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Original Article

Prognostic value of CD147 and HIF-2α expression in localized clear cell renal cell carcinoma

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Abstract: Objective: To identify the prognostic significance of CD147 and HIF-2α expressions in localized clear cell renal cell carcinoma (ccRCC). Methods: Tissue microarrays of 74 cases of ccRCC without distant metastasis or invasion were performed by CD147 and HIF-2α immunohistochemical staining. The expressions were qualified by intensity and percentage of tumor cells stained. Results: Increased expression of CD147 was positively correlated with high pT stages (P = .003) and high grade (P = .008). A positive correlation between CD147 and HIF-2α expressions (P = .002, *rho* = .359) was observed. Kaplan-Meier analysis indicated that ccRCC patients with high CD147 expression and high HIF-2α expression were significantly associated with poor overall survival (P = .001 and P = .002, respectively). Moreover, a significant difference in overall survival was found among the four coexpression subgroups, low CD147 & low HIF-2α, low CD147 & high HIF-2α, high CD147 & low HIF-2α, and high CD147 & high HIF-2α, of which patients with both high expressions of CD147 and HIF-2α had the poorest prognosis (P = .001). Multivariate survival analysis suggested that the combined expression of CD147 and HIF-2α, instead of CD147 and HIF-2α individually, was an independent prognostic factor. Conclusions: The expressions of CD147 and HIF-2α were significantly correlated with poor survival of patients with localized ccRCC, and combined expression of CD147 and HIF-2α may serve as an independent prognostic factor in localized ccRCC.

Keywords: CD147, HIF-2α, localized ccRCC, prognosis

Introduction

Renal cell carcinoma (RCC) is a universal disease in adults, with a higher incidence of 11.8 per 100,000 in males than 5.8 in females [1]. Patients with RCC were more than 350,000 worldwide in 2013, with more than 140,000 deaths per year, which made it the seventh most common site for tumors [2]. The principle treatment for localized RCC is nephrectomy, which benefits RCC patients limitedly with locally advanced or metastatic RCC [3]. Given the situation that the current management of RCC mainly depends on imaging technologies, identifying the safe and accurate biomarkers to distinguish patients with poor prognosis in the early stage of RCC is urgently needed [2].

Clear cell renal cell carcinoma (ccRCC), representing about 80% of RCC subtypes, is characterized as the inactivation of the von Hippel-Lindau tumor-suppressor gene and the reduced degradation of hypoxia inducible factor (HIF), of which HIF-2α, but not HIF-1α seems to be necessary and sufficient for tumor growth [4].

CD147, also known as extracellular matrix metalloproteinase inducer (EMMPRIN) or basigin, has been reported to be associated with tumor progression and prognosis in various cancers such as hepatocellular carcinoma, breast cancer, and RCC [5, 6]. Previous study has validated the prognostic value of CD147 and vascular endothelial growth factor (VEGF) in advanced RCC [7]. However, the prognostic role of CD147 in localized ccRCC patients is not fully established.

In the study, we examined the correlation and prognostic value of CD147 and HIF-2α by immunohistochemistry staining in localized ccRCC patients. Expression of CD147 was significantly higher in ccRCC tissues compared with adjacent tissues. High expression of CD147 was

Table 1. The correlation between CD147 and HIF-2α expressions and clinicopathological parameters in localized ccRCC*

