Biochemical and Biophysical Research Communications xxx (2018) 1–8

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

MicroRNA-630 inhibitor sensitizes chemoresistant ovarian cancer to chemotherapy by enhancing apoptosis

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ARTICLE INFO

Article history:
Received 5 February 2018
Accepted 7 February 2018
Available online xxx

Keywords:
MicroRNA
Ovarian cancer
miR-630
Apoptosis

ABSTRACT

MicroRNA-630 (miR-630) has been implicated in the development and progression of multiple cancers. The current study aimed to investigate the role of miR-630 in chemoresistant epithelial ovarian cancer. MiR-630 expression levels were detected in ovarian cancer cell line SKOV3 and paclitaxel-resistant SKOV3 (SKOV3-TR) via microarray and qRT-PCR. MiR-630 inhibitors and negative controls were transfected into SKOV3 and SKOV3-TR cells. Wound healing, invasion, chemosensitivity, and cell apoptosis assays were performed to determine proliferation and migration rates. Chemoresistant patient-derived xenograft (PDX) models were established and utilized to verify the effect of miR-630 on chemoresistant ovarian cancer. Inhibition of miR-630 decreased cell proliferation and enhanced the sensitivity of SKOV3-TR and SKOV3 cells to paclitaxel. In the chemosensitivity assay, we observed that the miR-630 inhibitor exhibited a synergistic effect with paclitaxel on SKOV3-TR cells. Inhibition was correlated with enhanced expression of apoptosis-related proteins. APAF-1 was predicted to be a potential target of miR-630. An *in vivo* PDX study showed that the miR-630 inhibitor sensitized chemoresistant ovarian cancer to paclitaxel. Thus, miR-630 inhibitor sensitizes chemoresistant epithelial ovarian cancer to chemotherapy by enhancing apoptosis. Our findings suggest that miR-630 might be a potential therapeutic target for chemotherapy-resistant ovarian cancer.

1. Introduction

Ovarian cancer is the most lethal gynecologic malignancy. Due to aggressive metastasis, recurrence, and drug resistance, the five-year survival rate is only about 30% [1]. Specifically, resistance to chemotherapy is a major obstacle to long-term remission [2]. Hence, novel therapeutic strategies to re-sensitize chemoresistant ovarian cancers and overcome drug resistance would have a significant clinical impact.

MicroRNAs (miRNAs) are small, noncoding, single-stranded RNAs that are highly conserved among plants, animals, and even some viruses. MiRNAs regulate gene expression epigenetically [3–5], and the dysregulated expression of specific miRNAs is correlated with several malignancies, including epithelial ovarian cancers [6]. Previous studies showed that several miRNAs, including miR-21, miR-125a, miR-125b, and miR-99a, are differentially expressed in epithelial ovarian cancer and correlated with ovarian cancer prognosis [7,8]. In addition, miR-630, miR-370, and miR-575 have been shown to be significantly upregulated in recurrent serous ovarian carcinoma [9]. Specifically, miR-630 overexpression is reported to promote epithelial ovarian cancer proliferation and migration, while anti-miR-630 attenuates this effect both *in vitro* and *in vivo* [10]. Recent research has suggested that the dysregulated expression of specific miRNAs may also influence the development of drug resistance in ovarian cancer [11,12]. Nonetheless, to date, little is known regarding the role of miR-630 in chemoresistance in ovarian cancer.

In this study, we explored the effect of a miR-630 inhibitor on chemoresistant epithelial ovarian cancer. Results from this analysis revealed that miR-630 inhibition suppresses chemoresistant ovarian cancer cell proliferation and migration, probably by targeting APAF-1. Our research suggests that miR-630 is a promising therapeutic target for chemoresistant ovarian cancer.

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https://doi.org/10.1016/j.bbrc.2018.02.062
0006-291X/

2. Materials and methods

2.1. Cell culture

Human epithelial ovarian cancer cell lines SKOV3 and paclitaxel-resistant SKOV3 (SKOV3-TR) were cultured in RPMI-1640 medium (Gibco) containing 10% fetal bovine serum (FBS; Gibco) and 1% penicillin/streptomycin (P/S, Gibco). Cells were maintained at 37 °C in a humidified atmosphere of 5% CO_2. Cells were detached using 0.25% trypsin-EDTA solution (Gibco) and transferred to culture flasks at a 1:4 split ratio.

2.2. Cell transfection with microRNA inhibitor

SKOV3 and SKOV3-TR cells were transfected with miR-630 inhibitor (Product No. 4104647-101, Exiqon) or miRNA inhibitor negative control (Product No. 199006-101, Exiqon). These were used at a final concentration of 30 nM and transfected using Lipofectamine RNAiMAX reagent (Invitrogen). Cells were harvested 48 h post-transfection for RNA and protein extraction and functional assays.

