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Expression of microRNA-3133 correlates with the prognosis in patients with clear cell renal cell carcinoma

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Abstract

Clear cell renal cell carcinoma (ccRCC) represents a prevalent urological malignancy among men worldwide. MicroRNAs (miRNAs) are involved in the progression of diverse human cancers. The aim of this study was to explore the expression profile and prognostic value of microRNA-3133 (miR-3133) in ccRCC.

The expression of *miR-3133* in ccRCC tissues and non-cancerous tissues was measured by quantitative real-time polymerase chain reaction (qRT-PCR). Chi-square test was applied to evaluate the relationship between *miR-3133* expression and clinical characteristics. Overall survival curve was constructed by Kaplan–Meier with log-rank test. The prognostic value of *miR-3133* in ccRCC was estimated by Cox regression analysis.

MiR-3133 was downregulated in ccRCC samples compared to the matched noncancerous samples ($P < .01$). Moreover, its expression level was correlated with T stage, vascular invasion and lymph node metastasis (all $P < .05$). Survival curves demonstrated that patients with low level of *miR-3133* underwent lower overall survival than those with high level (log rank test, $P = .002$). *MiR-3133* might be an independent prognostic biomarker in ccRCC patients (HR = 2.802, 95% CI = 1.391–5.646, $P = .004$).

MiR-3133 is downregulated, and plays inhibitory roles in aggressive progression of ccRCC. *MiR-3133* may be an independent prognostic biomarker for ccRCC.

Abbreviations: ccRCC = Clear cell renal cell carcinoma, miR-3133 = microRNA-3133, miRNAs = microRNAs, nccRCC = non-clear cell renal cell carcinoma, qRT-PCR = quantitative real-time polymerase chain reaction, RCC = renal cell carcinoma.

Keywords: clear cell renal cell carcinoma, *MiR-3133*, prognosis

1. Introduction

Renal cell carcinoma (RCC) represents one of the most common malignancies and a common cause of cancer-related deaths in male in developed countries.[1] Based on cytogenetic and histological signatures, RCC can be divided into non-clear cell renal cell carcinoma (nccRCC) and clear cell renal cell carcinoma (ccRCC).[2,3] The cancer is characterized by aggressive metastasis. Most of the patients are at metastatic stage when diagnosed, missing the optimal operation chance. Despite of surgery, some of the patients still develop postoperative recurrence or metastasis.[4,5] Metastatic stage represents a challenge for ccRCC patients, due to its high resistance to chemotherapy and radiotherapy.[6,7] Although great progress has been made in

the therapeutic strategy, the 5-year survival rates of patients with ccRCC are still not ideal.[8,9] Until now, the progression of ccRCC cannot be sufficiently predicted by clinical characteristics or common molecular biomarkers.[10] Therefore, novel and effective prognostic biomarkers are urgently needed for prognosis evaluation and improving the managements of ccRCC.

MicroRNAs (miRNAs) are a class of short and non-coding RNA molecules. They have been reported to negatively regulate gene expression, thus degradation of their targeted mRNAs and suppression of transplantation.[11,12] Accumulated evidences suggest that miRNAs play important roles in various malignancies via controlling tumor cell proliferation, invasion, migration, and apoptosis.[13–16] It is considered that the abnormal expression of miRNAs is significantly associated with tumorigenesis that can act as the diagnostic and prognostic biomarker in human cancers, including ccRCC.[17–19] In the previous tumor investigation, a variety of miRNAs were confirmed as oncogenes or tumor suppressor in the pathogenesis of ccRCC. ccRCC related oncogenic miRNAs included *miR-630*, *miR-155*, *miR-106b*, and so on,[20–22] while *miR-182*, *miR-206*, and *miR-335* played suppressive roles in progression of ccRCC.[23–25] MicroRNA-3133 (*MiR-3133*), a common member of cancer-related miRNAs, has ever been reported to be located at the cancer-related cytogenetic defined common fragiles site (CFSs).[26] It was a novel identified miRNA, and its functional roles in progression of ccRCC remained poorly known.

