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Original Article

Up-regulation of miR-630 in clear cell renal cell carcinoma is associated with lower overall survival

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Abstract: Introduction: MicroRNAs (miRNAs) are noncoding RNAs that regulate multiple cellular processes during cancer progression. MiR-630 has recently been identified to be involved in tumorigenesis of several cancers such as lung cancer and gastric cancer. However, the regulation of miR-630 in clear cell renal cell carcinoma (ccRCC) has not yet been reported before. Methods: Expression of miR-630 was evaluated by quantitative real-time PCR in tumour and their normal matched tissues in n = 92 ccRCC patients, and its association with overall survival of patients was analyzed by statistical analysis. Results: The expression level of miR-630 was significantly higher in renal cancer in comparison to normal matched tissue ($P < 0.05$). It is also proved that miR-630 expression was to be associated with renal cancer histologic grade, lymphnode metastasis, distant metastasis ($P < 0.05$). In addition, the Kaplan-Meier survival curves revealed that high miR-630 expression was associated with poor prognosis in ccRCC patients. miR-630 expression was an independent prognostic marker of overall ccRCC patient survival in a multivariate analysis. Conclusions: The study proves for the first time that miR-630 is upregulated in a majority of ccRCC patients. It also shows that miR-630 expression is an independent prognostic factor for patients with renal cancer, which might be a potential valuable biomarker for ccRCC.

Keywords: MiR-630, clear cell renal cell carcinoma, quantitative real-time PCR, prognosis

Introduction

Renal cell carcinoma (RCC) is the most lethal urologic tumor and the sixth leading cause of cancer deaths in Western countries. Each year, around 200,000 patients are diagnosed with this malignancy resulting in approximately 100,000 deaths, and its incidence is increasing steadily in recent years [1, 2]. RCC is represented by 80% by clear cell RCC (ccRCC), originating from the renal proximal tubule [3]. Nearly 25-30% of patients with RCC have evidence of metastases at initial presentation [4, 5]. Although radical nephrectomy is effective to cure early and local RCCs, 30% of patients develop metastatic disease after surgery [6]. Patients with metastatic RCC face a dismal prognosis and have limited therapeutic options. Median survival in a recent cohort was only 1.5 years with fewer than 10% of patients surviving to 5 years [7]. Therefore, the goal to attain a more thorough understanding of the molecular biology, genetic causes, and cellular origin of ccRCC is of great significance in the development of improved therapeutic strategies and in the identification of prognostic markers.

MicroRNAs (miRNA) are small noncoding RNA molecules that inhibit gene expression at the post-transcriptional levels. Thus, miRNA binding to the 3' untranslated region of target mRNAs can result in mRNA degradation or translation inhibition, depending on the degree of complementary base pairing [8]. It has been widely accepted that miRNAs play pivotal roles in various biological processes, including tumor progression and metastasis through regulation of cell migration, invasion, proliferation, epithelial-to-mensenchymal ransition, angiogenesis and apoptosis [9]. Aberrant miRNA expression has been associated with oncogenesis, and some miRNAs act as tumor suppressors,

Table 1. Association of miR-630 with clinicopathological characteristics of ccRCC patients

Parameters	Group	Total	MiR-630 expression		*P value*
			High	Low	
Gender	Male	43	24	19	0.178
	Female	49	34	15	
Age (years)	< 65	39	25	14	0.857
	≥ 65	53	33	20	
Histological grade	I-II	32	28	4	0.000
	III-IV	60	30	30	
Tumor size (cm)	< 4 cm	45	29	16	0.785
	≥ 4 cm	47	29	18	
Tumor stage	T_1-T_2	59	38	21	0.717
	T_3-T_4	33	20	13	
Lymph nodes metastasis	Absence	77	55	22	0.000
	Presence	15	3	12	
Distant metastasis	Absence	80	56	24	0.000
	Presence	12	2	10	

whereas others as oncogenes, depending on their targets, which may provide insight into the diagnosis and prognosis of human malignancies [10]. Recent studies have shown that expression of miRNAs is deregulated in various types of human cancer like renal cancer, prostate cancer, breast cancer, lung cancer and pancreas cancer [11]. In human renal cancer, lots of miRNAs with aberrant expression have been identified, such as miR-21, miR-26a, miR-106, miR-126 and miR-141 which played oncogenic or suppressive role [12, 13]. These results showed that miRNAs can play diverse and crucial roles in human malignancies including renal cancer. However, the expression profile of miRNAs is highly tissue and cancer type specific and miRNA specific, thus demonstrating the functional and clinical significance of a specific miRNA may provide clinically relevant insights into miRNA function and efficacious cancer management [14]. Galluzzi study on non-small cell lung found that miR-630 could regulate cisplatin-induced cancer cell death, indicating miR-630 was a potential modulator of the cisplatin response in non-small cell lung cancer [15]. Chu indicated that upregulation of miR-630 levels are associated with worse outcome in gastric cancer [16]. However, the clinical and prognostic significance of miR-630 expression in renal cancer has not been reported yet.

