European **Rev**iew for **Med**ical and **Pharmacol**ogical **Sci**ences 2017; 21: 4566-4576

MicroRNA-21 functions as an oncogene and promotes cell proliferation and invasion via TIMP3 in renal cancer

J. CHEN[1], Y. GU[2], W. SHEN[3]

[1]Department of Urology, Tongde Hospital of Zhejiang Province, Hangzhou, Zhejiang Province, China
[2]Department of Urology, The first People's Hospital of Aksu Prefecture of Xinjiang, Aksu, Xinjiang, China
[3]Department of Nephrology, Zhejiang Provincial People's Hospital, People's Hospital of Hangzhou Medical College, Hangzhou, Zhejiang Province, China

Abstract. – OBJECTIVE: Renal cell carcinoma (RCC) displays an increasing incidence and mortality rate worldwide in recent years. More and more evidence identified microRNAs function as positive or negative regulatory factors in many cancers, but the role of miR-21 in RCC remains unclear.

PATIENTS AND METHODS: Relative expression levels of miR-21 in human RCC tissue samples and RCC-derived cell lines were measured using quantitative real-time Polymerase Chain Reaction (PCR). Clinical features were collected to further study the relationship between the miR-21 level and clinicopathologic variables. Loss- and gain- of miR-21 experiments were employed to measure the influence of miR-21 in cell proliferation, apoptosis, invasion and migration. Downstream target gene was confirmed by using luciferase and Western blotting assays.

RESULTS: MiR-21 significantly over-expressed in RCC tissues and cell lines than normal groups. Higher miR-21 expression level indicated larger tumor sizes, more lymph metastasis and advanced tumor node metastasis (TNM) stage. Knocking down miR-21 inhibited the cell growth, invasion and migration abilities but promoted the cell apoptosis, while over-expressing miR-21 promoted cell growth and metastasis. Furthermore, TIMP3 was confirmed as a direct target of moR-21 and inhibition of TIMP3 reserved the effect of down-regulating miR-21 in RCC cells.

CONCLUSIONS: Our study demonstrated miR-21 was significantly over-expressed and functioned as a tumor oncogene via TIMP3 in RCC, which could provide a potential target for RCC diagnosis and therapy.

Key Words:
MicroRNA, Renal cell carcinoma, Prognosis, TIMP3, *In vitro.*

Introduction

In recent years, renal cell carcinoma (RCC) continues to be the most common type of kidney tumor along with the highest mortality in urological tumors[1]. Because of lacking early stage symptoms and clinical evidence, RCC patients often develop an advanced Tumor Node Metastasis (TNM) stage when found incidentally[2]. Also, the prognosis of patients after surgery still has room for improvement[3]. Therefore, more studies are required to explain the underlying mechanism of RCC, which may provide specific targets for early stage diagnosis and treatment as well as for prognosis prediction[4].

MicroRNA (miRNA) is a class of non-coding single-stranded RNA molecules consisting of 20-24 nucleotides. They are widely present in eukaryotic cells and are highly conserved in evolution[5]. They are one of the most popular biomolecules in recent years. They play a negative regulator of gene expression at post-transcriptional levels, affecting cell growth, proliferation, differentiation and apoptosis[6]. Several studies have revealed that miRNAs were involved in the tumorigenesis and progression of many cancers[7]. In addition, some miRNAs were reported having the potential to predict tumorigenesis and prognosis and be a target for therapy[8]. For instance, in RCC, miR-141 regulated cell proliferation and metastasis by controlling EphA2 expression[9]; Let-7d suppressed cell proliferation, invasion, and tumor macrophage infiltration via regulating CCL7 and COL3A1[10]; microRNA-34a could suppress

Corresponding Author: Wei Shen, MD; e-mail: gongzuogong@163.com

malignant transformation of RCC by targeting c-Myc transcriptional complexes[11]. In addition, miR-221/222 could enhance the sensitive of sunitinib treatment in metastatic RCC[12].

MiRNA-21 located at 17q23.1 has been reported to regulate many carcinoma progressions. MiR-21 could specifically target clusterin in head and neck squamous carcinoma and had proto-oncogenic properities[13]. In breast cancer, miR-21 could promote cell proliferation and invasion activities via phosphatase and tensin homolog deleted on chromosome ten (PTEN), which indicated a poor prognosis[14]. In addition, micro-RNA-21 regulated cancer-associated fibroblast-mediated frug resistance in pancreatic cancer, which increased the difficulty of treatment[15]. In laryngeal carcinoma, oral cancer, and colorectal cancer, miR-21 also acted as an oncogene and promoted the tumor progression[16-18]. In RCC, miR-21 up-regulation was reported to be related with lower kidney tumor survival[19]; however, the specific relationship between miR-21 and RCC and underlying specific mechanism still remains unclear.

In our study, we first analyzed the expression level of miR-21 in 104 pairs of RCC and adjacent normal tissues as well as in cancer cell lines. Then, the relationship between the miR-21 and clinical pathological variables was studied. Furthermore, we conducted the model of over-expressing and knocking down miR-21 cells *in vitro*. Several functional experiments confirmed that over-expressing miR-21 promoted cell growth, invasion, migration and inhibited cell apoptosis abilities of RCC though down-regulating TIMP3. These findings might indicate a novel target for biological treatment for RCC.

Patients and Methods

Patients

A total of 104 pairs human RCC and adjacent normal tissues were collected from Tongde Hospital of Zhejiang Province. Before the surgery, none of patients accepted any chemotherapy or radiotherapy strategies. The tissues were stored in liquid nitrogen immediately after surgical resection. The clinical pathological features were collected based on AJCC standard. All patients had signed the written consent of their information and the study got approval of the Ethics Committee of Tongde Hospital of Zhejiang Province.

Cell Lines and Culture

Five renal cell cancer cell lines ACHN, A498, Caki-1, Caki-2 and 786-O and human embryonic kidney 293T cell using as normal control were bought from the Cell Bank of Type Culture Collection of Chinese Academy of Sciences (Shanghai, China). The six cell lines were maintained in Dulbecco's Modified Eagle Medium (DMEM) (Gibco, Grand Island, NY, USA) containing 10% fetal bovine serum (FBS) (Gibco, Grand Island, NY, USA), 100 U/mL penicillin and 50 ug/mL streptomycin (Corning, Corning, NY, USA). All the cells were incubated at 37°C in humidified atmosphere containing 5% CO_2.

