Original Article
High CHD9 expression is associated with poor prognosis in clear cell renal cell carcinoma

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Abstract: Clear cell renal cell carcinoma (ccRCC) is the most common subtype of renal cell carcinoma. The chromo-helicase-DNA binding proteins (CHDs), containing nine members named CHD1-9, act as regulators of the chromatin remodeling process and gene expression. To determine the correlation between CHD9 expression and ccRCC, we performed an immunohistochemical staining in a tissue microarray (TMAs) containing tissue samples from 88 ccRCC patients. The results showed that cytoplasm CHD9 expression was statistically decreased in tumor tissues compared to adjacent tissues (8.54±2.90 vs 12.61±2.05, P=0.000), while nuclear CHD9 expression was upregulated in the tumor tissues (1.47±2.93 vs 0.29±1.24, P=0.000). A univariate analysis found that cytoplasm CHD9 expression in cancer tissues was correlated with the patients' pathological grading (P=0.002, r=0.330), the clinical stages (P=0.02, r=0.250) and the T grading (P=0.024, r=0.241) significantly. In addition, cytoplasm CHD9 expression in non-tumor tissues was correlated with the ccRCC patients' pathological grading (P=0.031, r=-0.231) significantly. Patients with high cytoplasm CHD9 expression had a significantly worse prognosis than those with low cytoplasm CHD9 expression levels (59.7% vs 85.7%, P=0. 042). In conclusion, our study indicated the important role of CHD9 in ccRCC and suggested CHD9 may be a potential biomarker for prognostic prediction and a new target for therapy.

Keywords: CHD9, clear cell renal cell carcinoma (ccRCC), tissue microarray, immunohistochemistry, survival time, chemotherapy

Introduction

Renal cell carcinoma (RCC), which comprises a heterogeneous group of four epithelial neoplasms with diverse biologic behaviors and variable clinical outcomes, is the most lethal of the common urological cancers. The most common subtype is the clear cell renal cell carcinoma, which makes up 75-80% of all RCC cases. The other three subtypes include papillary carcinoma (PRCC, 10% of RCC), chromophobic carcinoma (ChRCC, 5% of RCC), and collecting duct carcinoma (2% of RCC) makes up the other 20%-25% of RCC cases [1, 2]. Although a number of targeted drugs have emerged in recent years, the overall survival times of patients with metastatic kidney cancer remain short [3].

The chromo-helicase-DNA binding proteins (CHDs) act as regulators of the chromatin

remodeling process and gene expression. The CHD proteins contain two basal tandem chromo domains and different additional domains, according to which the CHDs are divided into three sub-groups: CHD1-2, CHD3-5 and CHD6-9 [4, 5]. Chromatin remodeling plays a critical role in regulating gene expression throughout ontogenesis, and the CHDs associated with many human diseases and cancers include lymphoma, liver cancer, renal cancer, colorectal cancer, and stomach cancer [6-8]. For example, CHD1 deficiency may contribute to prostate cancer metastasis [9]. CHD4 and CHD3 bind ZGPAT (zinc finger, CCCH-type with G patch domain) could inhibit tumor development [10]. CHD5 expression could serve as an independent prognostic marker of survival for patients with ovarian cancer, neuroblastoma, glioma. and gallbladder carcinoma [8, 11]. Respectively, the third sub-family CHD enzymes are orthologs

Table 1. Details of the ccRCC patients' clinical information

	Clinical index	N	Lost	Total N
Gender	Male	57	1	88
	Female	30		
Age	≤60 years	49	2	88
	>60 years	37		
Tumor size	≤5 cm	52		88
	>5 cm	36		
T staging	T1	57		88
	T2	25		
	T3	6		
N staging	N0	84		88
	N1-3	2		
M staging	M0	86		88
	M1	2		
Clinical staging	Stage I	55	2	88
	Stage II	23		
	Stage III	6		
	Stage IV	2		
Pathological grading	I-II	34		88
	III-IV	24		

of the drosophila kismet enzyme and are characterized by the brahma and kismet domains at C termini. The mutant of CHD7, 8 could lead to the distinct disease states of CHARGE syndrome and Kallman Syndrome [12, 13]. There few studies on the relationship of CHD9 and human disease, especially on renal disease [14, 15].

