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DUSP1 induces paclitaxel resistance through the regulation of p-glycoprotein expression in human ovarian cancer cells

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ABSTRACT

The heterogeneity and genetic instability of ovarian cancer cells often lead to the development of drug resistance, closely related with the increased cancer-related mortality. In this study, we investigated the role of dual-specificity phosphatase 1 (DUSP1) in the development of the resistance in human ovarian cancer cells against paclitaxel. Overexpression of DUSP1 in HeyA8 human ovarian cancer cells (HeyA8-DUSP1) up-regulated the expression of the drug efflux pump, p-glycoprotein. Consequently, HeyA8-DUSP1 cells are highly resistant to paclitaxel, with the resistance comparable to that of a multi-drug resistance cell line (HeyA8-MDR). Moreover, over expression of DUSP1 significantly increased the activation of p38 MAPK, leaving the activation of ERK1/2 and JNK1/2 unaffected. Pharmacological suppression of p38 MAPK activity prevents the up-regulation of p-glycoprotein expression and the consequent resistance against paclitaxel in HeyA8-DUSP1 cells. By contrast, HeyA8-MDR cells expressed a significantly higher level of DUSP1, but treatment with small interference RNA against DUSP1 significantly suppressed the expression of p-glycoprotein and the resistance against paclitaxel in HeyA8-MDR cells. Ectopic expression of MKK3, an upstream activator of p38 MAPK, significantly up-regulated the expression of p-glycoprotein and increased the consequent resistance against paclitaxel in HeyA8 cells. Collectively, these data indicated that DUSP1 may induce the resistance against paclitaxel through the p38 MAPK-mediated overexpression of p-glycoprotein in human ovarian cancer cells.

1. Introduction

Ovarian cancer is the most lethal gynecologic malignancy, with an estimated incidence of 22,280 new cases accounting for 14,240 deaths for 2016 in the United States [1]. In spite of high incidence and mortality rates, the etiology and involvement of molecular markers of this lethal disease are not completely understood [2]. Efforts to develop screening or diagnostic tools for early detection of ovarian cancer have not been successful; therefore, a significant number of patients are diagnosed in advanced stages, requiring cytoreductive and systemic therapies such as palliative surgery and chemotherapy, respectively. The standard chemotherapy approach often relies on a combination of highly toxic platinum-based compounds such as cisplatin and paclitaxel. However, for ~60% of patients with advanced-stage disease, improvements in outcome are limited by intrinsic and acquired chemoresistance because of tumor heterogeneity and the genetic instability of ovarian cancer cells. Accordingly, advanced-stage ovarian cancer patients show 5-year survival statistics of only 20%–30%, and the disease usually recurs within six months [3].

Dual-specificity phosphatases (DUSPs) are negative regulators of mitogen-activated protein kinases (MAPKs) by dephosphorylating both the threonine/serine and tyrosine residues of their substrates. DUSP1 is a nuclear MAPK phosphatase that preferentially dephosphorylates p38 MAPK and JNK1/2, and to a lesser extent ERK1/2 [4], and is induced by oxidative stress, hypoxia, nutritional deprivation and chemotherapeutic drugs [5–7]. Several reports

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indicate that DUSP1 expression is increased in a number of human tumors, including those associated with gastric, pancreatic, breast, early-stage prostate and non-small-cell lung cancer [8–13]. However, the clinical outcome of DUSP1 expression appears to be cancer-type-specific because expression of DUSP1 in ovarian carcinoma is associated with poor patient outcomes [11], whereas increased DUSP1 levels in hepatocellular carcinoma correlate with improved prognosis [14] by inhibiting the proliferation of cells through the negative regulation of ERK signaling [14]. Moreover, DUSP1 expression has been reported to be associated with chemoresistance in many human cancers, including breast, non-small lung and prostate carcinoma, whereas targeting DUSP1 enhances chemo-sensitivity to these agents (for review, [4]). In ovarian cancer, ERK-dependent expression of DUSP1 protein was reported to play a critical role in acquired cisplatin resistance, possibly through the down-regulation of anti-apoptotic bcl-2 proteins [15]. Recently, DUSP1 has been reported to be an important mediator for the impaired response to chemotherapy driven by adrenergic pathways [16]. However, the functional roles and molecular mechanism of DUSP1 underlying the induction of paclitaxel resistance in human ovarian carcinoma cells remain to be elucidated.

In this study, we investigated the biological role of DUSP1 in paclitaxel resistance and found for the first time that DUSP1 may play a critical role in paclitaxel resistance through the p38 MAPK-mediated expression of p-glycoprotein, thereby enhancing drug efflux in human ovarian carcinoma cells.

2. Materials and method

2.1. Human ovarian cancer cell lines

The human ovarian cancer cell lines HeyA8, HeyA8-MDR and SKOV3ip1 were kindly provided by Dr. Isaiah J. Fidler (The University of Texas MD Anderson Cancer Center, Houston, TX, USA). Cells were maintained in Roswell Park Memorial Institute (RPMI)-1640 medium supplemented with 15% FBS (HyClone) at 37 °C in 5% CO_2.