Cell Transfection

ACN or 786-O cells were seeded in six-well plates and maintained in normal medium to a density of 60%. Using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA), cells were co-cultured with appropriate amount miR-21 mimics, miR-21 inhibitors or relatively negative controls according to manufactory instructions. Also, siRNA-TIMP3 was co-transfected into 786-O cells with miR-21 inhibitors using Lipofectamine 3000. All the sequences were synthesized by GenePhama (Shanghai, China). Using qRT-PCR, the efficiency of miR-21 expressing regulation was confirmed.

RNA Isolation and qRT-PCR

Total RNAs of cells were extracted by using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). For miR-21 detection, Taqman assays were employed, and U6 was using as internal control. For mRNA analysis, total RNAs were reverse transcribed into cDNAs by using TaKaRa Reverse Transcription Kit (TaKaRa, Shiga, Otsu, Japan). Then, SYBR Green Premix Kit was used to perform the qRT-PCR and GAPDH using as the control. All the relative expression RNA levels were calculated using the $2^{-\Delta\Delta CT}$ method.

CCK8 Assay

CCK8 (Dojindo, Kumamoto, Japan) assay was applied to study the cell proliferation. The cells were planted in a density of $1X10^3$ cells with 100 uL medium, and then were cultured for 24, 48, 72, 96 h after transfection. CCK8 reagent (10 µL per well) was added into the wells and absorbance of 450 nm was measured.

Cell Apoptosis Detection

Flow cytometry was obtained to measure the cell apoptotic rate with using a fluorescein isothiocyanate (FITC) and propidium iodide (PI) kit (Vazyme, Nanjing, China). Cells were harvested after miR-21 mimics or inhibitors treatment and washed with pre-cooling phosphate-buffered saline (PBS). Then cells were resuspended in 1000 µL of binding buffer mixing 10 µL of FITC and PI, respectively. Then ,cell apoptotic rate was measured by flow cytometry (FACS, Partec AG, Arlesheim, Switzerland), and the percentage of apoptotic cells was counted. Each measurement was repeated three times.

Transwell and Matrigel Assay

8 um transwell inserts (Millipore, Billerica, MA, USA) was used to measure the cell invasion and migration ability. For invasion assay, a total of $4x10^4$ treated cells in 10% FBS medium were seeded into the top chamber of the insert, which had plated Matrigel (BD Biosciences, Franklin Lakes, NJ, USA). The lower chamber was added with 500 µL FBS-free medium. After 36 h incubation, the membranes containing cells on its lower surface were fixed with methanol and stained with 0.5% crystal violet. Then, the cells stained were calculated after pictures taken using a microscope in five random visions. For migration assay, the top chamber of the insert plated nothing before cell seeding. The other steps were the same as the invasion assay.

Dual-luciferase Assay

The Dual-Luciferase reporter system (Promega, Madison, WI, USA) was recruited to study the activity of luciferase. The TIMP3 3'-UTR cDNA sequences including the mutant or wild type miR-21 binding region was amplified and cloned into pGL3 luciferase vector (Promega, Madison, WI, USA). Next, ACHN cells were co-treated with the established pGL3 vector and miR-21mimics or NC using lipofectamine 3000. Then, the activity of luciferase was determined using luminometer (Promega, Madison, WI, USA) and measured based on the basic pGL3 vector.

Western Blotting

To investigate the expression of TIMP3, protein of conducted cells was isolated using RIPA reagent (Beyotime, Shanghai, China) containing protease inhibitor. Proteins mixed with loading buffer (Beyotime, Shanghai, China) were separated using 8-10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), and then transferred to polyvinylidene fluoride (PVDF) membranes (Millipore, Billerica, MA, USA). The membranes were immersed in 5% fat-free milk to block non-specific protein interactions in Tris-buffered saline and Tween 20 (TBST) buffer at 4 °C for one hour. Then, the membranes loaded with proteins were maintained in 5% fat-free milk with the primary antibody against TIMP3 or GAPDH (CST, Danvers, MA, USA). The membranes were then incubated at room temperature with secondary antibody conjugated with horseradish peroxide (HRP) for one hour after washing with TBST buffer 10 min × 3 times. Then, we detected the membranes using ECL Kit (Millipore, Billerica, MA, USA).

Statistical Analysis

All the statistical analysis was handled by using SPSS 19.0 software (SPSS Inc., Armonk, NY, USA). All quantitative results were displayed as mean± SD. One-way ANOVA test was used to measure the comparison between groups followed by least significant difference (LSD). $p<0.05$ indicated significant difference.

Results

MiR-21 was Over-Expressed in RCC Tissues and Cell Lines

To examine the expression level of miR-21 in human renal carcinoma, we examined miR-21 expression level in 104 RCC tissue samples and adjacent normal samples. As clearly shown in Figure 1A, miR-21 expression levels in RCC group were significantly higher than that in normal group. Next, we divided 104 samples into high miR-21 level group and low miR-21 level group according the median expression to further evaluate the relationship between miR-21 and RCC clinical pathological features. Table I showed that higher miR-21 level signified larger tumor size, more lymph node metastasis and advanced TNM stage, but not related with age, gender, or laterality. Also, in RCC-derived cell lines miR-21 expressed higher level than HEK-293T cells (Figure 1B). These data showed miR-21 might act as a tumor oncogene in RCC.

To further characterize the miR-21 function, ACHN cells were treated with miR-21 to overexpress miR-21 while 786-O cells were transfected with miR-21 inhibitors to knockdown miR-21 expression. The efficiency of treatments was measured by qRT-PCR (Figure 1C-D).

Table I. Correlation between miR-21 level and clinicopathological features in RCC.

