

# Gene expression profiles related with overcoming cisplatin resistance in human cancer cell lines

## Research Article

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**Abbreviations:** superoxide dismutase, (SOD); high-mobility group protein, (HMG)

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## Summary

Gene expression profiles were analyzed using cDNA microarray for cisplatin-sensitive and their resistant cancer cell lines. Sensitive cells included the cervical ME180, leukemia K562, and ovarian A2780. Their corresponding resistant cell lines to cisplatin were ME180/PDD, K562/PDD and A2780/PDD, respectively. All three cisplatin-resistant cell lines showed small changes in gene expression profiles between them. Genes of cell adhesion & matrix, DNA binding & response, and specific enzymes, were up- or down-regulated depending on the cell type. However genes involved in cell cycle, oncogenes and SOD (superoxide dismutase) were up-regulated only in cisplatin-resistant cell lines. In order to investigate changes in gene expression linked with overcoming cisplatin resistance, we have treated A2780/PDD cells with pegylated liposomal *trans*(±)-1,2-diaminocyclohexane glutamato-platinum(II) complex [L-Pt(dach)(glu)], which is known to overcome cisplatin resistance. This treatment resulted in upregulation of cytokine, tumor suppressor and carbohydrate-modifying enzymes, whereas oncogene, DNA binding & response, cell membrane redox- and calcium-related enzymes, and SOD were down-regulated. All these changes in gene expression profiles between drug-treated and untreated A2780/PDD cells seemed to be related with the cytotoxicity to L-Pt(dach)(glu) in resistant cells, arising from overcoming cisplatin resistance. We show that microarray analysis is useful for evaluating the overcoming in cisplatin resistance and can efficiently be custom applied as an indicator of maintenance or lack of cisplatin resistance in chemotherapy for predicting therapeutic efficacy.

## I. Introduction

Cisplatin [*cis*-diamminedichloroplatinum(II)] is very effective in the treatment of various types of human cancers (Carter et al, 1984). The cytotoxicity of this drug is believed to result from the platinum binding to DNA. The reaction between cisplatin and DNA produces several types of platinum-DNA adducts; monoadducts and bifunctional intrastrand or interstrand crosslinked adducts. These DNA-platinum adducts prevent efficient DNA replication and transcription to exert the cytotoxic effect of cisplatin. Though cisplatin has excellent cytostatic effect in tumors as a result of DNA binding, the success of cisplatin-based chemotherapy is limited due to its

significant toxic side effects such as acute nephrotoxicity and neurotoxicity, and its pre-existed or acquired drug resistance. Cisplatin resistance is multifactorial. It consists of mechanisms such as decreased drug accumulation (Andrews et al, 1988; Kelland et al, 1992), increased drug detoxification (Behrens et al, 1987; Mistry et al, 1991; Godwin et al, 1992), and an enhanced ability to repair (Lai et al, 1988; Masuda et al, 1988; Parker et al, 1991; Zhen et al, 1992) and tolerate (Johnson et al, 1997) DNA damage. Up-regulation of DNA repair genes including XPB, XPD, XPA and ERCC-1 have been implicated in the development of cisplatin resistance in human tumor cells (Wood, 1997; Dabholkar et al, 2000; Aloyz et al, 2002; Xu et al, 2002).

Recently, cDNA microarrays have been successfully used to study global patterns of gene expression in human cancer research field (DeRisi et al, 1996; Golub et al, 1999; Alizadeh et al, 2000; Perou et al, 2000). Microarray can detect gene expression changes between two samples about the known and unknown huge number of genes by a single experiment. Thus, to investigate the change of gene expression concerned with cisplatin sensitivity and resistance, gene expressions was compared by using microarray analysis. Through the reports of gene expressions in cisplatin resistant cells and tissues, many kinds of genes were included such as apoptosis, cell adhesion, motility, cell cycle, cell development regulators, receptors, growth factors, invasion regulators, oncogenes, as well as DNA damage and repair genes (Sakamoto et al, 2001), whereas previous studies before microarray on cisplatin resistance mechanism only focused on the genes related with the DNA damage and repair mechanisms. Therefore, we tried to use cDNA microarray analysis to identify diverse gene expressions in cisplatin-resistant cells compared to sensitive ones. First, we tried to analyze the gene expression profiles concerned with cisplatin sensitivity and resistance using three different cancer cell lines. Those are human cervical ME180, leukemia K562, and ovarian A2780 cancer cell lines; and their corresponding cisplatin-resistant cell lines, ME180/PDD, K562/PDD and A2780/PDD, respectively.