Parameters	n (%)	CD147			*P*	HIF-2α			*P*
		-	+	++		-	+	++	
		(n = 32)	(n = 28)	(n = 14)		(n = 32)	(n = 25)	(n = 17)	
Sex									
Male	45 (60.8)	21 (28.4)	14 (18.9)	10 (13.5)	.309	22 (29.7)	13 (17.6)	10 (13.5)	.430
Female	29 (39.2)	11 (14.9)	14 (18.9)	4 (5.4)		10 (13.5)	12 (16.2)	7 (9.5)	
Age (years)									
≥ 60	37 (50.0)	12 (16.2)	17 (23.0)	8 (10.8)	.168	14 (18.9)	11 (14.9)	12 (16.2)	.154
< 60	37 (50.0)	20 (27.0)	11 (14.9)	6 (8.1)		18 (24.3)	14 (18.9)	5 (6.8)	
Size (cm)									
< 4	32 (43.2)	16 (21.6)	12 (16.2)	4 (5.4)	.140	15 (20.3)	10 (13.5)	7 (9.5)	.513
4-7	18 (24.3)	10 (13.5)	6 (8.1)	2 (2.7)		9 (12.2)	7 (9.5)	2 (2.7)	
> 7	24 (32.4)	6 (8.1)	10 (13.5)	8 (10.8)		8 (10.8)	8 (10.8)	8 (10.8)	
pT stages									
T1a	32 (43.2)	17 (23.0)	11 (14.9)	4 (5.4)	.003	15 (20.3)	10 (13.5)	7 (9.5)	.472
T1b	23 (31.1)	12 (16.2)	8 (10.8)	3 (4.1)		12 (16.2)	8 (10.8)	3 (4.1)	
T2a	13 (17.6)	3 (4.1)	8 (10.8)	2 (2.7)		2 (2.7)	6 (8.1)	5 (6.8)	
T2b	3 (4.1)	0 (0)	0 (0)	3 (4.1)		2 (2.7)	0 (0)	1 (1.4)	
T3	3 (4.1)	0 (0)	1 (1.4)	2 (2.7)		1 (1.4)	1 (1.4)	1 (1.4)	
Grade									
I	29 (39.2)	17 (23.0)	9 (12.2)	3 (4.1)	.008	15 (20.3)	9 (12.2)	5 (6.8)	.117
I-II	10 (13.5)	4 (5.4)	3 (4.1)	3 (4.1)		2 (2.7)	4 (5.4)	4 (5.4)	
II	24 (32.4)	10 (13.5)	12 (16.2)	2 (2.7)		13 (17.6)	8 (10.8)	3 (4.1)	
II-III	2 (2.7)	0 (0)	0 (0)	2 (2.7)		0 (0)	0 (0)	2 (2.7)	
III	8 (10.8)	0 (0)	4 (5.4)	4 (5.4)		2 (2.7)	3 (4.1)	3 (4.1)	
III-IV	1 (1.4)	1 (1.4)	0 (0)	0 (0)		0 (0)	1 (1.4)	0 (0)	

*Cases of group "+++" were assigned to group "++".

positively correlated with high pT stages and high grade. High expressions of CD147 and HIF-2α, especially combined expression of CD147 and HIF-2α, were positively associated with poor prognosis.

Materials and methods

Tissue microarrays (TMAs) and immunohistochemical staining

The TMAs were purchased from Shanghai Biochip Company, Ltd. (Shanghai, China), which contained a total of 90 pairs of ccRCC tissues and matched adjacent tissues. The patients underwent nephrectomy between July 2006 and February 2008, and were followed up to September 2013. The median follow-up period was 70.5 months (range 2-83).

Immunohistochemical staining was conducted for detection of CD147 and HIF-2α. Briefly, TMA slides (4 μm thickness) were deparaffinaged and dehydrated by xylene and alcohol infusion followed by antigen retrieval and blocking endogenous peroxidase activity. Primary antibodies including anti-CD147 HAb18 antibody (produced in our laboratory, 1:100 dilution), anti-HIF-2α antibody (Bioss, Beijing, China; 1:300 dilution) were incubated at 4°C overnight. Following incubation of immunoperoxidase, the staining was conducted by a streptavidin-peroxidase kit (ZSGB-BIO, Beijing, China). And the slides were visualized by 3,3'-diaminobenzidine (ZSGB-BIO) and counterstained with hematoxylin.

