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LncRNA MIR22HG negatively regulates miR-141-3p to enhance DAPK1 expression and inhibits endometrial carcinoma cells proliferation

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ABSTRACT

Emerging evidence has indicated that long non-coding RNAs (lncRNAs) play critical roles in tumor development and progression. Recent studies reported that lncRNA MIR22HG could play important roles in hepatocellular carcinoma and lung cancer progression. However, the expression and underlying mechanism of MIR22HG in endometrial cancer (EC) remain unclear. In the present study, qRT-PCR showed that MIR22HG expression was significantly downregulated in EC tissues. In vitro function assays showed that increased MIR22HG expression significantly inhibited EC cells proliferation, induced EC cells apoptosis, and arrested EC cells in G0/G1 phase. Furthermore, miR-141-3p was identified and confirmed to be the target of MIR22HG. Subsequently, DAPK1 was confirmed to be regulated by MIR22HG and miR-141-3p, and could play a positive role in inhibiting EC cells proliferation. Collectively, these data demonstrated that lncRNA MIR22HG could act as a tumor suppressor and inhibited EC cells proliferation through regulating miR-141-3p/DAPK1 axis, which provides a new therapeutic target for EC treatment.

1. Introduction

Endometrial cancer is the sixth most common cancer in women worldwide [1]. Endometrioid endometrial carcinoma (EEC), occurring from epithelial cells lining the endometrium, constitutes ~85%–90% of EC [2]. Primary surgical treatment is the mainstay of therapy, but the effectiveness and extent of lymphadenectomy have been challenged [3]. Therefore, investigate the molecular mechanisms underlying EC tumorigenesis is very important.

Long non-coding RNAs (lncRNAs) are a type of non-coding transcripts from genome with more than 200 nucleotides [4]. Recently, mounting evidence indicated that lncRNAs are involved in diverse biological processes in tumor initiation, growth and metastasis through epigenetic, transcriptional and post transcriptional mechanisms [5,6]. In EC, a few of lncRNAs have been well characterized in the development of endometrial carcinoma. For example, Yang et al found that lncRNA HAND2-AS1 inhibited invasion and metastasis in endometrioid endometrial carcinoma through inactivating neuromedin U [7]. Sun et al. showed that lncRNA MEG3 inhibited endometrial carcinoma tumorigenesis through PI3K pathway [8]. Li et al. revealed that LINC00672 contributed to p53 protein-mediated gene suppression and promoted endometrial cancer chemosensitivity [9].

LncRNA MIR22HG, which is located in chromosome 17p13.3, a chromosomal region that is frequently deleted, hypermethylated, or shows loss of heterozygosity in liver cancer [10,11]. Recently, Dong et al revealed that MIR22HG was down-regulated in hepatocellular carcinoma and under-expression of MIR22HG was an independent risk factor associated with the prognosis of patients [12]. Liu et al showed that MIR22HG repressed hepatocellular carcinoma cell invasion by deriving miR-22 and targeting HMGB1 [13]. Su et al indicated that MIR22HG inhibition triggered cell survival signaling via oncogenes YBX1, MET, and p21 in lung cancer [14]. However, the function and underlying mechanism of MIR22HG in EC remain unclear. In the present study, we explored the role and underlying mechanism of lncRNA MIR22HG in the progression of EC. We verified that MIR22HG/miR-141-3p/DAPK1 axis could play critical roles in EC tumorigenesis.

2. Material and methods

2.1. Sample collection

33 EC tissues and 14 endometrial tissue specimens were collected

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from patients who had undergone surgical resection at Affiliated Hospital of Hebei University of Engineering. These patients were diagnosed based on histopathological evaluation. None of the patients received local or systemic treatment before the operation. These tissue specimens were immediately snap frozen in liquid nitrogen, and stored at −80 °C until use. The study was approved by the ethics committee of Hebei University of Engineering, and all patients signed informed consent.

2.2. Cell culture and transfection

EC cell lines HEC-1 A and KLE cells were purchased from American Type Culture Center (ATCC, USA). All cell lines were maintained in DMEM medium (Gibco, USA) supplemented with 10% fetal bovine serum (FBS, Hyclone, USA) and 1% penicillin-streptomycin solution in a humidified 5% CO_2 atmosphere. miR-141-3p mimics, pcDNA3.1/MIR22HG plasmid were chemically synthesized by Shanghai Integrated Biotech Solutions Co., Ltd. (Shanghai, China). The siRNA sequences targeting DAPK1 (si-DAPK1) was purchased from RiboBio Co. Ltd (Guangzhou, China). All transfection reactions were performed by using Lipofectamine 2000 (Invitrogen), according to the manufacturer's protocol.