		***miR-21* expression**		
Characteristics	**Total**	**Low**	**High**	***p*-value**
Age				
<60 years	57	31	26	0.431
>60 years	47	21	26	
Gender				
Male	58	28	30	0.841
Female	46	24	22	
Laterality				
Left				
Right	41	17	24	
	63	35	28	0.229
Tumor size				
<3 cm	66	41	25	0.002*
>3 cm	38	11	27	
Lymph node metastasis				
Yes	19	3	16	0.002*
No	85	49	36	
TNM stage				
I	15	13	2	0.0008*
II	49	27	22	
III	31	11	20	
IV	9	1	8	

The expression level of miR-21 was cut off by median expression level and *indicated $p<0.05$.

Ectopic miR-21 Expression Effected the Cell Proliferation and Apoptosis in RCC

To evaluate the cell proliferation ability, CCK8 assay was employed. ACHN cells over-expressing miR-21 performed increased cell growth activity (Figure 2A), while 786-O cells knocking down miR-21 showed reduced cell growth rate (Figure 2B). Next, we detect the effects of miR-21 in cell apoptosis using Flow Cytometer. Up-regulation of miR-21 using mimics reduced cell apoptotic rate of ACHN cells (Figure 2C), however, down-regulation of miR-21 using inhibitors accelerated 786-O cells apoptosis (Figure 2D) comparing to relative control groups. These findings indicated that miR-21 accelerated RCC cell proliferation and inhibited cell apoptosis.

Down-Regulation of miR-21 Reduced Cell Invasion and Migration of RCC.

Next, we probed whether miR-21 influenced cell metastasis of RCC using transwell assays. We observed that over-expressing miR-21 in ACHN cells increased the cell invasion ability obviously (Figure 3A), on contrast, down-regulating miR-21 in 786-O cells markedly impaired cell invasion activity when comparing with negative control group (Figure 3B). Consistent with this result, migration ability of ACHN cells was enhanced by miR-21 over-expressing (Figure 3C) while 786-O cells reduced by miR-21 silencing (Figure 3D). All these data suggested miR-21 could promote cell invasion and migration in RCC.

TIMP3 was a Direct Target of miR-21

According to microRNA target analysis of several database (targetscan, miRanda, PicTar), we speculated TIMP3 as a candidate target for miR-21. The biding site of miR-21 with TIMP3 3'-UTR was shown in Figure 4A. To further verify our assumption, we performed dual-luciferase assay. Using conducted wild type or mutant TIMP3 3'-UTR vector, we found that luciferase activity significantly decreased in wild type group but no difference in mutant group (Figure 4B). In addition, we next measured the TIMP3 protein level of established cell lines by Western blotting.

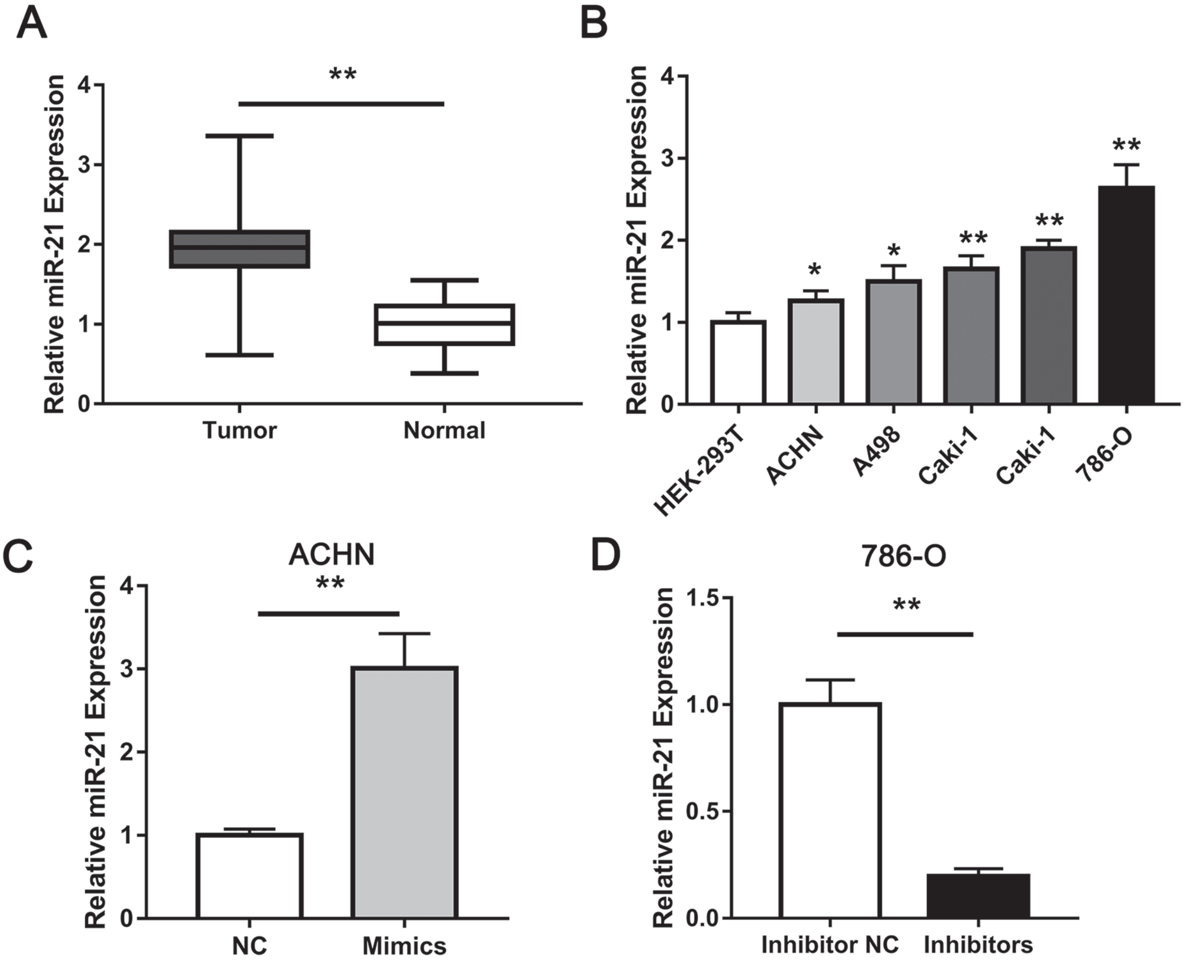

Figure 1. MiR-21 was over-expressed in RCC tissues and cell lines. ***A,*** Expression level of miR-21 in 104 pairs RCC tumor and adjacent normal tissues. ***B,*** miR-21 expression level in RCC cell lines (ACHN, A498, Caki-1, Caki-2, 786-O) and human embryonic kidney 293T cell (HEK-293T). ***C,*** Expression of miR-21 in miR-21 mimics treated ACHN cells. D: Level of miR-21 in miR-21 inhibitors treated 786-O cells. U6 was used as an internal control. $^{*}p< 0.05$, $^{**}p< 0.01$.

