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Expression and correlation of PBRM1 and P53 in clear cell carcinoma of kidney

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Prevalence of clear cell renal cell carcinoma (ccRCC) among human population though common among adults, it occurs in children and young adults as well. The prognostic value of P53 expression in ccRCC is well known. Recently, PBRM1 has also acquired attention for its prognostic and predictive value in ccRCC. Here, we investigated the expression and correlation of PBRM1 and P53 in ccRCC. Renal tissues were collected from 70 patients who have undergone radical nephrectomy for clear cell carcinoma of the kidney in our hospital and 24 healthy volunteers for the study. We used immunohistochemical approach to determine the expression of PBRM1 and P53 in clear cell carcinoma of the kidney and normal kidney tissues and to analyze the correlation between them. Clinicopathological parameters and prognosis of patients were also studied. The positive expression rate of PBRM1 in clear renal cell carcinoma tissues was significantly higher (62.86%) compared to the normal renal tissues 8.33%. Similarly, positive expression rate of P53 in clear renal cell carcinoma tissues was 40%, while it was no expression in normal renal tissues. The expression level of PBRM1 was correlated with pathological grade and clinical stage of ccRCC patients, but not with age, sex and tumor size. P53 and expression levels were independent of age, sex, tumor size, pathological grade, and clinical stage of patients with clear cell carcinoma of the kidney. The 5-year survival rate of PBRM1 positive expression patients was 40.91% significantly lower than that of PBRM1 negative expression patients (84.62%), whereas in P53 it was 50 and 61.90%, respectively. Clinical stage, pathological grade and PBRM1 were all independent risk factors affecting the prognosis of patients with clear cell carcinoma of the kidney. Overall, the results suggest that PBRM1 is positively correlated with P53 in clear cell carcinoma of kidney (r=0.781, *P*=0.012). PBRM1 and P53 are both highly expressed in ccRCC and play an important role in the development of the disease. PBRM1 can also be used as an independent risk factor affecting the prognosis of ccRCC patients.

Keywords: Clear cell Renal carcinoma, Kidney cancer

Clear cell renal cell carcinoma (ccRCC) is a pathological type of kidney cancer, accounting for about 85% of kidney cancer. With the extension of human life span and the continuous improvement of diagnostic methods, its incidence has been increasing year by year[1]. Globally, 0.431 million people are affected by kidney cancer, and approximately 0.179 million people have succumbed to this disease in 2020[2]. The early symptoms of clear cell carcinoma of the kidney are mainly systemic, such as fever and fatigue, which are not specific and easy to be ignored, and are more than found in physical examination[3,4]. Reports show that about 25% of the patients in the diagnosis for the first time there has been a tumor volume increase, to some extent, diffusion and transfer, more performance for blood in the urine and kidney area pain and other symptoms, it belongs to a type of malignant degree is low in the kidney, but it's in clinical practice with more granular cell carcinoma, spindle cell carcinoma, lead to the microscope classification is more difficult[5,6]. Most clear cell carcinoma of the kidney is treated by radical nephrectomy, but the rate of tumor metastasis with blood transport is high. Once lymph node metastasis occurs, the survival time of most patients is less than 5 years, and the prognosis is worse if metastasis to important organs occurs[7].

Renal clear cell carcinoma development process is complex, often recognized as tumor for its genetic disorders or tumor-suppressor gene inactivation closely linked. However, the occurrence and development of its relevant molecular biology foundation has not been elucidated till now[8].. Relevant studies have shown that PBRM1 is abnormally expressed in breast cancer and has the function of tumor inhibition. Other studies have shown that P53 gene mutation is related to the biological behaviour of renal cell carcinoma to a certain extent[9,10]. Therefore, in the present study, we explored the

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correlation between PBRM1 and P53 by detecting their expressions in clear cell carcinoma of the kidney.