Immunohistochemical scores

Each TMA slide was evaluated by two independent pathologists. Staining was scored by the intensity and percentage of tumor cells stained. The staining intensity was graded as

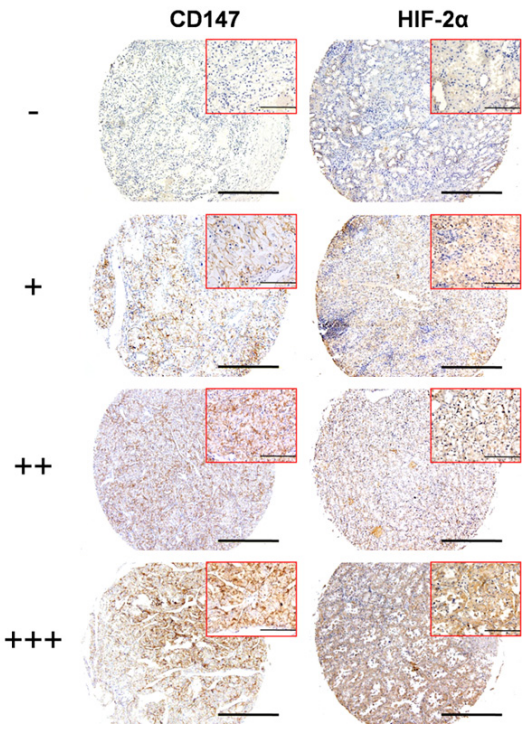

Figure 1. Representative immunohistochemical staining of CD147 and HIF-2α in tissue microarrays of localized ccRCC. -, +, ++, +++ indicated different staining levels of CD147 and HIF-2α. Long scale bar = 500 μm, short scale bar = 200 μm.

0 (no staining), 1 (weak), 2 (moderate), and 3 (strong). The percentage of positive staining was graded as 0 (0-5%), 1 (6-30%), 2 (31-70%), and 3 (71-100%). Then these two scores were summed, giving a resultant score of 0-6. Finally, the levels of staining were classified into four groups by "-" (score 0-1), "+" (score 2-3), "++" (score 4-5), and "+++" (score 6).

Statistical analysis

The correlations between the patients' clinicopathological parameters and the expressions of CD147 and HIF-2α were analyzed by Pearson's chi-squared test (χ^2). Spearman correlation analysis was used to analyze the correlation between CD147 and HIF-2α expressions. Overall survivals (OS) were analyzed using Kaplan-Meier curves and prognostic significance was assessed by the log-rank test. Multivariate survival analysis was done by using the Cox regression methods. All statistical analyses were performed using IBM SPSS 19.0 (SPSS, Inc., Chicago, IL, USA). Statistical significance was defined as two-sided ($P < .05$).

Results

Patients

Seventy-four of 90 samples were involved in the analysis except for patients with metastasis (3 cases), loss of follow-ups (9 cases), and absence of information of TNM phase (4 cases). The clinical features of patients were summarized in **Table 1**. The study involved 74 patients who were diagnosed as ccRCC without metastasis, including 45 men (60.8%) and 29 women (39.2%), with a median age of 69.5 years (range, 29-82 years). Tumor size was divided into 3 groups, of which 32 patients were less than 4 cm (43.2%), 18 were 4-7 cm (24.3%), and 24 were over 7 cm (32.4%). Furthermore, 32 patients were diagnosed as T1a (43.2%), 23 were T1b (31.1%), 13 were T2a (17.6%), 3 were T2b (4.1%), and 3 were T3 (4.1%). For pathology grade, 29 patients were diagnosed as grade I (39.2%), 10 were grade I-II (13.5%), 24 were grade II (32.4%), 2 were grade II-III (2.7%), 8 were grade III (10.8%), and only one patient was grade III-IV (1.4%).

