Environmental Toxicology and Pharmacology 62 (2018) 227–233

Contents lists available at ScienceDirect

Environmental Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/etap

MicroRNA-375 inhibits the proliferation, migration and invasion of kidney cancer cells by triggering apoptosis and modulation of PDK1 expression

Jinling Wang, Xiuju Sun*

Department of Kidney, The Affiliated Hospital of Weifang Medical University, Weifang, Shandong Province, China

ARTICLE INFO

Keywords:
Kidney cancer
PDK1
miR-375
Proliferation
Cell migration

ABSTRACT

Kidney cancer is one of the deadly cancers and is the cause of significant number of deaths worldwide. The treatments used for the treatment of kidney cancer are limited and associated with number of side effects. Therefore, there is need for the development of new drug options or to identify novel therapeutic targets for the treatment of kidney cancer. In this study we investigated the potential of miR-375 as the therapeutic target for the treatment of Kidney cancer. The results revealed that miR-375 is significantly downregulated in the Kidney cancer cells. To investigate the role therapeutic potential of miR-375, one kidney cancer cell line (A-498) was selected for further experimentation. It was observed that overexpression of miR-375 inhibits A-498 kidney cancer proliferation by induction of apoptosis. In addition, overexpression of miR-375 causes suppression of migration and invasion of the A-498 kidney cancers cells. Bioinformatic analysis revealed PDK1 to be putative target of miR-375 in Kidney cancer cells. The western blot analysis revealed the expression of PDK1 to be significantly upregulated in Kidney cancer cells and overexpression of miR-375 in A-498 cells caused inhibition of PDK1 preventing the phosphorylation of AKT (Thr^{308} and Ser^{473}). Additionally, inhibition of PDK1 had similar effects as that of miR-375 overexpression on cell proliferation of A-498 kidney cancer cells. The inhibition of miR-375 expression could not rescue the effects of PDK-1 suppression on A-498 cell proliferation. In contrary, overexpression of PKD1 in A-498 cells transfected with miR-375 mimics could nullify the effects of miR-375 on proliferation of the A-498 cells. Taken together, we conclude that miR-375 regulates cell proliferation, migration and invasion of A-498 kidney cancer cells and may prove to be an important therapeutic target.

1. Introduction

Kidney cancer in adults generally arises from the renal parenchyma or renal pelvis. The pelvis cancers include around 10% of all the diagnosed kidney cancer cases and the rest of 90% kidney carcinomas are adenocarcinomas which originate in the renal parenchyma (Alsaiari et al., 2017; Nayan et al., 2017; Fabrizio et al., 2017). Around the globe, kidney cancer is responsible for significant mortality and its incidence is still increasing every now and then. Although there are treatments available for this type of cancer, however there are frequent relapses and the treatments have a number of side effects (Motzer et al., 2015; Gallardo et al., 2018). Therefore, there is a need to explore new drugs or to identify specific therapeutic targets for kidney cancer treatment. Over the years microRNAs (miRNAs) have been found to be potential therapeutic targets for the treatment of cancers (Garzon et al., 2009). The miRNAs include a highly conserved non-coding RNAs that have been found to play vital roles in a diversity of cellular processes (Ambros, 2004) Interestingly, the expression of miRNAs has been found to dysregulated in cancer cells as such they could be utilized as therapeutic targets (Lu et al., 2005). In the current study, we investigated the potential of miR-375 as the therapeutic target for the treatment of kidney cancer. The miR-375 has been reported to be differentially expressed several types of cancers such as gastric and colorectal cancers (Guled et al., 2009). Moreover, in a recent study miR-375 was reported to inhibit the growth of pancreatic cancer cells (Zhang et al., 2012). The miR-375 has also been reported to regulate the proliferation of several types of cancers. For instance, miR-375 inhibits the proliferation of gastric cancer cells by targeting JAK2 (Ding et al., 2010). In cervical cancer cells, miR-375 targets transcription factor SPI to inhibit their migration and invasion (Wang et al., 2011). It also inhibits the tumor properties of liver cancer cells by targeting the hippo-signalling factor YAP (Liu et al., 2010). In glioma and colon cancer, miR-375 has been reported to inhibit the proliferation and migration by targeting CTGF-EGFR signaling pathway (Zhang et al., 2018; Alam et al., 2017). Besides

* Corresponding author at: Department of Kidney, The Affiliated Hospital of Weifang Medical University, Weifang Kuiwen District, Yuhe Road 2428, Shandong Province, China.
E-mail address: SchoeneTaur@yahoo.com (X. Sun).

https://doi.org/10.1016/j.etap.2018.08.002
Received 4 March 2018; Received in revised form 28 July 2018; Accepted 1 August 2018
Available online 02 August 2018
1382-6689/

this miR-375 has been reported to regulate the expression of other genes as well (El Ouaamari et al., 2008; Liu et al., 2013). Given this background, the expression of miR-375 was investigated in four Kidney cancer and one normal Kidney cell lines. It was observed that the expression of miR-375 is significantly downregulated in all the Kidney cancer cell lines and overexpression of miR-375 expression leads to the inhibition of proliferation through induction of apoptosis and suppression migration and invasion of A-98 kidney cancer cells. Takentogether, we propose that miR-375 could prove to be an important therapeutic target for the treatment of Kidney cancer and deservers further research endeavours.