Materials and Methods

A total of 70 cases of cancer tissues were removed by radical nephrectomy from December 2018 to January 2019 in our hospital were selected; and 24 cases of normal renal tissue from healthy volunteers were collected. Inclusion criteria: (i) Age 6-18; (ii) clear cell carcinoma of the kidney was diagnosed by pathological examination and professional pathologist; (iii) all had complete clinicopathological data; (iv) all knew the content of this study and signed the informed consent; and (v) all met the approval requirements of the ethics committee of our hospital. Exclusion criteria: (i) patients with malignant tumor; and (ii) chemoradiotherapy. By age, 36 cases were aged 6 years and below, and 34 cases were aged over 6 years. By sex, there were 30 males and 40 females. According to the tumor size, 35 cases were 5 cm or below, 35 cases were above 5c, and 25 cases were stage I~II and 45 cases were stage III~IV according to clinical stages. According to pathological classification, there were 46 cases of G1, 12 cases of G2 and 12 cases of G3.

Materials

Rabbit anti-human PBRM1 and P53 were purchased from Shanghai Huangring Biotechnology Co., Ltd. Immunohistochemical kit was purchased from Changzhoubeiyuanxin Biotechnology Co., Ltd. Goat serum working fluid was purchased from Anhuijingke Biotechnology Co., Ltd. PBS powder was purchased from Beijing Kailiki Biotechnology Co., Ltd. Neutral gum was purchased from Shanghai Yihui Biotechnology Co., Ltd. Citric acid antigen repair buffer solution was purchased from Beijing Solebo Technology Co., Ltd.

Paraffin slicing machine was purchased from Shenzhenruiwode Life Technology Co., Ltd. Table high speed cryogenic centrifuge purchased from Hangzhouaosheng Instrument Co., Ltd. Booth sheet baking machine was purchased from Hubeihuida Instrument co., Ltd. Anti-stripping machine was purchased from Shanghai Aladdin Biochemical Technology Co., Ltd.

Immunohistochemical method and interpretation criteria

Specimens of clear cell carcinoma of the kidney and normal kidney tissues were selected, placed in 10% forfalin, embedded in paraffin, then placed in a slicer, and the thickness of sections was set to 5 μm. Immunohistochemical kit prepared in advance was taken and immunohistochemical staining was carried out according to its operating procedures. PBRM1 is mainly expressed in the cytoplasm, cell membrane, P53 mainly in the nucleus. The proportion of positive cells was determined by cell sex status and the proportion of positive cells: no positive cells were found to score 0, the proportion of positive cells <1% to score 1, 1~10% to score 2, 10~50% to score 3, and more than 50% to score 4. Score 0, light yellow 1, brown 2, brown 3, negative expression ≤3, positive expression >3. The patients were followed up by telephone, and the survival and prognosis of the patients were analyzed.

Statistical methods

SPSS22.0 software package was used for statistical analysis. The expression of PBRM1 and P53 in the sample tissues was tested by χ^2 test. The survival curve of patients with renal clear cell carcinoma was plotted by Kepaln-Meier method. COX proportional risk regression model was established to analyze the prognosis of patients with clear cell carcinoma of the kidney. Value of P <0.05 was considered significant.

Results

Expression of PBRM1 and P53 in clear cell carcinoma of kidney and normal kidney tissue

The positive expression rate of PBRM1 in clear renal cell carcinoma tissues was 62.86%, significantly higher than that in normal renal tissues 8.33% (P <0.05). The positive expression rate of P53 in clear renal cell carcinoma tissues was 40% (28/70) whereas there was no expression in normal renal tissues (P <0.05) (Fig. 1).

Correlation between PBRM1 and P53 and clinicopathological parameters of clear cell carcinoma of the kidney

The expression level of PBRM1 was correlated with pathological grade and clinical stage of patients with clear cell carcinoma of the kidney (P <0.05), but not with age, sex and tumor size (P >0.05). P53 and expression levels were independent of age, sex, tumor size, pathological grade, and clinical stage of patients with clear cell carcinoma of the kidney (P >0.05) (Table 1).

