MicroRNA-20b-5p functions as a tumor suppressor in renal cell carcinoma by regulating cellular proliferation, migration and apoptosis

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Abstract. Renal cell carcinoma (RCC) is the most common type of kidney cancer in adults and is associated with a poor prognosis due to a lack of early-warning signs, protean clinical manifestations, and resistance to radiotherapy and chemotherapy. Recently, increasing evidence has suggested that microRNAs (miRNAs) are involved in the proliferation, invasion and apoptosis of various types of human cancer cells. In a previous study, miRNA expression profiles from renal cell carcinoma (RCC) revealed that expression of miR-20b-5p was significantly downregulated in RCC tissues. The aim of this study was to investigate the expression and functional significance of miR-20b-5p in RCC. The expression of miR-20b-5p was quantified in 48 paired RCC tissues and cell lines, and compared with adjacent normal tissues and the 293T cell line by reverse transcription-quantitative polymerase chain reaction. The functional impact of miR-20b-5p on cell proliferation, cell migration and apoptosis in the 786-O and ACHN RCC cell lines, was determined by an 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay, a scratch assay and flow cytometry. To the best of our knowledge, the present study was the first to reveal that miR-20b-5p was downregulated in RCC tissues and cell lines. It also demonstrated that upregulation of miR-20b-5p inhibited cellular migration and proliferation, and promoted cellular apoptosis, suggesting that miR-20b-5p functioned as a potential tumor suppressor. However, further studies are required to fully determine the effects of miR-20b-5p and the miR-20b-5p-mediated molecular pathway in RCC and other types of cancer. In conclusion, these results imply that miR-20b-5p may be a biomarker for early detection and prognosis prediction, as well as a therapeutic target for RCC.

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Introduction

Renal cell carcinoma (RCC) is the most common type of kidney cancer in adults, accounting for ~90% of all renal tumors and 3.9% of all cancers (1,2). In the United States, renal cancer is the 6th and 8th leading malignancy among men and women, respectively. In 2014, 63,920 novel cases and 13,860-related fatalities from renal cancer are estimated to have occurred (3). In China, kidney cancer incidence has increased with an average annual growth rate of 6.5% over the past 20 years, and kidney cancer-related mortality has surpassed bladder cancer as the most common cancer of the urinary system (4,5). RCC is characterized by a lack of early-warning signs, protean clinical manifestations, and resistance to radiotherapy and chemotherapy (6). As a result, 25% of patients present with advanced disease when initially diagnosed with RCC, and 33.3% of the patients who undergo resection of localized disease have a recurrence (7). Survival of patients with localized tumors who undergo radical nephrectomy is significantly longer than patients with regional and distant metastasis, thus underscoring the importance of early detection (8). Therefore, it is critical to identify novel molecular biomarkers for early detection, diagnosis and targeted therapy.

MicroRNAs (miRNAs) are 21-25 nucleotide endogenously produced non-coding RNAs, which regulate the expression of numerous genes, and are significant role in a wide range of biological processes, including animal and plant development, cell proliferation, cell differentiation, apoptosis and metabolism. Generally, miRNAs, as negative regulators, bind to a partially complementary sequence usually located in the 3'-untranslated region (3'-UTR) of their target mRNA and inhibit its translation (9). Due to partial complementation, a

specific miRNA can regulate multiple genes and a single gene can be modulated by hundreds of miRNAs at the post-transcriptional level (10).

Recently, miR-20b-5p has been reported to be dysregulated in malignancies of the breast (11-13), stomach (14), cervix (15), blood (16), oropharynx (17) and colorectum (18). A recent miRNA microarray chip analysis showed that miR-20b-5p was downregulated in ccRCC (19). Thus, the expression and function of miR-20b-5p in renal cancer requires further investigation. The aim of the present study was to identify miR-20b-5p as a tumor suppressor in RCC. Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) was performed to determine the downregulation of miR-20b-5p in RCC tissues and cell lines compared with paired normal tissues and cell lines and the effects of miR-20b-5p on cell migration, proliferation and apoptosis were analyzed in RCC cell lines.

Materials and methods

Specimens. A total of 48 paired fresh RCC samples and adjacent normal tissue samples (located 2.0 cm outside of visible RCC lesions) were obtained from the Peking University Shenzhen Hospital (Shenzhen, China). Written informed consent was obtained from all patients. The study was approved by the ethics committee of the Peking University Shenzhen Hospital (Shenzhen, China). The clinical specimens were collected between September 2012 and November 2014. All fresh tissue samples were immediately immersed in RNAlater® RNA Stabilization agent (Qiagen, Inc., Hilden, Germany) following surgical resection, snap-frozen in liquid nitrogen and stored in a cryo freezer at -80˚C for further use. The clinicopathological information of the patients is shown in Table I. Stage classification was performed according to the 2010 American Joint Committee on Cancer staging system (20).

