Expression of the Cisplatin Resistance Phenotype in a Human Ovarian Carcinoma Cell Line Segregates with Chromosomes 11 and 16

Beloo Mirakharu, Hemant K. Parekh, and Henry Simpkins

Department of Pathology, Temple University School of Medicine and Fels Institute of Molecular Biology and Cancer Research, Philadelphia, Pennsylvania 19140

Abstract

Many mechanisms have been proposed to explain cisplatin resistance, suggesting that this phenomenon is multifactorial. In an attempt to define the chromosomes responsible for cisplatin resistance, a cisplatin-resistant human ovarian carcinoma cell line was fused with a cisplatin-sensitive rodent fibroblast cell line. The hybrids were then analyzed for segregation of the cisplatin-resistant phenotype. Chromosomes 11 and 16 were present in all of the resistant cell hybrids, with the highest concordance for chromosome 16. The role of both of these chromosomes was further established with microcell hybrids. Microcell hybrids of A9 cells with chromosome 16 from the cisplatin-resistant 2008/C13* cells did not exhibit cisplatin resistance, but the presence of a normal chromosome 11 with chromosome 16 (derived from cisplatin-resistant 2008/C13* cells and not from the cisplatin-sensitive 2008 cells) resulted in increased resistance to cisplatin. In addition, loss of chromosome 11 from a resistant somatic cell hybrid resulted in the hybrid becoming sensitive to cisplatin, implicating this chromosome in maintaining the resistant phenotype. The results demonstrate that resistance to cisplatin is a dominant trait in the 2008/C13* human ovarian cells, and both chromosomes 11 and 16 are required for its expression.

Introduction

Ovarian carcinoma is one of the most common of cancer deaths in women (1). Cisplatin (cis-diaminedichloroplatinum (II)) is an effective agent in the treatment of ovarian, testicular, head, neck, and bladder cancers (2). The cytotoxic effects of cisplatin are thought to result from the covalent binding of the cisplatin to DNA (reviewed in Refs. 3–5). The resultant adducts are cross-links of three types: intrastrand, interstrand, and DNA-protein. The most common adduct is the intrastrand cross-link bridging adjacent guanines at the N(7) position (4, 6, 7). The use of cisplatin, however, is limited due to the emergence of resistance (1, 8). Several biochemical mechanisms of cisplatin resistance have been elucidated (9–11) and include reduced intracellular cisplatin accumulation (12–14), increased intracellular levels of glutathione (15–17), increased dTMP synthase activity (18), decreased DNA binding (19), enhanced drug inactivation by metallothionein (20–23), and increased cisplatin-DNA adduct removal (24, 25). However, the precise genes involved in the development of cisplatin resistance remain unknown, but the list of possible mechanisms suggest that the acquisition of the resistant phenotype is multifactorial.

To analyze the gene(s) responsible for cisplatin resistance, a cisplatin-resistant human ovarian cell line, 2008/C13*, which had been generated by the in vitro selection of the parental human ovarian carcinoma cell line 2008 (26), was used. Studies with the 2008/C13* resistant cell line have demonstrated unique characteristics, such as reduced accumulation of intracellular cisplatin (13), an enhanced replicative bypass of platinum-DNA adducts (27), and reduced cyto-keratin 18 (28) and membrane associated β-tubulin (29). In addition, the mitochondria appear morphologically aberrant, and these cells are hypersensitive to lipophilic cations (30). Variations in protein kinase activity (31, 32) and the CAMP signal transduction pathway (33) have also been reported in the 2008/C13* cell line. These studies suggest that there are multiple mechanisms by which 2008/C13* cells became drug resistant.

To identify the chromosomes involved in cisplatin resistance, a panel of somatic cell hybrids was established by the fusion of a cisplatin-sensitive rodent cell line, A9, with the drug-resistant 2008/C13* cell line. The individual human chromosomes in the somatic cell hybrids were then characterized and correlated to the resistant phenotype.