Correlation between PBRM1 and P53 and prognosis of clear cell carcinoma of kidney

The 5-year survival rate of PBRM1-positive expression patients was 40.91% significantly lower than that of PBRM1-negative expression patients 84.62% (P <0.05).As shown in Fig. 2A.The 5-year survival rate of patients with positive expression

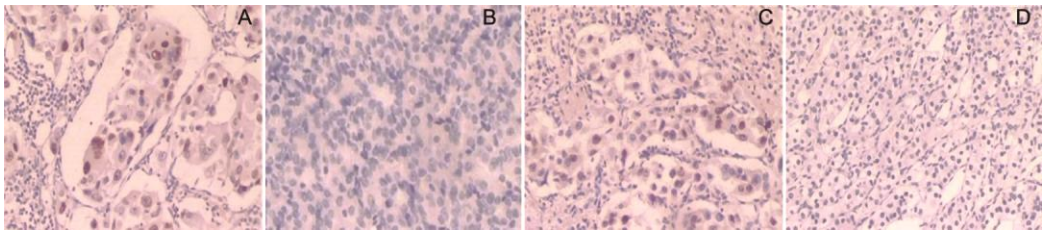

Fig. 1 — Expression of PBRM1 and P53 in clear cell carcinoma and normal renal tissue. (A) positive expression of PBRM1 in clear cell carcinoma of kidney; (B) negative expression of PBRM1 in normal renal tissue; (C) positive expression of P53 in clear cell carcinoma of kidney; and (D) negative expression of P53 in normal renal tissue

Table 1 — Correlation of clinicopathological parameters of clear cell carcinoma of the kidney between PBRM1 and P53

Clinicopathological parameters	No.	PBRM1		χ^2	*P*	P53		χ^2	*P*
		NE (n=26)	PE (n=44)			NE (n=42)	PE (n=28)		
Age (year)				1.693	0.193			5.042	0.999
≤18	36	16	20	-	-	17	19	-	-
>6	34	10	24	-	-	25	9	-	-
Gender				3.717	0.054			2.188	0.110
Male	30	15	15	-	-	15	15	-	-
Female	40	11	29	-	-	27	13	-	-
Tumor size (cm)				2.143	0.143			0.952	1.000
≤5	35	17	18	-	-	19	16	-	-
>5	35	9	26	-	-	23	12	-	-
Clinical stage				7.540	0.006			0.259	1.132
I~II stage	25	16	9	-	-	14	11	-	-
III~IV stage	45	10	35	-	-	28	17	-	-
Pathological grade				12.984	0.002			0.686	0.710
G1	46	24	22	-	-	26	20	-	-
G2	12	1	11	-	-	8	4	-	-
G3	12	1	11	-	-	8	4	-	-

[NE:Negative expression; PE: Positive expression]

of P53 was 50 % compared with 61.90% of patients with negative expression of P53 (P >0.05) (Fig. 2B).

COX model analysis of patients with clear cell carcinoma of the kidney

As revealed by Table 2, the clinical stage, pathological grade and PBRM1 were all independent risk factors affecting the prognosis of patients with clear cell carcinoma of the kidney.

Correlation analysis between PBRM1 and P53

PBRM1 was positively correlated with P53 in clear cell carcinoma of kidney (r=0.781, P=0.012).

Table 2 — COX model analysis of patients with clear cell carcinoma of the kidney

Parameters	SE	Wald	*P*	95% CI
Gender	0.555	0.651	0.435	0.543~4.493
Age	0.571	5.59	3.501	1.176~2.448
Tumor size	0.611	11.937	0.082	2.462~5.803
Clinical stage	0.572	8.331	0.005	1.691~15.168
Pathological grade	1.672	9.551	0.003	1.846~15.117
PBRM1exprssion	0.602	6.767	0.021	1.463~14.629
P53 expression	0.609	3.093	0.090	0.896~9.217

Discussion

PBRM1 is a new type of gene. Clinical studies have shown that it has a high mutation rate and plays an inhibitory role in tumor onset and progression. However, in a special period, PBRM1 loses its activity due to damage, which eventually leads to the occurrence of tumors[11,12]. Relevant literature has shown that PBRM1 is an extremely critical member in the development of clear cell carcinoma of the kidney, and can play a role through cell apoptosis, proliferation and other links[13]. However, the inactivation mechanism of PBRM1 and its tumor regulation mechanism are still not clear, and hence, it needs to be further explored for clear understanding of clinical prevention and treatment of clear cell carcinoma of kidney and the assessment of prognosis. Previous studies have shown that the expression of PBRM1 in renal cancer is significantly increased, and the positive expression rate is correlated with the degree of tumor differentiation and malignancy[14]. P53 gene change is a common type in human solid tumors, and it plays a significant role in regulating cell cycle and cell differentiation. Currently, there are many studies on P53, and it has been reported that the