Cell culture and transfection. 786-O and ACHN human RCC cell lines and the 293T normal kidney human embryo kidney cell used in the present study were obtained from the Guangdong and Shenzhen Key Laboratory of Male Reproductive Medicine and Genetics (Shenzen, China). The human RCC cell lines, including 786-O and ACHN, were originally obtained from the American Type Culture Collection (Manassas, VA, USA). The human embryo kidney cell line 293T (293T) was purchased from the Type Culture Collection of the Chinese Academy of Medical Sciences (Beijing, China). All cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco; Thermo Fisher Scientific Inc., Waltham, MA, USA), with 10% fetal bovine serum (Gibco), 1% antibiotics (100 μ/ml penicillin and 100 mg/ml streptomycin sulfates) and 1% glutamate (Gibco), and then incubated at 37˚C in a humidified chamber containing 5% CO_2. For the upregulation of miR-20b-5p, synthesized miR-20b-5p mimics (GenePharma, Shanghai, China) and Lipofectamine 2000 (Invitrogen; Thermo Fisher Scientific Inc.) were mixed into the Gibco™ Opti-MEM I Reduced Serum medium (Thermo Fisher Scientific, Inc.) for transfection, after the cells were seeded and cultured for 24 h. Then, fluorescence microscopy (using a fluorescence microscope obtained from Carl Zeiss Pte. Ltd., Oberkochen, Germany) and RT-qPCR were used to verify transfection efficiency.

Extraction of total RNA and RT-qPCR. Total RNA was extracted from 48 paired RCC samples and normal tissue using TRIzol reagent (Invitrogen) and were purified using the RNeasy Maxi kit (Qiagen), according to the manufacturer's instructions. 786-O, ACHN and 293T cells ($4x10^5$ cells/well) were plated into 6-well plates (BD Biosciences, USA) with three replicate wells, respectively. The cells were trypsinized, using Gibco™ trypsin (Thermo Fisher Scientific Inc.) to extract the total RNA using the miRNeasy Mini kit (Qiagen, Valencia, CA, USA) 24 h later. The RNA samples with 260/280 ratios of 1.8-2.0 were used for further experiments. Total RNA was converted into cDNA using the miScript II RT kit (Qiagen, Valencia, CA, USA).

The expression level of miR-20b-5p was confirmed with the miScriptSYBR green PCR kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions on the Roche lightcycler 480 Real-Time PCR system (Roche). U6 was used as the endogenous control to normalize the data. Their expression levels were shown as fold differences relative to U6, which was based on the equation relative quantification (RQ)=$2^{-\Delta\Delta Cq}$ [$\Delta\Delta Cq=(meanCq_{tumor}-meanCq_{control})-(meanCq_{normal}-meanCq_{control})$]. The miR-20b-5p forward primer was 5'-CAAAGTGCTCATAGTGCAGGTAG-3' and reverse primer was provided by the miScriptSYBR® green PCR kit (Qiagen, Valencia, CA, USA). The forward primer of U6 was 5'-CTCGCTTCGGCAGCACA-3' and reverse primer was 5'-ACGCTTCACGAATTTGCGT-3'. The reaction conditions for PCR were as follows: 95˚C for 15 min, followed by 40 cycles of 94˚C for 15 sec, 55˚C for 30 sec and 72˚C for 30 sec.

Cell scratch assay. The migratory ability of 786-O and ACHN cells was assessed by a wound scratch assay *in vitro*. Approximately $3x10^5$ cells were seeded per 12-well dish and transfected after 24 h with 100 pmol of either the miR-20b-5p mimics or a negative control, using Lipofectamine 2000. After 6 h of transfection, a sterile 200 μl pipette tip was used to scrape a clear line through the cell layer. The cells were then rinsed with phosphate-buffered saline (PBS) and cultured in serum-free DMEM. Images of the scratches were acquired using a digital camera system (Olympus Optical Co., Ltd., Tokyo, Japan) 0, 24 and 48 h after the scratches were made. The experiments were performed in triplicate and repeated ≥3 times.

Cell proliferation assay by MTT. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (MTT, 5 mg/ml; Sigma-Aldrich) was used to analyze the cell proliferation. 786-O and ACHN (5,000 cells/well) were plated into 96-well plates with 5 replicate wells of each condition. Each well was transfected with 5 pmol miR-20b-5p mimics or a negative control and assessed at 0, 24, 48 or 72 h post-transfection. The blank control wells were just set up with DMEM. Before measurement, 20 μl MTT was added to the cells and incubated at 37˚C in a humidified chamber containing 5% CO_2 for 6 h. Then, the MTT medium mixtures were discarded and 120 μl dimethyl sulphoxide (DMSO;

Table I. Clinicopathological features in RCC patients.

Characteristic	Number of cases
Mean age range (years)	52 (27-72)
Gender	
Male/female	30/18
Histological type	
Clear cell/papillary	39/9
pT-stage	
T1/T2/T3+T4	27/19/2
Fuhrman grade	
I/II/III/IV	15/22/8/3
AJCC clinical stages	
I/II/III+IV	27/18/3

pT, primary tumor; AJCC, American joint committee on cancer.

