Biomedicine & Pharmacotherapy 99 (2018) 299–305

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

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

miR-152 inhibits proliferation of human endometrial cancer cells *via* inducing G2/M phase arrest by suppressing CDC25B expression

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ARTICLE INFO

Keywords:
miR-152
CDC25B
Cell proliferation
Cell cycle
Endometrial cancer

ABSTRACT

microRNA-152 (miR-152) is a tumor suppressor that is down-regulated in many cancers including endometrial cancer (EC). However, the underlying mechanism of action of miR-152 in EC is unclear. The aim of the present study was to evaluate the role of miR-152 on proliferation of human endometrial cancer cells. Herein, we found that miR-152 overexpression and CDC25B knockdown inhibited proliferative ability and induced G2/M phase arrest in KLE and HEC-1B cells. CDC25B was a target of miR-152. In addition, CDC25B overexpression rescued miR-152-induced proliferation inhibition and G2/M phase arrest in human endometrial cancer cells. The results indicated that miR-152 was a tumor suppressor in EC that inhibited proliferation of human endometrial cancer cells via inducing G2/M phase arrest by suppressing CDC25B expression.

1. Introduction

Endometrial cancer (EC) is one of the most common gynecological cancers in women [1]. The main treatment methods of EC are surgery, external radiotherapy, brachytherapy and chemotherapy based on estimation of the patient's condition [1]. It has been reported that the occurrence of EC is related to the accumulation of multiple genetic abnormalities, which activates oncogenes or inactivates tumor suppressor genes. Researchers have performed genome-wide screenings to explore the tumor suppressor genes involved in EC [2]. Genomic alterations in microRNAs (miRNAs) have been discovered [2,3]. The identification of abnormal genes may be useful for the prevention, diagnosis, and treatment of EC.

miRNAs are a group of endogenous small non-coding RNAs with 19–22 nucleotides that are reported to play crucial roles in many cellular processes, such as cell proliferation, division, differentiation and death [4,5]. Recently, multiple miRNAs have been demonstrated to be involved in many kinds of cancers, including EC [6–8]. The tumor suppressor microRNAs in EC have recently been identified. Among them, miR-152 was epigenetically silenced in EC and notably inhibited tumor cell growth both *in vitro* and *in vivo* [3]. The role of miR-152 in EC suggested its potential utility in therapeutic strategies of EC.

The CDC25 phosphatase family is responsible for dephosphorylating specific tyrosine/ threonine residues on cyclin dependent kinases (CDKs) [9]. The CDC25 family plays an important role in regulating the cell cycle [9]. There are three CDC25 genes in mammals: *CDC25A*, *CDC25B*, and *CDC25C* [10]. Accumulating evidence indicates that CDC25A and CDC25B are oncogenes. CDC25B is overexpressed in a variety of human malignancies, such as endometrial and prostate cancers [10,11]. Thus the inhibitors of CDC25B might be critical for the treatment of the related cancers [11].

In the present study, the role of miR-152 in human endometrial cancer cells and its targeted gene was studied. The results indicated that miR-152 inhibited cell proliferation via inducing G2/M phase arrest by suppressing the expression of its target gene *CDC25B* in human endometrial cancer cells.

2. Materials and methods

2.1. Cell culture and treatment

Human endometrial cancer cell lines, HEC-1B and Ishikawa, were purchased from the Chinese Academy of Sciences Committee on Type Culture Collection Cell Bank (Shanghai, China). Human endometrial cancer cell lines, RL95-2 and KLE, were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). The cells were cultured in DMEM/F12 media (Invitrogen, Carlsbad, CA, USA) with 10% fetal bovine serum (FBS; Invitrogen), 100 U/ml penicillin, and 100 μg/ml streptomycin. Cells were grown in 5% CO_2 at 37 °C.

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https://doi.org/10.1016/j.biopha.2018.01.046
Received 13 November 2017; Received in revised form 25 December 2017; Accepted 5 January 2018

2.2. Quantitative RT-PCR (qRT-PCR)

Total RNAs were extract from HEC-1B, Ishikawa, RL95-2 and KLE cells using the Trizol reagent (Invitrogen) according to the manufacturer's protocol. Expression of miR-152 was normalized to U6 snRNA. qRT-PCR assay was performed on a 7900-HT Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) using a SYBRPrimeScript™ miRNA RT-PCR Kit (TAKARA Biotechnology, Dalian, China). The relative changes in gene expression were analyzed by the $2^{-\Delta\Delta CT}$ method. The primers used in the study are: miR-152 forward, 5'-GATC ATTG GCCT TGCC AGTA-3'; reverse, 5'-GTGT GTAG AGGT CAGG AAGT-3', and U6 forward, 5'-GGTA GCCG CGGT TGAA ATGG-3'; reverse, 5'-CAGT AAGC AGTA AAGT CGA-3'.

2.3. Transfection

The hsa-miR-152 mimics and the scramble control were purchased from RiboBio (Guangzhou, China). The siRNA targeting CDC25B and control siRNA were designed and synthesized by Shanghai GenePharma Co., Ltd. (Shanghai, China). The CDC25B overexpression vector pcDNA3.1-CDC25B and empty vector pcDNA3.1 were obtained from RiboBio. The transfection was performed using Lipofectamine 2000 (Invitrogen) according to the instructions of the manufacturer.

