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ScienceDirect
Cancer Letters 270 (2008) 295–301
CANCER Letters
www.elsevier.com/locate/canlet

Alterations in the glycoform of cisplatin-resistant human carcinoma cells are caused by defects in the endoplasmic reticulum-associated degradation system

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Received 28 February 2008; received in revised form 28 February 2008; accepted 13 May 2008

Abstract

Cisplatin, *cis*-diamineplatinum-(II) dichloride (CDDP), is one of the most common and valuable chemotherapeutic reagents for various cancers. However, it is well known that tumor cells gain acquired or intrinsic resistance to treatment by this anti-cancer reagent. In spite of extensive efforts using genetic and proteomic approaches, the mechanism underlying CDDP resistance remains unclear. In the present study, we report drastic structural changes in the *N*-glycans of glycoproteins in CDDP-resistant tumor cells (the KCP-4 cell line obtained from KB-3-1 human carcinoma cells). It was suggested that the CDDP-resistant cells exhibited an increase in one of the high-mannose-type glycans, particularly M8.1. This *N*-glycan is well known as a tag for the transport of unfolded protein from the endoplasmic reticulum to the lysosome, a process known as endoplasmic reticulum-associated degradation (ERAD) system. The revertant cells (KCP-4R) obtained from the KCP-4 cell line showed almost the same glycoform profile as that of the parental cells, suggesting that *N*-glycan biosynthesis in tumor cells clearly corresponds to the alteration in the sensitivity against CDDP. Gene expression analysis using a cDNA microarray showed a decrease in the expression of major histocompatibility complex (MHC) proteins in the resistant cells. MHC proteins form a complex with lysosome-degradated proteins and are presented on the cell surface. These results suggest that CDDP tolerance in KCP-4 cells is caused by a defect in the ERAD system.

Keywords: Cisplatin; resistant/revertant cell; *N*-glycan profile; Gene expression profile

Abbreviations: CDDP, *cis*-diamineplatinum-(II) dichloride; ER, endoplasmic reticulum; ERAD, ER-associated degradation; Fuc, fucose; Gal, galactose; GlcNAc, *N*-acetyl-D-glucosamine; GnT, *N*-acetyl-D-glucosaminyltransferase; MHC, major histocompatibility complex; PA, pyridylaminated.

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doi:10.1016/j.canlet.2008.05.019

1. Introduction

Cisplatin, *cis*-diamineplatinum-(II) dichloride (CDDP), is a chemotherapeutic drug used for the treatment of neuroblastomas, and testicular, bladder, ovarian, and other cancers [1]. However, it is well known that its efficiency is restricted by serious side effects and tolerance. As a result, more effective and less debilitating reagents must be designed and developed. CDDP binds DNA and eliminates cancer cells, which replicate their DNA faster than normal cells. Although the effects of CDDP resistance have been described in terms of DNA repair, apoptosis, the cell cycle and ion pumps [2,3], and investigated extensively using genomic and proteomic approaches [4,5], no plausible mechanism for drug resistance during cancer treatment by CDDP has yet been clarified. In a previous paper, we established a method for the preparation and isolation of the CDDP-resistant cell line, KCP-4, from the human epidermoid carcinoma KB-3-1 cell line, and subsequently the revertant cell line KCP-4R from the KCP-4 cells [6]. These cell sets provide an effective tool for the study of the mechanisms underlying CDDP resistance. Using KB-3-1, KCP-4, and KCP-4R cells, it was suggested that the mechanisms of CDDC resistance in KB carcinoma cells are associated with the glutathione *S*-conjugate export pump [7,8] and p73, which regulate apoptosis [9]. However, it seems likely that other crucial molecules may be required to mediate the multiple mechanisms involved in the resistance of cancer cells to CDDP.

Cell surfaces are covered by a variety of glycoconjugates such as glycoproteins, sphingoglycolipids, and proteoglycans. It is well documented that the cell surface glycans of these glycoconjugates function in numerous biological processes including metastasis, malignancy, and have been reported as important markers reflecting survival rates [10,11]. We speculated that the *N*-glycans of the cell surface glycoproteins may play an important role in sensitivity to CDDP, as tunicamycin, a recognized inhibitor of *N*-glycan biosynthesis, enhances sensitivity to cisplatin in cultured cells [12] and glycoforms are altered in epirubicin- and mitoxantrone-resistant cell lines [13]. It was also reported β1,6GlcNAc branching in *N*-glycans in α5β1 integrin was decreased in a cisplatin-resistant human squamous cell carcinoma cell line and this might have led to a reduction in cell adhesion activity [14]. In the present study, we analyzed the glycoforms of *N*-glycans and 5000 gene expressions from parent, CDDP-resistant, and revertant cells.

2. Experimental procedures

2.1. Materials, cell lines, and culture method

The human epidermoid carcinoma cell line, KB-3, was obtained from Dr. M.M. Gottesman (National Cancer Institute, Bethesda, MD). The parental cell line, KB-3-1, the cisplatin resistant line, KCP-4, and the sensitive revertant cell line, KCP-4R, were isolated as previously described [6,7]. These cell lines were cultured in MEM (Nissui Seiyaku, Tokyo) containing 10% heat-inactivated newborn calf serum (Cell culture laboratories, Cleveland, OH), 1 mg/ml bactopeptone (Difco Laboratories, Detroit, MI), 2 mM glutamine and 100 U/ml penicillin (MEM medium) in a 5% CO_2 atmosphere at 37 °C. Cells were plated onto 90-mm culture dishes at a density of 1×10^6 cells/dish and incubated for 48 h. KCP-4 cells were cultured with or without 30 μM cisplatin, and their growth rates under either condition were similar. Cultured cells were harvested using EDTA and trypsin, and pelleted by centrifugation. Ten dishes per line were cultured, and cells from five dishes were pooled to make one sample. Cells were washed twice with PBS, resuspended in water, and then heated at 90 °C for 15 min and lyophilized.

2.2. Glycoform analysis by 2D-LC mapping technique

Lyophilized cells (20 mg) were suspended in 0.1 M Tris–HCl buffer (200 μl, pH 8.0) containing 200 μg each of trypsin and chymotrypsin (Sigma–Aldrich Co., St Louis, MO), incubated for 16 h at 37 °C, and then heated at 90 °C for 10 min to stop enzymatic reactions. *N*-Glycans were released from glycopeptide-containing digests by treatment with *N*-glycosidase F (20 U, Roche Diagnostics, Mannheim, Germany) for 20 h at 37 °C in the same solution. Finally, pronase (200 μg, Calbiochem, Merck, Darmstadt, Germany) was added to the crude mixture and incubated for 16 h at 37 °C. Oligosaccharides were purified on a Bio-Gel P-4 column (1.0×38 cm, Bio-Rad, Hercules, CA) with water as the eluant, and the sugar-containing fractions were collected and lyophilized. Oligosaccharides were reductively aminated with 2-aminopyridine in the presence of sodium cyanoborohydride (Sigma–Aldrich Co.) according to the method reported by Hase et al. [15,16]. Crude pyridylaminated (PA)-oligosaccharides were purified by gel-filtration on a Sephadex G-15 column (Amersham Biosciences, Piscataway, NJ) with 10 mM ammonium bicarbonate as the eluant. Sialic acid residues at the non-reducing terminal positions were selectively released from oligosaccharides by acid hydrolysis at pH 2.0 and 90 °C for 60 min. PA-oligosaccharides were further puri-