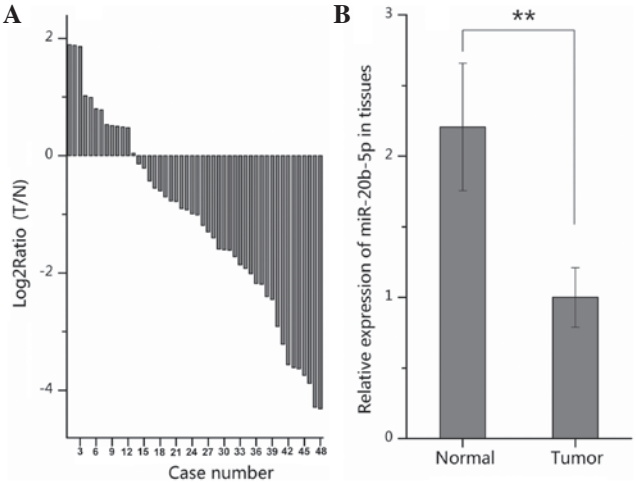

Figure 1. Expression of miR-20b-5p in 48 paired RCC tissues and adjacent normal tissues. (A) Log 2 ratios of miR-20b-5p expression in 48 paired RCC tissues vs. normal kidney tissues (T/N). (B) The relative expression of miR-20b-5p in RCC (tumor) and normal tissues (normal). **P<0.001. RCC, renal cell carcinoma.

Sigma-Aldrich, Shanghai, China) was added. After agitating for 30 min at room temperature, the absorbance was measured by the ELISA microplate reader (Bio-Rad, Hercules, CA, USA) at a wavelength of 490 nm (with 630 nm as the reference wave length).

Cell apoptosis assay. 786-O or ACHN cells ($3x10^5$) were plated in 6-well plates for the cell apoptosis assay. The cells were transfected with 200 pmol of miR-20b-5p mimics or the negative control (GenePharma) for 6 h. The sequence of the negative control RNA was as follows: Forward, 5'-UCC AUAAAGUAGGAAACACUACA-3'; and reverse, 5'-CAG UACUUUUGUGUAGUACAA-3'. After 48 h transfection, the cells, including floating cells, were harvested, washed twice with 4˚C PBS, resuspended in 100 μl of 1X binding buffer and stained with 3 μl of propidium iodide (PI) and 5 μl of Annexin V-fluorescein isothiocyanate (Invitrogen) for

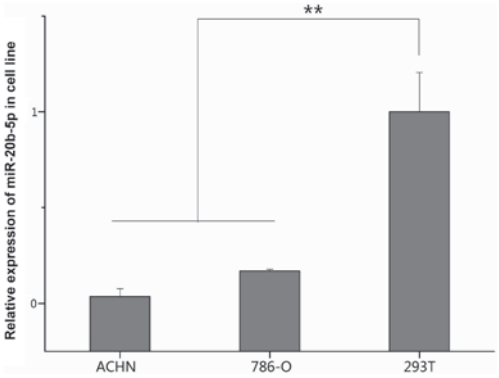

Figure 2. Expression of miR-20b-5p in RCC cell lines (786-O and ACHN) and the normal kidney human embryo kidney cell line (293T). **P<0.001, compared with the ACHN cells.

15 min at room temperature. Flow cytometry (EPICS, Xl-4, Beckman Coulter Inc., Brea, CA, USA) was used to quantify the percentage of apoptotic cells within 30 min of staining and 400 μl 1X binding buffer was added to each sample prior to measurement. Each experiment was conducted at least three times.

Bioinformatics. Predictions of potential targets of miR-20b-5p were performed using computational algorithms based on 'seed regions' between miRNAs and target genes. miRanda (http://mirdb.org/miRDB/index.html), TargetScan Release 6.2 (http://www.targetscan.org), microRNA (http://www.microrna.org) and miRWalk (http://www.umm.uni-heidelberg.de/apps/zmf/mirwalk) were used to explore the association between miR-20b-5p and long non-coding RNAs.

Statistical analysis. All data are presented as the mean ± standard deviation from the three independent experiments. Statistical analysis was conducted with SPSS 19.0 statistical software package (IBM, Armonk, NY, USA). Statistical significance was determined with Student's t-test. For the comparison of miR-20b-5p expression levels between matched tumor and normal samples a paired t-test was used. P<0.05 was considered to indicate a statistically significant difference.

Results

miR-20b-5p is downregulated in human RCC clinical specimens and RCC cell lines. A recent miRNA microarray chip analysis showed downregulation of miR-20b-5p in RCC tissues (19). To confirm downregulation of miR-20b-5p, RT-qPCR was performed in 48 paired RCC tissues and adjacent normal tissues. Relative expression of miR-20b-5p [Log2(T/N)] is shown in Fig. 1A. The present study demonstrated that the expression of miR-20b-5p in RCC tissues was significantly lower compared with adjacent normal tissues (P<0.001), as shown in Fig. 1B.

