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miR-137 suppresses cell growth in ovarian cancer by targeting AEG-1

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ABSTRACT

Astrocyte elevated gene-1 (AEG-1) is an oncogene overexpressed in multiple types of human cancers including ovarian cancer (OC). However, the underlying mechanism of AEG-1 up-regulation in OC is not well understood. In this study, we showed that miR-137 downregulated AEG-1 expression through interaction with its 3′ untranslated region (3′UTR) and that miR-137 expression was inversely correlated with AEG-1 levels in OC specimens. Similar to the downregulation of AEG-1, overexpression of miR-137 in OC cell lines decreased *in vitro* cell growth, clonogenicity, and also induced G1 arrest. Importantly, miR-137 overexpression suppressed *in vivo* tumor growth in nude mice models. Furthermore, we found that restoring the AEG-1 (without the 3′UTR) significantly rescued miR-137-induced cell growth inhibition and cell-cycle arrest. Taken together, these findings indicate that miR-137 functions as a tumor suppressor by inhibition of AEG-1. These molecules might be targets for prevention or treatment of OC.

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1. Introduction

Ovarian cancer (OC) is one of the most common and lethal gynecologic malignancies, accounting for more than 15,000 deaths per year [1,2]. Despite advances in the diagnosis and treatment of this cancer, only 30% of patients survive 5 years after initial diagnosis [3,4]. Therefore, there is an urgent need to discover novel diagnostic biomarkers and therapeutic targets for OC patients.

Astrocyte elevated gene-1 protein (AEG)-1, also known as metadherin (MTDH) [5], is the product of a novel gene induced in primary human fetal astrocytes (PHFA) infected with human immunodeficiency virus type 1 (HIV-1) or treated with tumor necrosis factor-α (TNF-α) [6]. Previous studies have shown that AEG-1 was significantly elevated in several kinds of cancers, including breast cancer, glioma, prostate cancer, and esophageal squamous cell carcinoma [7–10]. Overexpression of AEG-1 promoted tumor cell proliferation, invasion, metastasis and chemoresistance [11–13]. In particular, our team previously detected high expression of AEG-1 in OC and its up-regulation was significantly associated with OC recurrence [14]. However, the functional mechanisms of AEG-1 in OC remains unclear.

MicroRNAs (miRNAs) are a class of single-stranded, small non-coding RNAs, which negatively regulate gene expression through base pairing with the 3′ untranslated region (3′UTR) of target mRNAs, causing mRNA degradation and/or translational repression [15]. miRNAs can direct a wide repertoire of biological mechanisms, such as cell cycle control, cell proliferation, apoptosis, senescence, cell migration and metastasis [16]. In OC, multiple miRNAs including miR-15a, miR-16, miR-31 and miR-125b have been identified as tumor-suppressor genes [17–19]. On the other hand, miR-187 and miR-182 have been shown to act as oncogenes in OC [20,21]. These findings suggest the involvement of miRNAs in OC tumorigenesis.

miR-137 has attracted much attention because it is frequently down-regulated and functions as a tumor suppressor in gastric cancer, glioblastoma, lung cancer, colorectal cancer and neuroblastoma [22–26]. However, whether miR-137 is involved in the progression of OC remains poorly understood. Intriguingly, online bioinformatic analysis showed that miR-137 has a conserved binding site in the AEG-1 3′UTR. Therefore, the objective of the current study was to validate the effect of miR-137 on AEG-1 in OC cells and to explore the role of this mechanism in the tumorigenesis and progression of OC.

2. Materials and methods

2.1. Cell lines and culture conditions

Ovarian cancer cell lines SKOV3, OV2008 and HEK293T were obtained from the Institute of Biochemistry and Cell Biology of the Chinese Academy of Sciences (Shanghai, China). All cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS), 100 IU/mL penicillin, and 100 mg/mL streptomycin (Invitrogen, Carlsbad, CA, USA). Cells were cultured at 37 °C in a humidified chamber supplemented with 5% CO_2.

