Sustained suppression of Fas ligand expression in cisplatin-resistant human ovarian surface epithelial cancer cells

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Although cisplatin derivatives are first line chemotherapeutic agents for the treatment of ovarian epithelial cancer, chemoresistance is a major therapeutic problem. Although the cytotoxic effect of these agents are believed to be mediated through the induction of apoptosis, the role of the Fas/FasL system in chemoresistance in human ovarian epithelial cancer is not fully understood. In the present study, we have used cultures of established cell lines of cisplatin-sensitive human ovarian epithelial tumours (OV2008 and A2780-s) and their resistant variants (C13* and A2780-cp, respectively) to assess the role of Fas/FasL system in the chemoresponsiveness of ovarian cancer cells to cisplatin. Cisplatin was effective in inducing the expression of cell-associated Fas and FasL, soluble FasL and apoptosis in concentration and time-dependent fashion in both cisplatin-sensitive cell lines (OV2008 and A2780-s). In contrast, while cisplatin was effective in increasing cell-associated Fas protein content in C13*, it failed to up-regulate FasL (cell-associated and soluble forms) and induce apoptosis, irrespective of concentration and duration of cisplatin treatment. Concentrated spent media from OV2008 cultures after cisplatin treatment were effective in inducing apoptosis in C13* cells which was partly inhibited by the antagonistic Fas monoclonal antibody (mAb) suggesting that the soluble FasL present in the spent media was biologically active. In the resistant A2780-cp cells, neither Fas nor FasL up-regulation were evident in the presence of the chemotherapeutic agent and apoptosis remained low compared to its sensitive counterpart. Activation of the Fas signalling pathway, by addition to the cultures an agonistic Fas mAb, was equally effective in inducing apoptosis in the cisplatin-sensitive (OV2008) and -resistant variant C13*, although these responses were of lower magnitude compared to that observed with cisplatin in the chemosensitive cells. A significant interaction between cisplatin and agonistic Fas mAb was observed in the apoptotic response in OV2008 and C13* when cultured in the presence of both agents. Immunohistochemistry of human ovarian epithelial carcinomas reveals the presence of Fas in low abundance in proliferatively active cells but in high levels in quiescent ones. Although the expression pattern of FasL in the tumour was similar to that of Fas, the protein content was considerably lower. Taken together, these data suggest that the dysregulation of the Fas/FasL system may be an important determinant in cisplatin-resistance in ovarian epithelial cancer cells. Our results are also supportive of the notion that combined immunotherapy (i.e., agonistic Fas mAb plus cisplatin) may provide added benefits in the treatment of both chemosensitive and -resistant ovarian tumours.

Keywords: Apoptosis; chemoresistance; cisplatin; Fas; FasL; ovarian cancer.

Introduction

Ovarian cancer is the most lethal gynaecological cancer in the Western world and ranks fifth among the most common female cancers. Epithelial ovarian tumours account for over 85% of all human ovarian malignancies and originate from the simple epithelium covering the surface of the ovary. Recent studies have suggested that cancer is characterized by enhanced cell proliferation and reduced physiological cell death, often referred as apoptosis. Many chemotherapeutic agents as well as irradiation activate the apoptotic death program in susceptible target cells.

Although cisplatin derivatives (e.g., carboplatin) and paclitaxel (taxol) are first line chemotherapeutic agents for the treatment of ovarian epithelial cancer, chemoresistance is major therapeutic problem and the molecular mechanisms involved are poorly understood. The development of resistance may be cell-type specific and related to the dosing schedule. The mechanisms of chemoresistance appear to be multifactorial and are generally thought of in terms of altered pharmacodynamics and gene expression (including multi-drug resistance gene), modified drug target, increased rate of DNA repair or decreased rate of drug-induced DNA or macromolecule damage.
Cisplatin (cis-diaminedichloroplatinum II) is a widely used anti-cancer agent with a broad range of anti-tumour activity. The activity of cisplatin is thought to be due to its ability to form inter- and intra-strand DNA crosslinks, predominantly at the N7 position of adjacent guanines. The cytotoxic effect appears to result from inhibition of replication by cisplatin-DNA adducts and G2 arrest with subsequent induction of apoptosis. While our knowledge on the events leading to chemoresistance is incomplete, failure to activate apoptosis in these cancer cells may confer resistance to these agents.