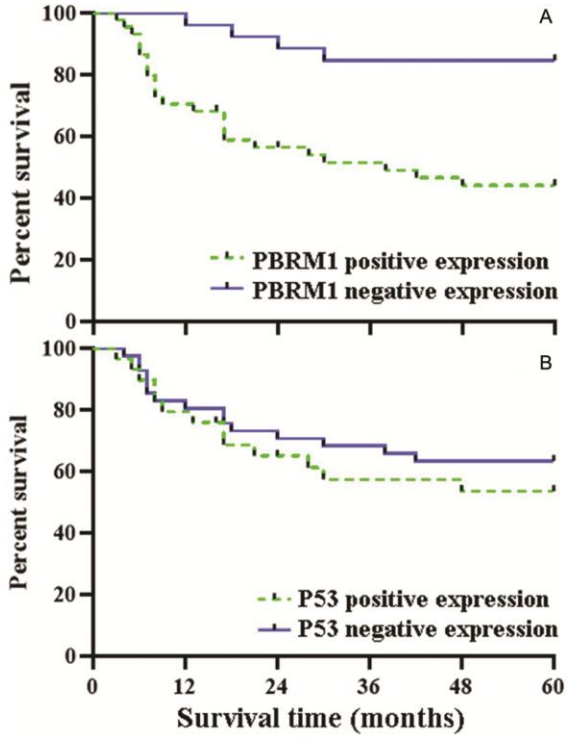

Fig. 2 — Correlation between (A) PBRM1; and (B) P53 and the prognosis of clear cell renal clear cell carcinoma.

positive expression rate of P53 gene in kidney tumor is 30~60% when it is changed[15]. There are different opinions about P53 in renal clear cell carcinoma, and some scholars believe that its expression level is related to the degree of tumor differentiation, invasion depth and lymphatic metastasis, and it can be used as an independent prognostic indicator[16]. Few others have reported that the expression level of P53 is independent of lymph node metastasis and its ratio can be used as an independent prognostic indicator.

In this study, immunohistochemical approach was used to determine PBRM1 and P53 in clear cell carcinoma of kidney and normal kidney tissues. The results have shown that the positive expression rate of PBRM1 in clear cell carcinoma of kidney and normal kidney tissues was 62.86%, significantly higher than that of PBRM1 in normal kidney tissues (8.33% (P <0.05). The positive expression rate of P53 in clear renal cell carcinoma tissues was 40 % while there was no expression in normal renal tissues (P <0.05). These results suggest that PBRM1 and P53 play an important role in the pathogenesis and progression of clear cell carcinoma of the kidney, in which P53 plays a role as an anticancer gene. PBRM1 expression level was correlated with pathological grade and clinical stage of patients with clear cell carcinoma of the kidney (P <0.05). It suggested that the increased PBRM1 positive expression rate may be closely associated with clear cell carcinoma of the kidney, which is of important reference value for the prediction of disease progression and disease severity. The 5-year survival rate of patients with PBRM1 positive expression was 40.91% significantly lower than that of patients with PBRM1 negative expression (84.62% (P <0.05). Similarly, the 5-year survival rate of patients with positive expression of P53 was 50% compared with 61.90% of patients with negative expression of P53 (P >0.05). The results suggest that both PBRM1 and P53 can be used as effective indicators to evaluate the survival of patients. Clinical stage, pathological grade and PBRM1 were all independent risk factors affecting the prognosis of patients with clear cell carcinoma of the kidney.PBRM1 was positively correlated with P53 in clear cell carcinoma of kidney (r=0.781, P=0.012).

Conclusion

PBRM1 and P53 are both highly expressed in clear cell carcinoma of the kidney and play an important role in the occurrence and development of the disease. At the same time, PBRM1 can also be used as an independent risk factor affecting the prognosis of patients with clear cell carcinoma of the kidney. It is expected to be an important indicator for evaluating the progress and prognosis of patients with clear cell carcinoma of the kidney and a target for antitumor therapy.

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Conflict of Interest

All authors declare no competing interests.

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