Gynecologic Oncology 130 (2013) 354–361

Contents lists available at SciVerse ScienceDirect

Gynecologic Oncology

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

WAVE1 gene silencing via RNA interference reduces ovarian cancer cell invasion, migration and proliferation

Jing Zhang, Suiyang Zhou, Liangdan Tang *, Liyuan Shen, Lin Xiao, Zhaoning Duan, Li Jia, Yi Cao, Xiaoling Mu

Department of Obsterics and Gynecology, the First Affiliated Hospital of Chongqing Medical University, Chongqing 400016, People's Republic of China

HIGHLIGHTS

- Our previously study has identified overexpression of WAVE1 in epithelial ovarian cancer (EOC) tissues is associated with a poor prognosis.
- WAVE1-silencing had a significant effect on cell morphological changes, and decreased cell migration, invasion, adhesion, colony formation and proliferation.
- Our results demonstrated WAVE1 promotes proliferative and invasive malignant behaviors through activation of PI3K/AKT and p38MAPK signaling pathways in EOC.

ARTICLE INFO

Article history:
Received 8 January 2013
Accepted 5 May 2013
Available online 13 May 2013

Keywords:
WAVE1
Ovarian cancer
RNA interference
Proliferation
Invasion

ABSTRACT

Objective. Wiskott–Aldrich syndrome protein family verprolin-homologous protein 1 (WAVE1) has been implicated in cancer cell migration and invasion. We have previously shown that the overexpression of WAVE1 in epithelial ovarian cancer (EOC) tissues is associated with a poor prognosis. However, the mechanism of WAVE1 regulating the malignant behaviors in EOC remains unclear.

Methods. In the present study, we knocked down WAVE1 expression in SKOV3 and OVCAR-3 cells through RNA interference to detect the cell biology and molecular biology changes. Moreover, western-blot was used to investigate the underlying mechanism of WAVE1 regulating the proliferative and invasive malignant behaviors in ovarian cancer cells.

Results. The down-regulation of WAVE1 had a significant effect on cell morphological changes. WAVE1 silencing decreased cell migration, cell invasion, cell adhesion, colony formation and cell proliferation in vitro. In addition, we found that down-regulation of WAVE1 inhibited malignant behaviors in vivo. Furthermore, our study also indicated that the PI3K/AKT and p38MAPK signaling pathways might contribute to WAVE1 promotion of ovarian cancer cell proliferation, migration, and invasion.

Conclusions. WAVE1 might promote the proliferative and invasive malignant behaviors through the activation of the PI3K/AKT and p38MAPK signaling pathways in EOC.

Introduction

Epithelial ovarian cancer (EOC) is the most lethal gynecological malignancy. In the majority of cases, patients are diagnosed at an advanced stage and suffer unfavorable outcomes [1]. Although there are advanced surgical and cytotoxic therapies, 80% of late-stage patients develop recurrent disease, and <30% of patients survive 5 years after diagnosis [2,3]. Tumor invasion and metastasis are the primary causes for the high rate of EOC morbidity and mortality [4].

Wiskott–Aldrich syndrome protein (WASP) family verprolin-homologous protein 1 (WAVE1) has been identified as inducing lamellipodia formation [5,6]. Rho family small GTPases, WASP family proteins, actin-related protein 2/3 (Arp2/3) complex, and myosin are involved in the reorganization of actin filaments. WASP family proteins have direct effects on actin filament reorganization [7,8]. WAVE1 has been identified as being sequestered in an inactive state via the formation of a complex with abelson interactor protein-1 (Abil), Nck-associated protein 1 (Nap1), hematopoietic stem progenitor cell 300 (Hspc300), and a p53-inducible mRNA (PIRl21) [9,10]. Recent reports have demonstrated that WAVEs also play a critical role in cancer cell migration and invasion [11–16]. Another study demonstrated the overexpression, compared to non-invasive cells levels, of WAVE1 in highly malignant metastatic melanoma cells [17]. In prostate cancer cells, WAVE1 knockdown decreases tumor cell invasion [18]. Moreover, WAVE1 plays a role in invasion and multi-drug resistance through the regulation of MMP-2 and Bcl-2 expression levels in leukemia cells [19,20]. The above findings indicated that WAVE1 acts as an enhancer gene in cancer cells.

* Corresponding author. Fax: +86 23 89011082.
E-mail address: ldtang2002@yahoo.com.cn (L. Tang).

0090-8258/$ – see front matter
http://dx.doi.org/10.1016/j.ygyno.2013.05.005

Our previous studies indicated that WAVE1 expression is significantly elevated in the plasma of EOC patients, and overexpression of WAVE1 in EOC tissues is associated with a poor prognosis [1,21]. Here, we focused on the functions and mechanisms of WAVE1 in the proliferative, migratory, and invasive malignant behaviors of ovarian cancer cells.

Materials and methods

Cell culture and reagents

The human epithelial ovarian cancer cell lines SKOV3, OVCAR-3, ES-2 and 3AO were obtained and cultured as described previously [21]. Goat anti-WAVE1, rabbit anti-Abi1, rabbit anti-VEGF, rabbit anti-E-cadherin, mouse anti-cyclinD1, mouse anti-Arp2, rabbit anti-p38, and rabbit anti-GAPDH antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Rabbit anti-AKT, rabbit anti-phospho-AKT, rabbit anti-ERK1/2, rabbit anti-phospho-ERK1/2, and rabbit anti-phospho-p38 MAPK antibodies were obtained from Cell Signaling Technology (Beverly, MA, USA). Rabbit anti-Nap1, mouse anti-MMP-2, and rabbit anti-MMP-9 antibodies were purchased from Abcam (Cambridge, UK).

