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S100P sensitizes ovarian cancer cells to carboplatin and paclitaxel *in vitro*

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Abstract

Objective: To investigate the relationship between the S100P and the sensitivity of ovarian cancer to chemotherapeutics.
Method: We established stable cell lines of ovarian cancer cells, SKOV3 and OVCAR3, that overexpress human S100P. We also transiently transfected the parent cell lines with S100P-targeted siRNA for down-regulation of S100P expression. The sensitivity of all transfected and untransfected cell lines to carboplatin and paclitaxel was detected by MTT assay.
Results: For both cells, IC_{50s} decreased to carboplatin and paclitaxel ($p < 0.05$), with overexpression of S100P compared to untransfected cells. Alternatively, with down-regulation of S100P by siRNA, the IC_{50} to carboplatin and paclitaxel increased in each case ($p < 0.05$), which was significantly higher compared to untransfected cells.
Conclusion: Changes in expression levels of S100P in SKOV3 and OVCAR3 cells results in variable susceptibility to carboplatin and paclitaxel. These data suggest that S100P contributes to chemosensitivity to carboplatin and paclitaxel in ovarian cancer cells.

Keywords: S100P; Drug-resistance; MTT assay; Ovarian cancer

1. Introduction

Ovarian cancer has a 5-year survival rate of 25–30%, making it the leading cause of gynecological cancer-related deaths in developing countries. Resistance to multiple chemotherapeutic agents remains the primary cause of treatment failure. Alterations in drug uptake/efflux [1] and DNA repair [2] are the major contributing factors to acquired drug-resistant phenotypes of cancer cells. However, the underlying mechanism of initial drug-resistance is unclear.

In our previous study, we demonstrated that expression of the S100P is reduced in colon cancer cells with an acquired drug-resistance phenotype [3]. It is unclear whether reduced levels of S100P expression is related to drug-resistance, or is the result of slo-

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wed cell growth. Surowiak et al. reported that S100P overexpression is generally associated with a poor prognosis for patients with drug-resistant ovarian cancers established by pathologic investigation [4]. These results are in contrast to the data obtained from our assays using an *in vitro* colon cancer cell line. Therefore, to investigate the relationship between S100P and the initial drug-resistant phenotype of ovarian cancer, we established ovarian cancer cell lines that overexpress S100P, and transfected ovarian cancer cells with S100P-targeted siRNA to down-regulate S100P expression.

Sl00P primarily functions to mediate Ca^{2+}-dependent signal transduction pathways involved in cell growth and differentiation, cell cycle regulation, and metabolic control [5,6]. S100P is an EF-hand calcium-binding protein consisting of 95 amino acids that was originally purified from placenta tissue [7]. It has since been detected in gastric, gall bladder, bladder epithelium [8], and in esophageal epithelial cells during differentiation [9]. This molecule has recently attracted attention as a potential tumor marker based on its up-regulation detected in malignant versus benign tumors [10–14]. However, it is not clear if the presence of S100P is a causative factor, or merely a passenger changes in tumor progression. In this study we examine the effects of increased and decreased expression of S100P on the sensitivity of SKOV3 and OVCAR3 cells to carboplatin and paclitaxel.

2. Materials and methods

2.1. Cell lines and cell culture

The ovarian cancer cell lines, SKOV3 and OVCAR3, were obtained from the TianJin Life Science Research Center. Cells were maintained in RPMI-1640 containing 10% fetal bovine serum (FBS; Sigma Chemical, St. Louis, MO, USA), 2 μM L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin (GIBCO, Grand Island, NY). Cells were incubated at 37 °C in 5% CO_2 at high humidity, and passaged every 2–3 days using 0.05% trypsin (Gibco BRL) and 0.01% EDTA (Sigma).

2.2. Cytotoxic drugs

Stock solutions of carboplatin and paclitaxel were obtained from Jiangsu Hengrui Medicine Co., Ltd. and stored at 4 °C. The drugs were dissolved in RPMI 1640 without FBS or antibiotics immediately before use.

2.3. Amplification of the S100P gene and vector construction

Sl00P cDNA was amplified using polymerase chain reactions with cDNA of HEK293 cells (a generous gift from Professor Hua Tang, TianJin Life Science Research Center) as a template. Forward (5′-GTCTGAATTCAGCACCATGACGGAACT AGAG-3′) and reverse (5′-GGCTCGAGTTGA GTCCTGCCTTCTCAAAG-3′) primers were used to generate EcoRI and XhoI restriction sites, respectively, for directed cloning of the coding region of Sl00P into a pcDNA3.0 expression vector for fusion with a hemagglutinin (HA) tag. PCR was performed using 1 pmol of each primer, 5 ng of template (cDNA), 10× Taq buffer (10 mM KCl, 2 mM Tris–HC1 (pH 8.8), 2 mM $MgSO_4$, 10 mM $(NH4)_2SO_4$, 0.1% Triton X-100), 1U Taq polymerase, 200 pM dNTP in a 50 μl reaction volume. PCRs were run on a Perkin-Elmer thermal cycler for 35 cycles [5 min at 94 °C, 1 min at 65 °C, 1 min at 72 °C] and followed by a final incubation at 72 °C for 10 min.

PCR fragments were digested with XhoI and EcoRI, gel purified by Qiaquick Gel Extraction, and ligated into pcDNA3.0 linearized by digest with XhoI and EcoRI. This vector was modified in our laboratory to include three tandem copies of the HA tag at the C-terminus of the cloning region.

2.4. Generation of stable cell clones: SKOV3/S100P and OVCAR3/S100P

Lipofectin 2000 (Promega) was used to transfect SKOV3 and OVCAR3 cells. Cells were transfected at 50–70% confluence with S100P plasmid (2 μg) and pcDNA3.0 (2 μg) after pre-incubation for 20 min with 20 μg/ml lipofectin in serum-free OPTI-MEM (Promega) according to a protocol provided by Promega. After 4 h, the media containing oligonucleotides and lipofectin was replaced with MEMα (10% fetal bovine serum 1% penicillin/streptomycin) supplemented with 500 μg/ml G418 (Sigma). Selection with G418 was maintained for 5 weeks to obtain stable clones. Expression of S100P in selected clones was analyzed by Western blot.

2.5. siRNA transfection

OVCAR3 and SKOV3 cells at 1.4×10^5 and 8×10^4 cells/ml, respectively, were seeded into 24-