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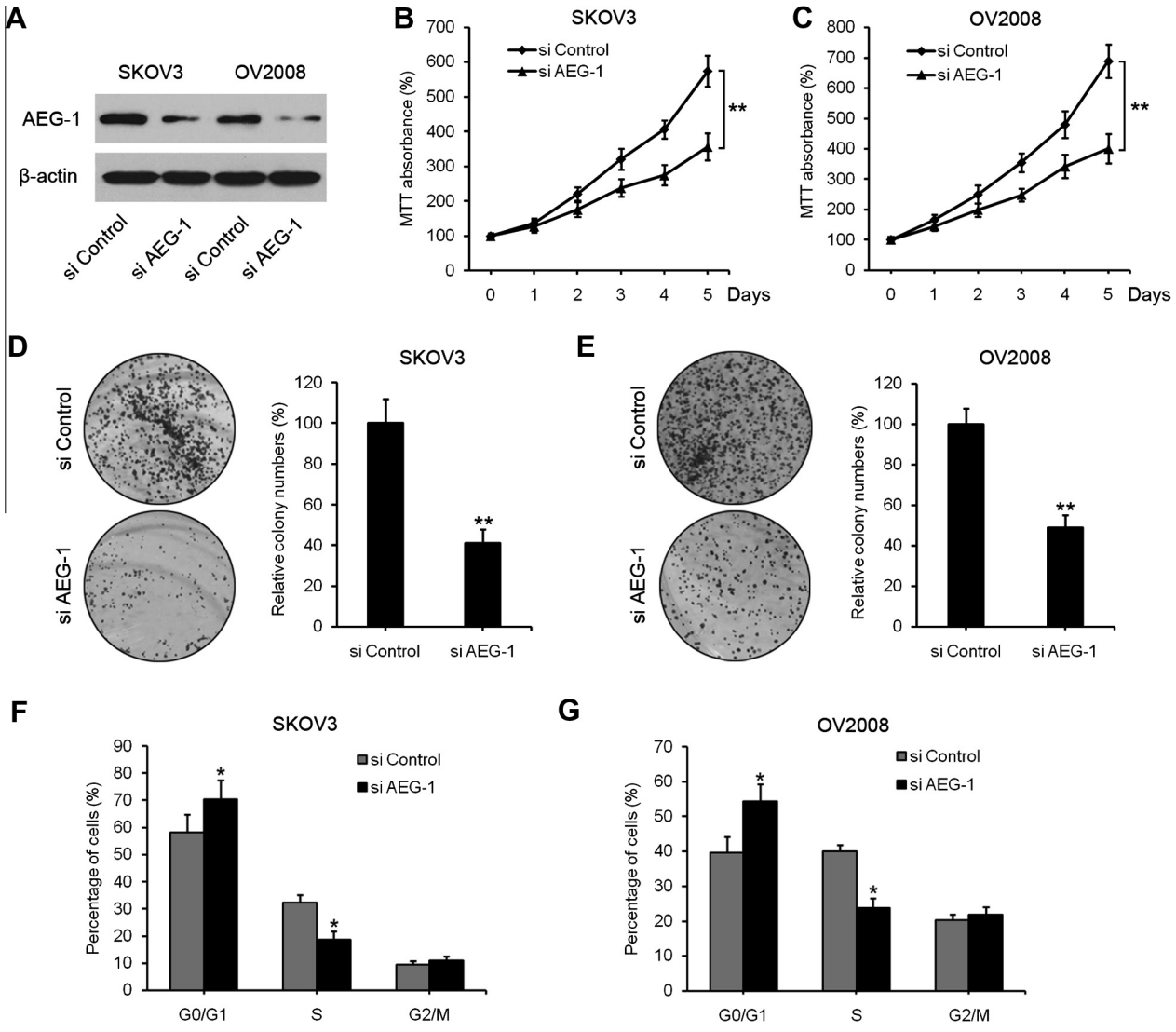

Fig. 1. Downregulation of AEG-1 inhibits OC cell growth. (A) Western blot analysis of AEG-1 expression in SKOV3 and OV2008 cells infected with AEG-1 siRNA (si AEG-1) or negative control (si Control). (B and C) Effect of AEG-1 down-regulation on OC cell growth was measured by MTT assay after si AEG-1 or si Control infection in SKOV3 and OV2008 cells. The line charts showed the relative MTT absorbance, which indicated the cellular viability. (D and E) Effect of AEG-1 down-regulation on the colonigenic ability of SKOV3 and OV2008 cells. The relative percentage of colony numbers from si Control group is designated as 100%. (F and G) Typical results of cell cycle analysis of SKOV3 and OV2008 cells infected with si AEG-1 or si Control. The bar chart represents the percentage of the cells in the G0/G1, S or G2/M phase of the cell cycle. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. si Control group.

2.2. Plasmid construction

Human miR-137 precursor and AEG-1 siRNA were purchased from GeneChem (Shanghai, China). The pre-miR-137 sequences were cloned into the lentiviral vector pCDH-CMV-MCS-EF1-GFP (System Biosciences, California, USA). AEG-1 siRNA sequences were subcloned into the pLKO.1 vector (Addgene, Cambridge, MA, USA). The coding sequences of AEG-1 (OriGene Technologies, Rockville, MD, USA) were cloned into pcDNA3.1 (+) to generate AEG-1 expression vector. The wild-type 3′UTR segment of AEG-1 was cloned into the *Not*I/*Xho*I sites downstream of the stop codon of Renilla luciferase in the psi-check2 vector (Promega, Madison, WI, USA). The corresponding mutant constructs were created by mutating the seed regions of the miR-137-binding sites. All constructs were verified by direct sequencing.

2.3. Lentivirus production and transduction

Virus particles were harvested 48 h after pCDH-miR-137 or pLKO.1-AEG-1 siRNA transfection with the packaging plasmid into HEK-293T cells as previously described [27,28]. OC cell lines SKOV3 and OV2008 were infected with recombinant lentivirus-transducing units plus 10 mg/ml Polybrene (Sigma–Aldrich, St. Louis, Missouri, USA). An empty lentiviral vector was used as negative control.

2.4. Western blot analysis

Proteins were extracted with RIPA buffer (PBS, 1% NP40, 0.1% SDS, 5 mM EDTA, 0.5% sodium deoxycholate, 1 mM sodium orthovanadate) with protease inhibitors and quantified using a Bradford reagent (Bio-Rad, Hamburg, Germany). Equivalent amounts of protein were subjected to 10% SDS–PAGE separation and then electrotransferred to polyvinylidene difluoride membranes (Millipore, Schwalbach, Germany). After blocking, the membranes were immunoblotted overnight at 4 °C with anti-AEG-1 or anti-β-actin antibody (Abcam, Cambridge, MA, UK), followed by their respective horseradish peroxidase-conjugated secondary antibodies. Signals were detected using an enhanced chemiluminescence.

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