Western blot analysis

Cells and tissues were lysed on ice in lysis buffer (Beyotime, Jiangsu, China). The protein concentrations of the samples were determined using a BSA protein assay kit (Beyotime, Jiangsu, China). For the blots, 40 μg of total protein was loaded onto a 6% to 12% SDS-PAGE gel. Immunoblot blot analysis was performed as described previously [21]. The dilution rates of the primary antibodies were 1:100 to 1:1000. GAPDH was used as an internal control.

Quantitative real-time PCR (Q-RT-PCR)

Total RNA was isolated from cells with TRIzol reagent (Takara, Japan), and first-strand DNA was synthesized using a cDNA reverse transcription kit (Takara, Japan), according to the manufacturer's protocol. Then, a 25-μl reaction volume from each well was examined using a CFX96™ real-time PCR Detection System (BioRad, USA). The primers sequences were as follows: WAVE1, sense primer: 5′-TCAACTTGAG ACGTGTGGTCAG-3′ and anti-sense primer: 5′-TCATGTGTCGCTATCG CTCC-3′ and GAPDH, sense primer: 5′-GGTCGGAGTCAACGGATTT G-3′ and anti-sense primer: 5′-GGAAGATGGTGATGGGATTTC-3′. The expression level was determined using the $2^{-\triangle\triangle Ct}$ method.

RNA interference

To knockdown WAVE1 in the SKOV3 cells, two small hairpin RNA (shRNA) sequences were designed to target human WAVE1 as follows: 5′-AATGTTGCAAGA TACAGAG-3′ (shRNA-1) and 5′-GTAGAAG AGCAGCGTGAAC-3′ (shRNA-2). Non-target shRNA (5′-GTGGATATTG TTGCCATCA-3′) transfected cells were used as negative control. The shRNA transfections were performed using lentiviruses according to the manufacturer's protocol. Infected cells were subjected to 2 μg/ml puromycin, and survived clones were isolated. The expression level of WAVE1 was confirmed by Western blot analysis and Q-RT-PCR.

Invasion and migration assays

To investigate the invasion ability of the cells, 24-well transwell inserts with 8-μm pores (BD Bioscience, CA, USA) coated with a 1:5 dilution of Matrigel (BD Bioscience, CA, USA) were used. For the cell migration assay, 24-well transwell inserts without a Matrigel coating were used. Cells (1×10^4), suspended in 200 μl of RPMI1640 medium containing 5% FBS, were seeded in triplicate into the upper chamber, and 600 μl of RPMI1640 (10% FBS) medium was added to the lower well as a chemoattractant. After 24 and 48 h of incubation, the non-motile cells on the upper surface of the membrane were wiped off, whereas the cells on the lower side were fixed by 4% paraformaldehyde and stained with hematoxylin. The number of cells was counted in five random fields per well under a light microscope.

Cell adhesion assay

Cells (2×10^4) were plated in triplicate in 96-well plates coated with Matrigel and incubated at 37 °C for 1 h. After rinsing, the adherent cells in each well were fixed with 4% formaldehyde and then stained with crystal violet. The adherent cell stain was extracted by acetic acid and quantified using a microplate reader at 490 nm absorbance.

Proliferation assay

Cells (0.5×10^3) were seeded into 96-well plates in triplicate and harvested on days 1, 2, 3, 4, and 5. At the start of harvesting, 20 μl of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution was added into each well. After incubation for 4 h at 37 °C, 150 μl of dimethyl sulfoxide (DMSO) was added to dissolve any present formazan crystals. The absorbance was measured at 490 nm using a microplate reader.

Soft agar assay

For the soft agar assay, 5 ml of 0.6% agar solution in RPMI1640 medium containing 10% FBS was solidified in 6-cm dishes in triplicate. The 4 ml of 0.3% agar solution, mixed with 1×10^4 cells, was layered on top of a base agar layer. After incubation for 3 weeks at 37 °C, each colony formation containing over 50 cells was counted.

Tumor formation in nude mice

For the tumor xenograft experiments, each 6-week-old athymic female nude mouse was injected subcutaneously with 4×10^6 cells in 100 μl of PBS in the flanks: SKOV3-Ri1 (8 implants) and SKOV3-NC (8 implants). The tumor sizes were measured weekly. Five weeks after injection, the mice were euthanized, and the xenografts were removed for analysis. All procedures were approved by the Institution Animal Care Committee at Chongqing Medical University, Chongqing, China.

Confocal immunofluorescence microscopy

The cells were seeded on sterile glass coverslips overnight and then fixed in methanol at −20 °C for 10 min, permeabilized with Triton-X at 37 °C for 10 min, and blocked with 10% normal goat serum at 37 °C for 30 min. Subsequently, the cells were incubated with actin (1:50) primary antibody overnight at 4 °C. After washing, the cells were incubated with secondary antibody at 37 °C for 1 h. All images were taken with a laser scanning confocal microscope.

Statistical analysis

The continuous data from the experiments are presented as the mean ± standard deviation (SD). Statistical evaluation was performed using one-way ANOVA analysis and Student's t-test. SPSS Version 17.0 for Windows was used for statistical analysis. All tests were two-tailed and considered to be significant when $P < 0.05$.