Expressions of CD147 and HIF-2α in localized ccRCC and adjacent tissues

The representative images of CD147 and HIF-2α expressions in ccRCC tissue array were shown in **Figure 1**. Immunohistochemical staining displayed that CD147 localized in the cytoplasm and membrane of RCC cells, but not found in stromal cells, with a positive rate of 56.8%. Whereas the positive rate of CD147 was 10.8% in the tissues adjacent to cancer. The expression of CD147 in ccRCC was significantly higher than that in tissues adjacent to cancer ($P < .001$). HIF-2α staining was detected in the cytoplasm and nuclear of ccRCC cells, with positive rates of 56.8% and 58.1% in ccRCC and adjacent tissues, respectively. Spearman correlation analyses indicated a positive correlation between CD147 and HIF-2α expressions ($P = .002$, $rho = .359$).

Association of expression of CD147 and HIF-2α with clinicopathological parameters in localized ccRCC

The expression of CD147 in ccRCC tissues was associated with pT stage ($P = .003$) and grade ($P = .008$), whereas HIF-2α did not show significant correlation with these clinicopathological

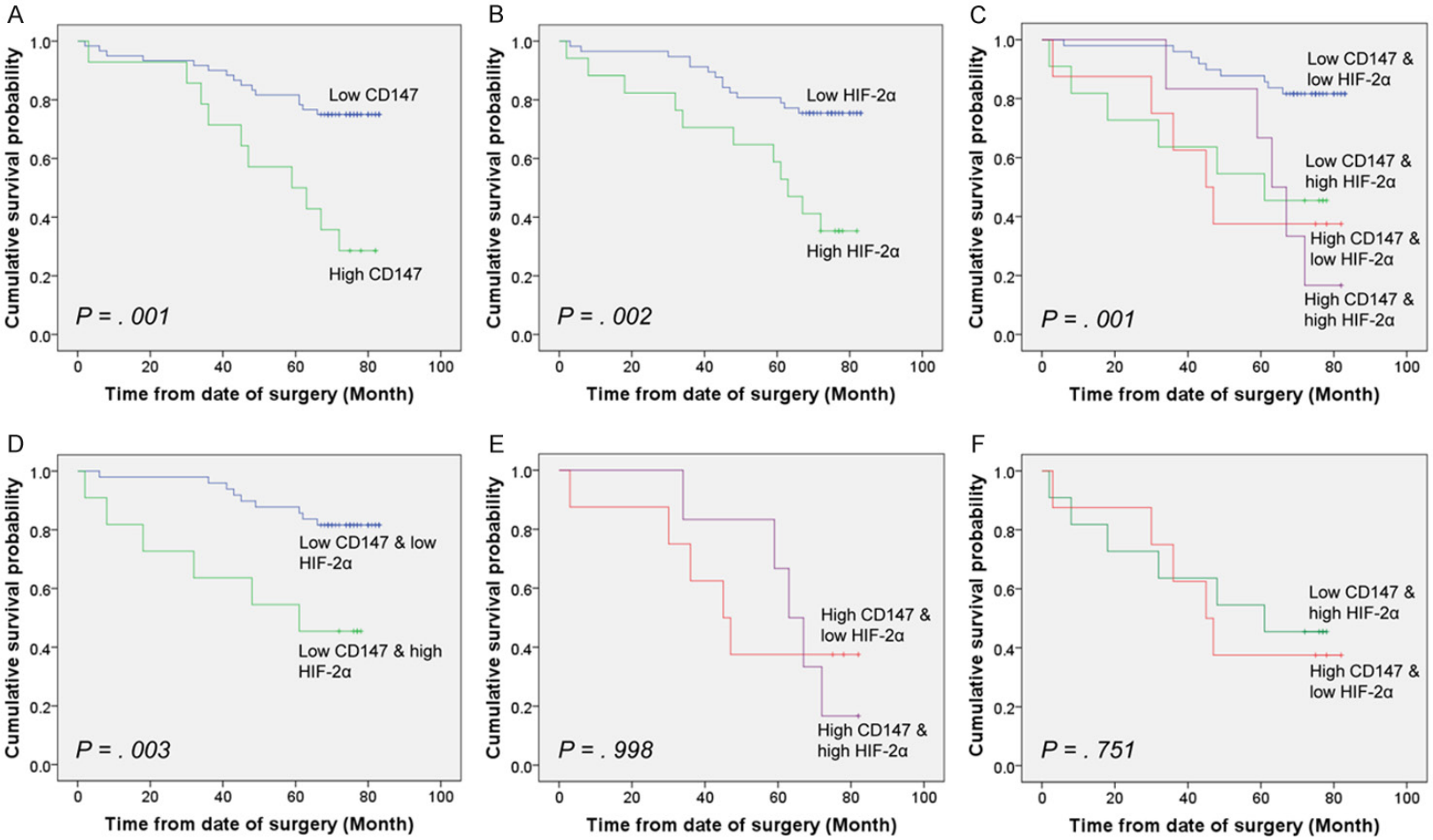

Figure 2. Kaplan-Meier survival analysis of CD147 and HIF-2α expressions in localized ccRCC. A. OS of ccRCC patients with high and low CD147 expressions. B. OS of ccRCC patients with high and low HIF-2α expressions. C. OS of ccRCC patients with combined expression of CD147 and HIF-2α. D. OS of ccRCC patients with low CD147 & low HIF-2α and low CD147 & high HIF-2α. E. OS of ccRCC patients with high CD147 & low HIF-2α and high CD147 & high HIF-2α. F. OS of ccRCC patients with low CD147 & high HIF-2α and high CD147 & low HIF-2α.