Materials and methods

Clinical material

Tumor samples from 88 clear cell renal cell carcinoma patients and their paired adjacent carcinoma tissues were assembled in a tissue microarray (HKidE180Su03, Shanghai Outdo Biotech Co., Ltd). All the typical pathological sites on the HE slices were labeled by two pathologists, and then drilled on the blank recipient paraffin (diameter was 1.5 mm) using a tissue microarray instrument. The patients' clinical information, including pathological information, such as their age, gender, tumor size, tumor differentiation, stage, TNM stage, distant metastasis and clinical stage, was detailed in **Table 1**.

The patients' operations took place from October 2006 to February 2008. The final follow-

up date was in August 2015, so follow-up times ranged from 90 to 106 months. During this follow-up time, 30 of the 88 ccRCC patients died of ccRCC (the overall survival time ranged from 2 to 79 months), and the other 58 survived. All patients were clinicopathologically diagnosed as having ccRCC and received no additional treatment before surgery.
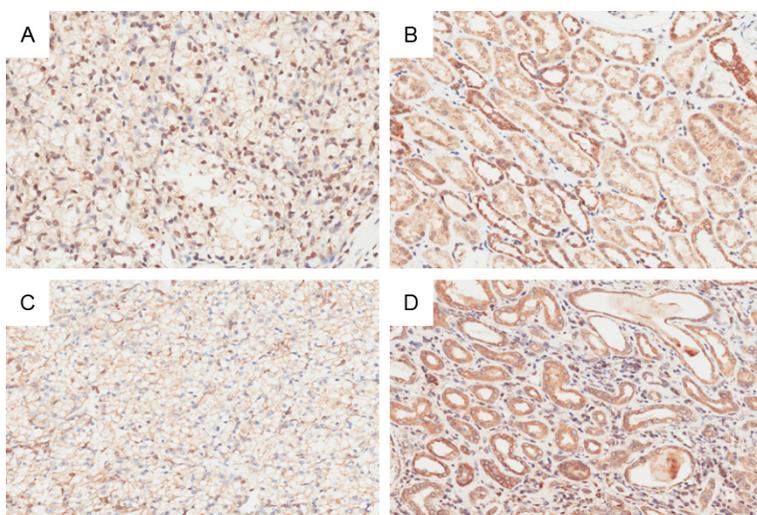
Immunohistochemistry

As previously described, two-step Immunohistochemical staining was performed on a tissue microarray in this study [16, 17]. Firstly, after they were treated with an EDTA buffer to retrieve the antigen, the tissue sections were blocked with goat serum. Then the tissue sections were incubated with a primary antibody (Proteintech, 13402-1-AP, 1:1500) at 4°C overnight.

Next, the tissue sections were incubated with a secondary antibody, washed with PBS, and then, examined using a diaminobenzidine (DAB) system and hematoxylin re-dying, observed and analyzed under microscope. The CHD9 expression was scored and the tissues grouped according to their "positive staining rate score" multiplied by their "staining intensity score". The positive staining rate was defined according to the proportion of positively stained cancer cells: "Negative" is 0, "1%-20%" for 1, "21%-40%" for 2, "41-60%" for 3, "61-80%" for 4. The staining intensity score was defined as follows: "Negative" is 0, "1+" for 1, "2+" for 2, "3+" for 3. Thus, patients were placed into the low expression group when the score was ≤6, while the patients with a score >6 were placed into the high expression group.

Statistical analysis

Firstly, we statistically analyzed the positive staining rate and the intensity of immunohistochemical staining. The different expression of CHD9 in cancer and in the adjacent tissues was evaluated by NPar tests. Spearman's correlation analysis was used to calculate the CHD9 expression in cancer and adjacent tissues. The relationships between the clinical indicators of the ccRCC patients and CHD9 expression were also analyzed by Spearman's correlation analysis. In this study, the Kaplan-Meier survival analysis and a log-rank statistical test were used to analyze the survival factors. Finally, statistically significant variables in univariate analysis were also included in a COX

Figure 1. Representative immunohistochemistry images of CHD9 expression in ccRCC tissues and their para-carcinoma tissues: A. Nucleus CHD9 expression in tumor tissues; B. Nucleus CHD9 expression in adjacent tissues; C. Cytoplasm CHD9 expression in cancer tissues; D. Cytoplasm CHD9 expression in adjacent tissues (Magnification times: ×200).

expression and ccRCC patients' clinical index including gender, age, tumor size, pathological grading, TNM stage. and clinical stages. The results showed that the cytoplasm CHD9 expression in cancer tissues correlated significantly with patients' pathological grading (P=0.002, r=0.330), the clinical stages (P=0.02, r=0.250), and T grading (P=0.024, r=0.241). In addition, the cytoplasm expression of CHD9 in non-tumor tissues correlated significantly with ccRCC patients' pathological grading (P=0.031, r=-231). The results are shown in **Table 2**.