Clearly, up-regulating miR-21 increased TIMP3 expression in ACHN cells (Figure 4C, 4D) and down-regulating miR-21 decreased TIMP3 level (Figure 4C, 4E) compared to each control group. These findings suggested miR-21 could inhibit TIMP3 expression.

MiR-21 Regulated RCC cell Proliferation and Metastasis via TIMP3

To further investigate the mechanism of miR-21 in RCC, we next conducted siRNA for TIMP3 to inhibit TIMP3 expressing. After co-treating 786-O cells with miR-21 inhibitors and siRNA-TIMP3, we analyzed the TIMP3 expression level, and showed decrease in treated cells (Figure 5A, 5B). CCK8 assay, apoptosis assay and invasion assay revealed that silence of TIMP3 remarkably rescued the effect of knocking down miR-21 in 786-O cells on cell proliferation, apoptosis, and invasion (Figure 5C-F). These data indicated that inhibition of TIMP3 reversed the effect of miR-21 silence.

Discussion

Though RCC patients who underwent surgical treatment have a relative optimistic prognosis, the metastasis still limits the improvement of 5-year-survival rate due to lack of early fo-

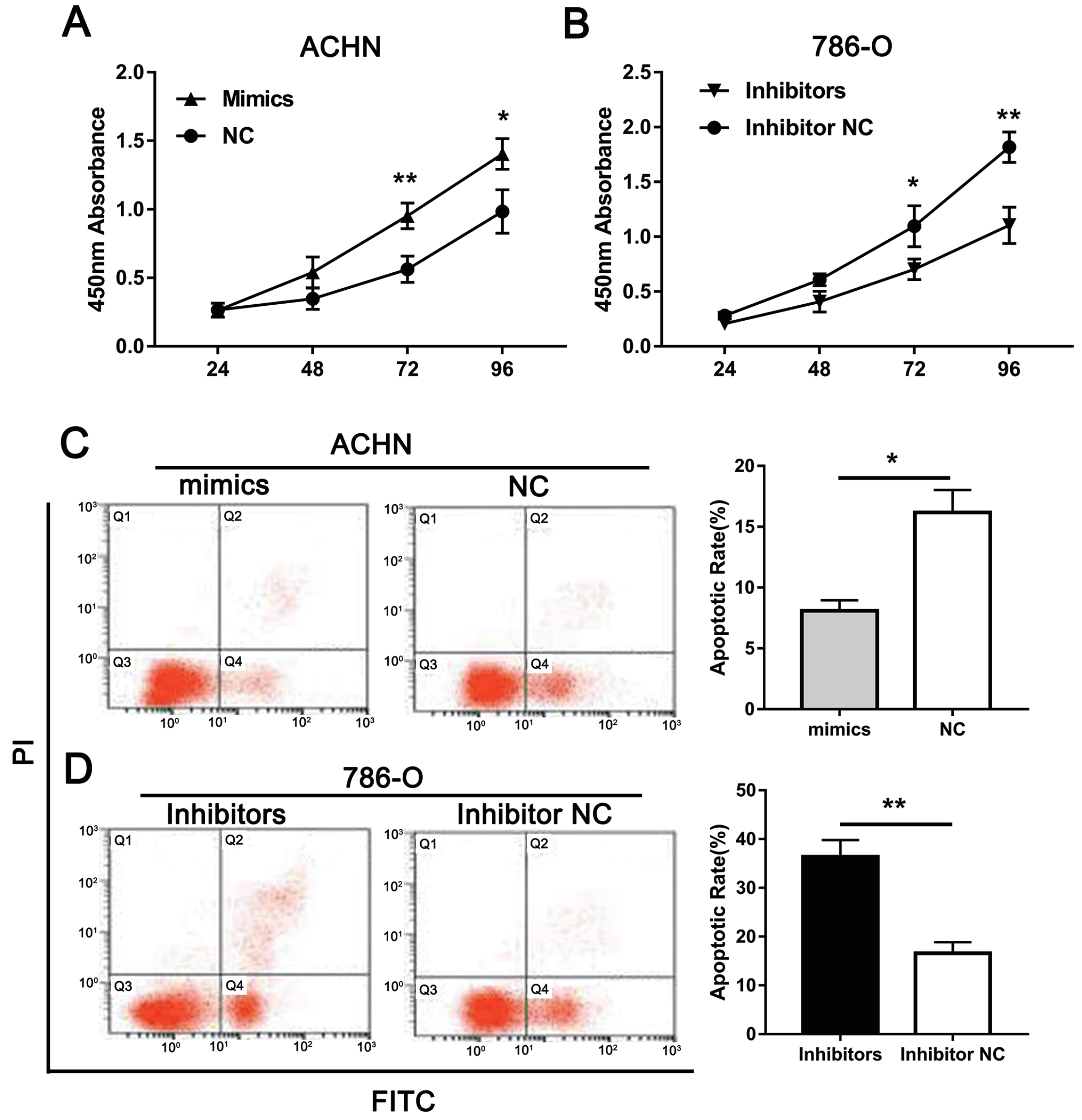

Figure 2. **MiR-21 effected the proliferation and apoptosis of RCC cells.** ***A-B***, CCK8 assay was employed to show proliferation of ACHN (A) or 786-O (B) cells treating with miR-21 mimics or inhibitors compared to relative negative control. ***C, D,*** Apoptosis assay performed by Flow cytometry to determine the apoptotic rate of ACHN (C) or 786-O (D) cells transfected with mimics or inhibitors, respectively. Q4: early apoptosis cells, Q2: late apoptosis cells. $^{*}p < 0.05$, $^{**}p < 0.01$.

recasting means[20]. Many studies in recent decades have proved some biomarkers such as miRNAs, which could act as predicting factors of renal cancer in aspects of cancer development and progression[8,21].