Several strategies have been developed to both overcome cisplatin resistance (Canon et al, 1990) and reduce cisplatin-induced toxicity on extra-tumoral normal tissues (Konno et al, 1992). Among the strategies in platinum drug, the dach-platinum(II) complex have attracted significant attention for many years because they are not cross-resistant to cisplatin (Jennerwein et al, 1989). Another strategy was the application of liposome to overcome the cisplatin resistance. Liposomes offer a versatile drug-carrier technology with great potential for improving the pharmacokinetics of anti-cancer drugs. They have been widely used as a means to reduce the toxicity of drugs (Gabizon et al, 1998) and enhance their therapeutic indexes (Sharma et al, 1993). In the case of cytostatic drugs, increasing local tumor exposure by liposomes has been reported to be a useful strategy for overcoming the resistance of cancer cells to chemotherapy (Khokhar et al, 1991; Ho et al, 1997). Our results also proved the effect of the encapsulation of Pt(dach)(glu) in liposomes to increase cytostatic activity and overcome the cisplatin resistance in several cancer cell lines. Thus, we have applied cDNA microarray analysis to evaluate the overcoming cisplatin resistance in A2780/PDD cells by the treatment of liposomal (L-) Pt(dach)(glu). By comparing the changes of gene expressions in A2780/PDD cells by the treatment of L- Pt(dach)(glu), we have tried to figure out which genes are important to overcome the cisplatin resistance.

## II. Materials and methods

**A. Platinum drug and** Cisplatin was purchased from Dong-A Pharmaceutical Co. (Ahnyang, Korea) and dach-glutamato platinum drug [Pt(dach)(glu) in **Figure 1**] was obtained

from Dr. Sohn in Ewha Womans University, Korea. Lipids like DMPC, PEG2000-DMPE, cholesterol were purchased from Avanti Polar Lipids (Alabaster, Ala).

### B. Preparation of liposomal platinum drug

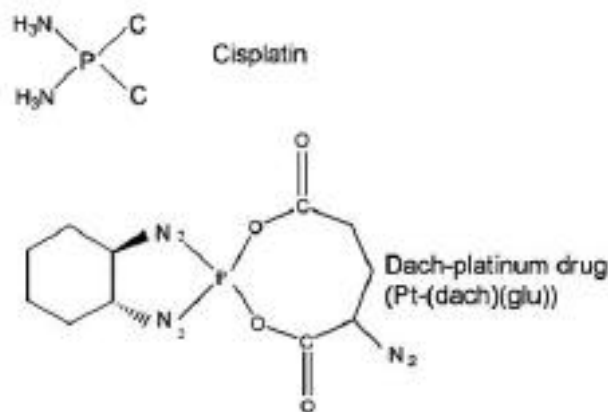
Liposomes containing Pt(dach)(glu) complex were prepared by lyophilization-rehydration method (Han et al, 2002). Briefly, lipids in chloroform were mixed at the desired molar ratio (DMPC/PEG2000-DMPE/CH=50/5/45), and the chloroform was removed in a rotary evaporator. To the dried lipid film, the platinum drug dissolved in methanol were added at the weight ratio of drug to lipid (1:20) and subsequently the methanol solvent was removed by rotary evaporator. Then, *tert*-butanol was added and the solutions was shaken at 40-50°C for 10-30 min to obtain the clear solutions. Aliquoted samples in vials were frozen in dry ice/acetone bath, and *tert*-butanol was removed by lyophilization overnight to give the lyophilized preliposomal powders. To reconstitute the preliposomes, saline or PBS solution was added at the concentration of 50 mg/ml, and the resulting suspension was shaken at 40°C for 60 min with vortexing and sonication.