2.4. MTT and 5-bromodeoxyuridine (BrdU) incorporation assays

Cell proliferation was evaluated by MTT and BrdU incorporation assays. MTT assay was performed at 0, 24, 48, and 72 h after transfection. Briefly, the transfected KLE and HEC-1B cells (1.5×10^3 cells/well) were seeded into 96-well plate. Then 20 μl MTT solution (5 mg/ml) was added to each well and incubated for 4 h. Dimethyl sulfoxide (DMSO; 150 μl) solution was added to dissolve formazan. Finally, the absorbance at 570 nm was examined. BrdU assay was carried out using a BrdU Cell Proliferation Assay Kit (Millipore, Billerica, MA, USA). Briefly, the transfected KLE and HEC-1B cells (1.5×10^3 cells/well) were seeded into a 96-well plate. Then 10 μM BrdU was added and incubated for 1 h. Subsequently, cells were incubated with peroxidase-conjugated anti-BrdU antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Finally, the absorbance was measured at a wavelength of 450 nm.

2.5. Flow cytometry

Cell cycle was detected by flow cytometry 48 h after transfection. Briefly, KLE and HEC-1B cells were fixed with 80% ethanol overnight at 4 °C. Then, 500 μl propidium iodide (PI, 50 μg/ml; Sigma-Aldrich, St. Louis, MO, USA) was added and incubated for 20 min. Cell cycle assay was performed with a FACSCalibur (BD Biosciences, Bedford, MA, USA).

2.6. Western blot

Total protein was extracted from KLE and HEC-1B cells and separated on 10% SDS gel. The protein was transferred on to a nitrocellulose membrane and blocked with 5% non-fat milk solution. Subsequently, the membrane was incubated with primary antibodies against CDC25B (dilution 1: 500; ab70927, Abcam, Cambridge, UK) and β-actin (dilution 1: 1000; ab6276, Abcam). After washing three times, horseradish peroxidase-conjugated rabbit anti-mouse (ab6728, Abcam) or goat anti-rabbit secondary antibody (ab97051, Abcam) was added. Finally, protein bands were visualized using enhanced chemiluminescence reagent (GE Healthcare, Chalfont, UK) and quantified using Image J software (NIH, Bethesda, MD, USA). β-actin was used as an internal reference.

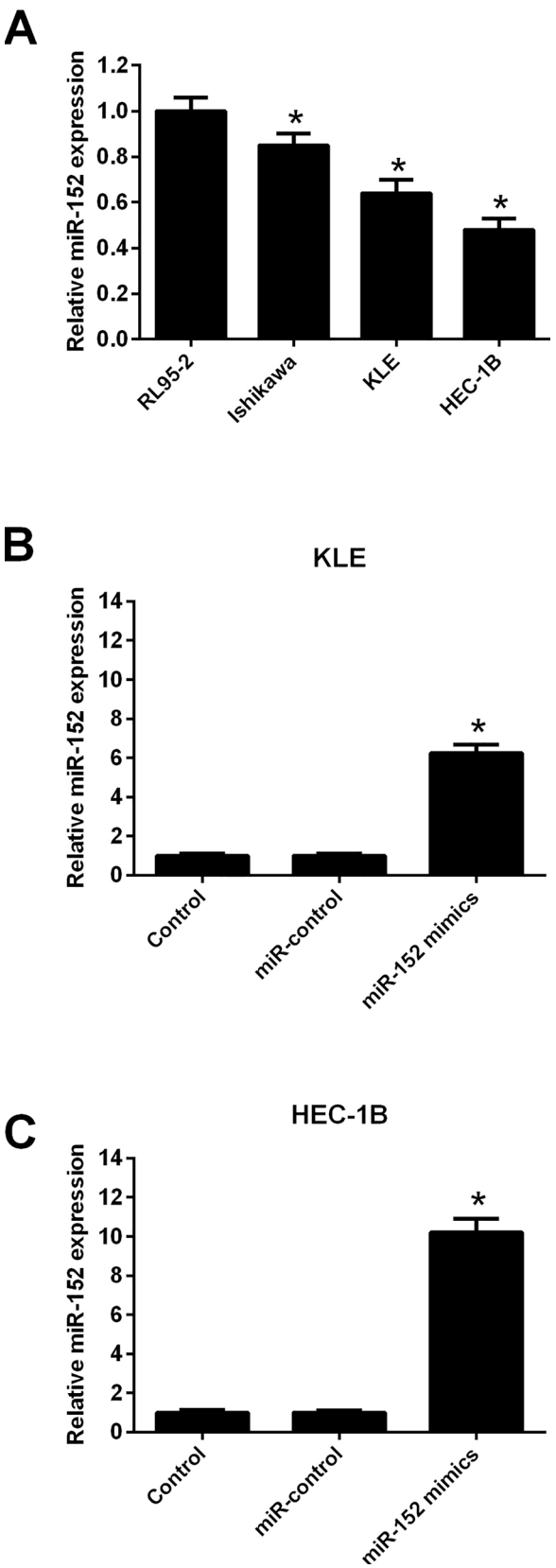

Fig. 1. Establishment of miR-152-overexpressing endometrial cancer cells.The miR-152 levels in the four human endometrial cancer cell lines (HEC-1B, Ishikawa, RL95-2 and KLE) were detected by qRT-PCR. To investigate the role of miR-152 in endometrial cancer, KLE and HEC-1B cells were transfected with miR-152 mimics or control mimics (miR-control). (A) miR-152 levels in the four cell lines. $^*p < 0.05$ compared to RL95-2 cells. (B) miR-152 levels in KLE cells after transfection for 48 h. $^*p < 0.05$ compared to miR-control. (C) The miR-152 levels in HEC-1B cells after transfection for 48 h. $^*p < 0.05$ compared to miR-control.