Our study first displayed the expression of miR-21 in RCC tissues were significantly higher than in the adjacent normal samples. And the expression level of miR-21 in RCC-derived cell lines also over-expressed in normal HEK-293T cells. More importantly, we next identified miR-21 correlated with advanced tumor progression in RCC patients including larger tumor volume, more metastatic lymph nodes, and severer TNM stage. These results performed over-expression of miR-21 could promote renal cancer progression.

MiR-21 has been reported to down-regulate several genes expression in different tumors, such as PTEN in breast cancer, Sce23a in colorectal carcinoma[14,22]. Here, we verified TIMP3, a member of TIMP gene family, as a direct target

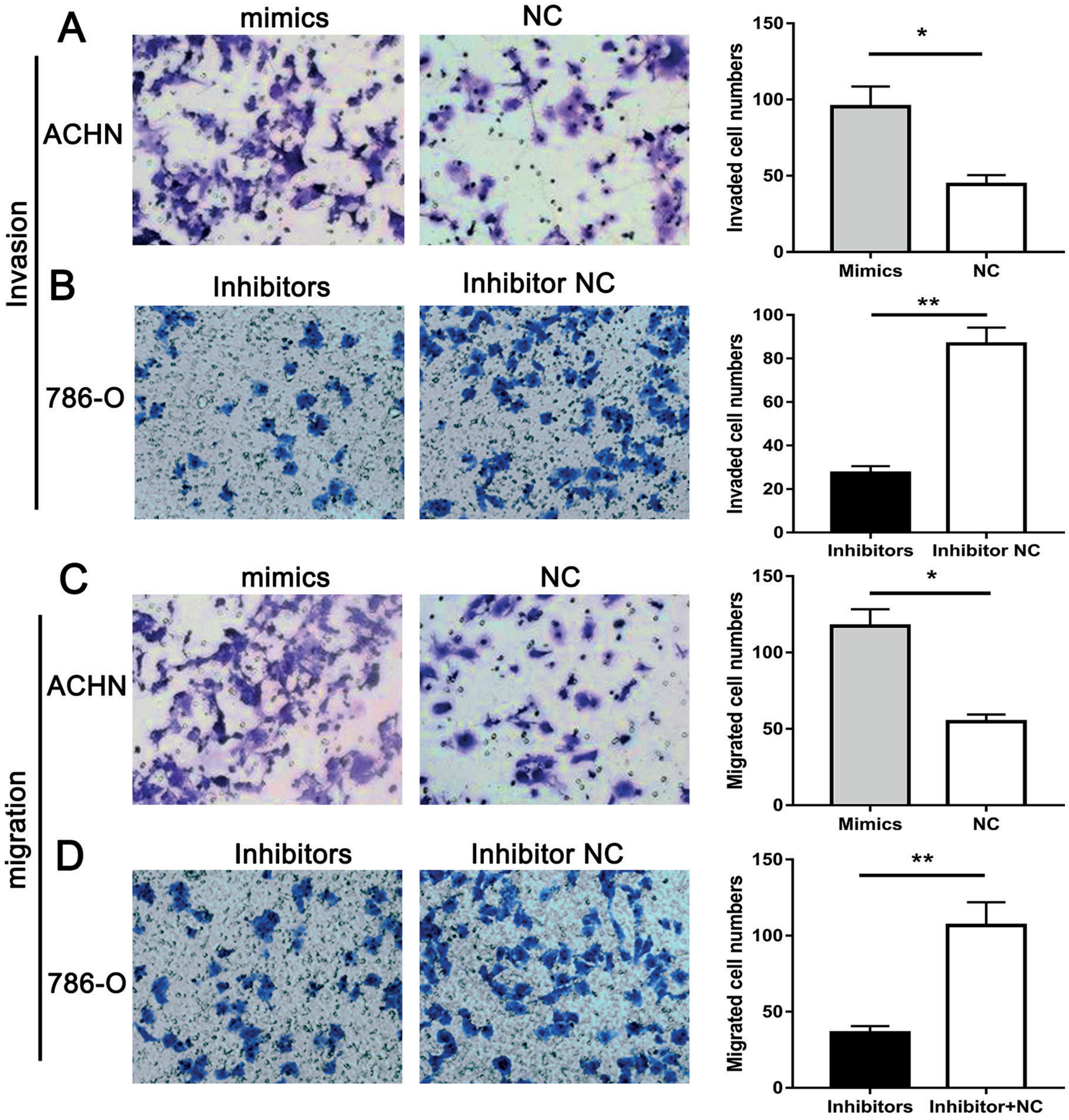

Figure 3. MiR-21 effected the invasion and migration of RCC cells. ***A-B***, Transwell assay was performed to determine invasion of ACHN (A) or 786-O (B) cells treating with miR-21 mimics or inhibitors compared to each negative control. ***C-D,*** Matrigel assay was used to detect the migration ability of miR-21 mimics treated ACHN cells (C) or miR-21 inhibitors treated 786-O cells (D). Data are presented as the mean ± SD of five random vision. $^{*}p< 0.05$, $^{**}p< 0.01$.

gene for miR-21 in RCC. The protein encoded by TIMP3 is inhibitor of the matrix metalloproteinases (MMPs)[23]. TIMP3 could inhibit the function of MPPs, which could promote cell migration and invasion. Several studies have proved TIMP3 function as tumor suppressors in different carcinomas[24-27]. Anania et al[28] reported TIMP3 inhibited cell metastasis and *in vivo* tumorigenicity in thyroid tumor. Das et al[29] performed in melanoma, TIMP3 expression decreased and inhibited melanoma cell migration. In renal cancer, decrease of expression of TIMP3 was identified by Masson et al[30]. What is more, TIMP3 acted as downstream molecule of some miRNAs to mediate the role of different miRNAs in different tumors including miR-221/222 in non-small cell lung cancer[31], miR-17 in prostate cancer[32], and miR-181b in hepatocarcinoma[33]. These studies confirmed TIMP3 might be a potential factor for cancer therapy.

