Int J Clin Exp Pathol 2016;9(8):8254-8260
www.ijcep.com /ISSN:1936-2625/IJCEP0026179

Original Article

MicroRNA-129-5p inhibits the proliferation and migration in renal cell carcinoma via targeting IGF2BP1

Hai-Liang Xu[1], Yun-Yun Feng[2], Zhan-Kui Jia[3], Yong-Gang Zhang[4], Jun Li[1], Ming-Liang Xia[1]

[1]*Department of Urologic Surgery, Zhumadian Central Hospital, Zhumadian 463000, China;* [2]*Department of Pediatrics, Zhumadian Central Hospital, Zhumadian 463000, China;* [3]*Department of Urologic Surgery, The First Affiliated Hospital of Zhengzhou University, Zhengzhou 450000, China;* [4]*Department of Emergency, Zhumadian Central Hospital, Zhumadian 463000, China*

Received February 18, 2016; Accepted June 27, 2016; Epub August 1, 2016; Published August 15, 2016

Abstract: MicroRNAs (miRNAs) are a class of small non-coding RNAs that have been suggested to play an essential role in tumorigenesis. Accumulating evidences indicated that miR-129-5p could function as a tumor suppressor in some malignancies. However, its role and mechanism in renal cell carcinoma (RCC) remains unclear. Here, we found that miR-129-5p was significantly decreased in ccRCC tissues and cell lines. Ectopic overexpression of miR-129-5p remarkably suppressed RCC cell proliferation, migration, and invasion. IGF2BP1 was identified as a target of miR-129-5p in RCC cells. Furthermore, down-regulation of IGF2BP1 by siRNA performed similar effects with overexpression of miR-129-5p in RCC cells. Collectively, our findings indicate that miR-129-5p may suppress renal cancer cell growth and motility partially by targeting IGF2BP1.

Keywords: miR-129-5p, renal cell carcinoma, IGF2BP1

Introduction

Renal cell carcinoma (RCC) is the most lethal urological malignancy, with about 65,150 new cases and 13,680 deaths estimated for 2013 in the United States, and clear cell renal cell carcinoma (ccRCC) accounts for 70-80% of primary renal cancer [1, 2]. Although RCC diagnosis and treatment have greatly improved over the past ten years, the 5-year survival rate of RCC patients is still poor [3]. Therefore, understanding molecular mechanisms of RCC in detail and searching for molecular therapeutic targets become more and more important for RCC treatment.

MicroRNAs (miRNAs) are small non-coding RNAs which regulate a variety of cellular processes via binding to the 3'-untranslated region (3'-UTR) of target mRNAs, resulting in the degradation of the mRNAs or inhibition of mRNAs translation [4]. Accumulating evidence showed that miRNAs play critical roles in the regulation of cancer initiation and progression [5, 6]. MiRNAs can function as oncogenes or tumor suppressors depending on their specific target genes and different cancers [7]. For example, Ma et al. found that miR-483-3p play an oncogenic role in esophageal squamous cell carcinoma by targeting tumor suppressor EI24 [8]. Minna et al. suggested that miR-451a acted as a tumor suppressor by targeted AKT/mTOR pathway in papillary thyroid carcinoma [9]. Wang et al. found that miR-135b inhibited tumour metastasis in prostate cancer by targeting STAT6 [10]. However, the role of miR-129-5p in RCC remains poorly understand.

In the present study, we found that miR-129-5p was significantly down-regulated in ccRCC tissues and cell lines, and overexpression of miR-129-5p significantly inhibited the proliferation, migration and invasion of RCC cells. IGF2BP1 was found to be a target of miR-129-5p, and down-regulation of IGF2BP1 by siRNA performed similar effects with overexpression of miR-129-5p in RCC cells.

