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Oncogene miR-187-5p is associated with cellular proliferation, migration, invasion, apoptosis and an increased risk of recurrence in bladder cancer

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ABSTRACT

Background: Bladder cancer, the ninth-most-common malignancy worldwide with an estimated 356,000 new cases and 145,000 deaths annually, has a propensity to relapse, requiring lifelong monitoring after diagnosis. 75% patients diagnosed with BC are non-muscle invasive BC and over 50% of them experience recurrences within 6–12 years of initial diagnosis. miRNA are small, noncoding RNA and shown to be oncogenes or anti-oncogenes in bladder cancer, contributing to numerous BC cell processes, including cell proliferation, differentiation, migration and apoptosis.

Methods: RT-qPCR were performed to detect the expression of miR-187-5p in tissues and cell lines, After which, clinicopathological variables and the prognostic value of altered miR-187-5p expression in BC was analyzed with the 48 formalin-fixed paraffin-embedded BC samples. Moreover, Cell functional assays (wound healing assay, CCK-8 assay, transwell assay and flow cytometry assay) were performed to explore the relationship between miR-187-5p expression and cell proliferation, migration, invasion and apoptosis in BC.

Results: Up-regulation of miR-187-5p was observed in BC tissues and BC cell lines. Cox proportional hazard regression analysis demonstrated that the patients with low expression of miR-187-5p experience lower risks of recurrence in the univariate and multivariate analysis. The Kaplan-Meier recurrence-free curves suggested that the patients with low expression of miR-187-5p experience lower risks of recurrence. Up-regulation of miR-187-5p promotes cell proliferation and mobility and inhibits the apoptosis of 5637 and UM-UC-3 cell, while down-regulation of miR-187-5p reverses these effects.

Conclusions: The results of our study demonstrated that oncogene miR-187-5p is associated with cellular proliferation, migration, invasion, apoptosis and an increased risk of recurrence in bladder cancer.

1. Introduction

Bladder cancer(BC), the ninth-most-common malignancy and the thirteenth deadliest worldwide with an estimated 356,000 new cases and 145,000 deaths annually, has a propensity to relapse, requiring lifelong monitoring after diagnosis [1–4]. According to current literature, 75% patients diagnosed with BC are non-muscle invasive BC and over 50% of them experience recurrences within 6 to 12 years of initial diagnosis [5,6]. However, little progress on the treatment for BC was made in the over 30 years [7]. Range of therapeutics provided by clinicians are limited, resulting in the 5-year survival rates being flat for 30 years [1]. Worse still, BC imposes the highest medical expense to the patients from diagnosis to death compared with other types of cancers [8]. Recent studies have shown that early diagnosis holds the key to a positive prognosis [9,10]. Molecular markers for early diagnosis, stratifying patient treatment and prognosis detection are urgently needed.

MicroRNAs (miRNAs) are small, noncoding RNA, ranging from 21 to 25 nucleotides in length and playing pivotal roles in the regulation of

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gene expression [11,12]. Since firstly discovered in 1993, miRNAs were found to be implicated in the regulation of more than 50% of mRNAs in mammalian genomes [13,14]. As such, miRNAs serve as critical cogs and are essential for various biological processes, and dysregulation of miRNAs are associated with numerous human diseases, including cancer [15,16]. miRNAs are shown to be oncogenes(such as miR-92 and miR-194) or anti-oncogenes(such as miR-126 and miR-99a) in bladder cancer, contributing to numerous BC cell processes, including cell proliferation, differentiation, migration and apoptosis [17–21].

More recently, miR-187-5p, a promising tumor-associated microRNA located on chromosome18q12.2, has been reported to play significant roles in the initiation and progression of certain types of tumors, including ovarian cancer [22], renal cell carcinoma [23], prostate cancer [24], osteosarcoma [25]. However, the expression and functions of miR-187-5p in BC have not yet been definitely settled, that is to say, our experiments were conducted firstly to study the role of miR-187-5p in BC. In present study, the amounts of miR-187-5p in BC tissues and cell lines was quantified by RT-qPCR, after which, clinicopathological variables and the prognostic value of altered miR-187-5p expression in BC was analyzed with the 48 formalin-fixed paraffin-embedded (FFPE) BC samples. Moreover, transwell assay as well as wound scratch assay, cell counting Kit-8 (CCK-8) assay and flow cytometry assay were performed to clarify the effects of the miR-187-5p on cell mobility, proliferation and apoptosis, respectively.