In our study, we investigated the expression level of miR-630 in clinical renal cancer specimens and adjacent normal tissues, and examined relationship between miR-630 expression and clinical and pathohistological parameters in renal cancer patients.

Materials and methods

Patients and specimens

This study was approved by the Research Ethics Committee of Affiliated Hospital Of Hebei University Of Engineering (Hebei, China), Written informed consent was obtained from all of the patients. All specimens were made anonymous and handled according to the ethical and legal standards.

Fresh clinical ccRCC specimens and adjacent normal tissues were collected from 92 patients who underwent radical nephrectomy between 2006 and 2008 in Affiliated Hospital of Hebei University of Engineering (Hebei, China). None of the patients had received chemotherapy or radiotherapy before surgery. The histomorphology of all tissue specimens were confirmed by the Department of Pathology, Affiliated Hospital of Hebei University of Engineering (Hebei, China). Specimens were put immediately into liquid N_2 after surgical resection for 10 min, then into a -70°C ultra-freezer for mRNA isolation. Clinical data of all the patients were collected from hospitalisation and subsequent records. Detailed information is listed in **Table 1**. All patients were followed up until September 2012 with a median observation time of 48 months.

Quantitative real-time PCR

Total RNA was purified from all the 92 renal cancer and matching adjacent normal specimens by the manufacturer using Trizol reagent (Invitrogen, CA, USA). Only those total RNA samples with OD A260/A280 ratio close to value of 2.0, which indicates that the RNA is pure, were subsequently analyzed. The miR-630 and RNU44 internal control specific cDNA were synthesized from total RNA using gene-specific primers according to the TaqMan MicroRNA assays protocol (Applied Biosystems, CA, USA).

The reverse transcription products were then amplified and detected by real-time PCR using Taqman MicroRNA Assay (Applied Biosystems) specific for hsa-miR-630. Each sample was examined in triplicate and the raw data were presented as the relative quantification of miR-630 expression evaluated by the comparative cycle threshold (CT) method, normalized with respect to RNU44. Mean normalized miR-630 expression ± standard deviation (SD) was calculated from triplicate analysis. Real-time PCR was performed using an ABI 7900 system (Applied Biosystems) and comparative $2^{-\Delta\Delta Ct}$ analysis was performed using SDS 2.2.2 software (Applied Biosystems) as described previously [17].

Statistical analysis

All computations were carried out using the SPSS software version 17.0 for Windows (IBM Corporation, NY, USA). Data were expressed as mean ± SD. Paired Student's t-test was conducted to compare miR-630 expression in paired clinical samples. The association between miR-630 expression and clinicopathologic characteristics of RCC patients was assessed by Mann-Whitney U and Kruskal-Wallis tests. Survival curves were obtained by using the Kaplan-Meier method and compared by using the log-rank test. Multivariate survival analysis was performed using the Cox proportional hazards model. The factors selected from univariate analysis, based on a $P < 0.05$, was entered into the Cox proportional hazards model. $P < 0.05$ was considered statistically significant.

Results

Increased expression of miR-630 in human ccRCC

The expression of miR-630 was detected in 92 human renal cancer specimens and matched adjacent normal tissues by quantitative real-time PCR. Relative expression of miR-630 normalized to RNU44 in renal cancer was found to be 7.36 ± 1.27, while that in matching adjacent normal specimens was 2.86 ± 0.57. Statistical results showed that the expression of miR-630 in renal cancer was significantly higher than that in adjacent normal specimens. These results indicated that miR-630 might play an oncogenic role in renal cancer. ccRCC patients who expressed miR-630 at levels less than 3.53 (mean ± SD expression of miR-630 in adjacent normal specimens) were assigned to the low expression group, and those with expression above than 3.53 assigned to the high expression group.