Materials and methods

Patients and specimens

A total of 33 paired ccRCC and adjacent non-tumor tissues were obtained from patients

undergoing radical nephrectomy between 2008 and 2009 at the Department of Urologic Surgery, Zhumadian Central Hospital. This work was approved by the Ethics Committee of Zhumadian Central Hospital. The study protocol was approved by the Institutional Ethics Committee. Written, voluntary, informed consent was taken from all the patients. The specimens were obtained after surgical resection, immediately frozen in liquid nitrogen, and stored in liquid nitrogen until use.

Cell culture and cell transfection

Three RCC cell lines (ACHN, 786-O, and Caki-1) and immortalized normal human proximal tubule epithelial cell line HK-2 were obtained from American Type Culture Collection and cultured in DMEM supplemented with 10% FBS (Invitrogen), and incubated in 5% CO_2 humid atmosphere at 37°C.

The miR-129-5p mimics and negative control miRNA mimics (miR-NC) were synthesized by RiboBio (China). Small interfering RNA against IGF2BP1 (si-IGF2BP1) and negative control (si-NC) were designed by Genepharma (China). Transfection was carried out using Lipofectamine 2000 (Invitrogen) method. The final concentration was 200 nm for mimics and 100 nm for siRNA.

Cell proliferation assay

Cell proliferation was quantified using the Cell Counting Kit-8 (CCK-8). Briefly, 100 μl of cells were seeded into a 96-well plate at a concentration of 1000 cells per well and incubated at 37°C. At daily intervals (0 h, 24 h, 48 h and 72 h), the optical density was measured at 450 nm using a microtiter plate reader (Quant BioTek Instruments). The results represent the average of three replicates under the same conditions.

Cell migration and invasion assays

Cell migration ability was assessed using 6.5-mm transwell chambers with a pore size of 8 μm. Cell invasion was assessed using the chamber with 100 μg Matrigel (BD Biosciences). Theassays were performed according to the manufacturer's instructions. Briefly, 1×10^4 cells from each group were suspended in serum-free medium and were seeded into the upper chamber. The lower chamber was filled with medium containing 10% FBS. After incubation for 24 h, the migrated/invaded cells in the lower chamber (below the filter surface) were fixed in 4% paraformaldehyde, stained with 0.1 mg/ml crystal violet solution, and counted under a microscope. The cells on the lower surface were photographed and five random fields were counted. Three independent experiments were performed.

Quantitative real-time PCR

Total miRNA from tissues or cells was extracted using a mirVana miRNA Isolation Kit (Ambion) according to the manufacturer's instructions. We synthesised cDNA from 10 ng total RNA using a TaqMan miRNA reverse transcription kit (Applied Biosystems), and quantified the expression levels of miR-129-5p using a miRNA-specific TaqMan MiRNA Assay Kit (Applied Biosystems). Therelative expression levels of miR-129-5p were calculated and normalized using the $2^{-\Delta\Delta Ct}$ method relative to U6. Three independent experiments were performed.

Western blotting

Cultured cells were washed twice and lysed in RIPA buffer with 1% PMSF. Proteinwas loaded and seperated by SDS-PAGE gel and transferred onto PVDF membrane. The blots were probed with primary antibodies at 4°C overnight and subsequently incubated with HRP-conjugated secondary antibodies. Signals were visualized using ECL Substrates (Pierce). GAPDH was used as anendogenous control.

Luciferase reporter assay

To observe the binding of miR-129-5p to IGF2BP1 mRNA, the 3'-UTR segment of IGF2BP1 mRNA was amplified by PCR and inserted into the pGL3/luciferase vector (Promega). The mutant (Mut) 3'-UTR of IGF2BP1 mRNA was cloned using the wild type (Wt) 3'-UTR as a template and inserted into pGL3/luciferase vector. Co-transfections of Wt IGF2BP1 3'-UTR or Mut IGF2BP1 3'-UTR plasmid with miR-129-5p mimics into the cells were accomplished by using Lipofectamine 2000 (Invitrogen). Luciferase activity was measured 48 h after transfection by the Dual-Luciferase Reporter Assay System (Promega). Data are presented as the mean value for triplicate experiments.