The relationships between the overall survival time of ccRCC patients and the CHD9 expression

The relationship between CHD9 expression and the prognosis of ccRCC was defined according to the Kaplan-Meier method and the log-rank test. The results showed that patients with high cytoplasm CHD9 expression had a worse prognosis than those with a low cytoplasm CHD9 expression (59.7% vs 85.7%, P=0.042). The results are shown in **Figure 2**.

Subsequently, the COX multi-factor survival analysis showed that cytoplasm CHD9 expression was not an independent predictive factor for ccRCC. The analysis results in detailed are shown in **Table 3**.

Discussion

The Chromo Helicase DNA-binding proteins (CHDs) act as regulators of the chromatin remodeling process and gene expression in humans. It has been found that the CHD family contains nine members divided into three subgroups according to the structural domains and features: CHD1-2, CHD3-5 and CHD6-9. The CHDs are associated with many human diseases and cancers including lymphoma, liver cancer, colorectal cancer, and stomach cancer [11]. Few studies have reported on the relationship between CHD9 and human renal disease or cancer.

multivariate regression survival analysis. Consider P<0.05 as statistically significant.

Results

The expression of CHD9 in the cancer tissues and their adjacent cancer

The NPar Tests showed that the cytoplasm CHD9 expression was statistically decreased in the cancer tissues compared to their adjacent tissues (8.54±2.90 vs 12.61±2.05, P=0.000) while nuclear CHD9 expression in cancer tissues was upregulated (1.47±2.93 vs 0.29± 1.24, P=0.000). The detailed images are shown in **Figure 1**.

The correlation of CHD9 expression between cancer tissues and their adjacent tissues

The Spearman's correlation analysis showed that the relationships between cytoplasm CHD9 expression and the nuclear CHD9 expression in cancers or in non-tumors positively correlated with the nuclear CHD9 expression in adjacent tissues (P=0.002; r=0.330).

The relationship between the CHD9 expression and ccRCC patients' clinical index

The Spearman's correlation analysis was used to analyze the relationship between CHD9

Table 2. The relationship between CHD9 expression and the ccRCC patients' clinical index

				Gender	Age	Pathological grading	Tumor size	T	N	M	Clinical staging
Spearman's rho	Cytoplasm CHD9 expression	Tumor	Correlation Coefficient	-0.084	0.027	0.330**	0.057	0.241*	0.029	0.026	0.250*
			Sig. (2-tailed)	0.437	0.805	0.002	0.596	0.024	0.792	0.808	0.020
		Adjacent	Correlation Coefficient	0.024	-0.138	-0.231*	-0.188	0.017	-0.028	-0.119	0.008
			Sig. (2-tailed)	0.826	0.207	0.031	0.081	0.879	0.802	0.274	0.945
	Nucleus CHD9 expression	Tumor	Correlation Coefficient	-0.004	0.027	-0.108	-0.056	-0.046	0.111	-0.092	-.012
			Sig. (2-tailed)	0.969	0.804	0.318	0.606	00.672	0.307	0.395	0.913
		Adjacent	Correlation Coefficient	-0.045	-0.069	-0.005	0.021	-0.081	-0.039	-0.038	-0.091
			Sig. (2-tailed)	0.682	0.530	0.961	0.850	0.455	0.723	0.728	0.412