Relationship between miR-630 expression and ccRCC patients' clinicopathologic variables

In our ccRCC cohort, the relationship between the expression of miR-630 and patient clinical characteristics was shown in **Table 1**. High expression of miR-630 was found to significantly correlate with higher histological grade ($P = 0.001$), lymph node metastasis ($P = 0.015$) and tumor distant metastasis ($P = 0.004$). No significant difference in miR-630 expression was observed with gender, age, tumor size, tumor stage ($P > 0.05$).

Relationship between clinicopathologic features, miR-630 expression, and ccRCC patients' survival: univariate survival analysis

In univariate survival analyses, cumulative survival curves were calculated according to the Kaplan-Meier method. Differences in survival times were assessed using the log-rank test. First, to confirm the representativeness of the ccRCC in our study, we analyzed established prognostic predictors of patient survival. Kaplan-Meier analysis demonstrated a significant impact of well-known clinical pathological prognostic parameters, such as histological grade, lymph node status and tumor distant metastasis on patient survival ($P < 0.05$, **Table 2**). Assessment of survival in ccRCC patients revealed that higher expression of miR-630 was correlated with adverse survival of ccRCC patients ($P = 0.008$, **Table 2**, **Figure 1**).

Independent prognostic factors for ccRCC: multivariate Cox regression analysis

Since variables observed to have a prognostic influence by univariate analysis may covariate, the expression of miR-630 and those clinicalopathological parameters that were significant in univariate analysis (histological grade, lymph node status and tumor distant metastasis) were further examined in multivariate analysis. The results showed that the expression of miR-

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	1.173	0.619-1.587	0.594			
Male vs Female						
Age (years)	1.439	0.517-1.894	0.264			
≥ 65 vs < 65						
Tumor stage	2.642	1.784-3.921	0.149			
T_{3-4} vs T_{1-2}						
Histological grade	3.264	1.976-6.043	< 0.001	2.781	1.867-4.638	0.006
I-II vs III-IV						
Lymph node	5.108	2.974-8.265	0.015	3.681	2.171-4.472	0.031
Presence vs Absence						
Distant metastasis	5.979	3.165-10.392	0.004	3.936	2.675-6.894	0.013
Presence vs Absence						
miR-630	3.378	2.219-6.448	0.008	3.021	2.074-5.726	0.016
low vs high						

Abbreviations: CI, confidence interval; miR, microRNA.

630 was an independent prognostic factor for overall patient survival (relative risk: 3.021, CI: 2.074-5.726, $P = 0.016$, **Table 2**). With regard to other parameters, histological grade, lymph node status and tumor distant metastasis status were also shown to be an independent prognostic factorfor overall survival ($P < 0.05$, **Table 2**).

Discussion

Clinical management of ccRCC has changed in recent years with increased incidental diagnosis and by initiating therapy in localized stages and the establishment of antiangiogenic agents. While tumour size at time of diagnosis has decreased, mortality rate for ccRCC has not suggesting an impact of differential tumour biology in morphologically similar tumours [18]. Therefore, identification of patients at high risk for cancer progression is warranted to tailor adjuvant treatment.

Recent studies showed more than 1,900 human miRNAs have been identified, which are estimated to regulate over 60% of genes in mammals [19]. Due to their great importance in the regulation of gene expression. It has been widely accepted that miRNAs are involved in multiple cellular functions such as differentiation, proliferation and apoptosis, thus, have been implemented in diverse physiological and pathological processes ranging from development to cancer [20, 21]. Patnaik investigation showed expression profiles of miRNA in stage I non-small cell lung cancer to identify patterns that might predict recurrence after surgical resection found that miR-630 was one of the most commonly miRNA among the 1,000 classifiers identified [22]. Another study demonstrated that miR-630 can regulate cisplatin-induced cancer cell death in lung cancer [15]. Farhana indicated that upregulation of miR-630 induces apoptosis in pancreatic cancer cells by targeting IGF-1R [23]. MiR-630 was also found to be upregulated in head and neck squamous cell carcinoma after cisplatin treatment and can modulate the protein expression of ATG5, ATG6, BECN1, ATG10, ATG12, ATG16L1 and UVRAG [24]. Chu found that miR-630 expression was an independent prognostic factor for patients with gastric cancer, which might be a potential valuable biomarker for gastric cancer [16].