2.2. Lentiviral transduction and transient transfection

Cells were plated at approximately 70–80% confluency in 100 mm culture dishes and incubated overnight. The media were replaced with serum-free media containing 5 μg/mL polybrene, and the cells were incubated for 1 h. Lentivirus particles containing pLenti6 V5/DEST vector (Invitrogen) or pLenti6 V5/DEST harboring DUSP1-coding gene were added drop by drop onto each plate. After 12 h of incubation, the cells were grown in RPMI-1640 with 15% FBS for a day, and the media were replaced with RPMI-1640 containing 15% FBS and 3 μg/mL blasticidin (Life Technology Gibco) until the blasticidin-resistant colonies were established. To construct HeyA8 cells with constitutively activated p38 MAPK, HeyA8 cells were plated at a density of 1×10^6 cells/100 mm dishes and transfected with pMKK3 vector using lipofectamin 2000 for 12 h in Opti-MEM. On the following day, the media were replaced with RPMI-1640 supplemented with 15% FBS. After 24 h, the cells were incubated with growth media containing 400 μg/mL G418 to select the transfectants.

2.3. Cell proliferation assay

Cells were seeded at a density of 1×10^3 cells/well in six-well plates, and the media were replaced every other day for 7 days. To test the effects of paclitaxel on the growth of ovarian cancer cells, 5×10^3 cells were plated in 96-well plates and treated with a range of concentrations of paclitaxel (Bristol-Myers Squibb), as indicated, for 48 h. MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium)] reagent (Promega) was added for 1 h, and the relative cell growth was quantified by measuring the absorbance at 490 nm using an ELISA reader according to the manufacturer's instructions.

2.4. Small interfering RNA (siRNA) transfection

The following siRNA against DUSP1 was synthesized by Bioneer (Daejeon, Korea): 5′-CUCAGUGUGUGAUCCGGUU(dTdT) and 5′-AACCGGAUCACACACUGAG(dTdT)-3′.

The HeyA8-MDR cells were plated at a density of 1×10^6 cells/well in six-well plates. On the next day, cells were transfected with siRNA against DUSP1 or scrambled siRNA (10 pmol) using lipofectamine 2000 reagent according to the manufacturer's instructions. The transfected cells were incubated for 6–10 h at 37 °C with 5% CO_2, and the media were then replaced. Cells were harvested to examine the expression level of DUSP1 using Western blotting.

2.5. P-glycoprotein function analysis

The cells were plated at a density of 2×10^5 cells/well in six-well plates overnight at 37 °C. Calcein AM (Invitrogen) stock solution was diluted in dimethyl sulfoxide (DMSO) to a concentration of 0.5 μM. On the following day, the plates were incubated for 30 min with serum-free RPMI-1640 media without phenol red in the presence of Calcein AM for cellular uptake. Cells were then incubated with RPMI-1640 containing 15% FBS to indicated time points for the extrusion of intracellular Calcein. The fluorescence intensity was measured by flow cytometry (FACSCalibur, BD Biosciences).

2.6. RNA extraction and quantitative real-time RT-PCR

Total RNA was purified using an RNA purification kit (Qiagen), and the RNA concentration was determined spectrophotometrically. For cDNA synthesis, 1 μg of RNA was reverse transcribed in a 20 μl mixture according to the manufacturer's instructions (Quantitect reverse transcription kit, Qiagen). Quantitative real-time PCR was performed with SYBR Green master according to the manufacturer's instructions (Roche Diagnostics) using the following primers: DUSP1-forward (5′-CTG CCT TGA TCA ACG TCT CA-3′) and DUSP1-reverse (5′-ACC CTT CCT CCA GCA TTC TT-3); p-glycoprotein/ABCB1-forward (5′-GCT CCT GAC TAT GCC AAA GC-3′) and p-glycoprotein/ABCB1-reverse (5′-TCT TCA CCT CCA GGC TCA GT-3′). Cycling conditions were 10 min of initial denaturation at 95 °C and 45 cycles of 95 °C for 1 min, annealing at 55 °C for 15 s and polymerization at 72 °C for 30 s. GAPDH was used as an internal control.

2.7. Apoptosis assay

Cells (1×10^6) were grown in RPMI-1640 media containing 15% FBS (without phenol red) in the presence of paclitaxel (20,000 ng/ml) for 24 h. Cells were then washed with PBS twice, and cell pellets were resuspended in 1× binding buffer to 1×10^5 cells/100 μl media. Cells were incubated with 5 μl of Annexin V-FITC and propidium iodide (BD Biosciences) solutions in the dark. After 15 min, 400 μl of 1× binding buffer was added to each tube, and samples were measured by flow cytometry (FACSCalibur).

2.8. Western immunoblotting

Cells were harvested in 500 μl lysis buffer (RIPA buffer, 100× EDTA solution, 100× phosphatase & protease inhibitor; Thermo