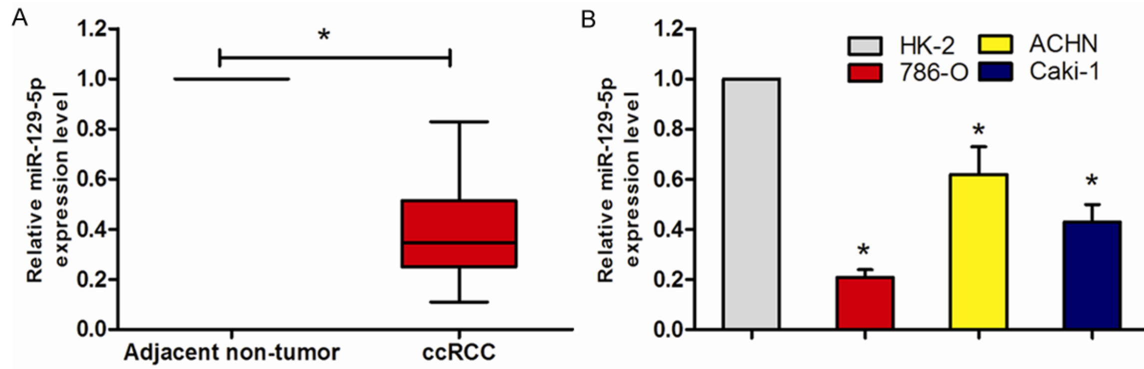

Figure 1. Decreased miR-129-5p expression in ccRCC tissuesand cell lines. A. qRT-PCR analysis of miR-129-5p expression in ccRCC tissues and adjacent non-tumor tissues from 33 patients. B. qRT-PCR analysis of miR-129-5p expression in three RCC cell lines and normal human proximal tubule epithelial cell line HK-2. *P < 0.05.

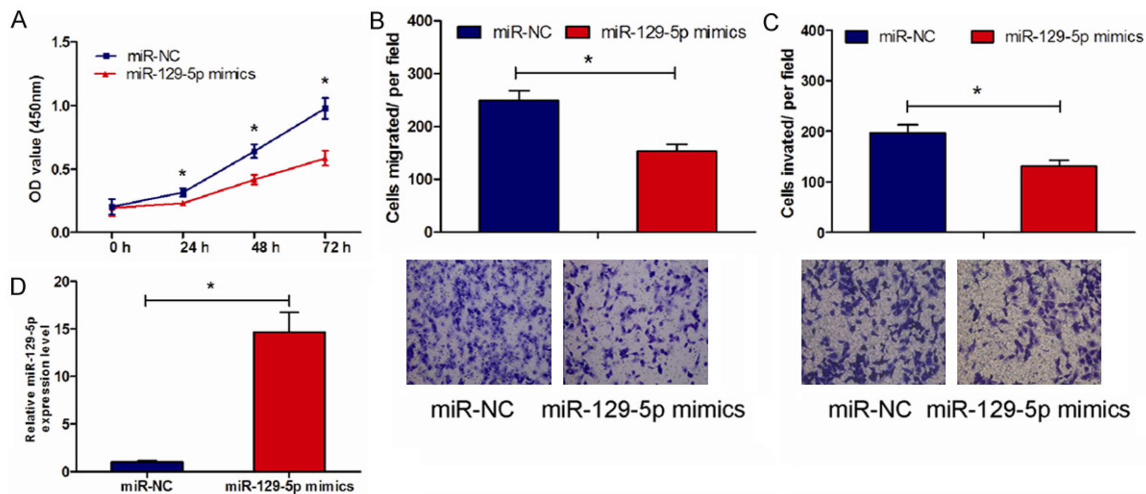

Figure 2. miR-129-5p inhibitedRCC cell proliferation, migration and invasion. A. CCK8 assay of 786-O cells transfected with miR-129-5p mimics or negative control miRNA mimics (miR-NC). B. Transwell migration assay of 786-O cells transfected with miR-129-5p mimics or miR-NC. C. Transwell invasion assay of 786-O cells transfected with miR-129-5p mimics or miR-NC. D. Expression of miR-129-5p in 786-O cells transfected with miR-129-5p mimics or miR-NCwasdetected by qRT-PCR. *P < 0.05.