Fas antigen (Fas), is a cysteine-rich transmembrane glycoprotein which belongs to the tumour necrosis factor (TNF)/nerve growth factor receptor superfamily. Upon ligand binding, Fas induces apoptosis in mammalian cells. The ligand (FasL) has been cloned and is a type II transmembrane protein belonging to the TNF family, which includes TNFα, TNFβ, lymphotoxin β and the ligands for CD27, CD30, and CD40. While Fas/FasL system is believed to play an important role in the regulation of ovarian follicular atresia, the expression of Fas has recently been reported in ovarian cancer cell lines and shown to be upregulated by chemotherapeutic agents. Whereas bleomycin and methotrexate are known to up-regulate FasL expression in hepatocytes, the regulation of this protein in human ovarian cancer cells has not been demonstrated. In addition, the role and regulation of the Fas/FasL system in chemoresistance remains unclear.

In the present studies, we have examined the regulation of Fas and its ligand (FasL) expression in cisplatin-mediated apoptosis in human ovarian surface epithelial cancer cells and its possible role in cisplatin resistance. Our studies demonstrate that increases in cell-associated and soluble form of FasL following cisplatin challenge are characteristic of cisplatin-sensitive cells and that the inability of ovarian cancer cells to express soluble FasL may be an important contributing factor to chemoresistance.

Materials and methods

Reagents

Cisplatin and Hoechst 33248 were supplied by Sigma (St. Louis, MO). Cisplatin solutions were prepared in the dark and used fresh each time. Human monoclonal anti-Fas and Fas Ligand antibodies used for Western analyses were purchased from Transduction Laboratories (Lexington, KY). Anti-Fas and anti-FasL antibodies (Ab) were generated against the extracellular amino terminal and carboxy terminal region of the proteins, respectively. Mouse IgM and a monoclonal anti-Fas antibody (Fas mAb) capable of binding to Fas and inducing apoptosis in most cell types were obtained from Upstate Biotechnology (CH-11; Lake Placid, NY). Polyclonal anti-Fas, FasL, monoclonal anti- Proliferating Cell Nuclear Antigen (PCNA; an auxiliary protein of DNA polymerase α highly expressed at the G1/S interphase) antibodies, rabbit and mouse IgG used for immunohistochemistry were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Protease K, biotinylated 16-dUTP and terminal deoxynucleotide transferase were purchased from Boehringer Mannheim (Indianapolis, IN). The Vector ABC Elite Kit containing the avidin-biotin peroxidase complex was purchased from Vector Laboratories (Burlington, CA).

Cell culture

Human ovarian surface epithelium cancer cells were gifts from Dr. R. Goal (OV2008 and C13*) and Dr. C.E. Ng (A2780-s and A2780-cp), both from the Ottawa Regional Cancer Centre, Ottawa, Canada. Cisplatin-sensitive cells and their resistant variants (OV2008 and C13*; A2780-s and A2780-cp) were cultured at 37°C and 5% CO2 in either RPMI 1640 (OV2008, C13*) or DMEM/F12 (A2780-s, A2780-cp) (Gibco/BRL, Burlington, ON). Media was supplemented with 1% non essential amino acids, 0.5% streptomycin-penicillin, 0.25% fungizone and 10% fetal bovine serum for cell plating. Cells in log growth phase were then treated with cisplatin and/or Fas mAb in serum free media for a up to 24 h.

Hoechst staining

At the end of the culture period, cells were subjected to trypsion treatment (0.05% trypsin, 0.53 mM EDTA; 37°C; 1 min), fixed (4% formalin in PBS) and washed in PBS. They were then resuspended in Hoechst staining solution (12.5 ng Hoechst 33248/ml PBS, 2 h) and spotted onto slides for microscopy. Nuclear staining was observed and photographed using a Zeiss fluorescence microscope (magnification 400X). Cells with typical apoptotic nuclear morphology (nuclear shrinkage, condensation, and fragmentation) were identified and counted, using randomly selected fields on numbered photographic slides to avoid bias. A minimum of 200 cells per treatment were counted in each experiment.

Protein extraction and western analysis

Cells were sonicated (1 min) in a lysis buffer (pH 7.4) containing 150 mM NaCl, 0.1% SDS, 0.5% sodium deoxycholate, 1% NP-40 in PBS and protease inhibitors [PMSF (1 mM), aprotinin (10 μg/ml)]. The sonicates were then incubated for 1 h and pelleted. Supernatant was retained and stored at −20°C, pending Western analysis. Spent