### C. Cancer cell lines

ME180, K562 and A2780 cancer cell lines were derived from the patients prior to chemotherapy and obtained from Dr. Perez-Soler in Albert Einstein College of Medicine. All these cell lines (ME180, K562, A2780) were made resistant to cisplatin *in vitro* by means of continuous stepwise exposure to cisplatin to produce the corresponding cisplatin-resistant cell lines; ME180/PDD, K562/PDD, and A2780/PDD, respectively.

### D. Cell cytotoxicity and resistance index

Cell cytotoxicity was determined by MTT (methylthiazolotetrazolium) dye reduction assay. Cells were seeded in 150  $\mu$ l of medium/well in 96-well plates, allowed to attach overnight, and then exposed to various concentrations of drugs for 48 h. After washing the cells with PBS twice, 40  $\mu$ l of a 5 mg/mL solution of MTT was added per well. After 4 h at 37°C, the cells were lysed by adding 100  $\mu$ l of dimethyl sulfoxide and incubated for 2 h. The cell survival fractions were determined by reading the absorbance at 570nm in a microplate reader (Model MCC/340, Titertek multiscan). All the IC<sub>50</sub> (50% inhibitory cell dose) values of liposomal platinum drug were normalized against those of the corresponding empty liposomes.



**Figure 1.** Chemical structure of anti-cancer platinum drug

The reported values are the averages of triplicate experiments. The resistance indexes were calculated as the ratio of IC<sub>50</sub> in resistant cells to IC<sub>50</sub> in sensitive cells.

## E. Preparation of total and messenger RNA

Total RNA was extracted from cultured cells using a modified acid phenol method. Briefly, the growth medium was removed and the cells lysed with Trizol (Life Technologies). The lysate was cleared and extracted with 1/10 volume of 1-bromo-3-chloropropane. The aqueous layer was collected in a new tube and precipitated with isopropanol. After 75% ethanol washing, the pellet was air dried and resuspended in DEPC-treated water, and quantified by A260/280 measurement using UV spectrometer (DU 530, Beckman, USA). For assessing the quality, 3~5 µg of total RNA was loaded onto denaturing 1.0% formaldehyde agarose gels and for electrophoresed. mRNA was then isolated from total RNA with oligotex mRNA midi kit (Qiagen, Chatsworth, CA, USA).

## F. cDNA microarrays

### 1. Preparation of fluorescent DNA probe from mRNA

Probes were made as described (DeRisi et al, 1996) with several modifications. The reverse transcriptase used here was Superscript II RNase H (Life Technologies). The Cy-3 dCTP and Cy-5 dCTP were purchased from Amersham Pharmacia Biotech. Each reverse transcription reaction contained 3 µg of mRNA and 0.5 µg of oligo(dT) primer. Following the reverse transcription step, samples were treated with each 1.0 µl of 1.5 M sodium hydroxide and 30 mM EDTA for 10 minutes at 65°C, then neutralized by adding 468 µl of TE buffer (pH 7.4). Using a Micron 30 (Millipore), the probe was purified and concentrated. Cy-3 and Cy-5 fluorescently labeled probes were mixed in 3 X SSC, 0.1 % SDS with 0.5 mg/ml poly A blocker (Amersham Pharmacia Biotech), and 0.5 mg/ml yeast tRNA (Life Technologies) to a final volume of 25 µl

### 2. Microarray hybridization

Arrays were prehybridized in 3.5 X SSC, 0.1% SDS, 10 mg/ml BSA in a Coplin jar for 20 minutes at 50°C, and washed by dipping in water and in isopropanol, and dried using centrifuge. The prepared probes was denatured by heating at 95-100°C for 2 minutes and added onto a array with cover slide. The hybridization was done in a CMT-Hybridization chamber (Corning) for 20 hours in a 50°C waterbath. Arrays were washed for 5 minutes at room temperature in low stringency wash buffer (0.1 X SSC/0.1% SDS), then twice for 5 minutes in high stringency wash buffer (0.1 X SSC) and dried using centrifuge.

