Biochemical and Biophysical Research Communications xxx (2018) 1–7

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

MiR-374b-5p-FOXP1 feedback loop regulates cell migration, epithelial-mesenchymal transition and chemosensitivity in ovarian cancer

Huanling Li*, [1], Jie Liang [1], Feng Qin, Yunfang Zhai

Department of Gynaecology, Second People's Hospital in Kashgar, Xinjiang Uyhur Autonomous Region. No.1, Health Road, Kashgar, Xinjiang, 844000, China

ARTICLE INFO

Article history:
Received 11 September 2018
Accepted 25 September 2018
Available online xxx

Keywords:
miR-374b-5p
FOXP1
EMT
Cisplatin
Ovarian cancer

ABSTRACT

MicroRNAs (miRNAs) are important regulators in tumorigenesis and progression of multiple human cancers, including ovarian cancer (OC). As a member of miRNAs family, miR-374b-5p has been reported to be a tumor suppressive gene in human cancers. In this study, the lower expression of miR-374b-5p was identified in OC tissues and cell liens using quantitative real time PCR (qRT-PCR). Forkhead box protein P1 (FOXP1) can act as an oncogene in human cancers. Mechanism experiments revealed that FOXP1 is a target of miR-374b-5p. Functionally, miR-374b-5p suppressed cell proliferation, migration and epithelial-mesenchymal transition (EMT) in ovarian cancer. Moreover, the sensitivity of OC cells to cisplatin was markedly enhanced by miR-374b-5p. However, FOXP1 reversed However, FOXP1 reversed miR-374b-5p-mediated biological functions. Previous reports demonstrated the inhibitory effect of FOXP1 on transcription FOXP1. Thus, we further examined the effect of FOXP1 on the transcription activity of miR-374b-5p in OC cells. The results showed that FOXP1 decreased miR-374b-5p expression by inhibiting the transcription activity of miR-374b-5p. Rescue assays revealed the regulatory effect of miR-374b-5p-FOXP1 feedback loop on ovarian cancer progression. In conclusion, miR-374b-5p-FOXP1 feedback loop regulates tumor progression and chemosensitivity in ovarian cancer.

1. Introduction

Ovarian cancer (OC) is one of the commonest gynecology malignancies all over the world [1]. Although some therapeutic methods such as surgical resection, chemotherapy and radiotherapy are efficient for OC patients at an early stage, the prognosis of patients at advanced stage is still unfavorable [2]. Therefore, it is necessary to investigate the molecular mechanism underlying the progression of ovarian cancer.

As a subtype of non-coding RNAs (ncRNAs), microRNAs (miRNAs) can act as an important participator or regulator in diverse biological processes of human cancer [3]. Mechanically, miRNAs commonly exert oncogenic or anti-oncogenic function by targeting the 3′UTR of their downstream mRNAs [4–10]. It has been reported that miR-374b can regulate tumor progression in multiple human malignant tumors by acting as a tumor suppressor [11–15]. However, the specific mechanism and function of miR-374b-5p in ovarian cancer are still marked. This study focused on the mechanism of miR-374b-5p involved in the progression and development of ovarian cancer.

At first, the expression pattern of miR-374b-5p in OC tissues or cell lines was determined. The potential relevance between miR-374b-5p expression and the prognosis of OC patients was analyzed by Kaplan-Meier method. Considering the common functional mechanism of miRNAs, we applied bioinformatics analysis to find the potential target mRNA of miR-374b-5p. The functional assays and rescue assays were conducted in OC cells to identify the influence of miR-374b-5p-FOXP1 axis on the cell proliferation, migration, EMT progress and cisplatin sensitivity of OC cells. Moreover, the expression of miR-374b-5p was negatively regulated by FOXP1. Since FOXP1 is known as a transcription inhibitor [16–18], we hypothesized that FOXP1 might inhibit miR-374b-5p transcription. Mechanism experiments such as ChIP assay and luciferase activity analysis revealed the inhibitory effect of FOXP1 on miR-374b-5p transcription. Rescue assays were designed and performed in two OC cells to validate the effects of miR-374b-5p-FOXP1 feedback loop on the biological processes and chemosensitivity of OC cells.

* Corresponding author.
E-mail address: HuanlingLI335@163.com (H. Li).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.bbrc.2018.09.161
0006-291X/

2. Materials and methods

2.1. Tissue samples

All tissue samples used in this study were collected and obtained from 84 patients with ovarian cancer which were diagnosed at the Second People's Hospital in Kashgar. All patients participated in this study had signed the informed consent. We had obtained the approval of the ethics committee of the Second People's Hospital in Kashgar before this study. We proclaimed that human subject research was made in accordance with the Declaration of Helsinki.

2.2. Cell lines

Four strains of human OC cell lines (OVCAR3, 3AO, A2780, SKOV3) and one normal ovarian epithelial cell line (HOSEpiC) were commercially obtained from American Type Culture Collection (ATCC, Rockville, MD, USA). Cell lines used in this study were cultured in Roswell ParkMemorial Institute (RPMI)-1640 medium in which a mixture of 10% fetal bovine serum (FBS, Invitrogen, Carlsbad, CA, USA), 100 U/ml penicillin and 100 μg/mL streptomycin (Sigma-Aldrich, St. Louis, MO, USA) was added. Cell lines were cultured in a humidified atmosphere with 5% CO_2 at 37 °C.