Statistical analysis

Data are expressed as mean ± SD, from at least three separate experiments. Differences between groups were analyzed using one-way ANOVA or Student's t-test. P < 0.05 was considered statistically significant.

Results

miR-129-5p expression was down-regulated in ccRCC tissues and cell lines

To explore the biological role of miR-129-5p in renal cancer carcinogenesis, the expression of miR-129-5p in 33 paired ccRCC tissues was examined by qRT-PCR. We found that miR-129-5p was significantly downregulated in ccRCC tissues compared with the adjacent non-tumor tissues (**Figure 1A**). Furthermore, we explored the expression of miR-129-5p in RCC cells, our data showed that miR-129-5p was also downregulated in three RCC cell lines compared with the normal human proximal tubule epithelial cell line HK-2 (**Figure 1B**). These results indicated that miR-129-5p might be involved in human RCC progression.

miR-129-5 pinhibited RCC cell growth and motility in vitro

To study the effect of miR-129-5p on RCC cell growth and motility, 786-O cells were transfect-

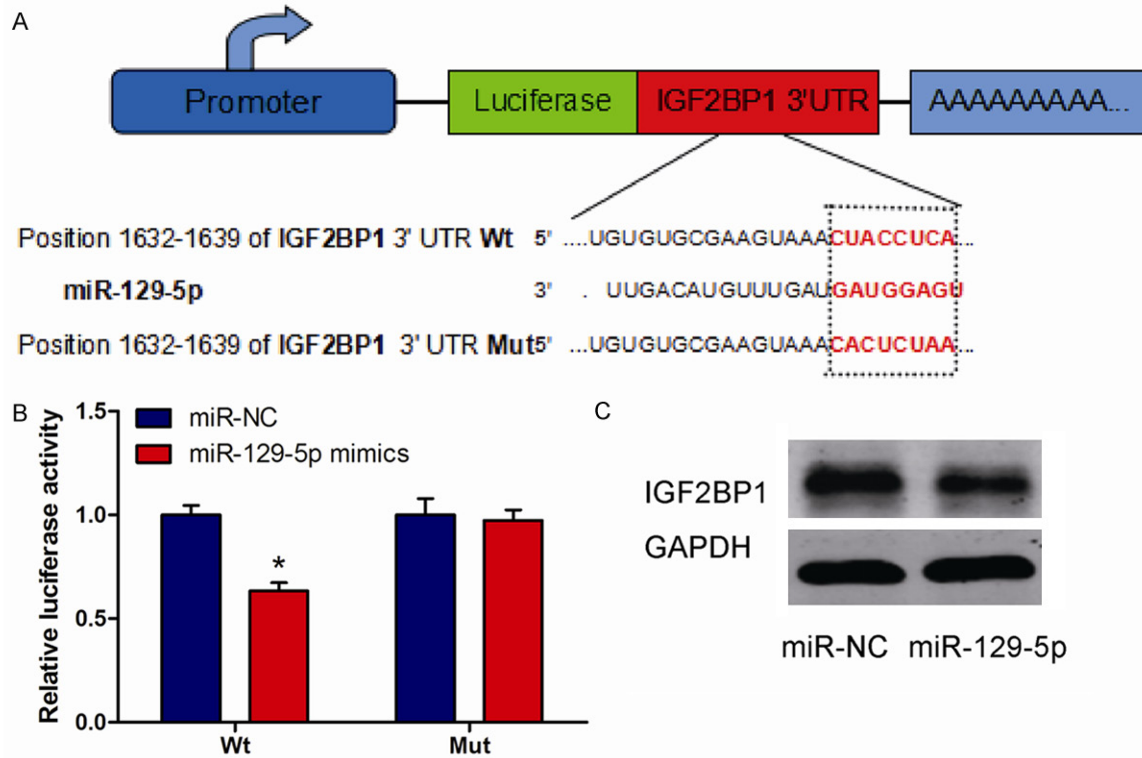

Figure 3. miR-129-5p targeted IGF2BP1 in RCC cells. A. IGF2BP1 was found to be the putative target of miR-129-5p using TargetScan. B. 786-O cells were co-transfected with miR-129-5p mimics or miR-NCwith Wt or Mut IGF2BP1 3'-UTR. Luciferase activity was detected. C. Overexpression of miR-129-5psuppressed IGF2BP1 protein level in 786-O cells using western blotting. $^{*}P < 0.05$.