The expression of miR-20b-5p in two RCC cell lines (786-O and ACHN) and the 293T normal kidney human embryo kidney cell line was analyzed. As shown in Fig. 2, miR-20b-5p expression was significantly lower in 786-O and

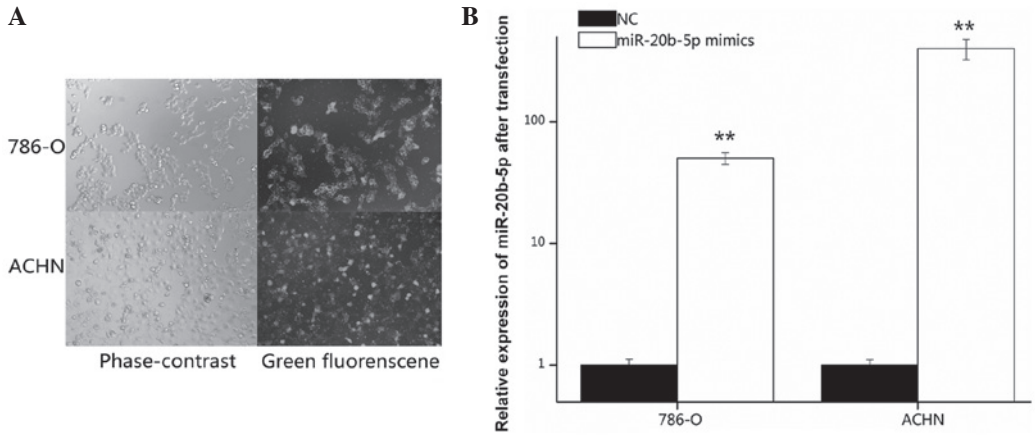

Figure 3. Validation of cell transfection efficiency. (A) Phase-contrast and green fluorescence images were taken from the same field (magnification, x500). (B) The relative expression of miR-20b-5p in 786-O and ACHN cells transfected with miR-20b-5p mimics or NC. **P<0.001, compared with NC. NC, negative control.

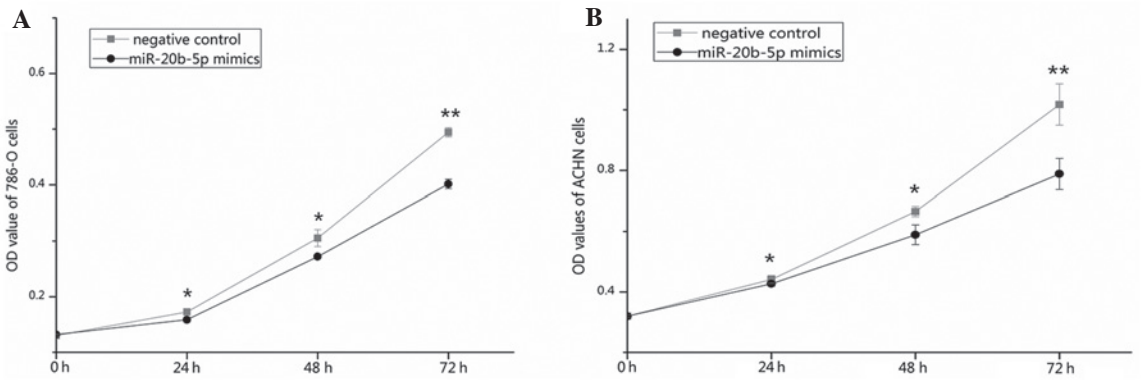

Figure 4. Cell proliferation of (A) 786-O and (B) ACHN cells was measured by a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay, at different time intervals. *P<0.05, **P<0.001, compared with the negative control. OD, optical density.

ACHN cells (P<0.001 and P<0.001) than that in the 293T cells, which is in accordance with the expression pattern of miR-20b-5p in tissues.

Validation of cell transfection efficiency. As shown in Fig. 3A, the transfection efficiency was >90% when the cells were transfected with fluorescein amidite-conjugated miRNA. RT-qPCR was also used to quantify the transfection efficiency, and revealed that expression of miR-20b-5p was 50.4 and 402.4 times that of negative control after transfection in 786-O (P<0.001) and ACHN (P<0.001), respectively (Fig. 3B).

miR-20b-5p inhibits cell proliferation. An MTT assay was used to determine whether overexpression of miR-20b-5p affected the proliferation of RCC cells. The outcomes revealed that the proliferation of 786-O cells was decreased by 8.11 (P<0.05), 10.92 (P<0.05) and 18.74% (P<0.01), and the proliferation of ACHN cells was decreased by 3.26 (P<0.05), 11.52 (P<0.05), and 22.53% (P<0.01) at 24, 48 and 72 h after transfection with miR-20b-5p mimics as compared with the negative control. The results indicated that the upregulation of miR-20b-5p expression significantly decreased the proliferation of renal cancer cells (Fig. 4).

