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Decreased expression of miR-495 is associated with poor prognosis in clear cell renal cell carcinoma.

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Abstract

Objective: The purpose of this study was to investigate whether miR-495 is a factor influencing survival in clear cell renal cell carcinoma (ccRCC) patients.
Methods: Quantitative real-time PCR (qRTPCR) was performed to evaluate the expression level of miR-495 in 186 participants. Then the association between tissue miR-495 expression level and clinical outcome was investigated. Overall survival was evaluated using the Kaplan-Meier method. Multivariate analysis of the prognostic factors was performed with Cox proportional hazards model.
Results: The expression level of miR-495 was significantly decreased in renal cancer tissues compared with that in normal matched tissues, and a high expression of miR-495 was found to be significantly associated with Histologic grade (p=0.000), Lymph nodemetastasis (p=0.000), and Distant metastasis (p<0.001). In addition, the results of Log-rank test indicated that ccRCC patients with low miR-495 expression experienced shorter overall survival. Multivariate analysis suggested that miR-495 expression was an independent prognostic factor for overall survival of patients with ccRCC.
Conclusions: Our results indicate that miR-495 relates to the prognosis of patients with ccRCC and may act as a promising predictor of ccRCC recurrence.

Keywords: MiR-495, Clear cell renal cell carcinoma, Quantitative real-time PCR, Prognosis.

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Introduction

Renal cell carcinoma (RCC) is responsible for approximately 3% of all cancers in adults and is the third most common urological cancer, with incidence rates increasing 2% per year [1,2]. Approximately 60,920 novel cases of RCC were diagnosed in the United States in 2011, with an estimated 13,120 mortalities [3]. Clear cell RCC (ccRCC) is the most type of RCC. Radical nephrectomy is effective to cure early and local ccRCC, but patients with metastatic RCC [4] face a poor prognosis and have limited therapeutic options. Therefore, investigating the pathogenesis and biological features of ccRCC is crucial to enhance early detection and treatment.

MicroRNAs (miRNAs) are short non-coding regulatory RNA involved in regulation of important cellular processes as differentiation, proliferation, cell cycle, or apoptosis [5,6]. Accumulating evidence has shown that miRNAs can participate in tumour genesis, progression and metastasis either as oncogenes or tumour suppressors [7,8]. MiR-495 was reported to act as a tumor suppressor gene or an oncogene in a lot of cancers including non-small cell lung cancer, breast cancer, glioblastoma, and gastric cancer [9-11]. Recently, Lv et al. found that miR-495 suppresses human renal cell carcinoma malignancy by targeting *SATB1* [12]. Those results informed that down-regulation of miR-495 levels are associated with worse outcome in ccRCC. However, to our knowledge, the relationship between the expression of miR-495 and survival in ccRCC patients remains to be determined.

In the present study, we explored the expression of miR-495 in human ccRCC tissues and investigated the correlation between the expression of miR-495 and clinicopathologic factors and survival in ccRCC patients.

Materials and Methods

Patients and tissue samples

This study was approved by the Institutional Review Board of the Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University. All patients written informed consent and are agreed to participate in this study. All samples were obtained with informed consent and approved by the hospital institutional review board.

Fresh clinical ccRCC specimens and adjacent normal tissues were collected from 166 patients who underwent radical nephrectomy between 2007 and 2010 in the Second Affiliated Hospital & Yuying Children's Hospital of Wenzhou Medical University. All patients did not receive anticancer treatment,

including chemotherapy, radiotherapy and biotherapy, prior to surgery resection. Samples were flash frozen in liquid nitrogen until use. The clinical and pathological information from patient records was gathered, and the details were listed in Table 1.

qRT-PCR of miR-495 expression

Total RNA was extracted from frozen tissue using TRIzol Reagent (Applied Invitrogen, Carlsbad, CA, USA). RNA concentration and purification were conducted using the NanoDrop ND-1000 spectrophotometer (Nanodrop, Wilmington, DE, USA). The expression level of miR-495 was analyzed using the Hairpin-it miRNA qPCR.

Quantitation Kit (GenePharma, Shanghai, China) following the manufacturer's instructions. Reverse transcription and quantitative PCR were performed using the One Step PrimeScript miRNA cDNA Synthesis Kit (Takara, Dalian, China) by using the ABI 7500 Real Time PCR system (Applied Biosystems, Foster City, CA, USA). Relative quantification of target miRNA expression was evaluated using the comparative cycle threshold (CT) method. U6small nuclear RNA was used as an internal control.