ed with miR-129-5p mimics or control mimics (miR-NC). Forced overexpression of miR-129-5p significantly suppressed the proliferation of 786-O cells (**Figure 2A**). We further investigated whether miR-129-5p could also inhibit migration and invasion of RCC cells. Our data indicated that overexpression of miR-129-5p dramatically inhibited the migration and invasion capacity of 786-O cells (**Figure 2B** and **2C**). The effect of miR-129-5p mimics was confirmed by qRT-PCR (**Figure 2D**). Taken together, these data indicated that miR-129-5p might act as a tumor suppressor that inhibit RCC cell growth and motility in vitro.

IGF2BP1 was a direct target of miR-129-5p

To identify the target of miR-129-5p in RCC, TargetScan 6.2 was used to screen the target gene of miR-129-5p. IGF2BP1 was predicted to be a target of miR-129-5p (**Figure 3A**). Luciferase activity assay showed that miR-129-5p significantly inhibited the Wt but not Mut luciferase activity in HEK293 cells (**Figure 3B**). Moreover, overexpression of miR-129-5p significantly inhibited IGF2BP1 protein levelin 786-O cells (**Figure 3C**).

Inhibition of IGF2BP1 showed similar effect with miR-129-5p overexpression

Further experiments were performed to explore whether downregulation of IGF2BP1 performed similar effects with overexpression of miR-129-5p in RCC cells. CCK8 assay, in vitro cell migration assay and cell invasion assay showed that inhibition of IGF2BP1 by siRNA significantly suppressed the proliferation (**Figure 4A**), migration (**Figure 4B**), and invasion (**Figure 4C**) ability of 786-O cells. The effect of IGF2BP1 siRNA was confirmed by Western blotting (**Figure 4D**). Taken together, these data indicated that IGF2BP1 was an important target of miR-129-5p that involved in the proliferation, migration and invasion of RCC cells.

Discussion

Over the past decades, researchers have identified numerous genes contribute to RCC