2.3. Transfection

The full-length sequence of FOXP1 was inserted into pcDNA3.1 (Invitrogen) plasmid to construct pcDNA3.1/FOXP1 expression vector. MiR-374b-5p mimics, miR-374b-5p inhibitors and miR-NC were provided by GenePharma Corporation (Shanghai, China). Moreover, sh-FOXP1 and sh-NC were also synthesized by GenePharma Corporation. Lipofectamine 2000 (Invitrogen) was used for cell transfection.

2.4. RNA extraction and qRT-PCR

RNAs extraction from tissues and cells were conducted using Trizol reagent (Takara, Otsu, Japan). The TaqMan™ Advanced miRNA cDNA Synthesis Kit (Waltham, MA, USA) or the reverse transcription kit (Takara, Otsu, Japan) was used to make RNAs reversely transcribe into cDNA. The RT-qPCR was performed by using SYBR Green PCR Kit (Takara, Otsu, Japan). GADPH acted as an internal control of FOXP1, while U6 acted as the internal control of miR-374b-5p. The relative expression levels were counted using the $2^{-\Delta\Delta Ct}$ method.

2.5. CCK-8 assay

Cell-Counting Kit 8 (CCK-8, Dojindo Molecular Technologies) was used for cell proliferation assay. After transfection, SKOV3 and A2780 cells (1×10^4 cells/well) were seeded in 96-well plates. When cells were incubated at 24, 48, 72 and 96 h, 10 mL of CCK-8 solution was added to each well. Incubation was conducted at 37 °C for 4 h. Absorbance (450 nm) was detected by using the microplate reader (EL340; Bio-Tek Instruments, Hopkinton, MA, USA).

2.6. Cisplatin sensitivity test

After transfection, Cells (1×10^4 cells/well) were seeded in 96-well plates. 0, 0.5, 1, 2, 4 and 8 μg/mL of cisplatin was respectively added to the culture medium, then incubated at 37 °C for 24 h. The percent survival was measured by CCK-8 (CCK-8, Dojindo Molecular Technologies). IC50 value was determined when the percent survival reached to 50%.

2.7. Transwell assay

The migratory abilities of cells were assessed by using Transwell chambers (8 mm pore filter, Corning Incorporated, Corning, NY, USA) which were not coated with matrigel. Transfected cells (1×10^4 cells/well) in 200 μl RPMI 1640 medium were seeded into the upper chambers. 800 μl RPMI 1640 medium supplemented with 10% FBS was added into the lower chambers. After 24 h of incubation, migratory cells were fixed with methanol and stained with 0.5% crystal violet (Amresco Co., Solon, OH, USA). Finally, these stained cells were counted by using a light microscope (Olympus Corporation, Tokyo, Japan).

2.8. Chromatin immunoprecipitation (ChIP) assay

EZ-ChIP Kit (Millipore, Billerica, MA, USA) was used for ChIP assays. Formaldehyde cross-linked chromatin was sonicated to generate different fragments. Then, these chromatin fragments were immunoprecipitated by using anti-FOXP1 and anti-Ago2 antibodies. qRT-PCR was conducted to analyze the precipitated chromatin DNA. IgG antibody was used as control.

2.9. Pull-down assay

MiR-374b-5p-WT, miR-374b-5p-MUT and miR-NC were transcribed by using TranscriptAid T7 High Yield Transcription Kit (ThermoFisher Scientific, USA). Bio-miR-374b-5p-WT, Bio-miR-374b-5p-MUT and Bio-miR-NC were produced by the Biotin RNA labeling mix (Roche Diagnostics, Indianapolis, IN, USA). 200 μg cell lysates (SKOV3 and A2780) was mixed with 50 pmol biotinylated RNA, then incubated with 50 μl streptavidin agarose beads (Invitrogen, Carlsbad, CA, USA) for 1 h at 4 °C. After washing, the enrichment of FOXP1 were measured by western blotting.

2.10. Immunofluorescence

Cells were seeded on glass cover slips, then fixed with 4% formaldehyde for 10 min and permeabilized with 0.5% Triton X-100 for 15 min. Then, cells were blocked with 5% goat serum for 30 min, followed by incubation with antibodies overnight. Antibodies for immunofluorescence are as follows: anti-E-cadherin (1:1000, Cell Signaling Technologies, Massachusetts, USA) and anti-N-cadherin (1:1000, Cell Signaling Technologies). DAPI (4, 6-diamidino-2-phenylindole) was used to stain cell nuclei. After immunostaining, a fluorescence microscope was used to observe the samples.

2.11. Luciferase reporter assay

The wild-type FOXP1 reporter (FXOP1-MT) and the mutant-type FXOP1 reporter (FXOP1-MUT) were produced by GeneArt™ Site-Directed Mutagenesis System (Thermo Fisher Scientific). MiR-374b-5p mimics or miR-NC was co-transfected with FXOP1-MT or FXOP1-MUT into SKOV3 or A2780 cells by using Lipofectamine 2000 according to the manufacturer's instructions. After 48 h of incubation, a dual-luciferase reporter assay system (Promega, Madison WI, USA) was used to detect the luciferase activities.

2.12. Western blot

RIPA lysis buffer (Beyotime Biotechnology, China) with protease inhibitor (Roche, China) was used to isolate proteins, then protein concentration was determined by the BCA protein assay kit (Beyotime Co., Shanghai, China). These proteins was separated by using 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). Then, transfer proteins into polyvinylidene

Please cite this article in press as: H. Li, et al., MiR-374b-5p-FOXP1 feedback loop regulates cell migration, epithelial-mesenchymal transition and chemosensitivity in ovarian cancer, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.09.161