### 3. Microarray hybridization

Fluorescence intensities at the immobilized targets were measured using Scanarray 4000 with a laser confocal microscope (GSI Lumonics, USA). The two fluorescent images (Cy-3 and Cy-5) were scanned separately from a confocal microscope, and color images were formed by arbitrarily assigning differentiated cell intensity values into the red channel and control intensity into the green channel and data were analyzed using Quantarray software (version 2.0.1, GSI Lumonics). Results were also analyzed by normalization between the images to adjust for the different efficiencies in labeling and detection with the two different fluors. This was achieved by matching of the detection sensitivities to bring a set of 32 internal control genes ( -actin and GAPDH) to nearly equal intensity. For this analysis, we used

a filter that included all genes exhibiting a minimum level of expression of intensity of >1,000 fluorescent units (on a scale of 0-65,535 fluorescent units) for both red and green channels for each pair of experiments.

## III. Results and discussion

### A. Preparation of cDNA microarray and hybridization

To screen the specific genes in cisplatin-resistant cancer cell lines, we tested with our in-house cDNA microarray, which contains 3,063 cDNA clones from 7 different cDNA libraries. Housekeeping genes, -actin (Accession No. NM $\mu$ 001101) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH; Accession No. NM $\mu$ 002046) was printed on the same array to serve as internal controls. The microarray was subsequently hybridized with cDNA probes labeled with fluorochromes. Probes were prepared with aRNA from cultured drug sensitive and resistant cells. The fluorescent targets were pooled and allowed to hybridize under stringent conditions to the clones on the microarray. Laser excitation of the incorporated targets yields an emission with a characteristic spectra, which is measured using a scanning confocal laser microscope. Monochrome images from the scanner (Scanarray 4000) were imported into software (GenePix) in which the images were pseudo-colored. The color images of the hybridization results were generated by representing the Cy-3 fluorescent image as green and the Cy-5 fluorescent image as red and then merging the two color images. To ensure reproducibility of the microarray results, we repeated each experiment twice with each RNA samples. The cDNA probe derived from sensitive cells was labeled with Cy-3 dUTP (green) and the cDNA probe from resistant cells was labeled with Cy-5 dUTP (red). Green and red fluorescent signals indicate greater relative expression in sensitive and resistant cells, respectively. The yellow fluorescent signal indicates that both of the RNA is equal expression level. The spots with signal intensities that were at least 1.5-fold different from control levels in both experiments were designated as genes that are differentially expressed.

### B. Analysis of gene expression pattern in cisplatin-resistant cancer cell lines

In order to examine the gene expression profiles in resistant cell lines to cisplatin, we analyzed three different human cancer cell lines; cervical ME180, leukemia K562, ovarian A2780 sensitive and their-resistant counterparts to cisplatin ME180/PDD, K562/PDD, and A2780/PDD cancer cell lines, respectively. The resistance index of ME180/PDD, K562/PDD and A2780/PDD cancer cell lines to cisplatin are 5.0, 3.7 and 6.0, respectively.

**Figure 2** shows tree view image of clustered data set of 3,063 genes in three human cancer cell lines. **Table 1** summarizes 30~40 of up-regulated and 10~15 of down-regulated genes with expression ratio in these cisplatin-resistant cancer cell lines. Compared to the sensitive cells, three resistant cells showed the up-regulated genes with