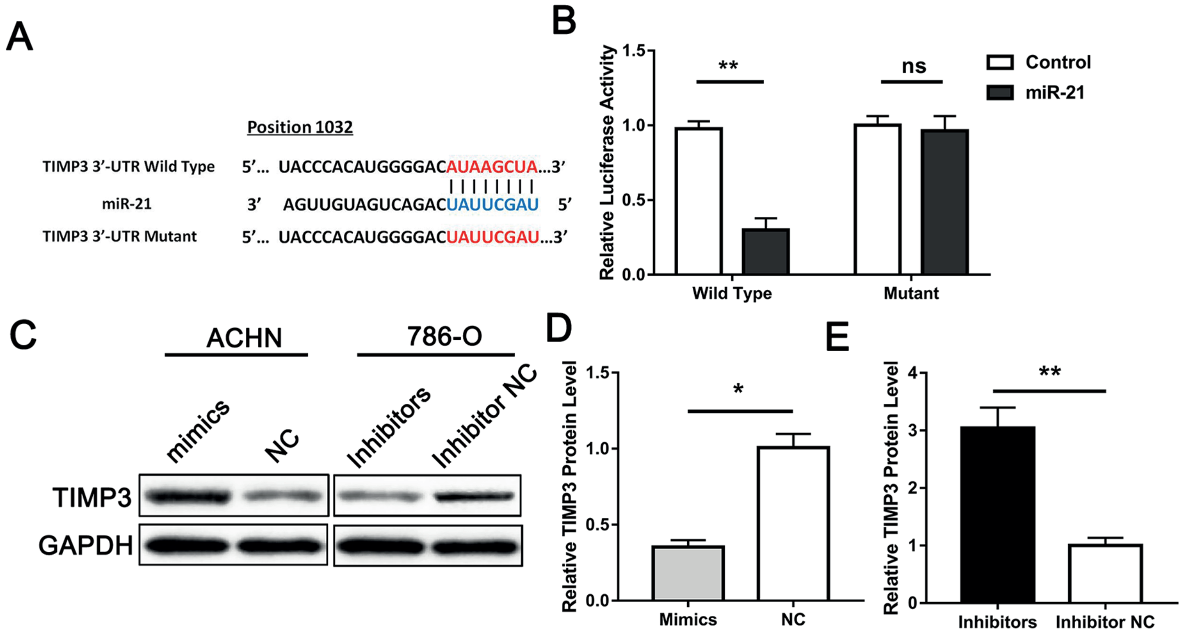

Figure 4. **TIMP3 was a direct target of miR-21.** ***A,*** The predicted binding region of miR-21 in the 3'-UTR of TIMP3. ***B,*** Dual-luciferase reporter assay showed relative activity change of luciferase in ACHN cells treated by mimics or NC were transfected with pGL3 construct containing the WT or mutant TIMP3 3'-UTR site. ***C-E,*** Levels of TIMP3 and GAPDH protein measured by Western-blot in miR-21 overexpression ACHN cells (C, D) and miR-21 knockdown 786-O cells (C, E). The protein levels were normalized to that of GAPDH. $^{*}p< 0.05$, $^{**}p< 0.01$, ns: not significant.

We performed several functional experiments and identified over-expressing miR-21 enhanced cell proliferation, invasion and migration of ACHN while silencing miR-21 in 786-O impaired cell growth and metastasis abilities. Furthermore, we conducted siRNA-TIMP3 and demonstrated TIMP3 silencing reversed the effects of inhibiting miR-21 in cells, which partly elucidated the mechanism of miR-21 in RCC.

Taken all together, our study demonstrated miR-21 was over-expressed in RCC tissues and cells and promoted cell proliferation, invasion, migration and inhibited cell apoptosis via TIMP3. As far as we know, this is the first study to explain the relationship between miR-21 and RCC metastasis. However, regulation mechanism of miRNAs is a large system of network. Further studies of miR-21 in RCC are still needed for explaining the complicated mechanism of tumor development and progression.

Conclusions

We observed that miR-21 was markedly up-regulated in renal cell cancer and the increased level of miR-21 was relative to larger tumor size, more metastatic lymph nodes and advanced TNM stage in RCC patients. Increased miR-21 expression could promote cell proliferation, invasion, migration and inhibited cell apoptosis abilities via targeting TIMP3. These findings indicated miR-21 could be a potential target for prognostic prediction and therapeutic strategies.

Acknowledgments

This study was supported by the General Project of the Medical and Health of Zhejiang Province (Grant Number 2015KYB035), the Project of Scientific Research Foundation of Chinese Medicine (Grant Number 2016ZQ007), the General Project of the Medical and Health of Zhejiang Province (Grant Number 2014KYA018).

Conflict of interest

The authors declare no conflicts of interest.

