Original Article

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

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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 E124 [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% CO₂ 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). The assays were performed according to the manufacturer’s instructions. Briefly, 1 × 10⁴ cells from each group 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 synthesized 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 2²⁻ΔΔCt method relative to U6. Three independent experiments were performed.

**Western blotting**

Cultured cells were washed twice and lysed in RIPA buffer with 1% PMSF. Protein was loaded and separated 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 an endogenous 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.
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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-129-5p inhibited RCC cell growth and motility in vitro

To study the effect of miR-129-5p on RCC cell growth and motility, 786-0 cells were transfect-
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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 level in 786-O cells using western blotting. *P < 0.05.

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
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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-
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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.

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Disclosure of conflict of interest

None.

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