miR-20b-5p suppresses cell migration. Cell migration was examined by a wound scratch assay. The results demonstrated that migratory distances of cells transfected with miR-20b-5p mimics were markedly shorter than that of the negative control group. The inhibition rates of migration of miR-20b-5p were 46.45 and 58.74% for 786-O cells (P<0.001), and 59.25 and 62.49% for ACHN cells (P<0.001) 2 and 48 h after transfection, compared with the negative control group. It is suggested that upregulation of miR-20b-5p inhibited the migratory ability in renal cancer cells (Fig. 5).

miR-20b-5p promotes cell apoptosis. The effects of miR-20b-5p on apoptosis were determined by flow cytometric analysis. After transfection with miR-20b-5p mimics or negative control for 48 h, 786-O and ACHN cells were harvested, and stained and the number of cells was quantified. The results demonstrated that the early apoptosis rate of 786-O cells was 11.77 and 7.21% (P<0.001) and of ACHN cells was 17.66 vs. 6.52% (P<0.05) when each were transfected with miR-20b-5p mimics or negative control, respectively. These data suggest that upregulation of miR-20b-5p promoted RCC cell apoptosis (Fig. 6).

Target gene prediction. miRanda, TargetScan Release 6.2, microRNA and miRWalk all predicted vascular endothelial

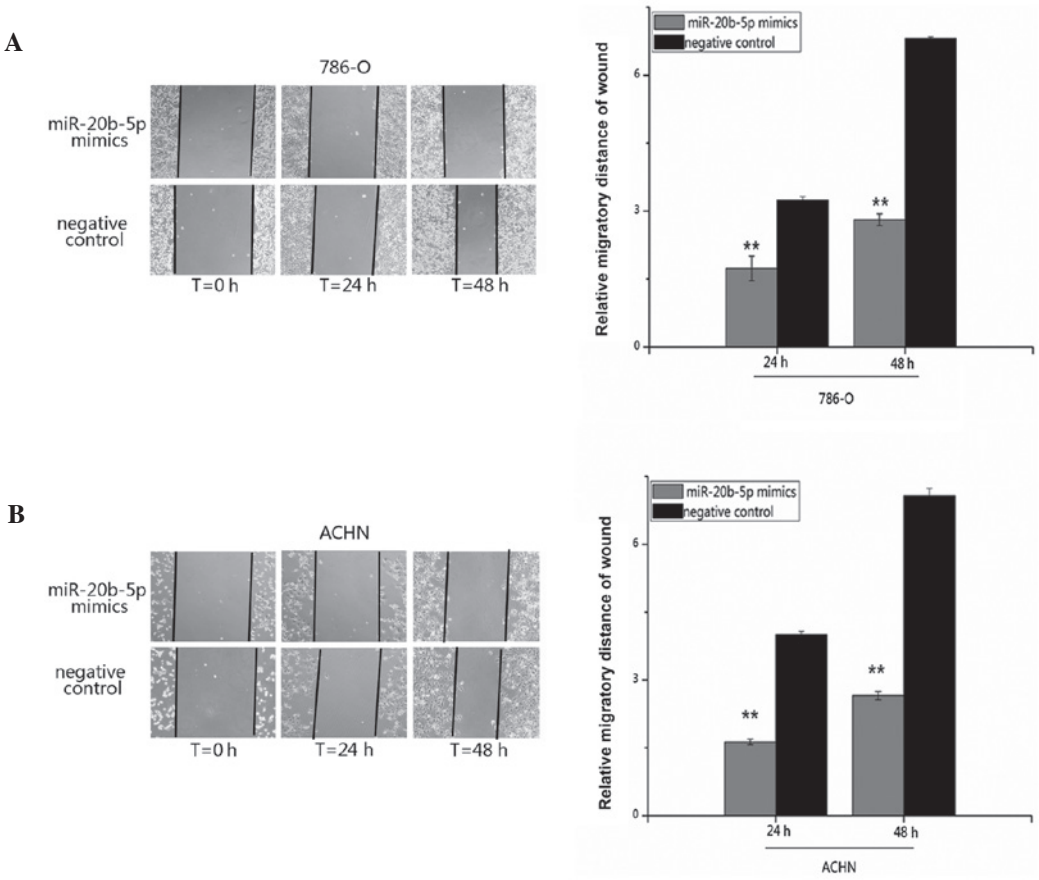

Figure 5. Cell scratch assay was used to examine the migration of (A) 786-O and (B) ACHN cells after transfection with miR-20b-5p mimics or negative control at 24 and 48 h. **P<0.001, compared with the negative control.