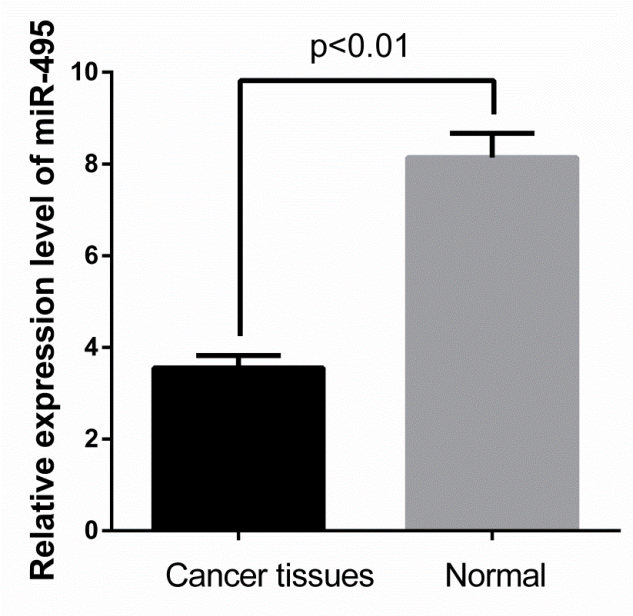

Figure 1. MiR-495 expression in 186ccRCC tissues samples and corresponding normal tissues.

Statistical analysis

Statistical analysis was conducted using the SPSS 18.0 for Windows (SPSS Inc., Chicago, IL, USA). The distinct expression of miR-495 between tumor tissues and normal tissues was examined by independent samples t-test. Chi-square test and t test were performed to explore the associations between miR-495 expression and clinical characteristics. The overall survival was analyzed by log-rank test, and survival curve was plotted based on Kaplan-Meier method. Multivariate analysis was performed using the Cox proportional hazard model. P values less than 0.05 were considered statistically significant.

Results

MiR-495 expression decreases in human ccRCC

To investigate the potential roles of miR-495 in ccRCC development, The expression levels of miR-495 in osteosarcoma and corresponding noncancerous biopsy samples were detected by qRT-PCR and normalized to U6. The results showed that ccRCC tissues had significantly lower miR-495 expression levels (P<0.01; Figures 1 and 2) compared to noncancerous tissues.

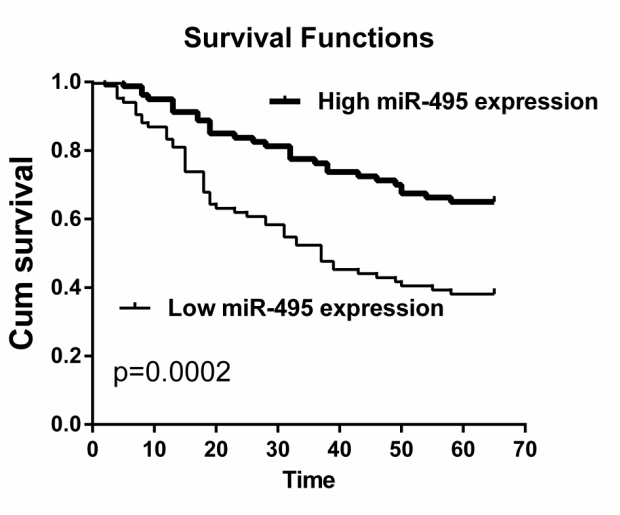

Figure 2. Overall survival of 186 ccRCC patients in relation to miR-495 expression levels.

Association of miR-495 expression with the linicopathological characteristics of human ccRCC

The correlation of miR-495 expression with different clinicopathological parameters in gliomas was illustrated in Table 2. Low expression of miR-495 was found to significantly correlate with higher histological grade (p=0.000), lymph node metastasis (p=0.000) and tumor distant metastasis (p=0.001). No significant correlations were observed between miR-495 expression and any other clinicopathological features, such as gender, age, tumor size, tumor stage (p>0.05).