Materials and Methods

Materials. Cisplatin was obtained from Aldrich Chemical Co. (Milwaukee WI). Cell culture reagents and geneticin (G418 sulfate) were purchased from Life Technologies, Inc. (Grand Island, NY). Immobilon-P membranes were purchased from Millipore (Bedford, MA). The anti-GST–m antibody was obtained from Tempe Research Laboratories, Inc. (West Grove, PA). Coatasome 11, a total human DNA probe, a chromosome 16-specific α satellite probe, and other supplies for the FISH analysis were purchased from Oncor (Gaithersburg, MD). PCR was performed with Taq polymerase from Perkin Elmer (Branchburg, NJ). The alkaline phosphatase detection kit was obtained from Bio-Rad (Hercules, CA). 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide and all other chemicals and reagents were obtained from Sigma Chemical Co. (St. Louis, MO) unless otherwise indicated.

Cell Lines and Culture Conditions. The human ovarian carcinoma cells sensitive and resistant to cisplatin, 2008 and 2008/C13*, respectively, were obtained from Dr. P. Andrews (Georgetown University, Washington, DC). 2008/C13* cells were grown in RPMI 1640 supplemented with 10% bovine calf serum and gentamicin at a final concentration of 10 μg/ml. 2008/C13* cells possess HPRT and APRT and therefore, survive in HAT and AAT media, but they are sensitive to ouabain (10–5 M). A9 is a mouse fibroblast cell line that was originally isolated from wild-type L cells (34), these cells were grown as a monolayer in D10. A9 cells are HPRT and APRT deficient; therefore, HAT and AAT media are cytotoxic; however, these cells are resistant to the ouabain and grow in the presence of 10–5 M ouabain. The A9 cell line was obtained from Dr. R. Athwal (Fels Institute, Temple University). AAT media is D10 containing 4 × 10–4 M adenine, 4 × 10–3 M aminopterin, and 8 × 10–3 M thymidine. HAT media is D10 containing 20 μg/ml hypoxanthine, 4 × 10–6 M aminopterin, and 20 μg/ml thymidine (35). The MCH 556.5 cell line consists of A9 cells, which contain a normal human chromo-

Received 12/1/95; accepted 4/2/96.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 To whom request for reprints should be addressed, at Department of Pathology, Temple University School of Medicine and Fels Institute of Molecular Biology and Cancer Research, Room 106, OMS, 3400 North Broad Street, Philadelphia, PA 19140. Phone: (215) 707-4353; Fax: (215) 707-2781.

2 The abbreviations used are: GST, glutathione S-transferase; FISH, fluorescence in situ hybridization; AAT, adenine-aminopterin-thymidine; APRT, adenosine phosphoribosyl transferase; D10, DMEM supplemented with 10% bovine calf serum; HAT, hypoxanthine-aminopterin-thymidine; HPRT, hypoxanthine phosphoribosyl transferase; IC50, 50% inhibitory concentration; TBS, Tris-buffered saline; LRP, lung resistance protein; MRPs, multidrug resistance-associated protein; MT-IIA, metallothionein.
some 11, tagged with the G418-resistant gene. The MCH 556.5 cell line was obtained from Dr. B. Weissman (University of North Carolina, Chapel Hill, NC) and were grown in RPMI with 10% FCS and further supplemented with 600 µg/ml G418 to ensure that it selectively retained the human chromosome.

**Somatic Cell Hybrids.** The derivation and use of somatic cell hybrids for mapping of several human genes have been described previously (36). Primary somatic cell hybrid clones were derived by fusion of the HPRT-deficient A9 cells with 2008/C13* cells. The 2008/C13* and A9 cells were fused in the presence of 47% polyethylene glycol (M, 1300–1600) for 1 min, followed by extensive washing with DMEM, and incubated overnight in D10. Following overnight incubation, the recipient cells were plated into HAT media containing 10−5 M ouabain. Somatic cell hybrid clones were visible within 2–3 weeks, and individual colonies were isolated using glass cloning cylinders. The colonies were grown in monolayers in the presence of HAT and ouabain, and after several passages, the selection medium was replaced by D10. This procedure was repeated twice to ensure an identical cell population within each hybrid clone. Each individual colony now represented a somatic cell hybrid clone and was tested for either sensitivity or resistance to cisplatin using the cytotoxicity assay.