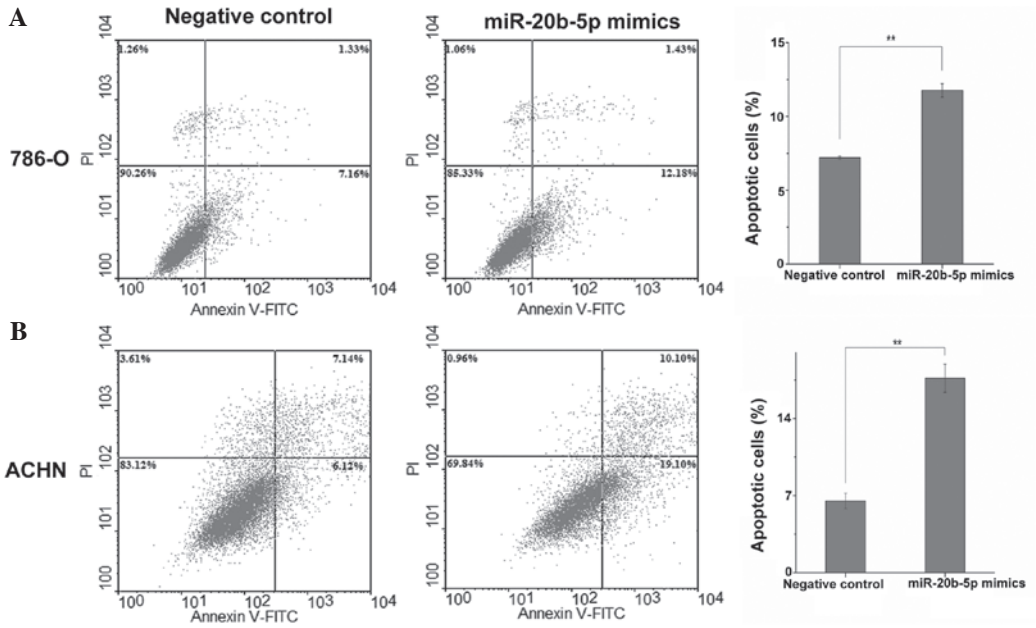

Figure 6. Cell apoptosis (A) 786-O and (B) ACHN cells was measured by flow cytometry. **P<0.001, compared with the negative control. PI, propidum iodide; FITC, fluorescein isothiocyanate.

growth factor A (VEGFA), proteinase-activated receptor 1 (PAR-1; also known as F2R), MAP3K8 and CAMP responsive element binding protein 1 (CREB1) to be putative targets of miR-20b-5p. VEGFA and PAR-1 have been demonstrated to be modulated by miR-20b in cancer (21,22), while MAP3K8 and CREB1 have not been investigated. microRNA also predicted that MALAT1 (lncRNA) is a target of miR-20b-5p.

Discussion

Cancer, a complex multistep process that involves the accumulation of sequential alterations of numerous genes, including the activation of oncogenes and dysfunction of anti-oncogenes, is characterized by unrestricted proliferation, invasion and metastasis (23). By inducing either mRNA degradation or translational repression, miRNAs regulate a large proportion of the transcriptome (~50% in humans) (10,24). Therefore, mRNAs function as potential oncogenes and tumor repressors and have the ability to affect all cellular processes. Thus, an aberrant miRNA expression signature is a hallmark of cancer, including kidney cancer (23). miRNA profiling in kidney cancers revealed that a number of miRNAs were up- or down-regulated, including miR-20b, and specific miRNA profiles could serve as a valuable tool for diagnosis (19,25).

miR-20b has been found to be upregulated in several types of human cancer, such as breast cancer (13,26), gastric cancer (14) and cervical neoplasms (15). miR-20b could function as an oncogene through downregulating tumor suppressor genes, such as PTEN, BRCA1 and p21 (13,27). Studies also demonstrated that upregulation of miR-20b was found in gastric and cervical cancer tissues and may be associated with the prognosis of patients and could serve as a diagnostic tool (14,15,26,28). However, certain studies have demonstrated that miR-20b is significantly downregulated in early T-cell precursor acute lymphoblastic leukemia, oropharyngeal carcinoma and colorectal tumors, and the downregulation of miR-20b-5p is important in metastasis and early recurrence of breast cancer (11,12,16-18). Perez-Rivas *et al*, identified a set of recurrence-related microRNAs, including miR-20b, with potential to identify patients that are likely to develop metastasis early after primary breast surgery (11). The results of another study (12) also confirmed that miR-20b-5p is important in metastasis and early recurrence of breast cancer. According to lymph node metastases, pathology and immunohistochemistry, patients in the study by Li *et al* (12) were divided into three groups: The high invasive and metastatic group (HIMG), the low invasive and metastatic group (LIMG) and the normal group. The authors detected the expression of miR-20b in the centre and at the edge of breast cancer tissues and normal tissues, and their results showed that the relative expression of miR-20a and miR-20b was lower in the center of the tumor than at the edge in the LIMG, lower at the edge of the tumor than in the center in the HIMG, and lower in breast cancer tissues than in normal tissues (12)