In the present study, we sought to investigate the expression level of *miR-3133* in ccRCC tissues samples, as well as its predictive significance for clinical outcomes of patients with ccRCC.

2. Materials and methods

2.1. Patients and tissue sample collection

Total of 135 ccRCC patient were enrolled in our study. They were diagnosed in Zhongnan Hospital, None of the patients received chemotherapy or radiotherapy before samples collection in this study. ccRCC tissue samples and matched noncancerous samples were collected from each patient and immediately stored in liquid nitrogen at −80°C for further experiments. Meanwhile, we investigated the clinicopathological characteristics of ccRCC patients, including the patients' age, gender, tumor size, T stage, vascular invasion, lymph node metastasis, and Fuhrman grade. The status of vascular invasion was detected by magnetic resonance imaging (MRI). When ccRCC invaded blood vessel, renal veins thickened, uneven density. The clinical characteristics of the patients were summarized in Table 1.

All the patients with ccRCC were followed up ranged from 6 to 60 months by telephone. The protocol of this study and the use of these clinical materials were approved by the ethics committee of Zhongnan Hospital. All the patients provided the informed consents before tissues collection.

2.2. RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNAs including miRNAs were isolated from the ccRCC tissue samples by using TRIzol reagent (Incitrogen, Carlsbad, CA) as per the manufacturer's protocols. The ratio of OD260/OD280 was measured to investigate the concentration and purity of RNAs.

The relative expression of *miR-3133* was examined by qRT-PCR. The cDNAs used in the PCR reaction were obtained by reverse transcription of RNAs with the AMV reverse transcription system (Promega, CA). qRT-PCR reaction was performed on the 7300 Real-Time PCR System (Applied Biosystems, MA) with the SYBR Green PCR master mix (Applied Biosystems). In this reaction, *U6* gene was selected to act as the endogenous control gene, and the relative expression of *miR-3133* was calculated with $2^{-\Delta\Delta Ct}$ method and normalized by the *U6* expression level.

2.3. Statistical analysis

All the statistical analyses were performed in SPSS 18.0 statistical software (SPSS, Chicago, IL). The continuous data in these analyses were expressed as mean±standard deviation (SD). The different expression of *miR-3133* between ccRCC samples and the paired noncancerous samples was analyzed by Student t test. The correlation of *miR-3133* expression with the clinicopathological data was examined with Chi-square test. Overall survival curve was built by Kaplan–Meier method with log-rank test. Cox regression analysis was carried out to analyze the prognostic value of *miR-3133* and the clinicopathological features in ccRCC. P values less than .05 were considered statistically significant.

3. Results

3.1. The expression level of miR-3133

In the present study, the expression level of *miR-3133* was calculated with the method of qRT-PCR. The result of qRT-PCR demonstrated that the expression of *miR-3133* was significantly downregulated in the ccRCC samples compared with the paired noncancerous samples ($P < .01$, Fig. 1).

3.2. Association between miR-3133 and clinicopathological features of ccRCC patients

The patients were divided into high expression group (n=60) and low expression group (n=75) based on their median expression value of *miR-3133*. Chi-square test was used to assess the correlation of *miR-3133* with the clinicopathological data of ccRCC patients. The results revealed that downregulated *miR-3133* expression was correlated with the advanced T stage ($P = .012$), positive vascular invasion ($P = .044$) and lymph node metastasis ($P = .010$). However, the significant relationship between *miR-3133* with patients' age,