**Microcell Hybrids.** Microcell-mediated chromosome transfer was carried out as described previously (37). Briefly, the donor cell was incubated in the presence of colcemid (0.1 µg/ml) in the D10 medium for a period of 48 h at 37°C. The cells were then microinjected in the presence of cytochalasin B (10 µg/ml) in DMEM. The microinjected cells were centrifuged at 25,000 g for 60 min at room temperature. The resulting preparation of microcell nuclei was suspended in 100 µg/ml phytohemagglutinin P and applied to the recipient cell monolayer. After allowing for agglutination at 37°C for 15 min, the cells and microcells were fused by 45% polyethylene glycol (M, 1300–1600) for 1 min, followed by extensive washing with serum-free medium. After an overnight incubation in a nonselective medium, the recipient cells were plated in selection media. Ouabain/AAT media were used for the selection of 2008/C13* microcells fused with the recipient A9 cells. The microcell hybrid thus obtained was designated MCH16. Fusion was performed with the MCH16 microcells and the recipient MCH556.5 cells, resulting in MCH11/16 hybrids, and microcells derived from the cisplatin-sensitive 2008 cells with MCH55.6 cells, resulting in the MCH11/16 hybrids, respectively (hybrids were selected using G418/AAT media).

**Chromosome Preparation.** Exponentially growing cells were treated with colcemid (0.1 µg/ml) for 3 h. The cells were harvested, hypotonically treated (0.56% KCl for 30 min at room temperature), and subsequently fixed in freshly prepared acetic acid:methanol (1:3). The cell suspension was dropped onto chilled slides and dried at room temperature. The slides were aged overnight and processed for FISH.

**FISH.** The procedure for FISH was carried out according to conditions defined by the manufacturer (ONCOR). Briefly, the slides were denatured in 70% formamide and 2X SSC (0.3 mM NaCl and 0.033 M sodium citrate [pH 7.2]) at 73°C for 2 min, immersed for 2 min in an ethanol series (70, 80, and 95%) at −20°C, and dehydrated. The slides were incubated with the denatured DNA probes in a humidified box at 37°C for 16–18 h. After washing, the slides were incubated with avidin-FITC (for the total human DNA probe) or with FITC-labeled antidigoxigenin (for the chromosome 11 cosomasome and chromosome 16 a satellite probe) at 37°C for 10 min and then counterstained with propidium iodide and antifade (0.6 µg/ml).

**PCR Analysis.** PCR reactions were carried out with 1 unit Tag enzyme, 1 µg genomic DNA, 150 µM of each deoxynucleotide, and 600 ng primers per reaction (total volume, 50 µl). The buffer and cycling conditions varied for all of the primers and were identical to those described by Abbott and Povey (38), Theune et al. (39), and Triputti et al. (40). The PCR products were electrophoretically separated on 2% agarose in 1X Tris-borate EDTA buffer (0.09 M Tris-borate and 0.001 M EDTA [pH 8.0]). A 0.2 µg/ml ethidium bromide (0.5 µg/ml) were added to each well during the last 6 h of drug exposure, and the plates were further incubated at 37°C in a 5% CO2 atmosphere. At the end of this exposure, the formazan crystals formed in the wells were dissolved in a solution containing 0.1 N HCl-isopropanol, and the absorbance of the colored product was measured at 570 nm in an automated spectrophotometer (model EL311s; BIO-TEK Instruments, Inc., Winooski, VT). Wells containing only the medium were used as blanks, and wells containing cells in the absence of the drug were used to determine the survival of the control untreated cells. A linear regression analysis of cell survival at the various drug concentrations was compared with the control, and the percentage of survival was calculated.

**Immunoblot Analysis.** For the detection of GST-7, the total cellular homogenate was prepared by lysing the cells in 10 mM Tris·HCl (pH 7.4), 100 mM NaCl, 0.5% Triton-X-100, and 0.1% SDS, and subjected to electrophoresis in a 15% separating gel according to previously published procedures (41). The separated proteins were electroeluted from the gel, transferred to Immobilon-P membranes. The membranes were preincubated with a blocking solution containing TBS (25 mM Tris·HCl [pH 7.5] and 150 mM NaCl) and 5% (w/v) nonfat dried milk for 60 min at room temperature with agitation. The membranes were then incubated with the primary antibody in TBS for 2 h, washed with TBS containing 0.05% (v/v) Tween-20 three times for 10 min, and then treated with the alkaline phosphatase-conjugated secondary antibody for 60 min. After thorough washing with TBS containing 0.05% (v/v) Tween-20, the membranes were stained with an alkaline phosphatase detection kit.