Table 2. Cox multivariate analysis of the prognostic significance of CD147 and HIF-2α expressions and clinicopathological parameters in patients with localized ccRCC

	Wald	df	P	Exp (B)	95% CI for Exp (B)	
					Lower	Upper
Sex	.073	1	.787	1.131	.462	2.769
Age (y)	2.494	1	.114	2.039	.842	4.939
Size (cm)	1.013	1	.314	.664	.299	1.475
pT stages	2.591	1	.107	1.984	.862	4.569
Grade	4.528	1	.033	1.441	1.029	2.017
CD147	.969	1	.325	1.679	.599	4.707
HIF-2α	.771	1	.380	1.558	.579	4.195
Sex	.580	1	.446	1.439	.564	3.670
Age (y)	3.237	1	.072	2.262	.930	5.503
Size (cm)	.150	1	.698	.855	.387	1.888
pT stages	1.753	1	.186	1.703	.774	3.747
Grade	6.917	1	.009	1.535	1.115	2.112
Low CD147 and low HIF-2α	10.245	3	.017			
Low CD147 and high HIF-2α	6.616	1	.010	4.361	1.420	13.398
High CD147 and low HIF-2α	7.263	1	.007	4.988	1.550	16.052
High CD147 and high HIF-2α	1.080	1	.299	1.890	.569	6.274

features (**Table 1**). Patients with higher pT stages and grades had significantly higher expression of CD147.

Correlation of CD147 and HIF-2α expressions with prognosis in localized ccRCC

Based on the different levels of CD147 and HIF-2α expressions in the tumor tissues, the OS analysis were conducted by Kaplan-Meier curves and analyzed by the log-rank test. As shown in **Figure 2A** and **2B**, patients with high CD147 expression (P = .001) and high HIF-2α expression (P = .002) were significantly associated with poor OS. The cumulative 5-year OS rates of patients with low and high expressions of CD147 were 81.7% and 50.0%, respectively. Similarly, patients with low and high expressions of HIF-2α were 80.7% and 58.8%, respectively.