In the present study, we sought to determine whether there was any difference in miR-630 expression between ccRCC and normal tissue samples which had not been studied previously. This study showed that miR-630 were upregulated in the ccRCC, and explored available evidence of close correlation of miR-630 expression and the total patients' survival during a five-year follow-up survey.

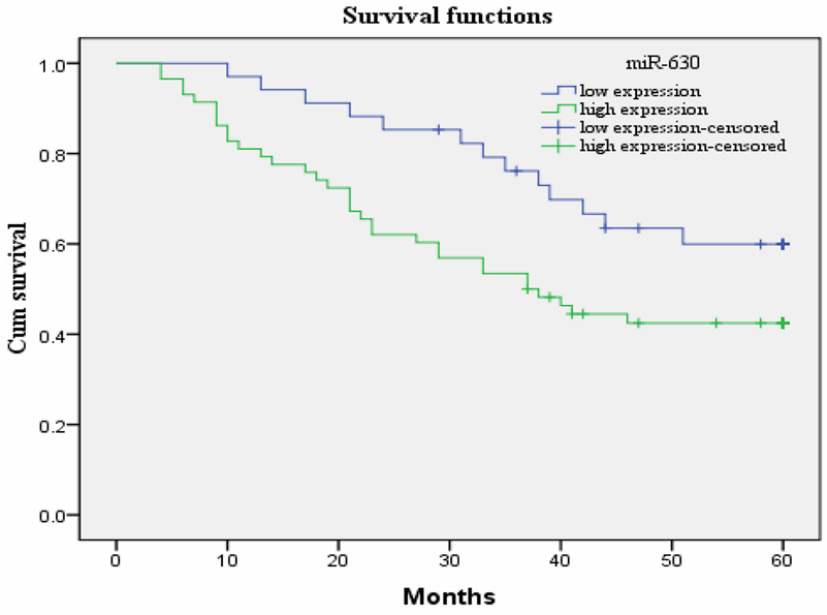

Figure 1. Kaplan-Meier postoperative survival curve for patterns of patients with renal cancer and miR-630 expression.

To directly address the potential roles for miR-630 in the occurrence and development of renal cancer, an elaborate experiment was conducted and a rigorous analysis was performed of human miR-630 expression on a renal cancer samples. Our results revealed that the miR-630 expression in renal cancer specimens was remarkably higher than that in normal renal tissues ($P < 0.05$). Chu's study also indicated that the abnormal expression of miR-630 might be correlated with gastric oncogenic event [16]. In the present study, we found the expression level of miR-630 was significantly associated with histological grade ($P < 0.001$), lymph node metastasis ($P = 0.015$) and distant metastasis ($P = 0.004$). It is suggested that miR-630 may play important roles in renal cancer carcinogenesis and progression and may affect tumor invasion and metastasis. We further imagined that miR-630 may affect the activation of cellular signal transduction pathway, cell division cycle and tumor angiogenesis to influence biological behavior of tumor, and this had just been unraveled in the latest relevant researches. Recent study showed that on non-small cell lung found that miR-630 could regulate cisplatin-induced cancer cell death and in pancreatic cancer cells miR-630 could induce apoptosis by targeting IGF-1R [15, 23].

Ultimately, A total of 92 patients histologically proven renal cancer with follow-up information were conducted a systematically analysis to confirm the relationship of miR-630 and outcome of patient initially. Our finding demonstrated that patients with higher expression of miR-630 in tumor tissue had a worse overall survival than patients with lower expression ($P < 0.05$, respectively), providing an evidence that elevated expression of miR-630 in renal cancer might facilitate an increased malignant and worse prognostic phenotype. It is noteworthy that by multivariate Cox analysis combining expression of miR-630 with other parameters, miR-630 was found as an independent prognostic factor ($P = 0.016$) for patient survival. The aberrant expression of miR-630 linked to a poor prognosis of patients has never been investigated in renal cancer before.

In conclusion, we have reported that miR-630 expression was increased in renal cancer and associated with tumor progression. Our results suggested for the first time that miR-630 expression was an independent prognostic factor of patients with renal cancer. Therefore, it is possible that miR-630 may play an important role in metastasis and invasiveness of renal cancer. It is also possible that miR-630 serves as prognostic marker in clinical practice. Apparently, a further understanding of the molecular mechanism by miR-630 in human ccRCC would help in the discovery of novel targeted agents and might also lead to the development of new approaches for efective therapy of human ccRCC.

Disclosure of conflict of interest

None.

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