2.3. RNA extraction and quantitative RT-PCR (qRT-PCR)

Total RNA was extracted using Trizol reagent according to the manufacturer's instructions (Invitrogen). The first strand cDNA was synthesized with 1 μg of RNA using the PrimerScriptTM RT Reagent Kit (Applied Biosystems). The expression of MIR22HG and DAPK1 mRNA was quantified on ABI 7500 real-time PCR system, and GAPDH were used as an internal control. The expression of miR-141-3p was quantified by using TaqMan miRNA assays (Applied Biosystems), and U6 was used as an internal control. The relative expression was calculated with the $2^{-\Delta\Delta CT}$ method.

2.4. Cell proliferation assay

Cell proliferation ability was measured by Cell Counting Kit-8 assay kits (CCK-8; Dojindo, Japan). The transfected cells (2×10^3 cells/well) were seeded in 96-well plates and cultured for 24, 48, 72 or 96 h, respectively. And then added 10 μl CCK-8 solutions in each well and incubated at 37 for 1 h. Microplate Reader was applied to determine the absorbance at 490 nm.

2.5. Flow cytometric assay

For the cell cycle analysis, the transfected cells were harvested and stained with PI using the Cell Cycle Analysis Kit (Biyuntian, China) followed by the assessment of flow cytometric. With Flowjo, the percentage of the cells in different phases was counted. Annexin V-FITC Apoptosis Detection Kit (BD Biosciences) was used for the apoptosis analysis. After harvesting the transfected cells and re-suspended in binding buffer, Annexin V-FITC and propidium iodide was used to stain the cells. Flow cytometric was performed according to the manufacturer' s instructions.

2.6. Dual luciferase activity assay

The dual luciferase assay was performed using dual-Luciferase® reporter assay kit (Promega, USA). Briefly, HEK293 T cells were cultured in 24-well plates and then co-transfected with miR-141-3p mimics or miR-NC mimics, pGL3-MIR22HG 3′UTR-WT or pGL3-MIR22HG 3′UTR-Mut using Lipofectamine 2000 (Invitrogen, USA). After 48 h, cells were collected and the dual-luciferase activity was examined with renilla luciferase as an internal control.

2.7. Western blot

Total proteins were extracted from cells, and then transfered into 10% SDS-PAGE. Separation of protein was then transferred onto PVDF membranes and blocked using 5% no-fat milk for 1 h. Then, PVDF membranes were incubated overnight with primary antibodies at 4 °C. Then, secondary antibody was incubated for 1 h. Finally, proteins expression was determined using a chemiluminescence detection kit (Amersham Pharmacia Biotech) correlated to GAPDH endogenous control.

2.8. Statistical analysis

All statistical analyses were performed with SPSS 16.0 software. The results were presented as the mean ± SD of three independent experiments. The differences between groups were analyzed by student's *t*-test or one-way ANOVA. Statistically significance was considered as $P < 0.05$.

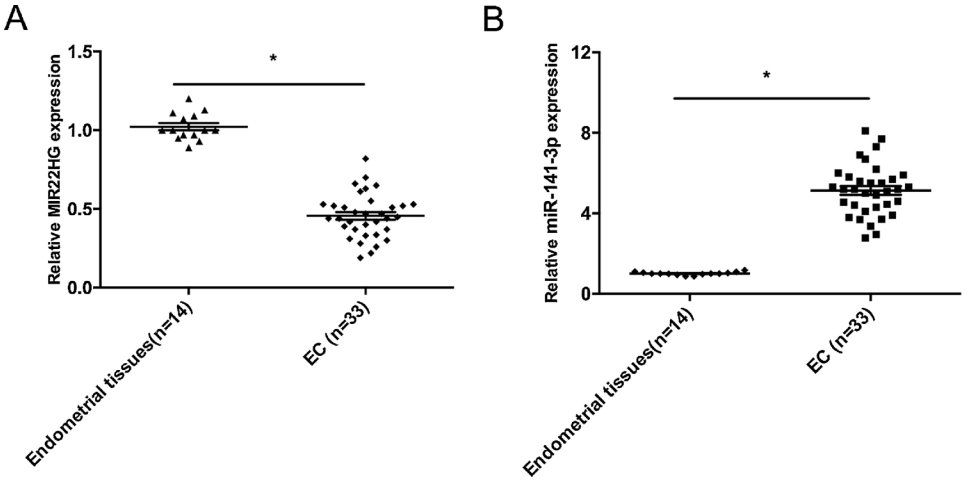

Fig. 1. MIR22HG was decreased in EC tissues. (A) qRT-PCR was used to measure MIR22HG expression in EC tissues and endometrial tissues. (B) qRT-PCR was used to determine miR-141-3p expression in EC tissues and endometrial tissues. *P < 0.05.