2.3. Wound healing assay

Transfected cells (SKOV3, 5.0×10^5 cells/well; SKOV3-TR, 7.0×10^5 cells/well) were seeded on 6-well cell culture plates with serum-containing medium and were cultured until the cell density reached ~90% confluence. The serum-containing medium was removed, and cells were serum-starved for 24 h. When the cell density reached ~100% confluence, an artificial homogeneous wound was created by scratching the monolayer with a sterile 200-μL pipette tip. After scratching, cells were washed with serum-free medium. Images of cells migrating into the wound were captured at 0, 24, and 48 h using a microscope.

2.4. Invasion assay

Invasion assays were performed using the Matrigel Invasion Chamber (pore size: 8.0 mm, 24-well; Corning) according to the manufacturer's protocol. Transfected cells (SKOV3, 1.2×10^5 cells/insert; SKOV3-TR, 2.0×10^5 cells/insert) were seeded in the upper compartment and allowed to migrate for 48 h. Cells were seeded in 100 μL serum-free RPMI-1640 in the upper chamber with 700 μL 10% FBS-containing medium in the well below the insert.

2.5. Chemosensitivity assay

Rates of sensitivity to paclitaxel were determined using the cell proliferation reagent CCK-8 (Dojindo). Transfected cells (SKOV3, 1.2×10^5 cells/well; SKOV3-TR, 2.0×10^5 cells/well) were seeded on 24-well cell culture plates. After overnight incubation, cells were treated with 0.2 μg/mL paclitaxel. Cell viability was measured after 48 h using CCK-8 according to the manufacturer's protocol. The number of viable cells was evaluated by measuring the absorbance at 450 nm.

2.6. Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was prepared from SKOV3 or SKOV3-TR cells using a TRIzol Kit (Bioline) according to the manufacturer's protocol. PCR was conducted as follows: 94 °C for 10 min; 40 cycles of 94 °C for 15 s, 60 °C for 1 min, and 72 °C for 1 min; followed by 72 °C for 5 min qRT-PCR was performed using the ABIStepOnePlus Real-Time PCR system (Applied Biosystems) with 2 μg of each cDNA sample in triplicate using SYBR® Select Master Mix (Applied Biosystems). Quantifications was performed using 18S rRNA as the internal standard. The PCR primers used are shown in Table 1. Relative gene expression was analyzed using the $2^{-\Delta\Delta CT}$ method, and the results were expressed as the extent of change relative to control values. All qRT-PCRs were run in triplicate.

2.7. TaqMan miRNA assay

Total RNA was prepared from SKOV3 or SKOV3-TR cells using a miRCURY™ RNA Isolation Kit (Exiqon) according to the manufacturer's protocol. For quantitative RT-PCR analysis of miRNA, cDNA was prepared from 200 ng total RNA with the TaqMan microRNA Reverse Transcription Kit (Applied Biosystems). TaqMan microRNA real-time PCR assays were performed according to the manufacturer's recommendations. The TaqMan U6 snRNA assay (Applied Biosystems) was used for normalization of expression values. Relative gene expression was analyzed using the $2^{-\Delta\Delta CT}$ method, and the results were expressed as the extent of change relative to control values. All qRT-PCRs were run in triplicates.

2.8. Cell apoptosis assays

Cells were plated on 6-well plates and transfected with siNC and miR-630 inhibitor. The apoptosis ratio was analyzed at 48 h after transfection using the FITC Annexin V Apoptosis Detection Kit (BD Biosciences) according to the manufacturer's instructions. Briefly, 1×10^5 treated cells were incubated with annexin V/propidium iodide for 15 min at room temperature. Cells were sorted using a FACS LSR II (BD Biosciences) and analyzed with BD FACSDiva (version 6.2) software. Apoptotic cells were calculated after FACS analysis. The tests were repeated in triplicate.

2.9. Western blot

Cells were lysed in ice-cold RIPA buffer (Thermo). After centrifugation at 13200 rpm for 40 min at 4 °C, proteins in the supernatant were quantified and separated with 10% SDS-PAGE. Then, proteins were transferred onto a polyvinylidene difluoride (PVDF) membrane (Millipore), which was incubated with 1 × TBS-T containing 5% skim milk at room temperature for 30 min. Membranes were incubated with primary antibodies against β-actin, Bax, Bcl-2, caspase-9, and PARP (Cell Signaling); caspase-3 (GeneTex); and APAF1 (Abcam) overnight at 4 °C, and then incubated with HRP-

Table 1
Real time PCR primers used in this study.

Gene	Primer sequence (5′→3′)		Product size (bp)	Annealing Tm (°C)	Accession number
House keepping gene					
18S rRNA	Forward	ATT AAG GGT GTG GGC CGA AG	111	60	NM_022551.2
	Reverse	GGT GAT CAC ACG TTC CAC CT			
Target gene					
APAF1	Forward	TGG AAT GTC TCA AAC GGT GA	117	60	AF149794
	Reverse	AAG CAT TTT GCC ATC TGG AG			

Please cite this article in press as: K.J. Eoh, et al., MicroRNA-630 inhibitor sensitizes chemoresistant ovarian cancer to chemotherapy by enhancing apoptosis, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.02.062