**Results.**

Human ovarian carcinoma cells resistant to cisplatin, 2008/C13*, with an IC50 of 27.5 µM (Table 1), were used as the chromosome donor cell line and were fused with the recipient mouse A9 cells, which are cisplatin sensitive, with an IC50 of 5.2 µM. The mouse A9 × human 2008/C13* somatic cell hybrids thus generated were selected in the presence of HAT and ouabain, which ensured selection of only true somatic cell hybrids (mouse A9 cells are sensitive to HAT, and human 2008/C13* cells are sensitive to ouabain). The somatic cell hybrids thus obtained were analyzed for expression of the cisplatin resistance phenotype by the cytotoxicity assay. Twenty somatic cell hybrid clones were analyzed, and those somatic cell hybrid clones exhibiting values similar to the A9 parental cell line were classified as the sensitive clones; alternatively, somatic cell hybrid clones exhibiting IC50 values similar to the 2008/C13* cell line were classified as resistant clones. Initial screening of 20 somatic cell hybrid clones for the drug-resistant phenotype established the presence of 6 resistant and 14 sensitive somatic cell hybrid clones (Table 1). PCR was performed to determine the presence of the individual human chromosomes in these somatic cell hybrids (38–40). Table 1 shows the segregation of the human chromosomes with the drug-resistant phenotype. The presence of the drug-resistant phenotype and the presence of a human chromosome defined the concordant fraction; alternatively, the absence of the chromosome and the drug-resistant phenotype also defined a concordant fraction. On the other hand, the absence of either the phenotype or the chromosome in the presence of the other defined the discordant fraction. The higher the concordance for an individual chromosome, the higher the probability that it is responsible for the observed phenotype; alternatively the lower the discordance of a chromosome, the greater the probability of its association with the observed phenotype (42). The ratio of the concordance to discordance was calculated for each chromosome. It was evident that there was no single chromosome that correlated to resistance to cisplatin, but chromosomes 11 and 16 were present in all of the cisplatin-resistant somatic cell hybrid clones, with chromosome 16 possessing the highest concordance:discordance ratio.
FISH studies performed on the resistant somatic cell hybrids confirmed the PCR results. Fig. 1 shows the FISH analysis of the drug-resistant somatic cell hybrids for the presence of chromosome 11 (Fig. 1A) in the N2p1 resistant somatic hybrid clone and chromosome 16 (Fig. 1B) in the K2 resistant somatic hybrid clone. The cosatellite probe for chromosome 16 could not be used, because this probe cross-reacted with the mouse genome; therefore, centromeric staining was performed with a chromosome 16-specific α satellite probe. Similar results were obtained for the other drug-resistant somatic cell hybrids (data not shown), and the results agreed with the PCR data (Table 1).

Because it was apparent that the either the presence of chromosome 16 (due to its high concordance:discordance ratio) or the absence of both chromosomes 11 and 16 (due to their universal presence in all the resistant somatic cell hybrid clones) was required for resistance, microcell hybrids containing either chromosome 16 of the 2008/C13* cell line or a combination of chromosomes 16 from 2008/C13* cells and a normal human chromosome 11 from rodent A9 cells were established. Chromosome 16 has the APRT gene; therefore, it can be selected for in the AAT media (43). A9 cells were fused with a microcell preparation from 2008/C13* cells, and the microcell hybrid was selected for in the presence of AAT and ouabain. The microcell hybrid clone thus obtained was designated MCH16. Similarly, another microcell hybrid was established using a MCH556.5 cell as the recipient cell (the MCH556.5 cell is an A9 cell with human chromosome 11 and is cisplatin sensitive, with an IC50 of 4.0 μM). This microcell hybrid, which now contained chromosome 11 and the cisplatin-resistant chromosome 16 from the MCH16 microcell hybrids, was designated MCH11/16. Total human biotinylated DNA was used as a probe for FISH analysis of these microcell hybrids to determine the presence of human chromosomes. MCH16 showed the presence of a single human chromosome (Fig. 1C), whereas both chromosomes 11 and 16 were observed in the MCH11/16 microcell hybrids (Fig. 1D). Chromosome 16 had fragmented in the MCH11/16 microcell hybrid, but both chromosome arms were present. These human chromosomes present in these microcell hybrids were further identified by PCR analysis using primers specific for DNA sequences on both arms of chromosomes 11 and 16. The PCR analysis of the microcell hybrids MCH16 and MCH11/16 is presented in Fig. 2. The PCR studies demonstrated the presence of chromosome 16 in MCH16 cells, and the microcell hybrid MCH11/16 possessed both chromosomes 11 and 16. Cytotoxicity assays were performed with these microcell hybrids to determine their resistance to cisplatin. The cell lines (A9 and MCH556.5) used as the recipient cells for the microcell-mediated chromosome transfer were used as controls to evaluate any change in drug sensitivity associated with the addition of human chromosome 16. The MCH16 microcell hybrid clone had an IC50 value similar to the recipient cell line A9 (Table 2). The microcell hybrid MCH11/16 was found to have an IC50 of 10.9 μM, compared with an IC50 of 4.0 μM in the recipient MCH556.5 cell line. To rule out the possibility that the increase in the IC50 for the MCH11/16 microcell hybrid was due to a gene dosage effect, chromosome 16 from the cisplatin-sensitive parental cell (2008) line was introduced into the recipient MCH556.5 cell. The resultant hybrid was labeled MCH11/S16. The presence of both chromosomes 11 and 16 was confirmed by FISH and PCR analysis (Table 2). However, the IC50 value for the MCH11/S16 hybrid was similar to that of MCH556.5 recipient cells. These results suggest that the increase in resistance to cisplatin observed in the MCH11/16 hybrids was not due to a gene dosage effect. Furthermore, these results indicate that chromosome 16 from the 2008/C13* cells and not the sensitive 2008 cells plays a role in cisplatin resistance.