**. Correlation is significant at the 0.01 level (2-tailed). *. Correlation is significant at the 0.05 level (2-tailed).
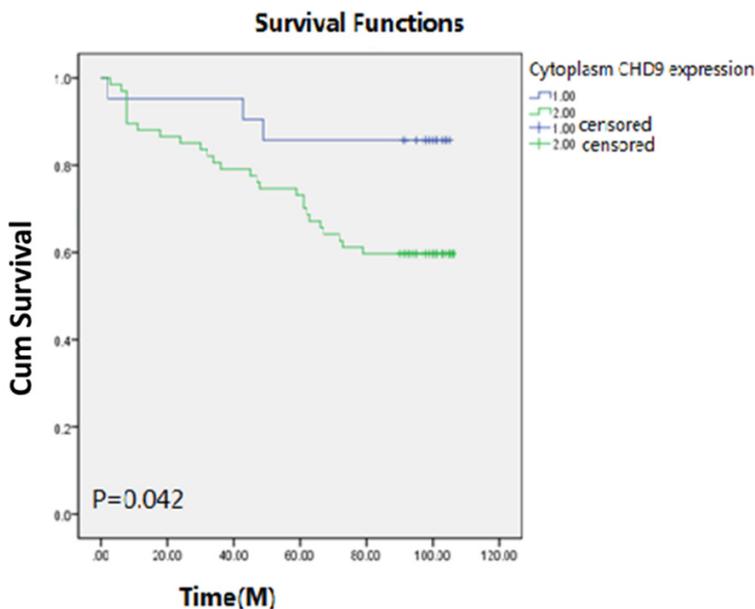
Figure 2. The relationships between the overall survival time of ccRCC patients and CHD9 expression: 1.00 represents the patients with low cytoplasm CHD9 expression; 2.00 represents the patients with high cytoplasm CHD9 expression.

We showed CHD9 expression in clear cell renal cell carcinoma through immunohistochemistry. The results show that the cytoplasm CHD9 expression was statistically significantly decreased, while nucleus CHD9 expression was upregulated in cancer tissues compared to their adjacent tissues. The nucleus CHD9 expression in cancer tissues were positively correlated with nucleus CHD9 expression in adjacent tissues. These alterations of the CHD9 expression in ccRCC indicate that CHD9 expression may be closely related to tumorigenesis

[18]. Additionally, the cytoplasm CHD9 expression in tumor tissues was significantly correlated with ccRCC patients' clinical index, including pathological grading, clinical grading and T stage. In line with previous studies, these findings indicate that cytoplasm CHD9 expression has a significant correlation with tumor cell progression, development, and invasiveness in ccRCC [19]. However, the cytoplasm expression of CHD9 in non-tumor tissue was correlated significantly negatively with the ccRCC patients' pathological grading, indicating that CHD9 might have different regulatory effects in cancer and adjacent tissues which should be validated in the future work.

It is reported that the loss of CHD8 expression may serve as a novel indicator in gastric cancer [20], and CHD7 may be a novel biomarker in pancreatic cancer patients [21]. Thus, as the other member of CHD6-9, the CHD9 expression must have some predictive value with the prognosis of ccRCC, even though the COX multifactor survival analysis showed that CHD9 expression was not an independent predictive factor. The statistical results showed that patients with high cytoplasm CHD9 expression in cancer tissues had an obviously worse prog-

Table 3. COX multivariate regression analysis of the independent predictors of CHD9 in ccRCC patients

	B	SE	Wald	Df	Sig	Exp (B)	95.0% Exp (B) confidence interval	
							Lower limit	Upper limit
Age	1.294	0.427	9.195	1	0.002**	3.649	1.581	8.425
Pathological Grading	0.613	0.277	4.917	1	0.027*	1.847	1.074	3.176
Tumor size	-0.068	0.462	0.021	1	0.884	0.935	0.378	2.312
T	0.675	0.369	3.343	1	0.067	1.964	0.953	4.050
N	1.508	0.860	3.074	1	0.080	4.519	0.837	24.392
M	1.283	0.941	1.858	1	0.173	3.608	0.570	22.839
Clinical stages			.	0	.			
Cytoplasm CHD9 expression in cancer	0.450	0.643	0.489	1	0.485*	1.568	0.444	5.532

A. The degrees of freedom were reduced because of the constant or linear variation. B. Constant or linear covariance. Clinical stages = T + N + M. **. Correlation is significant at the 0.01 level (2-tailed). *. Correlation is significant at the 0.05 level (2-tailed).

nosis than those with low cytoplasm CHD9 expression.

In summary, we found abnormal, upregulated expression of CHD9 to be associated with clinical index and overall survival in clear cell renal cell carcinoma. The findings are in line with CHD9 differential expression in the cytoplasm and nucleus, indicating that CHD9 expression correlates with survival and metastatic propensity in cancer. Though CHD9 expression could not serve as an independent prognostic marker, it still provides a reference for prognostic diagnosis. Our study indicated the important role of CHD9 in ccRCC and suggests that CHD9 is a potential biomarker for prognostic prediction and a new therapy target.

Disclosure of conflict of interest

None.

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