Correlation between miR-495 expression and overall survival

The prognostic value of miR-495 expression for overall survival in ccRCC patients was evaluated by comparing the patients with high and low miR-495 expression. Kaplan-Meier survival analysis and log-rank test demonstrated that patients with low expression of miR-495 had significantly worse overall survival rates compared with those who had cancers with low miR-495 expression (p=0.0002). Univariate and multivariate analyses were utilized to evaluate whether the miR-495 expression level was independent prognostic

parameters of patient outcomes. The results showed that the expression of miR-495 was an independent prognostic factor for overall patient survival (p=0.011, Table 2).

Table 1. *Clinicopathological features and miR-495 expression in ccRCCpatients.*

Variables	Cases (n=166)	miR-495 expression level		p value
		High expression	Low expression	
Age (years)				0.660
<55	106	51	55	
≥ 55	60	31	29	
Gender				0.762
man	79	40	39	
woman	87	42	45	
Tumor size (cm)				0.750
<4	79	38	41	
≥ 4	87	44	43	
Histologic grade				0.000
I-II	94	32	62	
III-IV	72	50	22	
Tumor stage				0.218
T1-T2	75	41	34	
T3-T4	91	41	50	
Lymph nodemetastasis				0.000
Absence	65	45	20	
Presence	101	37	64	
Distant metastasis				0.001
Absence	66	43	23	
Presence	100	39	61	

Table 2. *Prognostic factors in Cox proportional hazards model.*

Variable	Univariate analysis			Multivariate analysis		
	Risk ratio	95% CI	p	Risk ratio	95% CI	p
Gender Male *vs.* Female	1.412	0.771-1.498	0.617			
Age (years) ≥ 55 *vs.* <55	1.332	0.417-1.659	0.326			
Tumor stage T3-4 *vs.* T1-2	2.441	1.983-3.771	0.179			
Histological grade I-II *vs.* III-IV	3.389	2.169-5.881	< 0.001	2.817	1.741-4.441	0.004
Lymph node Presence *vs.* Absence	5.417	3.012-8.118	0.012	3.341	2.367-4.615	0.026
Distant metastasis Presence *vs.* Absence	5.361	3.016-9.558	0.002	3.561	2.871-7.554	0.018
miR-495 low *vs.* high	3.515	2.376-7.663	0.006	3.147	2.218-6.225	0.011

Discussion

Renal cell carcinoma remains to be one of the leading causes of death [13]. It is necessary to search for novel markers for ccRCC, which could improve the outcome of this lethal disease. MiRNAs are aberrant expressions in multiple tumors and play an important role in tumorigenesis and development and may be used as novel biomarkers for the prognosis and treatment of cancer. For instance, Bai et al. found that down-regulation of miR-32 predicted poor prognosis in human non-small cell lung cancer [14]. Zhang et al. showed that MicroRNA-377 suppressed proliferation and invasion of human glioblastoma cells by directly targeting specificity protein 1 [15]. Zhang et al. identified Serum miR-200c as a prognostic biomarker for gastric cancer [16]. Zhu et al. found that MiR-451 acts as an anti-oncogene in RCC and the down-regulation of miR-451 was correlated with lower survival rate of RCC patients [17]. In our present study, our attention focuses on miR-495.

In our present study, we showed that miR-495 was significantly down-regulated in ccRCC tissues for the first time. The relationship of the miR-495 with various clinical features of ccRCC was analyzed. The results showed that low expression of miR-495 was correlated with higher histological grade, lymph node metastasis and tumor distant metastasis. Suggesting that miR-495 might be involved in the carcinogenesis and metastasis of ccRCC. Furthermore, ccRCC patients with low miR-495 expression level had distinctly shorter OS than patients with high miR-495 expression level. The results of Cox regression analyses revealed that miR-495 may be an independent prognostic marker for ccRCC patients.

Several studies reported that miR-495 acted as a tumor suppressor gene or an oncogene in a lot of cancers. For instance, miR-495 functions as a tumor suppressor in acute myeloid leukemia (AML) by targeting essential leukemia-related genes [18]. Xu et al. found that MicroRNA-495 suppressed cell growth and migration in endometrial cancer by targeting *FOXC1* [19]. Another study revealed that miR-495 acts as an oncogene in breast cancer *via* down regulation of E-cadherin and *REDD1* [20]. To our interest, Lv et al. found that MicroRNA-495 served as a tumor suppressor in human ccRCC, suggesting that miRNA-495 could be promising biomarkers for ccRCC prognosis.

Conclusion

We provide the evidence that miR-495 was down regulated in ccRCC patients and may act as independent prognostic factors for ccRCC patients.

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