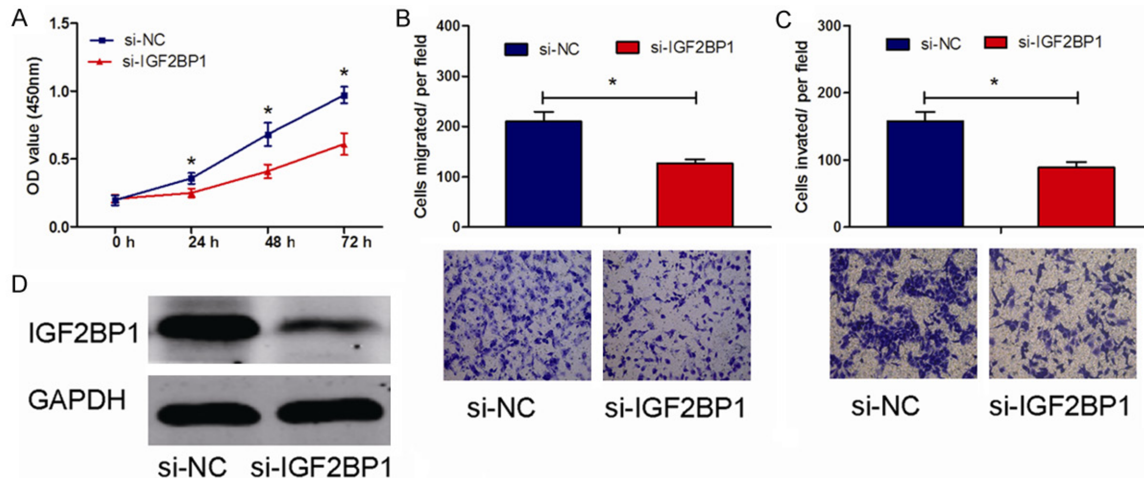

Figure 4. Inhibition of IGF2BP1 showed similar effect with miR-129-5p overexpression. A. The vitality of 786-O cells transfected with si-IGF2BP1 or negative control (si-NC) was detectedusing the CCK8 assay. B. Transwell migration assay of 786-O cells transfected with si-IGF2BP1 or si-NC. C. Transwell invasion assay of 786-O cells transfected with si-IGF2BP1 or si-NC. D. Protein level of IGF2BP1 was detected by Western blotting in 786-O cells transfected with si-IGF2BP1 or si-NC. *$P < 0.05$.

growth, invasion and migration. The dysregulated expression of miRNAs have been found to correlate with these phenotypes, thus reinforcing the importance of miRNA biology in RCC-associated tumorigenesis [11]. For example, Nakata et al. revealed that expression of miR-27a-3p was an independent predictive factor for recurrence in clear cell renal cell carcinoma [12]. Yang et al. indicated that miR-506 inhibited RCC cell growth and metastasis via targeting FLOT1 [13]. Jingushi et al. reported that miR-629 targeted TRIM33 to promote TGFβ/Smad signaling and metastatic phenotypes in ccRCC [14].

In this study, we explored the expression of miR-129-5p in RCC progression. Our data showed that the expression levels of miR-129-5p were significantly decreased in ccRCC tissues and cell lines. Ectopic overexpression of miR-129-5p significantly inhibited the proliferation, migration and invasion of RCC cells. By using luciferase activity assay and Western blotting, we found that IGF2BP1 was a direct target of miR-129-5p. Inhibition of IGF2BP1 showed similar effect with miR-129-5p overexpression in RCC cells. Thus, our data illustrated that the possible role of miR-129-5p and IGF2BP1 in the pathogenesis of RCC.

miR-129-5p has been found to play important roles in some cancers. For instance, Ma et al. showed that miR-129-5p inhibited hepatocellular carcinoma cell metastasis and invasion via targeting ETS1 [15]. Shen et al. suggested that upregulation of miR-129-5p inhibited laryngeal cancer cell proliferation, invasion and migration via targeting STAT3 [16]. Tan et al. found that miR-129-5p suppressed ovarian cancer cell proliferation and survival via direct suppression of transcriptional co-activators YAP and TAZ [17]. Yue et al. demonstrated that down-regulation of miR-129-5p via the Twist1-Snail feedback loop stimulates the epithelial-mesenchymal transition and was associated with poor prognosis in breast cancer [18]. In the present study, our findings expanded the function of miR-129-5p in RCC.

