 (SPSS, Chicago, IL, USA). The correlation between PGAM1 expression and clinicalpathological features was analyzed by χ^2 Test. The difference of PGAM1 expression in normal kidney and CCRCC tissues was analyzed with independent samples *t* test. P<0.05 was regarded as statistically significant.

Results

PGAM1 expression in normal kidney and CCRCC tissues

PGAM1 expression was detected in 192 cases of CCRCC and 80 cases of matched normal kidney tissues by IHC. Positive PGAM1 expression was observed in the cytoplasm of tumor and

normal cells (**Figure 1**). In CCRCC tissues, 45 cases (23.4%) with weak positivity, 71 cases (37.0%) with moderate positivity and 17 cases (8.9%) with strong positivity were observed, however, 59 cases (30.7%) didn't exhibit positive PGAM1 expression. In normal tissues, 34 cases (42.5%) with weak positivity, 20 cases (25.0%) with moderate positivity and 6 cases (7.5%) with strong positivity were recorded, and 20 cases (25.0%) didn't exhibit positive PGAM1 expression. A significant difference was observed in PGAM1 expression between normal tissues and CCRCC tissues (P=0.017, **Table 1**). In addition, the high-expression rate of PGAM1 in CCRCC tissues was 45.8%, which was significantly greater than those in normal tissues (P=0.044, **Table 2**). Moreover, in order to further investigate the expression of PGAM1, we quantitatively detected the expression of PGAM1 in 8 cases of CCRCC and matched normal tissues by Western blot assay. The results were showed in **Figure 2**, indicating that PGAM1 expression was markedly elevated in CCRCC tissues compared with those in normal tissues.

Clinical significance of PGAM1 expression in CCRCC

Subsequently, the correlation between PGAM1 expression and clinicalpathological features in CCRCC was assessed (**Table 3**). A significant association between PGAM1 expression and age, tumor size and T stage was observed. Patients less than age 57 years exhibited markedly higher level of PGAM1 than those aged greater than or equal to 57 years (P=0.005). Patients with stage T2-T3 also showed greater level of PGAM1 compared with those with stage T1 (P=0.008). Meanwhile, significant increase of PGAM1 was recorded in tumors tissues larger than 5 cm in diameter (P=0.027). However, PGAM1 expression was not significantly associated with gender, tumor location and smoke history (P>0.05).

Discussion

PGAM1 is an important enzyme in the glycolysis pathway. Nowadays, studies demonstrate that PGAM1 is upregulated in many cancer tissues, which may be implicated in cancer cell glycolysis [10-12]. However, the relationship between PGAM1 and CCRCC is unclear. In this study, we detected the expression of PGAM1 in normal kidney tissues and CCRCC tissues without lymph node metastasis and distant metastasis. IHC results exhibited that positive PGAM1 expression was found in both normal kidney and CCRCC, but the high-expression rate of PGAM1 in CCRCC was significantly higher compared with those in normal kidney. Meanwhile, significant increase of PGAM1 in CCRCC was validated by Western blot assay. These observations suggested that PGAM1 was expressed in normal kidney and might be implicated in renal glycolysis. Also, PGAM1 served as a major isozyme was found in normal brain and liver tissues [14]. However, PGAM1 high-expression might result in abnormal glycolysis and contribute to the formation of CCRCC. Also, PGAM1 was reported to be highly elevated in hepatocellular carcinoma, oral squamous cell carcinoma, non-small cell lung cancer, colorectal cancer and glioma tissues in comparison with adjacent normal tissues [8-11, 15]. Thus, our results were consistent with these reports and confirmed that PGAM1 was correlated with tumor formation. PGAM1 High-expression might be beneficial for the early diagnosis of CCRCC.

Subsequently, we evaluated the correlation between PGAM1 expression and clinicalpathological features in CCRCC. The results demonstrated that PGAM1 expression was associated with age, which was consistent with the observation in human testis [16]. In addition, PGAM1 expression was found to be positively correlated with tumor size, indicating that PGAM1 expression might be contributed to cell proliferation. Hitosugi et al. reported that PGAM1 coordinated glycolysis and biosynthesis to promote cell proliferation and tumor growth [12]. Ren et al. reported that PGAM1 down-regulation significantly inhibited cell growth in hepatocellular carcinoma [8]. Vander et al. reported that PGAM1 activity was higher in rapidly proliferating cells [17]. Moreover, our results revealed that patients with stage T2-T3 exhibited higher level of PGAM1 compared with those with stage T1, suggesting that PGAM1 expression might be correlated with the progression of CCRCC. Meanwhile, PGAM1 expression was reported to be correlated with the grade and patients' prognosis in glioma [15]. In hepatocellular carcinoma, PGAM1 was reversely correlated with patients' survival rates and could be served as an independent

prognostic factor [8]. Therefore, our observations were in line with the current reports, suggesting that PGAM1 might be related to patients' prognosis in CCRCC.

In conclusion, our observations demonstrate that PGAM1 is highly expressed in CCRCC and correlated with age, tumor size and T stage, which may contribute to tumor formation and progression. Our study may be beneficial for investigating the potential function and mechanism of PGAM1 in CCRCC. Also, our study further validates that the potential therapeutic implications of PGAM1 for cancer treatment. However, some limitations in this study need to be mentioned. For one thing, samples size is not large to exclude selection bias. For another, the relationship between PGAM1 expression and patients' prognosis remains unknown because of patients without follow-up data. Therefore, further studies are needed to validate our observations.

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Disclosure of conflict of interest

None.

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