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 cyto genetic and histological signatures, RCC can be divided into non-clear cell renal cell carcinoma (nccRCC) and clear cell renal cell carcinoma (ccRCC). 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. 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 miRNAs 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 while miR-182, miR-206, and miR-335 played suppressive roles in progression of ccRCC.[21–25] 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 $2^{-\Delta\Delta C_t}$ 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,
Table 2: Cox regression analysis for prognosis in ccRCC patients.

| Variables                          | Univariate analysis | Multivariate analysis |
|-----------------------------------|---------------------|-----------------------|
|                                   | HR                  | 95% CI                | P value  | HR                  | 95% CI                | P value  |
| miR-3133 (low vs high)            | 2.802               | 1.391–5.646           | 0.004    | 2.802               | 1.391–5.646           | 0.004    |
| 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)    | 1.858               | 1.746–2.905           | 0.002    | –                   | –                    | –        |

– = indicated no related data, CI = confidence interval, HR = hazard ratio.
patients, and showed obvious association with dismal disease-free survival and overall survival. 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.

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