Another approach taken to demonstrate the role of chromosomes 16 and 11 in cisplatin resistance was to attempt to correlate the loss of the resistant phenotype with the loss of a chromosome. Because the retention of human chromosomes in these somatic cell hybrids was not under any selective pressure, these hybrids had the tendency to lose human chromosomes over a period of time. Thus, the resistant somatic cell hybrid clones were maintained continuously in culture, and their resistance was determined periodically. N2p1, a resistant

---

**Table 1** Segregation of cisplatin resistance in human X mouse somatic hybrids

<table>
<thead>
<tr>
<th>Hybrid</th>
<th>IC50 (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1q</td>
</tr>
<tr>
<td>2008/C13*</td>
<td>27.4</td>
</tr>
<tr>
<td>J1</td>
<td>16.5</td>
</tr>
<tr>
<td>K2</td>
<td>21.4</td>
</tr>
<tr>
<td>N2p1</td>
<td>23.1</td>
</tr>
<tr>
<td>Q2</td>
<td>24.5</td>
</tr>
<tr>
<td>X3</td>
<td>23.7</td>
</tr>
<tr>
<td>Z13</td>
<td>20.9</td>
</tr>
</tbody>
</table>

**Resistant somatic cell hybrids**

**Sensitive somatic cell hybrids**

A9 5.2

C3 7.1

E2 6.4

M3 5.8

R1 6.1

S3 5.5

U1 6.6

W1 4.4

Y4 5.3

ZC4 4.8

ZE1 6.8

ZF3 5.7

ZH1 4.9

ZJ2 5.2

ZN4 6.4

Concordant:discordant 1.5 2.3 1.5 1.8 1.2 3 3 0.53 1 1.8 4 2.3 3 1.8 1.8 19 3 4 5.6 23 1.5 1.2 1.5