**Table 1:** List of up- and down-regulated genes in three human resistant cancer cell lines to cisplatin

Up-regulated genes	Exp. ratio	Down-regulated genes	Exp. ratio
<b><u>Cell adhesion &amp; matrix</u></b>		<b><u>Cell adhesion &amp; matrix</u></b>	
actin, alpha 2	3.7	elastin	0.1
integral type I protein	2.4	lumican	0.1
myosin, light chain	2.2	decorin	0.2
vimentin	2.1	cadherin 2	0.2
keratin 5 (KRT5)	1.9		
integrin-linked kinase(ILK)	1.7		
collagen, type V, alpha 3	1.6		
tubulin, alpha, brain-specific	1.6		
myosin (MYL6)	2.0		
<b><u>Cell cycle</u></b>		<b><u>DNA binding &amp; response</u></b>	
thymosin, beta 4	3.2	transcription factor Dp-2	0.4
thymosin beta-10	1.8	nuclear receptor BP	0.4
cyclin-dependent kinase 4	1.7		
<b><u>DNA binding &amp; response</u></b>		<b><u>Enzymes</u></b>	
high-mobility group protein	1.8	antizyme inhibitor	0.5
G protein (GNB2L1)	2.3	kinectin 1(kinesin receptor)	0.4
G-rich RNA sequence binding factor 1	2.0	thyroid receptor protein	0.5
PAI-RBP1	1.9		
<b><u>Oncogenes</u></b>		<b><u>Other genes</u></b>	
RAS oncogene family	1.9	Dickkopf-1	0.1
prostate tumor over expressed gene 1	1.8	ATP binding protein	0.5
<b><u>Enzymes</u></b>		netrin 4	0.5
B4GALT1	2.9	selenoprotein (SEP15)	0.5
PPAP2B	2.7	crystallin, alpha B	0.5
ubiquitin esterase L1	2.6		
aldo-keto reductase family 1	2.0		
Ca-independent phospholipase A2	1.9		
tissue inhibitor of metalloproteinase 1	1.7		
N-terminal acetyltransferase complex	1.7		
<b><u>Other genes</u></b>			