IGF2BP1, one of the VICKZ proteins, is a member of the RNA-binding IGF2BP protein family containing three members [19]. Previous studies reported that IGF2BP1 was elevated in several cancers and overexpression of IGF2BP1 was associated with poor prognosis of various cancers [20, 21]. Furthermore, IGF2BP1 was found to be regulated post-transcriptionally by several miRNAs, For example, Wang et al. showed that miRNA-873 inhibited glioblastoma tumorigenesis and metastasis by suppressing the expression of IGF2BP1 [22]. Qu et al. reported that miR-150 function as a tumor suppressor in osteosarcoma by targeting IGF2BP1 [23]. Zhou et al. suggested that miR-625 sup-

pressed tumour proliferation and invasion by targeting IGF2BP1 in hepatocellular carcinoma [24]. In this study, we found that miR-129-5p could inhibit the proliferation, migration and invasion in renal cell carcinoma via targeting IGF2BP1.

In conclusion, the present study identified for the first time that miR-129-5p could act as a tumor suppressor in RCC cells, which are largely mediated through inhibiting of IGF2BP1 expression. These findings suggesting that miR-129-5p might be a novel therapeutic strategy for the treatment of RCC.

Acknowledgements

This work was supported by the Key Departments Foundation of Zhumadian Central Hospital and the Innovation Team of Science and Education Health Project of Zhumadian in 2015 (Grant No. 20150149).

Disclosure of conflict of interest

None.

Address correspondence to: Hai-Liang Xu, Department of Urologic Surgery, Zhumadian Central Hospital, Zhumadian 463000, China. E-mail: hailiangxu7788@163.com

References

[1] Siegel R, Naishadham D, Jemal A. Cancer statistics, 2013. CA 2013; 63: 11-30.

[2] Cohen HT, McGovern FJ. Renal-cell carcinoma. N Engl J Med 2005; 353: 2477-2490.

[3] Ljungberg B, Bensalah K, Canfield S, Dabestani S, Hofmann F, Hora M, Kuczyk MA, Lam T, Marconi L, Merseburger AS, Mulders P, Powles T, Staehler M, Volpe A, Bex A. EAU guidelines on renal cell carcinoma: 2014 update. Eur Urol 2015; 67: 913-924.

[4] Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004; 116: 281-297.

[5] Esquela-Kerscher A, Slack FJ. Oncomirs-microRNAs with a role in cancer. Nat Rev Cancer 2006; 6: 259-269.

[6] Garzon R, Calin GA, Croce CM. MicroRNAs in cancer. Annu Rev Med 2009; 60: 167-179.

[7] Shenouda SK, Alahari SK. MicroRNA function in cancer: oncogene or a tumor suppressor?. Cancer Metas Rev 2009; 28: 369-378.

[8] Ma J, Hong L, Xu G, Hao J, Wang R, Guo H, Liu J, Zhang Y, Nie Y, Fan D. miR-483-3p plays an oncogenic role in esophageal squamous cell carcinoma by targeting tumor suppressor EI24. Cell Biol Int 2016; 40: 448-55.

[9] Minna E, Romeo P, Dugo M, De Cecco L, Todoerti K, Pilotti S, Perrone F, Seregni E, Agnelli L, Neri A, Greco A, Borrello MG. miR-451a is underexpressed and targets AKT/mTOR pathway in papillary thyroid carcinoma. Oncotarget 2016; 7: 12731-47.

[10] Wang N, Tao L, Zhong H, Zhao S, Yu Y, Yu B, Chen X, Gao J, Wang R. miR-135b inhibits tumour metastasis in prostate cancer by targeting STAT6. Oncol Lett 2016; 11: 543-550.

[11] Catto JWF, Alcaraz A, Bjartell AS, De Vere White R, Evans CP, Fussel S, Hamdy FC, Kallioniemi O, Mengual L, Schlomm T, Visakorpi T. MicroRNA in prostate, bladder, and kidney cancer: a systematic review. Eur Urol 2011; 59: 671-681.

[12] Nakata W, Uemura M, Sato M, Fujita K, Jingushi K, Ueda Y, Kitae K, Tsujikawa K, Nonomura N. Expression of miR-27a-3p is an independent predictive factor for recurrence in clear cell renal cell carcinoma. Oncotarget 2015; 6: 21645-21654.