Because it was apparent that the either the presence of chromosome 16 (due to its high concordance:discordance ratio) or the absence of both chromosomes 11 and 16 (due to their universal presence in all the resistant somatic cell hybrid clones) was required for resistance, microcell hybrids containing either chromosome 16 of the 2008/C13* cell line or a combination of chromosome 16 from 2008/C13* cells and a normal human chromosome 11 from rodent A9 cells were established. Chromosome 16 has the APRT gene; therefore, it can be selected for in the AAT media (43). A9 cells were fused with a microcell preparation from 2008/C13* cells, and the microcell hybrid was selected for in the presence of AAT and ouabain. The microcell hybrid clone thus obtained was designated MCH16. Similarly, another microcell hybrid was established using a MCH556.5 cell as the recipient cell (the MCH556.5 cell is an A9 cell with human chromosome 11 and is cisplatin sensitive, with an IC50 of 4.0 μM). This microcell hybrid, which now contained chromosome 11 and the cisplatin-resistant chromosome 16 from the MCH16 microcell hybrids, was designated MCH11/16. Total human biotinylated DNA was used as a probe for FISH analysis of these microcell hybrids to determine the presence of human chromosomes. MCH16 showed the presence of a single human chromosome (Fig. 1C), whereas both chromosomes 11 and 16 were observed in the MCH11/16 microcell hybrids (Fig. 1D). Chromosome 16 had fragmented in the MCH11/16 microcell hybrid, but both chromosome arms were present. These human chromosomes present in these microcell hybrids were further identified by PCR analysis using primers specific for DNA sequences on both arms of chromosomes 11 and 16. The PCR analysis of the microcell hybrids MCH16 and MCH11/16 is presented in Fig. 2. The PCR studies demonstrated the presence of chromosome 16 in MCH16 cells, and the microcell hybrid MCH11/16 possessed both chromosomes 11 and 16. Cytotoxicity assays were performed with these microcell hybrids to determine their resistance to cisplatin. The cell lines (A9 and MCH556.5) used as the recipient cells for the microcell-mediated chromosome transfer were used as controls to evaluate any change in drug sensitivity associated with the addition of human chromosome 16. The MCH16 microcell hybrid clone had an IC50 value similar to the recipient cell line A9 (Table 2). The microcell hybrid MCH11/16 was found to have an IC50 of 10.9 μM, compared with an IC50 of 4.0 μM in the recipient MCH556.5 cell line. To rule out the possibility that the increase in the IC50 for the MCH11/16 microcell hybrid was due to a gene dosage effect, chromosome 16 from the cisplatin-sensitive parental cell (2008) line was introduced into the recipient MCH556.5 cell. The resultant hybrid was labeled MCH11/S16. The presence of both chromosomes 11 and 16 was confirmed by FISH and PCR analysis (Table 2). However, the IC50 value for the MCH11/S16 hybrid was similar to that of MCH556.5 recipient cells. These results suggest that the increase in resistance to cisplatin observed in the MCH11/16 hybrids was not due to a gene dosage effect. Furthermore, these results indicate that chromosome 16 from the 2008/C13* cells and not the sensitive 2008 cells plays a role in cisplatin resistance.
Fig. 1. Presence of human chromosomes 11 and 16 in the cisplatin-resistant somatic cell and microcell hybrid clones. FISH was performed using cosasome 11, chromosome 16-specific α satellite, and total human biotinylated genomic DNA as described in "Materials and Methods." A, clone N2pl probed with cosasome 11; B, clone K2 probed with chromosome 16-specific α satellite; C, clone MCH16 probed with total human biotinylated genomic DNA; D, clone MCH11/16 probed with total human biotinylated genomic DNA. Arrow, chromosome 11; arrowhead, chromosome 16.
cisplatin resistance and is located on chromosome 11. Western blot
assay demonstrated the importance of chromosome 11 in cisplatin resistance. Unfortu-

nately, the chromosome 11 used in these microcell hybrid MCH 11/16
preparations was from a cisplatin-sensitive cell line and not from the
resistant 2008/C13* cells, which may explain why the IC50 value of
the MCH11/16 hybrids was never as great as that of the 2008/C13*
resistant 2008/C13* cells, which may explain why the IC50 value of
the MCH11/16 hybrids was never as great as that of the 2008/C13*
cell lines; and (b) chromosome 11 is essential for its expression. However, the presence of a cisplatin resistance gene
cannot be assigned to chromosome 11, because the concordance:
discordance ratio for this chromosome is not sufficiently high.

The microcell fusion experiments showed that chromosome 16
did not contribute to drug resistance (Table 2). The cytotoxicity
analysis of the MCH11/16 hybrids demonstrates that the presence of
chromosome 11 is a requirement for cisplatin resistance. Unfortunately,
the chromosome 11 used in these microcell hybrid MCH11/16
preparations was from a cisplatin-sensitive cell line and not from the
resistant 2008/C13* cells, which may explain why the IC50 value of
the MCH11/16 hybrids was never as great as that of the 2008/C13*
cell lines; and (b) chromosome 11 is essential for its expression. However, the presence of a cisplatin resistance gene
cannot be assigned to chromosome 11, because the concordance:
discordance ratio for this chromosome is not sufficiently high.