superoxide dismutase 1	1.8		
anti-oxidant protein 2	2.0		
fibroblast activation protein	2.1		
osteonectin	2.0		
HS1 binding protein (HAX1)	2.2		
prostatic binding protein (PBP)	1.7		

expression ratio higher than 1.60 including genes of cell adhesion & matrix such as actin, integrin, collagen, tubulin, and keratin; cell cycle such as thymosin and cyclin-dependent kinase; DNA binding & response such as G protein, RNA binding factor and high mobility group; oncogene such as RAS oncogene and prostate tumor overexpressed gene; various enzymes such as ubiquitin esterase, aldo-keto reductase, and phospholipase; and superoxide dismutase (SOD). They also showed the down-regulated genes with expression ratio lower than 0.5 including genes of cell adhesion such as elastin, cadherin, and decorin; DNA binding & response such as transcription factor Dp-2 and nuclear receptor binding protein; enzyme such as thyroid receptor protein, and antizyme inhibitor; and Dickkopf-1. ME180, K562 and A2780 resistant cell lines showed a little different gene

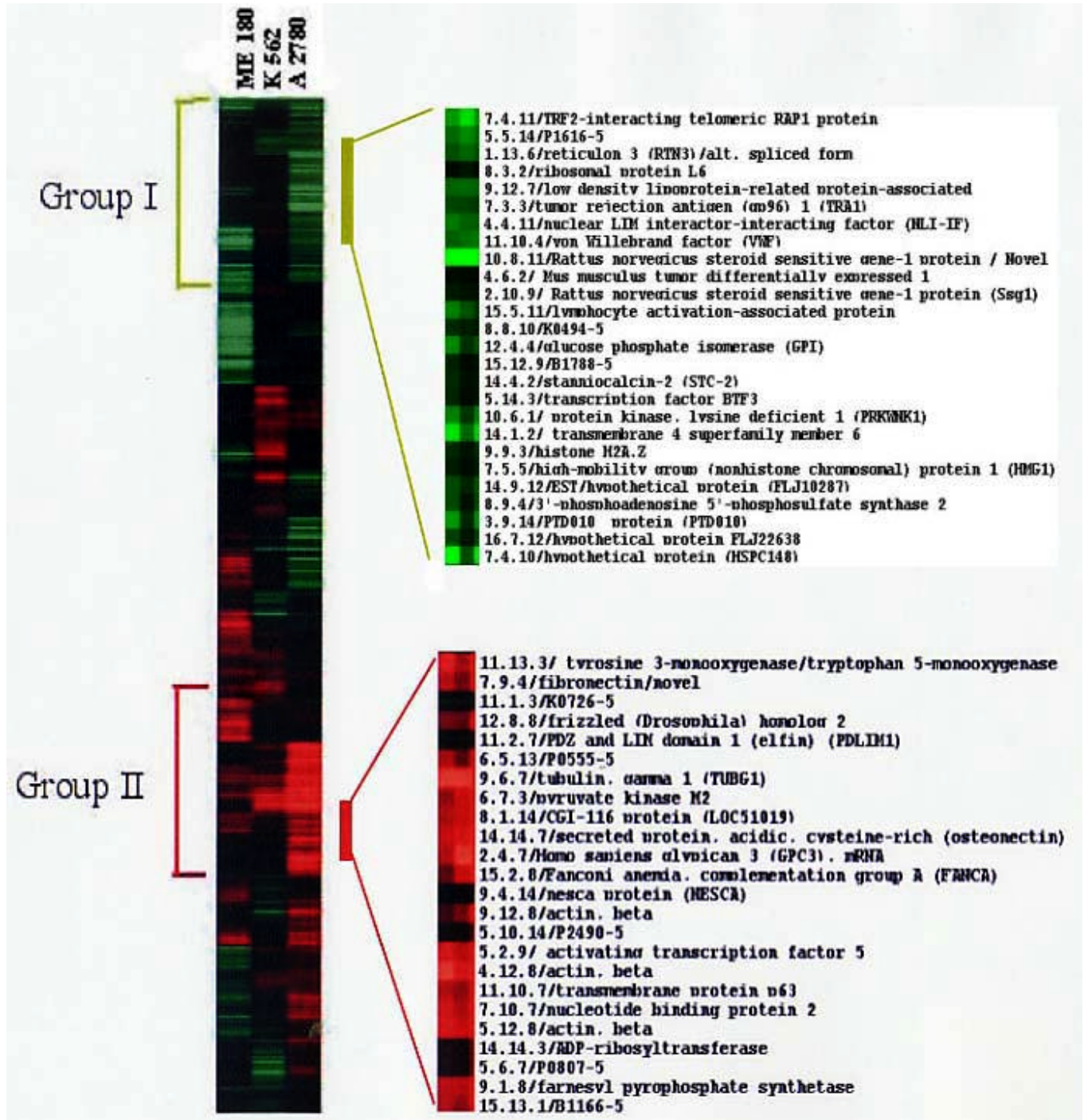
expression profiles, respectively, however majortrends in described genes in **Table 1** were similar. Characteristic point in **Table 1** is that several genes of cell adhesion & matrix, DNA-associated, and enzyme are up- or down-regulated depending on their types, however genes of cell cycle and oncogene were only shown in up-regulated genes of cisplatin-resistant cell lines.