[13] Yang F, Zhang H, Chen SJ, Yan Y, Zheng JH. MiR-506 is down-regulated in clear cell renal cell carcinoma and inhibits cell growth and metastasis via targeting FLOT1. PLoS One 2015; 10: e0120258.

[14] Jingushi K, Ueda Y, Kitae K, Hase H, Egawa H, Ohshio I, Kawakami R, Kashiwagi Y, Tsukada Y, Kobayashi T, Nakata W, Fujita K, Uemura M, Nonomura N, Tsujikawa K. miR-629 Targets TRIM33 to Promote TGFβ/Smad Signaling and Metastatic Phenotypes in ccRCC. Mol Cancer Res 2015; 13: 565-574.

[15] Ma N, Chen F, Shen SL, Chen W, Chen LZ, Su Q, Zhang LJ, Bi J, Zeng WT, Li W, Huang XH, Wang Q. MicroRNA-129-5p inhibits hepatocellular carcinoma cell metastasis and invasion via targeting ETS1. Biochem Biophys Res Commun 2015; 461: 618-623.

[16] Shen N, Huang X, Li J. Upregulation of miR-129-5p affects laryngeal cancer cell proliferation, invasiveness, and migration by affecting STAT3 expression. Tumor Biol 2016; 37: 1789-96.

[17] Tan G, Cao X, Dai Q, Zhang B, Huang J, Xiong S, Zhang Yy, Chen W, Yang J, Li H. A novel role for microRNA-129-5p in inhibiting ovarian cancer cell proliferation and survival via direct suppression of transcriptional co-activators YAP and TAZ. Oncotarget 2015; 6: 8676.

[18] Yu Y, Zhao Y, Sun XH, Ge J, Zhang B, Wang X, Cao XC. Down-regulation of miR-129-5p via the Twist1-Snail feedback loop stimulates the epithelial-mesenchymal transition and is associated with poor prognosis in breast cancer. Oncotarget 2015; 6: 34423-36.

[19] Stöhr N, Hüttelmaier S. IGF2BP1: a post-transcriptional "driver" of tumor cell migration. Cell Adh Migr 2012; 6: 312-318.
[20] Bell JL, Raseswari T, Liu T, et al. IGF2BP1 and MYCN cooperate in an oncogenic feedback loop, in high-risk neuroblastoma. Cancer Res 2014; 74 Suppl: 3103-3103.
[21] Gutschner T, Hämmerle M, Pazaitis N, Bley N, Fiskin E, Uckelmann H, Heim A, Groβ M, Hofmann N, Geffers R, Skawran B, Longerich T, Breuhahn K, Schirmacher P, Mühleck B, Hüttelmaier S, Diederichs S. Insulin-like growth factor 2 mRNA-binding protein 1 (IGF2BP1) is an important protumorigenic factor in hepatocellular carcinoma. Hepatology 2014; 59: 1900-1911.
[22] Wang RJ, Li JW, Bao BH, Wu HC, Du ZH, Su JL, Zhang MH, Liang HQ. MicroRNA-873 (miRNA-873) inhibits glioblastoma tumorigenesis and metastasis by suppressing the expression of IGF2BP1. J Biol Chem 2015; 290: 8938-8948.
[23] Qu Y, Pan S, Kang M, Dong R, Zhao J. MicroRNA-150 functions as a tumor suppressor in osteosarcoma by targeting IGF2BP1. Tumor Biol 2016; 37: 5275-84.
[24] Zhou X, Zhang CZ, Lu SX, Chen GG, Li LZ, Liu LL, Yi C, Fu J, Hu W, Wen JM, Yun JP. miR-625 suppresses tumour migration and invasion by targeting IGF2BP1 in hepatocellular carcinoma. Oncogene 2015; 34: 965-977.