2. Materials and methods

2.1. Sample collection

After approved by the ethics committee of Peking University Shenzhen Hospital, we collected altogether 44 paired BC tissues and adjacent normal bladder tissues (a distance of 3 cm from the BC tissues) from the patients who underwent BC tissues resection at Peking University Shenzhen Hospital (Shenzhen, Guangdong, China). We had obtained informed consent form each patient before collecting the specimens. All patients enrolled in our study were diagnosed with BC according to histopathological evaluation. All specimens were dipped in RNAlater (Qiagen GmbH, Inc., Hilden, Germany) in 30 min following surgery and afterwards stored at 80℃ refrigerator. Clinical-pathologic features of the patients with were recorded and summarized in Table 1.

2.2. Formalin-fixed paraffin-embedded tissue specimens

The formalin-fixed paraffin-embedded (FFPE) bladder cancer samples were obtained from Department of pathology of Peking University Shenzhen Hospital. All the BC patients of the FFPE samples underwent surgery at Peking University Shenzhen Hospital between 2013 and 2015. Clinical stage of tumors was defined by the 6th AJCC TNM staging system, are presented in Table 2. The total RNA from the FFPE samples was extract by using the miRNeasy FFPE Kit(Qiagen).

Table 1
Clinicopathological features of bladder cancer patients.

Characteristics	Number of cases
Mean age (Year)	72
Range (Year)	51-88
Gander	
Male	34
Female	10
Histological type	
Transitional cell	39
Squamous cell	5
Tumor size	
< 3 cm	28
≥ 3 cm	16
Pathologic stage	
Ta	28
T1	14
T2	2
Pathologic grade	
G1	12
G2	23
G3	9

Table 2
Association between miR-187-5p expression level[a] and Clinical information in FFPE bladder cancer samples.

Variable	Total	No. of patients(%)		P-value[b]
		High	Low	
Gender				
Male	41	23	18	1.000
Female	7	4	3	
Age(Years)				
≥ 60	33	19	14	0.761
< 60	15	8	8	
Smoking	39	22	17	1.000
No	9	5	4	
Yes				
Tumor size(cm)				
< 3	26	15	11	1.000
≥ 3	22	12	10	
Tumor number	34	18	14	0.749
Single	14	9	5	
Multiple				
Clinical stage				
Ta	30	15	15	0.369
T1	18	12	6	

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

2.3. Cell culture and cell transfection

The normal transitional epithelial cell (SV-HUC-1) and BC cell lines including RT4, J82, UM-UC-3, 5637 and T24, were maintained in Dulbecco's modified Eagle's medium (DMEM; Gibco; Thermo Fisher Scientific. Inc., Waltham, MA, USA) or RPMI-1640 (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with fetal bovine serum (FBS; 10% Gibco; Thermo Fisher Scientific, Inc.), antibiotics (1% 100 μl/ml penicillin and 100 mg/ml streptomycin sulfates) and glutamine(1%). These cell lines were purchased from Shanghai Institute of Biochemistry and Cell Biology (Shanghai, China) and maintained in 37 ˚C in a 5% CO2 incubator. For transfection, miR-187-5p mimic(F: 5′-GGCUACAACACAGGACCCGGGC-3′, R:5′-GCCCGGGUCCUGUGUUGUAGCC3′), inhibitor(5′- GCCCGGGUCCUGUGUUGUAGCC -3′), negative control(NC: F:5′-UCACAACCUCCUAGAAAGAGUAGA3′, F: 5′- UCACAACCUCCUAGAAAGAGUAGA 3) and inhibitor NC(5′-UCUACUCUUUCUAGGAGGUUGUGA 3′)(Sangon Biotech, Shanghai, China) were transfected into 5637 and UM-UC-3 cells by using Lipofectamine 3000 (Invitrogen Life Technologies) when the cells were at 60%–70% confluence. The transfection efficiency was authenticated by RT-qPCR.

2.4. RNA extraction, cDNA synthesis and RT-qPCR

The RNA in BC tissues and cell lines were extracted with Trizol (Invitrogen; Thermo Fisher Scientific, Inc.) and afterwards purified with the RNeasy Maxi kit (Qiagen GmbH) according to the provided instructions. The extractive RNAs with optical density ratio < 1.8 or > 2.0 (measured by NanoDrop 2000/2000c; 260/280) were excluded from our study. Reverse transcription reactions and RT-qPCR were performed using miScript Reverse Transcription kit (Qiagen GmbH) and miScript SYBR® Green PCR kit (Qiagen GmbH). The conditions of RT-PCR were: 95 ˚C for 1 min, and then, 40 cycles of 95 ˚C for 10 s, 55 ˚C for 30 s and 70 ˚C for 30 s. After normalization to U6, the