Pharmacological Reports 70 (2018) 963–971

Contents lists available at ScienceDirect

Pharmacological Reports

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

Original article

MiR-137 suppresses tumor growth and metastasis in clear cell renal cell carcinoma

Meizhi Wang[a], Hui Gao[b], Haijun Qu[c], Jing Li[c], Kaili Liu[b], Zhiwu Han[c,*]

[a] Department of Critical Care Medicine, The Affiliated Hospital of Qingdao University, Qingdao, China
[b] Department of Pharmacology, School of Pharmacy, Qingdao University, Qingdao, China
[c] Department of Pharmacy, The Affiliated Hospital of Qingdao University, Qingdao, China

ARTICLE INFO

Article history:
Received 2 December 2017
Received in revised form 9 April 2018
Accepted 20 April 2018
Available online 22 April 2018

Keywords:
miR-137
ccRCC
RLIP76

ABSTRACT

Background: The most frequent type of renal cell carcinoma is called clear-cell renal cell carcinoma (ccRCC) which is associated with a poor prognosis. It has been observed that miR-137 is aberrantly expressed in many different kinds of human malignancies including ccRCC. This research aims to examine the role of miR-137 in ccRCC.

Methods: Quantitative RT-PCR (qRT-PCR) was applied to measure miR-137 expression in ccRCC and adjacent noncancerous tissue. Gene expression was determined by western blot. Cell Counting Kit-8 (CCK-8) assay, flow cytometry and Transwell assay were used to determine the effects of miR-137 on cell growth, apoptosis and invasion, respectively. Moreover, xenograft and pulmonary metastasis animal models were established to investigate the role of miR-137 *in vivo*.

Results: Our findings show that there was significant downregulation of miR-137 in ccRCC tissue relative to corresponding non-cancerous tissue. Ectopic miR-137 expression in ccRCC cells led to suppression of cell growth and invasion, as well as apoptosis induction. In contrast, knockdown of miR-137 enhances proliferation and invasion, inhibits apoptosis. It also confirms that miR-137 plays a tumor supressor role *in vivo*. Mechanically, miR-137 directly targets the 3′-UTR of RLIP76 which is an established oncogene in ccRCC.

Conclusion: MiR-137 serves as a tumor suppressor, which can be considered a potential therapeutic target in ccRCC.

Introduction

As the 7th most frequent cancer in the developed world, renal cell carcinoma (RCC) represents 80% to 85% of all kidney cancers [1,2]. Statistics show that, internationally, there are about 209,000 new cases and 102,000 deaths from RCC annually [3]. About 20–30% of patients present with metastatic clear cell renal cell carcinoma (ccRCC), the most common histological subtype of RCC, at initial diagnosis. In addition, the percentage of ccRCC patients who develop metastases from localized disease has been reported to be up to 30% and the recurrence rate of post-surgery treatment cases is among 20–30% [4]. Therapies targeting the VHL/HIF/VEGF and the PI3K/AKT/mTOR pathways, two interacting pathways, have improved the prognosis of patients with ccRCC over the past decade [5–8]. As the clinical effects of these therapies are not ideal due to possible tumor metastasis and recurrence, developing more powerful therapeutic strategies against ccRCC is urgent.

As a protein which has many functions, DNP-SG ATPase (RLIP76) participates in the transportation of chemotherapy drugs and glutathione conjugates [9–11]. The participation of RLIP76 in the process of ligand-dependent receptor endocytosis, proliferation and metastasis have been evidenced in a number of studies [12–14]. Furthermore, over-expression of RLIP76 was observed in a majority of tumor cell lines and a number of human carcinomas [15]. Knockdown of RLIP76 by small interfering RNA (siRNA) has been found to suppress proliferation, induce apoptosis, and inhibit invasion of colon carcinoma cells *in vitro* [16]. Using shRNA, antibody or antisense in RLIP76-targeted therapy leads to long-term and complete response in xenografts of colon carcinoma [17]. In the context of ccRCC, a previous study has shown that RLIP76 has an overarching anti-apoptosis mechanism [10]. However, the regulatory mechanism of RLIP76 in ccRCC remains unclear.

* Corresponding author.
E-mail address: drzhiwuhan@163.com (Z. Han).

https://doi.org/10.1016/j.pharep.2018.04.006
1734-1140/

MicroRNAs (miRNAs), a class of small non-coding RNAs, mediate cell differentiation, proliferation, migration, apoptosis and invasion [18]. Interestingly, miR-137 is documented to be down-regulated in many human malignancies, such as non-small cell lung cancer [19], gastric cancer [20], colorectal cancer [21] and breast cancer [22]. In addition, it has been found that the restoration of miR-137 expression can inhibit cancer growth, induce apoptosis and suppress invasion [21,22]. As the role of miR-137 in ccRCC is still not clear, we examined the function of miR-137 in ccRCC and investigated the possible molecular mechanism underlying its function.

Materials and methods

Clinical samples

Forty-five patients diagnosed with ccRCC who received surgery were included in this study. Tissue samples were collected during surgery and maintained in liquid nitrogen (−70 °C). Two senior pathologists assessed random samples for diagnosis and histological classification in accordance with the 2011 Union for International Cancer Control TNM classification. RLIP76 expression in tissue was assessed by automated capillary western blot (WES) [23]. This clinical study protocol was approved by an ethics committee, and all patients or guardians provided informed consent.

