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Oncogene miR-154-5p regulates cellular function and acts as a molecular marker with poor prognosis in renal cell carcinoma

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ABSTRACT

Aims: In adult population, the renal cell carcinoma (RCC) is one of the most common urological malignancies. It is meaningful to research for the molecular markers which are involved in the occurrence and development of RCC. Therefore, we concentrate on illuminating the role of microRNA-154-5p in progression of RCC and explore its prognostic values.

Main methods: The real-time quantitative polymerase chain reaction (RT-qPCR) was applied to determine expression level of miR-154-5p in tissues. Afterwards, the transfected cell lines ACHN and 786-O were used for the CCK-8 assay, MTT assay, wound healing assay, transwell assay and flow cytometric assay to explore the role of miR-154-5p in regulating cellular function. In addition, formalin-fixed paraffin-embedded (FFPE) renal cancer samples were used for detecting the relationship between expression level of miR-154-5p and clinical information. Furthermore, univariate and multivariate Cox proportional-hazards regression analyses, and the Kaplan-Meier survival curves were performed to evaluate the prognostic value of miR-154-5p in RCC.

Key findings: The RT-qPCR indicated that miR-154-5p is up-regulated in RCC pathologic specimens and cell lines. Results of study also demonstrated that upregulation of miR-154-5p reduced cell apoptosis and promoted cell proliferation, viability, migration as well as invasion in RCC cells. The prognosis analyses indicated that the expression level of miR-154-5p is associated with the prognosis of renal cancer, and the overall survival of patients with low expression is longer.

Significance: The present study revealed that the oncogene miR-154-5p regulates cellular function and acts as a molecular marker with poor prognosis in renal cell carcinoma.

1. Introduction

Renal cell carcinoma (RCC) is considered as the most common malignant tumor in kidney, accounting for approximately 3% of all adult malignances [1–3]. There are five histological types of RCC, including clear cell RCC (65%), papillary RCC (15%), chromophobic RCC (1%) and collecting duct RCC (4%) and unclassified RCC(5%) [4]. In the past two decades, the incidence of RCC has increased gradually [5]. At present, up to 30% patients have suffered from cancer metastasis when they were primarily diagnosed with RCC [6]. In addition, approximately 20–40% RCC, which is localized, have metastasized distally after surgical treatment [7]. It could be said that RCC is still an extremely lethal disease, and timely detection and early diagnosis is extremely important for the treatment of RCC. Thus, it would be significant to illustrate the genesis and development of RCC and explore its potential molecular mechanisms.

In recent years, microRNA has been defined as the regulator and potential biomarker, playing a crucial part in oncogenesis, development and metastasis of RCC [8,9]. As one kind of small non-coding RNAs, through attaching to the complementary sequences in mRNA transcripts, microRNA regulates the post-transcriptional expression of specific genes to repress the translation of functional from those

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transcripts, even cause their degradation [10–12]. Thus, MircroRNA might function as a molecular marker of RCC on early diagnosis, prognosis prediction and targeted treatment.

Previous research have revealed that miR-154-5p have dysregulated expression in many tumors of different organs and tissues, such as prostate cancer [13], colorectal cancer [14] and hepatocellular carcinoma [15]. Nevertheless, to the best of our knowledge, there is still no relevant research in the kidney. Therefore, the present study systematically describes the function of miR-154-5p in RCC to investigate the roles of miR-154-5p in renal carcinogenesis. Furthermore, univariate and multivariate Cox proportional-hazards regression analysis, and the Kaplan-Meier survival curves were performed to predict the prognostic value of miR-154-5p in renal cell carcinoma.

2. Materials and methods

2.1. Human specimens and cell lines

All human pathologic specimens of Renal cell carcinoma (RCC) were collected and stored at −80 °C in RNAlater (Qiagen, Valencia, CA, USA) after permitted by Peking University Shenzhen Hospital Ethical Committee (Shenzhen, China). On the basic of the 2010 American Joint Committee on Cancer staging system (AJCC), all RCC tissues were classified,and results are given in the Table 1.

2.2. Paraffin specimens of renal carcinoma

All Formalin-fixed paraffin-embedded (FFPE) specimens of renal carcinoma were provided by the Peking University Shenzhen Hospital. As shown in Table 2, the clinicopathological characteristics were analyzed on the basis of the 2010 AJCC. The miRNeasy FFPE Kit (Qiagen) was used for extracting RNA from paraffin specimens.