The microcell fusion experiments showed that chromosome 16
alone cannot account for drug resistance (Table 2). The cytotoxicity
analysis of the MCH11/16 hybrids demonstrates that the presence of
chromosome 11 is a requirement for cisplatin resistance. Unfortunately,
the chromosome 11 used in these microcell hybrid MCH11/16
preparations was from a cisplatin-sensitive cell line and not from the
resistant 2008/C13* cells, which may explain why the IC50 value of
the MCH11/16 hybrids was never as great as that of the 2008/C13*
cell lines; and (b) chromosome 11 is essential for its expression. However, the presence of a cisplatin resistance gene
cannot be assigned to chromosome 11, because the concordance:
discordance ratio for this chromosome is not sufficiently high.

The microcell fusion experiments showed that chromosome 16
alone cannot account for drug resistance (Table 2). The cytotoxicity
analysis of the MCH11/16 hybrids demonstrates that the presence of
chromosome 11 is a requirement for cisplatin resistance. Unfortunately,
the chromosome 11 used in these microcell hybrid MCH11/16
preparations was from a cisplatin-sensitive cell line and not from the
resistant 2008/C13* cells, which may explain why the IC50 value of
the MCH11/16 hybrids was never as great as that of the 2008/C13*
cell lines; and (b) chromosome 11 is essential for its expression. However, the presence of a cisplatin resistance gene
cannot be assigned to chromosome 11, because the concordance:
discordance ratio for this chromosome is not sufficiently high.

The microcell fusion experiments showed that chromosome 16
alone cannot account for drug resistance (Table 2). The cytotoxicity
analysis of the MCH11/16 hybrids demonstrates that the presence of
chromosome 11 is a requirement for cisplatin resistance. Unfortunately,
the chromosome 11 used in these microcell hybrid MCH11/16
preparations was from a cisplatin-sensitive cell line and not from the
resistant 2008/C13* cells, which may explain why the IC50 value of
the MCH11/16 hybrids was never as great as that of the 2008/C13*
cell lines; and (b) chromosome 11 is essential for its expression. However, the presence of a cisplatin resistance gene
cannot be assigned to chromosome 11, because the concordance:
discordance ratio for this chromosome is not sufficiently high.

The microcell fusion experiments showed that chromosome 16
alone cannot account for drug resistance (Table 2). The cytotoxicity
analysis of the MCH11/16 hybrids demonstrates that the presence of
chromosome 11 is a requirement for cisplatin resistance. Unfortunately,
the chromosome 11 used in these microcell hybrid MCH11/16
preparations was from a cisplatin-sensitive cell line and not from the
resistant 2008/C13* cells, which may explain why the IC50 value of
the MCH11/16 hybrids was never as great as that of the 2008/C13*
cell lines; and (b) chromosome 11 is essential for its expression. However, the presence of a cisplatin resistance gene
cannot be assigned to chromosome 11, because the concordance:
discordance ratio for this chromosome is not sufficiently high.

The microcell fusion experiments showed that chromosome 16
alone cannot account for drug resistance (Table 2). The cytotoxicity
analysis of the MCH11/16 hybrids demonstrates that the presence of
chromosome 11 is a requirement for cisplatin resistance. Unfortunately,
the chromosome 11 used in these microcell hybrid MCH11/16
preparations was from a cisplatin-sensitive cell line and not from the
resistant 2008/C13* cells, which may explain why the IC50 value of
the MCH11/16 hybrids was never as great as that of the 2008/C13*
cell lines; and (b) chromosome 11 is essential for its expression. However, the presence of a cisplatin resistance gene
cannot be assigned to chromosome 11, because the concordance:
discordance ratio for this chromosome is not sufficiently high.

The microcell fusion experiments showed that chromosome 16
alone cannot account for drug resistance (Table 2). The cytotoxicity
analysis of the MCH11/16 hybrids demonstrates that the presence of
chromosome 11 is a requirement for cisplatin resistance. Unfortunately,
the chromosome 11 used in these microcell hybrid MCH11/16
preparations was from a cisplatin-sensitive cell line and not from the
resistant 2008/C13* cells, which may explain why the IC50 value of
the MCH11/16 hybrids was never as great as that of the 2008/C13*
cell lines; and (b) chromosome 11 is essential for its expression. However, the presence of a cisplatin resistance gene
cannot be assigned to chromosome 11, because the concordance:
discordance ratio for this chromosome is not sufficiently high.