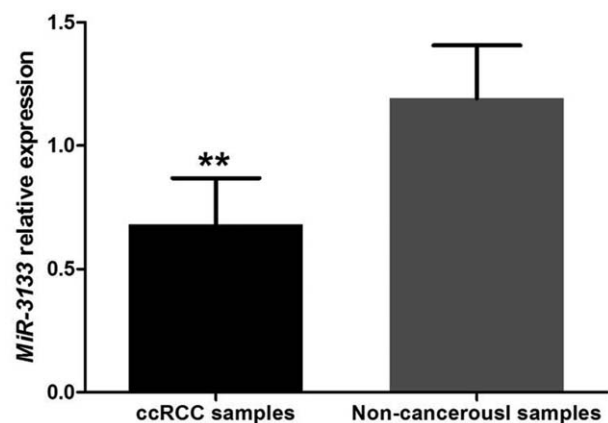| Features	No. n=135	*miR-3133* expression		P values
		Low (n=75)	High (n=60)	
Age, yr				
≤55	47	28	19	.492
>55	88	47	41	
Gender				
Female	55	30	25	.845
Male	80	45	35	
Tumor size, cm				
≤4	61	33	28	.757
>4	74	42	32	
T stage				
T1-T2	67	30	37	**.012**
T3-T4	68	45	23	
Vascular invasion				
Negative	59	27	32	**.044**
Positive	76	48	28	
Lymph node metastasis				
Negative	71	32	39	**.010**
Positive	64	43	21	
Fuhrman grade				
I-II	67	35	32	.441
III-IV	68	40	28	

Figure 1. The expression level of *miR-3133* in both ccRCC samples and matched non-cancerous samples. The expression of *miR-3133* was lower in ccRCC samples than that in the paired noncancerous samples. **: suggested $P < .01$.
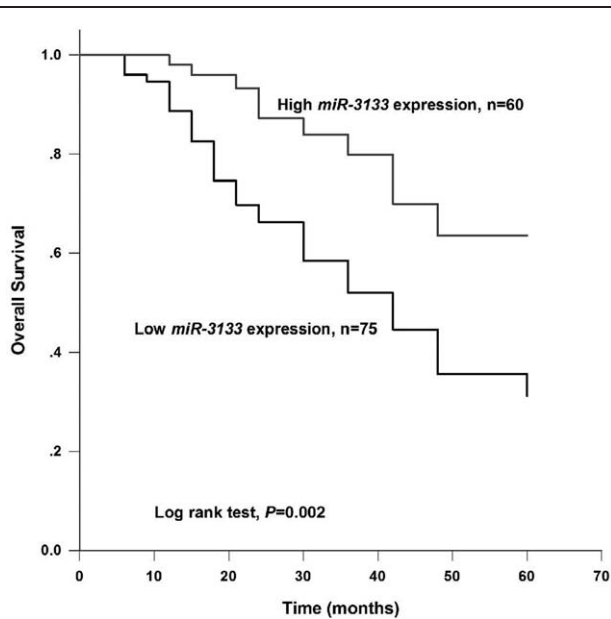
Figure 2. The Kaplan-Meier survival curves based on the expression of *miR-3133* in patients with ccRCC. The downregulated expression of *miR-3133* indicated poor survival rates for patients suffering from ccRCC (log rank test, *P* = .002).

gender, tumor size, and Fuhrman grade was not found in this analysis (all *P* > .05) (Table 1).

3.3. The prognostic value of miR-3133 in patients with ccRCC

The result of Kaplan–Meier survival analysis suggested that the patients with low expression of *miR-3133* had poorer survival rates than those with high expression (log-rank *P* = .002, Fig. 2). In order to examine the prognostic value of *miR-3133* in ccRCC, Cox analysis was performed. The results in Table 2 suggested that *miR-3133* was an independent prognostic factor in ccRCC (HR = 2.802, 95% CI = 1.391–5.646, *P* = .004).

4. Discussion

RCC is a most frequently diagnosed urological malignancy among men in the world.[27] ccRCC represents the most common subtype of RCC. Due to the non-specific symptoms at the early

stage, most of the patients appear metastasis at the time of diagnosis, contributing to high mortality of this disease.[28] Therefore, identification of novel and effective prognostic biomarker is crucial for patients suffering from ccRCC.