According to the positive correlation between CD147 and HIF-2α expressions, we wonder whether combined expression of CD147 and HIF-2α was more accurate to predict the prognosis than CD147 alone. Thus, patients were categorized into four groups: 1) low expression of CD147 and low expression of HIF-2α (low CD147 & low HIF-2α); 2) low expression of CD147 and high expression of HIF-2α (low CD147 & high HIF-2α); 3) high expression of CD147 and low expression of HIF-2α (high CD147 & low HIF-2α); 4) high expression of CD147 and high expression of HIF-2α (high CD147 & high HIF-2α). Kaplan-Meier analysis indicated a significant difference among the four subgroups (P = .001, **Figure 2C**). Patients with both low expressions of CD147 and HIF-2α had the best prognosis, while patients with both high expressions of CD147 and HIF-2α had the poorest prognosis. In the low CD147 expression group, patients with low expression of HIF-2α had better survival than those with high expression of HIF-2α (P = .003, **Figure 2D**). Whereas the survivals of patients with high CD147 & low HIF-2α and high CD147 & high HIF-2α were not statistically different (**Figure 2E**). Additionally, patients with low CD147 & high HIF-2α and high CD147 & low HIF-2α showed no statistical difference (**Figure 2F**).

To further evaluate the prognostic significance of CD147 and HIF-2α in localized ccRCC, we carried out Cox regression analysis. Covariates included age, sex, tumor size, pT stages, grade, CD147 expression, and HIF-2α expression. Only grade was found an independent prognostic factor for patients with ccRCC (**Table 2**). However, when combined expression of CD147 and HIF-2α was added, grade, low CD147 & low HIF-2α expression, low CD147 & high HIF-2α expression, and high CD147 & low HIF-2α expression were independent prognostic factors (**Table 2**). These results indicated that compared with CD147 and HIF-2α expression individually, combined expression of CD147 and HIF-2α was more accurate in prediction of ccRCC prognosis.

Discussion

In our study, we observed that expression of CD147 in ccRCC tissues was significantly higher than that in adjacent tissues. Increased

expression of CD147 was positively correlated with clinicopathological features including high pT stages and high grade. In the survival analysis, higher expressions of CD147 and HIF-2α were positively associated with poor OS. Moreover, combined expression of CD147 and HIF-2α further demonstrated their prognostic value in ccRCC. These results indicate that high expressions of CD147 and HIF-2α, especially combined expression of CD147 and HIF-2α, is significantly correlated with poor prognosis of patients with ccRCC.

We discovered that high CD147 expression was associated with clinicopathological features including high pT stages and high grade. This finding was comparable with previous studies showing that CD147 expression was correlated with clinicopathological parameters and poor survival rates in RCC [8, 9]. Moreover, Liang et al. explained the prognostic role of CD147/VEGF coexpression in patients with advanced RCC [7]. Here, we attempted to prove the prognostic role of CD147 in localized ccRCC and found that patients with high CD147 expression were significantly associated with poor OS.

Numerous researches in the prognostic role of HIF family made it clear that cytoplasmic expression of HIF-2α indicated unfavorable prognosis in RCC [10, 11]. Several studies have demonstrated the tight correlation between CD147 and HIF family. CD147 promotes melanoma cell malignant properties through a HIF-2α mediated upregulation of VEGFR-2 [12, 13]. Besides, hypoxia upregulates CD147 expression through a combined effect of HIF-1α and Sp1 to promote glycolysis and tumor progression in epithelial solid tumors [14]. However, the relationship between CD147 and HIF-2α in localized ccRCC is still yet unknown. In the present study, we revealed the correlation between CD147 and HIF-2α in localized ccRCC. Either CD147 or HIF-2α is reported as a good marker of ccRCC individually, thus we analyzed the combination of CD147 and HIF-2α expression in prognosis of ccRCC. Compared with CD147 and HIF-2α expression individually, combined expression of CD147 and HIF-2α indicated more accurate prediction in ccRCC prognosis. However, whether CD147 and HIF-2α coexpression can serve as an independent prognosis factor in localized ccRCC needs a further research involved in more patients. The specific role of the crosstalk between CD147 and HIF-2α also needs to be further investigated.

Our results indicate that high expression of CD147 is identified as a poor prognostic factor in localized ccRCC. Expression of CD147 and HIF-2α, especially combined expression of CD147 and HIF-2α, may guide us in predicting the prognosis of ccRCC. CD147 may be a practical prognostic factor and therapeutic target in ccRCC.

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Disclosure of conflict of interest

None.

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