The microcell fusion experiments showed that chromosome 16
alone cannot account for drug resistance (Table 2). The cytotoxicity
analysis of the MCH11/16 hybrids demonstrates that the presence of
chromosome 11 is a requirement for cisplatin resistance. Unfortunately,
the chromosome 11 used in these microcell hybrid MCH11/16
preparations was from a cisplatin-sensitive cell line and not from the
resistant 2008/C13* cells, which may explain why the IC50 value of
the MCH11/16 hybrids was never as great as that of the 2008/C13*
cell lines; and (b) chromosome 11 is essential for its expression. However, the presence of a cisplatin resistance gene
cannot be assigned to chromosome 11, because the concordance:
discordance ratio for this chromosome is not sufficiently high.
parental cell line. It is highly likely that expression of another gene present only on a chromosome 11 derived from the drug-resistant C13* cells.

The importance of chromosome 11 in cisplatin resistance was also documented by the evolution of the resistant N2p1 somatic cell hybrid clone into the sensitive N2p46 clone, which was accompanied by the loss of chromosome 11. This result supports the hypothesis that the presence of chromosome 11 alone is not sufficient for drug resistance, and that the presence of both chromosomes 11 and 16 is necessary for the resistance phenotype.

Chromosomes 11 and 16 have both been implicated previously in different aspects of drug resistance. Genes for MRP, LRP, excision repair cross-complementing protein 4, and MT-IIA have been reported on chromosome 16. MRP is a membrane glycoprotein, which is found in cell lines exhibiting multiple drug resistance but failing to show the presence of P-glycoprotein (44). The cisplatin-sensitive parental cell line 2008 and the cisplatin-resistant derivative 2008/C13* have been analyzed by reverse transcription-PCR for mRNA expression of the MRP gene, but no discernible differences were observed (45). LRP is another protein associated with drug resistance that has been mapped to the 16p chromosome (46). LRP is a vault protein, i.e., a cellular organelle mediating intracellular (nucleocytoplasmic) transport of a wide variety of substrates (47). It has been demonstrated that the level of LRP expression is directly correlated to the response of ovarian cancer cells to cisplatin and other alkylating agents in ovarian cancer cells (48). The role that this protein plays in 2008/C13* cells remains to be determined. There is also some evidence that overexpression of the MT-IIA gene in tumors may be associated with increased cisplatin resistance (23, 50, 51). The antioxidant properties of this protein have been proposed as possible mechanisms of cisplatin detoxification (52). Recent studies on tumor cells transfected with the human MT-IIA gene showed resistance to cisplatin as well as other alkylating agents (20). However, the role of the MT-IIA gene in 2008/C13* cells remains to be determined.

Genes present on chromosome 11 that have been implicated in cisplatin resistance, include GST-σ and the structure-specific recognizing protein. The former has been widely implicated in cisplatin resistance, but its expression in the sensitive somatic cell hybrid clones C3 and S2 was found to be higher than the resistant somatic cell hybrid N2p1 suggesting that the GST-σ enzyme does not play a major role in resistance to cisplatin in the 2008/C13* cell line. In the resistant and sensitive somatic cell hybrid clones, glutathione levels were not significantly different (41).

Recently, a protein named structure-specific recognition protein has been shown to specifically recognize platinum-DNA adducts and to be present on chromosome 11 (53, 54). Its function in cisplatin resistance of the 2008/C13* cells is presently unknown.

The results of this study have shown that: (a) the phenotype for cisplatin resistance is dominant, and it is also a result of mutations occurring in the genes of the sensitive cells; and (b) the expression of one or more genes on both chromosomes 11 and 16 is required for the expression of the cisplatin resistance phenotype in 2008/C13* cells.

Acknowledgments

We thank Dr. B. Weissman (University of North Carolina Lineberger Comprehensive Cancer Center) for providing the MCH556.5 cells and for his advice. We also thank Dr. E. Stanbridge (University of California, Irvine, CA) for his kind guidance and suggestions.

References


Expression of the Cisplatin Resistance Phenotype in a Human Ovarian Carcinoma Cell Line Segregates with Chromosomes 11 and 16

Beloo Mirakhur, Hemant K. Parekh and Henry Simpkins


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/56/10/2256

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link http://cancerres.aacrjournals.org/content/56/10/2256. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.