### C. Analysis of gene expressions of A2780/PDD cells treated with liposomal platinum drug

To examine the changes of gene expressions by overcoming the cisplatin resistance, we tried to compare the microarray results between untreated and treated A2780/PDD cells with liposomal Pt(dach)(glu),

respectively. **Table 2** shows cell cytotoxicity results of cisplatin and L-Pt(dach)(glu), in A2780 sensitive and A2780/PDD resistant cells. L-Pt(dach)(glu) was known to overcome the cisplatin resistance due to the dach group in platinum complex and cell fusion ability of liposome. As a result, the IC<sub>50</sub> values of cisplatin (20 µg/ml) and L-

Pt(dach)(glu) (10 µg/ml) were similar in sensitive cells, however those in resistant cells were 120 and 25 µg/ml, respectively. Therefore, the resistance indexes were calculated out to be 6.0 and 1.25 for cisplatin and L-Pt(dach)(glu), respectively. It means that L-Pt(dach)(glu) can overcome the cisplatin resistance in A2780/PDD cells.



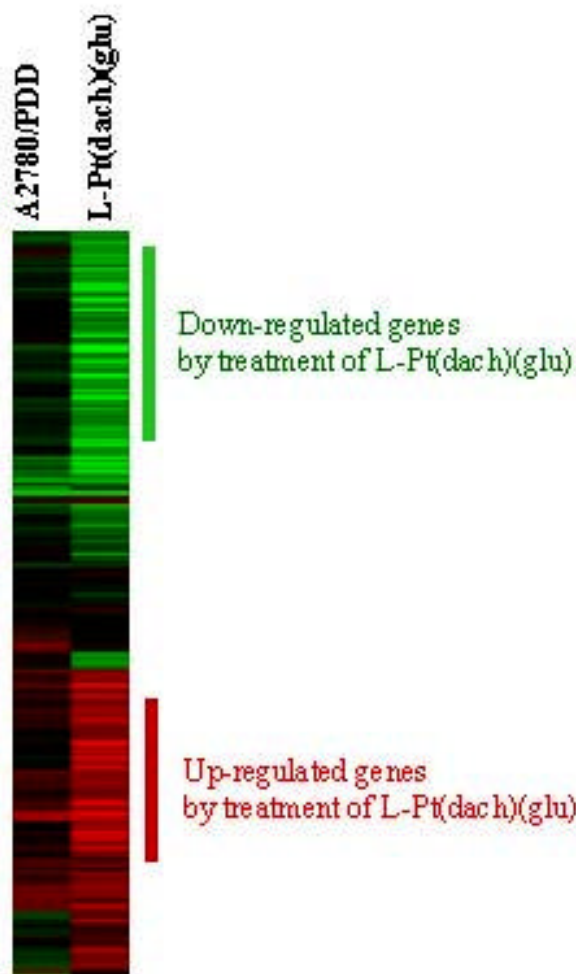
**Figure 2.** Tree view image of clustered data set of 3,063 genes in three human carcinoma cell lines. The vertical axis corresponds to genes, and the horizontal axis to cell lines. Red colors indicate up-regulated transcripts and green colors indicate down-regulated transcripts in each cell line.

**Table 2.** Cell cytotoxicity of cisplatin and liposomal dach-platinum drug (L-Pt(dach)(glu)) in human ovarian A2780 cancer cell lines<sup>a</sup>

Drug type	A2780	A2780/PDD	Resistance Index
Cisplatin	IC <sub>50</sub> <sup>c</sup> = 20	IC <sub>50</sub> = 120	6.0
Liposomal <sup>b</sup> platinum drug (L-Pt(dach)(glu))	10	25	1.25

**Table 3.** Lists the up- and down-regulated genes in A2780/PDD resistant cells treated with liposomal dach-platinum drug.

<b>Up-regulated genes</b>	<b>Exp. ratio</b>	<b>Down-regulated genes</b>	<b>Exp. ratio</b>
<b><u>Cell adhesion &amp; matrix</u></b>		<b><u>Cell adhesion &amp; matrix</u></b>	
integrin beta 1 BP1	2.5	collagen, type VI, alpha 3	0.2
cyclophilin B	2.4	aggrecan 1	0.4
actin, beta	2.3		
integrin beta 4 BP	2.1		
tubulin, beta 2	1.9		
fibrillarin	1.9		
		<b><u>DNA binding &amp; response</u></b>	
		high-mobility group protein 1	0.2
		translation initiation factor 3	0.2
		transcription factor BTF3	0.5
		methyl-CpG BP 2	0.5
<b><u>Enzymes</u></b>		<b><u>Enzymes</u></b>	
fructose aldolase A	4.8	topoisomerase II alpha	0.2
carboxypeptidase E	5.5	glutathione S-transferase,13	0.5
glucose phosphate isomerase	2.9	thioredoxin isolog	0.5
proteasome subunit, beta	4.1	peroxiredoxin 3	0.5
catechol-O-methyltransferase	3.4	glycyl-tRNA synthetase	0.1
enolase 1, alpha	2.8	asparaginyl-tRNA synthetase	0.4
NADH dehydrogenase	2.2		
cytochrome c oxidase 8	2.1		
isocitrate dehydrogenase	1.9		
<b><u>Cytokines</u></b>		<b><u>Ca<sup>2+</sup> related genes</u></b>	
IL-1 receptor-kinase 1	2.0	calumenin	0.2
IL-10	2.1	reticulocalbin 1	0.3
interferon-induced protein 1-8U	3.0	calnexin	0.5
<b><u>Tumor suppressor</u></b>		<b><u>Oncogenes</u></b>	
non-metastatic cells 2	2.6	hypoxia-inducible factor 1	0.2
BCL2-associated athanogene 3	2.6	ras homolog gene family, C	0.2
downregulated in ovarian cancer1	2.0	RAB7 (RAS oncogene)	0.5
		TGF beta 2	0.2
<b><u>Other genes</u></b>		<b><u>Other genes</u></b>	
heat shock protein 1	6.9	superoxide dismutase 1	0.5
dickkopf homolog 3	3.5	anti-oxidant protein 2	0.3
chromatin assembly factor 1	2.6	dynein, LIC-2	0.5
chloride intracellular channel 1	2.3	SMT3, homolog 2	0.5
clusterin (lusis inhibitor)	2.2		
annexin A5	1.9		



**Figure 3.** Tree view image of clustered data set of 3,063 genes in A2780/PDD cell lines treated with liposomal dach-platinum drug (L-Pt(dach)(glu)). The vertical axis corresponds to genes, and the horizontal axis to cell lines treated with the liposomal dach-platinum drug. Red colors indicate up-regulated transcripts and green colors indicate down-regulated transcripts.