Cell culture

Human ccRCC cell lines HK-2, 786-O, Caki1, Caki2, ACHN, RCC4 and A498 provided by ATCC (Shanghai, China) were maintained in DMEM medium including 2 mM glutamine, 10% (v/v) heat-inactivated FEB, 1% nonessential amino acids and 100 U/ml Penicillin-Streptomycin solution at 37 °C in a humidified atmosphere at 5% CO_2.

Construction of miR-137 mimic or anti- miR-137 and cell transfection

ccRCC cells were transfected with the lentiviral constructs of anti-miR-137, anti-miR-137 control (anti-miR-control), miR-137 mimic (miR-137) and miR-137 control (miR-control) obtained from Genepharma (Shanghai, China) in accordance with standard protocols.

Quantitative real-time PCR (qRT-PCR)

Total RNA was isolated from cultured ccRCC cells using TRIzol Reagent (Life Technology, Carlsbad, CA, USA), then transcribed to cDNA using a reverse transcription kit (Promega, Madison, WI, USA) in accordance with the manufacturer's instructions. RT-PCR was conducted using Power SYBR Green PCR Master Mix (Carlsbad, CA, USA) with GAPDH functioning as an internal control to detect the expression of RLIP76 mRNA. Sangon (Shanghai, China) synthesized the forward and reversed primer sequences as the following: forward primer, 5′-GATGAGAAGG-TATTTCTGCT-3′, and reverse primer, 5′-GAGAAATTGAAGGTCA-TAAA-3′. The $2^{-\Delta\Delta}$Ct method was applied to analyze the relative expression of the target gene. The miRVANA Kit (Ambion, Carlsbad, MA, USA) was employed to isolate miRNA, then miR-137 levels in cells and tissues were measured. Finally, cDNA was reversely transcribed using a small RNA specific stem-loop RT primer. Following TaqMan MicroRNA Assay (Thermo Fisher Scientific, Waltham, MA, USA), miR-137 levels were assessed with qRT-PCR. Data are expressed as Log 2-fold change in the respective miR/U6 snRNA levels with small nuclear RNA U6 serving as the internal control.

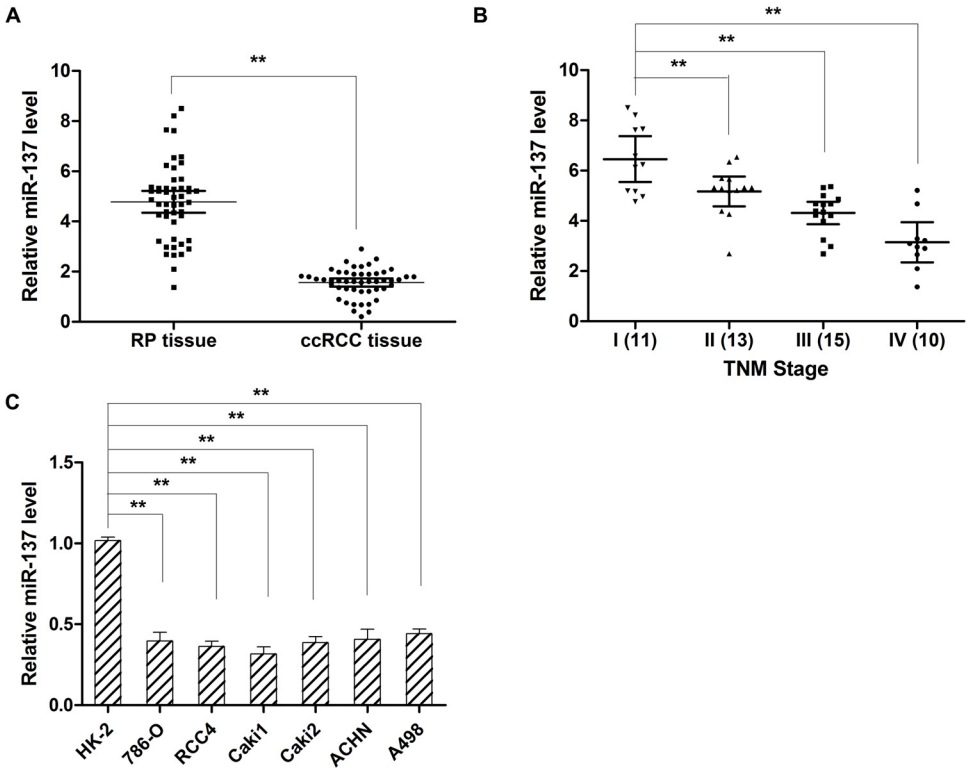

Fig. 1. MiR-137 expression in ccRCC tissue and cell lines. A). MiR-137 expression in ccRCC tissue is clearly lower than in RP. B). The association between miR-137 expression and TNM stage. C). MiR-137 expression is remarkablely lower in ccRCC cell lines compared to normal kidney cells. $^{**}p < 0.01$.