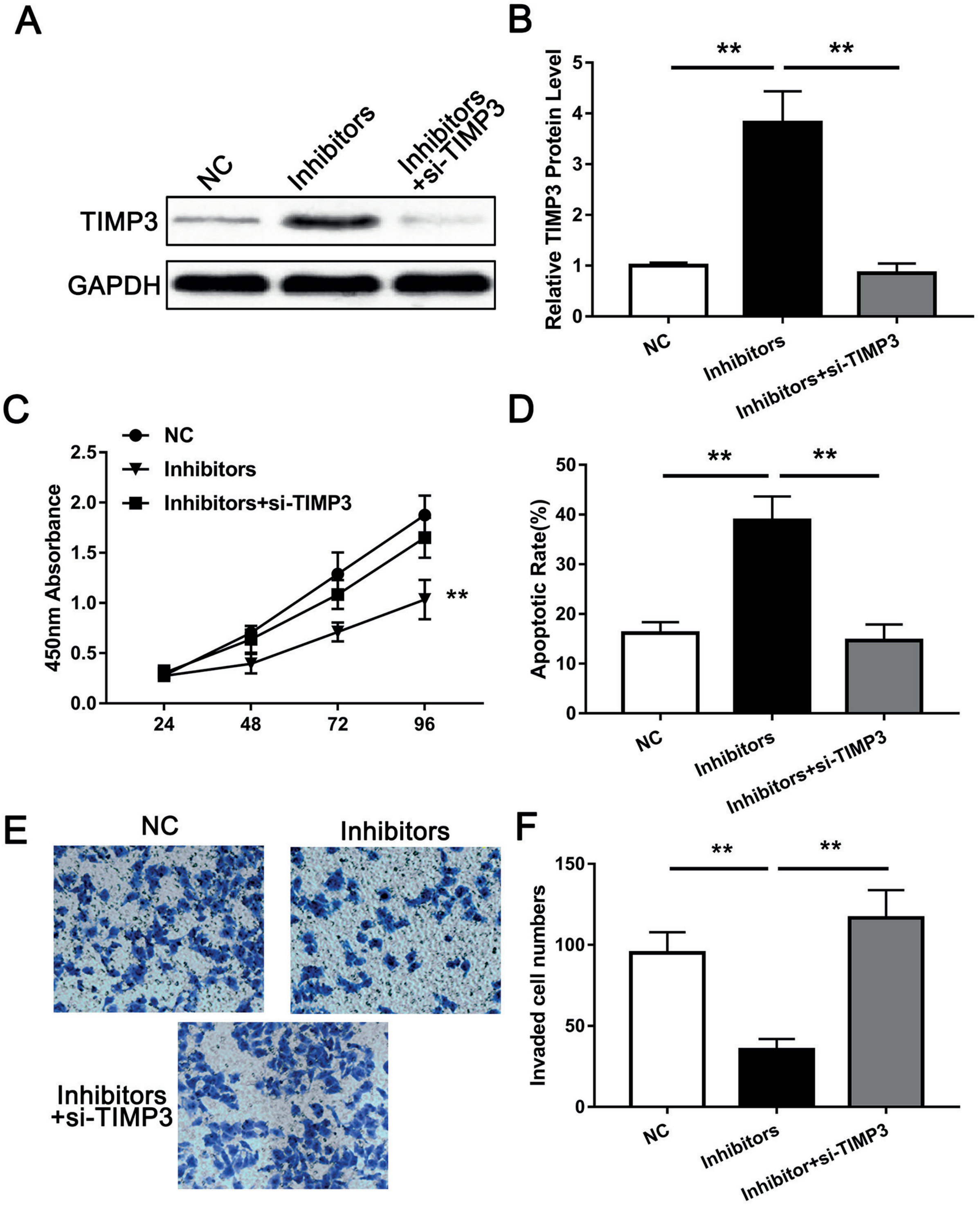

Figure 5. siRNA-TIMP3 reversed the effects of miR-21 inhibitors in 786-O cells. ***A,*** Western-blot analyses of TIMP3 and GAPDH expression level. ***B,*** Relative protein band densities of TIMP3. The GAPDH was used as an internal control. ***C,*** Analysis of the cell proliferation ability by CCK8 assay in miR-21 NC, inhibitors, or inhibitors + si-TIMP3 treated 786-O cells; ***E-F,*** Cell invasion ability was measured by transwell assay. $^{*}p< 0.05$, $^{**}p< 0.01$.