MiRNAs are a class of small non-coding RNAs that can regulate gene expression at post-transcriptional level. It is considered that miRNAs can bind to the 3'-untranslated region (3'-UTR) of their targeted mRNAs, thus repressing the translation of functional proteins.[29] Abnormal expression of miRNAs may contribute to disease, like malignancy. In addition, the expression patterns of miRNAs can be stably and accurately detected in archived tissue specimens and body fluids.[30] Therefore, miRNAs may provide a promising way for early detection and prognosis evaluation of cancer.

In the present study, we investigated the clinical significance of *miR-3133* in ccRCC. The expression level of *miR-3133* in ccRCC tissues was detected by using qRT-PCR analysis. The result suggested that the expression of *miR-3133* was decreased in ccRCC samples compared with the matched noncancerous samples. Moreover, the downregulated expression of *miR-3133* was significantly correlated with the advanced T stage, positive vascular invasion and lymph node metastasis of ccRCC patients. However, the expression profiles of *miR-3133* did not show significant association with patients' age, gender, tumor size, or Fuhrman grade. All the data revealed that *miR-3133* served as a tumor suppressor and played inhibitory roles in aggressive progression of ccRCC. The conclusion was consistent with the previous research carried out in other types of cancer. Lu et al found that the expression profiles of *miR-3133* were significantly different between laryngeal carcinoma tissues and adjacent normal tissues, suggesting its roles in early onset and development of the disease.[31] However, it was worthy noting that *miR-3133* was a novel identified cancer-related gene, and its functional roles in tumorigenesis remained poorly known. Therefore, further investigation was still needed to confirm the action of *miR-3133* in the pathogenesis of ccRCC.

Given their pivotal roles in tumor progression, accumulating evidence had proved that miRNAs could act as diagnostic and prognostic biomarkers in a wide of human cancers, including ccRCC. Vergho et al suggested that the expression of *miR-126* and *miR-21* might independently predict cancer-specific survival for ccRCC patients that could be employed as prognostic biomarkers for the cancer.[32] Nofech-Mozes et al reported that *miR-194* was down-regulated in ccRCC, and correlated with aggressive clinical characteristics and poor prognosis of the patients.[33] Khella et al suggested that the expression of *miR-126* was decreased in ccRCC

Table 2

Cox regression analysis for prognosis in ccRCC patients.

Variables	Univariate analysis			Multivariate analysis		
	HR	95%CI	P value	HR	955CI	P value
MiR-3133 (low vs high)	2.802	1.391–5.646	0.004	2.802	1.391–5.646	.004
Age, yr (>55 vs ≤55)	1.439	0.744–2.781	0.279	–	–	–
Gender (male vs female)	1.259	0.694–2.283	0.448	–	–	–
Tumor size (cm) (>4 vs ≤4)	1.266	0.695–2.306	0.440	–	–	–
T stage (T3-T4 vs T1-T2)	1.588	1.855–2.950	0.143	–	–	–
Vascular invasion (positive vs negative)	1.344	0.737–2.449	0.334	–	–	–
Lymph node metastasis (positive vs negative)	1.593	0.885–2.867	0.120	–	–	–
Fuhrman grade (III-IV vs I-II)	1.337	0.746–2.395	0.330	–	–	–

−= indicated no related data, CI = confidence interval, HR = hazard ratio.

patients, and showed obvious association with dismal disease-free survival and overall survival.[34] In the present study, we investigated the prognostic value of *miR-3133* expression in ccRCC patients. The survival curves demonstrated that patients with high expression of *miR-3133* had a prolonged overall survival than those with low level. Furthermore, cox regression analysis suggested that *miR-3133* had the potential to serve as an independent prognostic factor in patients with ccRCC. However, due to the relatively small sample in the study, further investigations were still required to identify the application value of *miR-3133* in predicting prognosis of ccRCC patients.

Taken together, *miR-3133* is down-regulated in the ccRCC samples and plays an inhibitory role in the aggressive progression of ccRCC. *MiR-3133* mat is a potential prognostic biomarker in patients with ccRCC.

Author contributions

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