Moreover, miR-20b-5p was reported to be downregulated in RCC tissues by next-generation small RNA-sequencing (19). However, the function and clinical significance of miR-20b-5p in RCC has yet to be explored in renal cancer. In this study, RT-qPCR was performed to quantify the relative miR-20b-5p expression in 48 paired RCC tissues and cell lines compared with adjacent normal tissues and 293T cells. The result, which showed that miR-20b-5p was downregulated significantly in RCC tissues and RCC cell lines, was in accordance with the previous sequencing. Furthermore, the role of miR-20b-5p in cellular process such as proliferation, migration and apoptosis were determined by an MTT assay, a scratch assay and flow cytometry after transfection with synthetic miR-20b-5p mimics or negative controls. In the present study, upregulation of miR-20b-5p decreased the ability of the cells to proliferate and migrate, and induced the apoptosis of RCC cells, significantly. The results of the present study demonstrated that the effects of miR-20b-5p on the migration, proliferation and apoptosis of the cells were more marked in ACHN cells than in 786-O cells. This phenomenon may have two alternative possible explanations: first, since the expression of miR-20b-5p is lower in ACHN than in 786-O cells, the same quantity of exogenous miR-20b-5p may lead to a more notable effect on the ACHN cells; second, the transfection efficiency experiments revealed that, comparatively, a much greater quantity of miR-20b-5p mimics could be transfected into the ACHN cells than into the 786-O cells. The function assay suggested that miR-20b-5p may be a tumor suppressor gene in RCC by inhibiting cell proliferation and migration, and promoting cell apoptosis.

miR-20b-5p is a negative regulator of numerous important genes in cancer progression. miR-20b has been demonstrated to cause downregulation of vascular endothelial growth factor (VEGF) at the mRNA and protein level under hypoxia-mimicking conditions, through reducing the levels of nuclear hypoxia inducible factor-1α subunit in breast cancer cells (21). VEGF is the key gene of angiogenic factors that induce angiogenesis, thereby ensuring the delivery of enough blood to support the growth of the tumor (29). RCC is a highly vascularized tumor that originates in the renal cortex. VEGF-targeted agents, such as sorafenib and axitinib have recently been tested in a phase III clinical trial and have been shown to improve prognosis in patients with metastatic RCC (30). Therefore, miR-20b-5p may be identified as an anti-VEGF agent for RCC patients. PAR-1 was also confirmed as a target gene of miR-20b by a luciferase reporter assay in melanoma (22). PAR-1 mediates angiogenesis and impacts the process of tumor growth and disease progression, and de Martino M *et al* reported that the AA genotype of the PAR-1 variation IVSn-14 A>T is associated with an increased risk of metastasis and poorer prognosis in patients with RCC (31).

To the best of our knowledge, this is the first study to identify that miR-20b-5p was downregulated in RCC tissue and cell lines and that it functioned as a tumor suppressor gene in RCC via inducing cellular migration reduction, proliferation inhibition and cell apoptosis. Further studies are required to explore the role of the miR-20b-5p-mediated molecular pathway in tumor suppression in RCC. The potential clinical significance of miR-20b-5p in early detection, prognosis prediction and therapeutic target is worthy of researching.