2.3. RNA extraction, reverse transcription and RT-qPCR

With the use of TRIzol Reagent (Invitrogen; Thermo Fisher Scientific, Inc.), total RNA was extracted, and refined by using the RNeasy Maxi kit (Qiagen GmbH). Subsequently, with the purpose of determining the expression of miR-154-5p, the qPCR was applied to reverse transcription using the miScript Reverse Transcription kit (Qiagen GmbH) in Roche Lightcycler 480 Real Time PCR system (Roche Diagnostics, Basel, Switzerland). The expression levels of miR-154-5p in pathologic specimens and cell lines were normalized to the expression levels of internal control U6.2-ΔΔCq method was the analytical method used in the study [16]. The primer sequences can be seen in Table 3.

Table 1
Clinicopathological features in RCC patients.

Characteristics	Number of cases
Mean age, range (years)	51(24–67)
Male	10
Female	09
Histological type	
Clear cell	17
Papillary	2
pT-stage	
T1	13
T2	4
T3 + T4	2
Fuhrman grade	
I	5
II	12
III	1
IV	1
AJCC clinical stages	
I	4
II	12
III + IV	2

pT, primary tumor; AJCC, American Joint Committee on Cancer.

Table 2
Relationship between miR-154-5p expression level[a] and Clinical information in FFPE renal cancer samples.

Variable	Total	No. of patients (%)		P-value[b]
		High	Low	
Gender				
Male	26	14	12	0.751
Female	16	7	9	
Age (years)				
≤60	33	17	16	1.000
> 60	9	4	5	
Tumor size(cm)				
≤4.0	17	9	8	1.000
> 4.0	25	12	13	
Tumor stage				
I + II	27	12	15	0.520
III + IV	15	9	6	

[a] Cut-off point: median.
[b] Calculated using Fisher's Exact test or Pearson Chi-square test.

Table 3
Sequences of primers and microRNAs.

Primer/microRNA	Sequence
miR-154-5p	Forward: 5′-TAGGTTATCCGTGTTGCCTTCG-3′
	Reverse: Universal primers (miScript SYBR Green PCR kit)
U6	Forward: 5′-CTCGCTTCGGCAGCACA-3′
	Reverse: 5′-ACGCTTCACGAATTTGCGT-3′
miR-154-5p mimic	Forward: 5′-UAGGUUAUCCGUGUUGCCUUCG-3′
	Reverse: 5′-AAGGCAACACGGAUAACCUAUU-3′
NC	Forward: 5′-UUCUCCGAACGUGUCACGUTT-3′
	Reverse: 5′-ACGUGACACGUUCGGAGAATT-3′
miR-154-5p inhibitor	5′-CGAAGGCAACACGGAUAACCUA-3′
NC inhibitor	5′-CAGUACUUUUGUGUAGUACAA-3′

miR, microRNA; NC, negative control; PCR, polymerase chain reaction.

2.4. Cell culture and transfection

ACHN and 786-O cell lines, were respectively raised with Dulbecco's modified Eagle's medium (DMEM; Gibco; Thermo Fisher Scientific Inc. Waltham, MA, USA) and Roswell Park Memorial Institute 1640 medium (Gibco), both them mixed together with 10% fetal bovine serum (Gibco), 1% antibiotics (penicillin, 100 μ/ml; streptomycin sulfates, 100 mg/ml) and 1% glutamate (Gibco). And then, all RCC cells were cultured in a humidified chamber at 37 °C and with 5% CO2. For preparation of transfection reagents, mimics and inhibitor, artificially synthesized by GenePharma Inc. (Shanghai, China), were mixed with Lipofectamine 2000 (Invitrogen Life Technologies) and added into Opti-MEM I Reduced Serum medium (Gibco, Thermo Fisher Scientific, Inc.). After that, RT-qPCR was performed to evaluate the expression of miR-154-5p and the transfection efficiency.

2.5. Cell proliferation assay

The ability of cell proliferation in cells were assessed with the Cell Counting Kit-8 (CCK-8) assay. About 5×10^3 RCC cells were seeded and transfected in 96-well plates. Those cells, after transfected 0 h, 24 h, 48 h and 72 h, were respectively incubated with 10 μl CCK-8 solution (US Everbright Inc.) for 30 min. In the end, ELISA microplate reader (Bio-Rad, Hercules, CA, USA) was performed at the wavelength of 490/630 nm to measure the optical density (OD) to estimate cell count.