2. Materials and methods

2.1. Cell lines, culture conditions and transfection assays

Kidney cancer cell lines A498 (ATCC number: HTB-44), Caki-1 (ATCC number: HTB-46), 769-P (ATCC number: CRL-1933), 786-O (ATCC number: CRL-1932) and non-cancerous Kidney cell line hTRET (ATCC number: CRL-4031-OAT1) were procured from American Type Culture Collection. All of these cell lines were maintained in Dulbecco's modified Eagle's medium containing fetal 10% bovine serum, antibiotics100 units/mL penicillin and 100 µg/mL streptomycin), and 2 mM glutamine. The Cells were cultured in CO_2 incubator (Thermo Scientific) at 37 °C with 98% humidity and 5% CO_2. MiR-375 mimics, miR-NC, miR-375 inhibitor, Inhibitor-NC, Si-PDK1 and pcDNAPDK1 were all procured from GenePharm (Shanghai, China). All transfection assays were carried out using Lipofectamine 2000 reagent (Invitrogen) according as per the manufacturer's guidelines.

2.2. cDNA synthesis and quantitative RT-PCR

Total RNA was extracted from all the kidney cancer cell lines by using TRIZOL reagent (Invitrogen). Thereafter, reverse transcription and real time PCR were performed with the help of PrimeScript RT reagent Kit (Takara, Dalian, China) and SYBR Premix Ex TaqII (Tli RNaseH Plus) kit (TaKaRa) respectively. Finally relative expression was determined by $2^{-\Delta\Delta CT}$ method and actin was used for normalisation.

2.3. Cell viability assay

The viability of Kidney cancer cells was determined by MTT assay. In brief, the cultured Kidney cancer cells were seeded at the density of 1.5×10^4 in 96-well microtiter plates. This was followed by the addition of MTT solution in all the wells and then the absorbance at 570 nm was taken at 0, 24, 48, 72 and 96 h using an ELISA plate reader.

2.4. Apoptosis assay

The Kidney cancer cells were seeded in 6-well plates (2×10^5 Cells per well). The cells were then DAPI stained to detect apoptosis by fluorescence microscopy as previously reported (Daniel and DeCoster, 2004). For percentage of the apoptotic cells an FITC-Annexin V/PI Apoptosis detection kit was employed as per the instructions of the manufacturer (Beijing Biosea Biotechnology, China). Alkaline comet assay and transmission electron microscopy were carried out as described previously (Møller, 2006; Mkandawire et al., 2015).

2.5. Cell migration and invasion assay

The cell invasion ability of the Kidney cancer cells was evaluated by transwell assay. Briefly, the Cells were seeded at 2×10^5 cells/mL density after 48 h transfection. Thereafter, 200 ml cell suspensions were added into the upper chamber and complete medium was added into the bottom wells. After 24 h culturing, the cells in the upper chambers were removed and cells migrated through the chambers were subjected to fixation with methyl alcohol followed by staining with crystal violet. Finally the number of cells that migrated was determined by counting the cells under an inverted microscope (Magnification 200×, 10 different fields). Invasion was determined using Matrigel®-coated invasion chambers (BD Biosciences). The cells that reached the lower surface of the membrane were stained with crystal violet (CV). Images of CV-stained cells were captured by a phase contrast microscope.

2.6. Western blotting

The Kidney cancer cells were harvested and lysed with lysis buffer (Tris-HCl, sodium-dodecyl sulfate (SDS), mercaptoethanol and glycerol).The extracts were boiled for 10 min in presence of loading buffer followed by separation of cell extracts using 15% SDS-PAGE gel. The samples were then put onto polyvinylidene fluoride membranes blocked using 5% skimmed milk powder. Membrane incubation with primary antibodies was performed overnight at 4 °C. The membranes were incubated with horseradish peroxidase-linked secondary biotinylated secondary antibodies at 1:1000 dilution for 2 h. Washing of the membranes with PBS was followed by visualization of the immunoreactive bands using the ECL-PLUS/Kit according to the manufacturer's instructions. The immune complexes development was carried out using an ECL detection kit according to the manual protocol (ECL GST western blotting detection kit, Pierce Biotechnology, Inc., Waltham, MA, USA). The bands were analyzed using GelGDoc2000 imaging system (Bio-Rad Laboratories GmbH, Munich, Germany).

2.7. Statistical analysis

The experiments were carried out thrice and the values represent mean of the three replicates ± SD. Student's *t*-test (for comparison between two samples) and one way ANOVA followed by Tukey's test (for comparison between more than two samples) was used for statistical analysis using GraphPad prism software 7. The values were considered significant at $p < 0.05$.

3. Results

3.1. MiR-375 is downregulated in Kidney cancer cells

The expression of miR-375 was examined in different Kidney cancer cell lines (A498, Caki-1, 769-P, 786-O) and one normal Kidney cell line (hTRET) by qRT-PCR. The results showed that miR-375 is highly downregulated in all the Kidney cancer cells in comparison to the normal Kidney cells. The expression of miR-375 was 4–7 lower in the Kidney cancer cells with lowest expression in 769-P and highest in 786-O cell line (Fig. 1). The Kidney cancer cell line A-498 with moderate

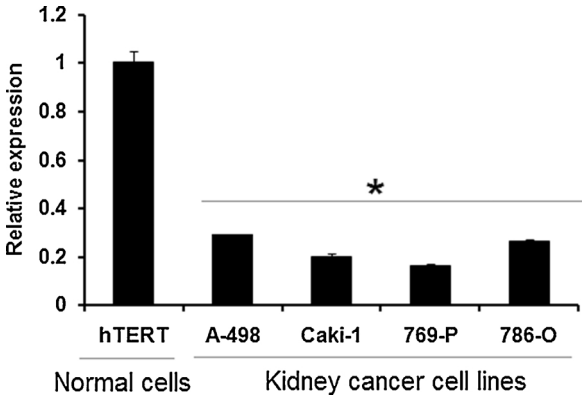

Fig. 1. Expression of miR-375 in Kidney cancer cell lines and normal cell line as determined by qRT-PCR. The values are mean of three biological replicates and expressed as mean ± SD ($p < 0.05$).