Acknowledgements

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References

1. Tavani A and La Vecchia C: Epidemiology of renal-cell carcinoma. J Nephrol 10: 93-106, 1997.

2. National Cancer Institute: Surveillance, epidemiology and end results program. SEER stat fact sheets: Kidney and renal pelvis cancer. http://seer.cancer.gov/statfacts/html/kidrp.html. Accessed March 30, 2014.
3. Siegel R, Ma J, Zou Z and Jemal A: Cancer statistics, 2014. CA Cancer J Clin 64: 9-29, 2014, 2014.
4. Zhejiang Channel XNA: World Kidney Day: Kidney Cancer incidence increased year by year become 'invisible killer'. Available at: http://www.zj.xinhuanet.com/news-center/science/2014-03/13/c_119759340.htm. Accessed 13 March 2014.
5. Chen W, Zheng R, Zhang S, Zhao P, Zeng H, Zou X and He J: Annual report on status of cancer in China, 2010. Chin J Cancer Res 26: 48-58, 2014.
6. Motzer RJ, Bander NH and Nanus DM: Renal-cell carcinoma. N Engl J Med 335: 865-875, 1996.
7. Cohen HT and McGovern FJ: Renal-cell carcinoma. N Engl J Med 353: 2477-2490, 2005.
8. Ridge CA, Pua BB and Madoff DC: Epidemiology and staging of renal cell carcinoma. Semin Intervent Radiol 31: 3-8, 2014.
9. Huntzinger E and Izaurralde E: Gene silencing by microRNAs: Contributions of translational repression and mRNA decay. Nat Rev Genet 12: 99-110, 2011.
10. Bartel DP: MicroRNAs: Target recognition and regulatory functions. Cell 136: 215-233, 2009.
11. Perez-Rivas LG, Jerez JM, Carmona R, de Luque V, Vicioso L, Claros MG, Viguera E, Pajares B, Sánchez A, Ribelles N, *et al*: A microRNA signature associated with early recurrence in breast cancer. PLoS One 9: e91884, 2014.
12. Li JY, Zhang Y, Zhang WH, Jia S, Kang Y and Zhu XY: Differential distribution of miR-20a and miR-20b may underly metastatic heterogeneity of breast cancers. Asian Pac J Cancer Prev 13: 1901-1906, 2012.
13. Li D, Ilnytskyy Y, Kovalchuk A, Khachigian LM, Bronson RT, Wang B and Kovalchuk O: Crucial role for early growth response-1 in the transcriptional regulation of miR-20b in breast cancer. Oncotarget 4: 1373-1387, 2013.
14. Guo J, Miao Y, Xiao B, Huan R, Jiang Z, Meng D and Wang Y: Differential expression of microRNA species in human gastric cancer versus non-tumorous tissues. J Gastroenterol Hepatol 24: 652-657, 2009.
15. Cheung TH, Man KN, Yu MY, Yim SF, Siu NS, Lo KW, Doran G, Wong RR, Wang VW, Smith DI, *et al*: Dysregulated microRNAs in the pathogenesis and progression of cervical neoplasm. Cell Cycle 11: 2876-2884, 2012.
16. Coskun E, Neumann M, Schlee C, Liebertz F, Heesch S, Goekbuget N, Hoelzer D and Baldus CD: MicroRNA profiling reveals aberrant microRNA expression in adult ETP-ALL and functional studies implicate a role for miR-222 in acute leukemia. Leuk Res 37: 647-656, 2013.
17. Hui AB, Lin A, Xu W, Waldron L, Perez-Ordonez B, Weinreb I, Shi W, Bruce J, Huang SH, O'Sullivan B, *et al*: Potentially prognostic miRNAs in HPV-associated oropharyngeal carcinoma. Clin Cancer Res 19: 2154-2162, 2013.
18. Yamaguchi T, Iijima T, Wakaume R, Takahashi K, Matsumoto H, Nakano D, Nakayama Y, Mori T, Horiguchi S and Miyaki M: Underexpression of miR-126 and miR-20b in hereditary and nonhereditary colorectal tumors. Oncology 87: 58-66, 2014.
19. Müller S and Nowak K: Exploring the miRNA-mRNA regulatory network in clear cell renal cell carcinomas by next-generation sequencing expression profiles. Biomed Res Int 2014: 948408, 2014
20. Edge SB, Byrd DR, Compton CC, Fritz AG, Greene FL and Trotti A (eds): AJCC Cancer Staging Manual. 7th edition. Springer Verlag, New York, NY, pp479-489, 2010.
21. Cascio S, D'Andrea A, Ferla R, Surmacz E, Gulotta E, Amodeo V, Bazan V, Gebbia N and Russo A: miR-20b modulates VEGF expression by targeting HIF-1 alpha and STAT3 in MCF-7 breast cancer cells. J Cell Physiol 224: 242-249, 2010.
22. Saleiban A, Faxälv L, Claesson K, Jönsson JI and Osman A: miR-20b regulates expression of proteinase-activated receptor-1 (PAR-1) thrombin receptor in melanoma cells. Pigment Cell Melanoma Res 27: 431-441, 2014.
23. Visone R and Croce CM: MiRNAs and cancer. Am J Pathol 174: 1131-1138, 2009.
24. Voinnet O: Origin, biogenesis and activity of plant microRNAs. Cell 136: 669-687, 2009.
25. Junker K, Ficarra V, Kwon ED, Leibovich BC, Thompson RH and Oosterwijk E: Potential role of genetic markers in the management of kidney cancer. Eur Urol 63: 333-340, 2013.
26. Katada T, Ishiguro H, Kuwabara Y, Kimura M, Mitui A, Mori Y, Ogawa R, Harata K and Fujii Y: microRNA expression profile in undifferentiated gastric cancer. Int J Oncol 34: 537-542, 2009.
27. Wu S, Huang S, Ding J, Zhao Y, Liang L, Liu T, Zhan R and He X: Multiple microRNAs modulate p21Cip1/Waf1 expression by directly targeting its 3' untranslated region. Oncogene 29: 2302-2308, 2010.
28. Wang X, Tang S, Le SY, Lu R, Rader JS, Meyers C and Zheng ZM: Aberrant expression of oncogenic and tumor-suppressive microRNAs in cervical cancer is required for cancer cell growth. PLoS One 3: e2557, 2008.
29. George DJ and Kaelin WG Jr: The von Hippel-Lindau protein, vascular endothelial growth factor and kidney cancer. N Engl J Med 349: 419-421, 2003.
30. Motzer RJ and Basch E: Targeted drugs for metastatic renal cell carcinoma. Lancet 370: 2071-2073, 2007.
31. de Martino M, Haitel A, Schatzl G and Klatte T: The protease activated receptor 1 gene variation IVSn-14 A>T is associated with distant metastasis and cancer specific survival in renal cell carcinoma. J Urol 190: 1392-1397, 2013.