References

1) Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015; 65: 87-108.
2) Capitanio U, Montorsi F. Renal cancer. Lancet 2016; 387: 894-906.
3) Liu TY, Li J, Wen XH, Zhang H, Gui Q. The efficacy of open nephron-sparing surgery in the treatment of complex renal cell carcinoma. Eur Rev Med Pharmacol Sci 2016; 20: 3959-3964.
4) Turajlic S, Larkin J, Swanton C. SnapShot: renal cell carcinoma. Cell 2015; 163: 1556-1556e1.
5) Iorio MV, Croce CM. MicroRNAs in cancer: Small molecules with a huge impact. J Clin Oncol 2009; 27: 5848-5856.
6) Chen CZ. MicroRNAs as oncogenes and tumor suppressors. N Engl J Med 2005; 353: 1768-1771.
7) Zhu SH, He XC, Wang L. Correlation analysis of miR-200b, miR-200c, and miR-141 with liver metastases in colorectal cancer patients. Eur Rev Med Pharmacol Sci 2017; 21: 2357-2363.
8) Silva-Santos RM, Costa-Pinheiro P, Luis A, Antunes L, Lobo F, Oliveira J, Henrique R, Jeronimo C. MicroRNA profile: a promising ancillary tool for accurate renal cell tumour diagnosis. Br J Cancer 2013; 109: 2646-2653.
9) Chen X, Wang X, Ruan A, Han W, Zhao Y, Lu X, Xiao P, Shi H, Wang R, Chen L, Chen S, Du Q, Yang H, Zhang X. MiR-141 is a key regulator of renal cell carcinoma proliferation and metastasis by controlling EphA2 expression. Clin Cancer Res 2014; 20: 2617-2630.
10) Su B, Zhao W, Shi B, Zhang Z, Yu X, Xie F, Guo Z, Zhang X, Liu J, Shen Q, Wang J, Li X, Zhang Z, Zhou L. Let-7d suppresses growth, metastasis, and tumor macrophage infiltration in renal cell carcinoma by targeting COL3A1 and CCL7. Mol Cancer 2014; 13: 206.
11) Yamamura S, Saini S, Majid S, Hirata H, Ueno K, Chang I, Tanaka Y, Gupta A, Dahiya R. MicroRNA-34a suppresses malignant transformation by targeting c-Myc transcriptional complexes in human renal cell carcinoma. Carcinogenesis 2012; 33: 294-300.
12) Khella HW, Butz H, Ding Q, Rotondo F, Evans KR, Kupchak P, Dharsee M, Latif A, Pasic MD, Lianidou E, Bjarnason GA, Yousef GM. MiR-221/222 are involved in response to sunitinib treatment in metastatic renal cell carcinoma. Mol Ther 2015; 23: 1748-1758.
13) Mydlarz W, Uemura M, Ahn S, Hennessey P, Chang S, Demokan S, Sun W, Shao C, Bishop J, Krosting J, Mambo E, Westra W, Ha P, Sidransky D, Califano J. Clusterin is a gene-specific target of microRNA-21 in head and neck squamous cell carcinoma. Clin Cancer Res 2014; 20: 868-877.
14) Fang H, Xie J, Zhang M, Zhao Z, Wan Y, Yao Y. MiRNA-21 promotes proliferation and invasion of triple-negative breast cancer cells through targeting PTEN. Am J Transl Res 2017; 9: 953-961.
15) Zhang L, Yao J, Li W, Zhang C. Micro-RNA-21 regulates cancer-associated fibroblast-mediated frug resistance in pancreatic cancer. Oncol Res 2017:
16) Shi C, Yang Y, Xia Y, Okugawa Y, Yang J, Liang Y, Chen H, Zhang P, Wang F, Han H, Wu W, Gao R, Gasche C, Qin H, Ma Y, Goel A. Novel evidence for an oncogenic role of microRNA-21 in colitis-associated colorectal cancer. Gut 2016; 65: 1470-1481.
17) Li ZB, Li ZZ, Li L, Chu HT, Jia M. MiR-21 and miR-183 can simultaneously target SOCS6 and modulate growth and invasion of hepatocellular carcinoma (HCC) cells. Eur Rev Med Pharmacol Sci 2015; 19: 3208-3217.
18) Yu EH, Tu HF, Wu CH, Yang CC, Chang KW. MicroRNA-21 promotes perineural invasion and impacts survival in patients with oral carcinoma. J Chin Med Assoc 2017; 80: 383-388.
19) Rasti A, Mehrazma M, Madjd Z, Keshtkar AA, Roudi R, Babashah S. Diagnostic and prognostic accuracy of miR-21 in renal cell carcinoma: a systematic review protocol. BMJ Open 2016; 6: e9667.
20) Nathan PD, Eisen TG. The biological treatment of renal-cell carcinoma and melanoma. Lancet Oncol 2002; 3: 89-96.
21) Motzer RJ, Bacik J, Schwartz LH, Reuter V, Russo P, Marion S, Mazumdar M. Prognostic factors for survival in previously treated patients with metastatic renal cell carcinoma. J Clin Oncol 2004; 22: 454-463.
22) Li C, Zhao L, Chen Y, He T, Chen X, Mao J, Li C, Lyu J, Meng QH. MicroRNA-21 promotes proliferation, migration, and invasion of colorectal cancer, and tumor growth associated with down-regulation of sec23a expression. BMC Cancer 2016; 16: 605.
23) Uria JA, Ferrando AA, Velasco G, Freije JM, Lopez-Otin C. Structure and expression in breast tumors of human TIMP-3, a new member of the metalloproteinase inhibitor family. Cancer Res 1994; 54: 2091-2094.
24) Bachman KE, Herman JG, Corn PG, Merlo A, Costello JF, Cavenee WK, Baylin SB, Graff JR. Methylation-associated silencing of the tissue inhibitor of metalloproteinase-3 gene suggest a suppressor role in kidney, brain, and other human cancers. Cancer Res 1999; 59: 798-802.
25) Brueckl WM, Grombach J, Wein A, Ruckert S, Porzner M, Dietmaier W, Rummele P, Croner RS, Boxberger F, Kirchner T, Hohenberger W, Hahn EG, Jung A. Alterations in the tissue inhibitor of metalloproteinase-3 (TIMP-3) are found frequently in human colorectal tumours displaying either microsatellite stability (MSS) or instability (MSI). Cancer Lett 2005; 223: 137-142.
26) Li SJ, Zhou J, Zhang L, Xiang W, Hu Q, He YY, Chen LG. The effect of miR-21 on SWOZ2 glioma cells and its biological mechanism. J BUON 2017; 22: 468-473.
27) Kornfeld JW, Meder S, Wohlberg M, Friedrich RE, Rau T, Riethdorf L, Loning T, Pantel K, Riethdorf S. Overexpression of TACE and TIMP3 mRNA in head and neck cancer: association with tumour development and progression. Br J Cancer 2011; 104: 138-145.

28) Anania MC, Sensi M, Radaelli E, Miranda C, Vizioli MG, Pagliardini S, Favini E, Cleris L, Supino R, Formelli F, Borrello MG, Pierotti MA, Greco A. TIMP3 regulates migration, invasion and in vivo tumorigenicity of thyroid tumor cells. Oncogene 2011; 30: 3011-3023.

29) Das AM, Bolkestein M, van der Klok T, Oude OC, Vermeulen CE, Rens JA, Dinjens WN, Atmodimedjo PN, Verhoef C, Koljenovic S, Smits R, Ten HT, Eggermont AM. Tissue inhibitor of metalloproteinase-3 (TIMP3) expression decreases during melanoma progression and inhibits melanoma cell migration. Eur J Cancer 2016; 66: 34-46.

30) Masson D, Rioux-Leclercq N, Fergelot P, Jouan F, Mottier S, Theoleyre S, Bach-Ngohou K, Patard JJ, Denis MG. Loss of expression of TIMP3 in clear cell renal cell carcinoma. Eur J Cancer 2010; 46: 1430-1437.

31) Garofalo M, Di Leva G, Romano G, Nuovo G, Suh SS, Ngankeu A, Taccioli C, Pichiorri F, Alder H, Secchiero P, Gasparini P, Gonelli A, Costinean S, Acunzo M, Condorelli G, Croce CM. MiR-221&222 regulate TRAIL resistance and enhance tumorigenicity through PTEN and TIMP3 downregulation. Cancer Cell 2009; 16: 498-509.

32) Yang X, Du WW, Li H, Liu F, Khorshidi A, Rutnam ZJ, Yang BB. Both mature miR-17-5p and passenger strand miR-17-3p target TIMP3 and induce prostate tumor growth and invasion. Nucleic Acids Res 2013; 41: 9688-9704.

33) Wang B, Hsu SH, Majumder S, Kutay H, Huang W, Jacob ST, Ghoshal K. TGFbeta-mediated upregulation of hepatic miR-181b promotes hepatocarcinogenesis by targeting TIMP3. Oncogene 2010; 29: 1787-1797.