Based on chemosensitivity results in **Table 2**, A2780/PDD resistant cells were treated with L-Pt(dach)(glu) at the concentrations of  $IC_{50}$  values for 24 hours. After analyzing the microarray results using drug-treated and -untreated cells, the clustered data set of 3,063 genes are shown in **Figure 3**. Red colors indicate up-regulated transcripts and green colors indicate down-regulated transcripts. **Table 3** shows up- and down-regulated genes in A2780/PDD cells when treated with L-Pt(dach)(glu). Gene expressions of cell cycle, DNA binding & response, oncogene, superoxide dismutase (SOD) and anti-oxidant protein those were found to up-regulated in cisplatin-resistant cell lines compared to sensitive cell lines (**Table 1**) were reduced or even found in down-regulated gene profile.

For example, DNA binding & response high-mobility group protein (HMG), oncogene (RAS), SOD and anti-oxidant protein were down-regulated from 1.8, 1.9, 1.8 and 2.0 expression ratio in A2780/PDD cells to 0.2, 0.2, 0.5 and 0.3 in drug-treated A2780/PDD cells, respectively. Most of cell adhesion and matrix genes were still found to up-regulated without significant changes by L-

Pt(dach)(glu). On the other hand, gene expressions of cytokines such as IL-10, IL-1 receptor kinase 1, and interferon-induced protein; and tumor suppressors such as non-metastatic related, Bcl2 associated, and down regulator of ovarian cancer 1 were newly appeared in list of up-regulated genes. In brief, L-Pt(dach)(glu) reduced gene expressions of oncogenic factors, DNA-associated, cell cycle and proliferation, and induced those of cytokine and tumor suppressor genes. These results proved the positive effect of cell cytotoxicity of L-Pt(dach)(glu) in A2780/PDD cell lines which indicates overcoming cisplatin resistance. Furthermore, some carbohydrate-modifying enzymes were newly added in up-regulated profiles. This suggests that this liposomal drug may go through different mode of action and cell-killing mechanism including several important carbohydrate modifications to overcome cisplatin resistance.

Among down-regulated genes by the treatment of L-Pt(dach)(glu) (**Table 3**), high mobility group protein 1, RAS, SOD, and anti-oxidant protein genes were found in up-regulated gene list in cisplatin-resistant profile (**Table 1**). Many other oncogenes such as hypoxia-inducible

factor, RAB7, and TGF- and DNA binding & response genes such as transcription factor and methyl CpG binding protein genes were also down-regulated in L-Pt(dach)(glu) treatment. In addition, topoisomerase and redox-related enzymes such as glutathion, thioredoxin, and peroxiredoxin; and calcium-related genes such as calumenin, reticulocalbin, and calnexin were appeared in down-regulated profiles.

In summary, L-Pt(dach)(glu) up-regulated gene expressions of cytokines, tumor suppressors and carbohydrate-modifying enzymes; and down-regulated those of oncogene, DNA binding & response, redox- and calcium-related enzymes, and SOD in cisplatin-resistant A2780/PDD cells. Therefore, some gene expression profiles of cisplatin resistant cells were changed to the opposite direction by the treatment of L-Pt(dach)(glu) which may explain their losing cisplatin resistance in A2780/PDD cells. Up-regulation of cytokine and tumor suppressor and down-regulation of oncogene definitely related with the cytotoxicity of anticancer-drug to kill the cancer cells. However, up-regulations of carbohydrate-modifying enzymes; and down-regulations of redox- and calcium-related enzymes would give a clue that L-Pt(dach)(glu) have overcome cisplatin resistance through different mode of action and cell killing mechanism compared to previous anti-cancer drug, cisplatin. All these results proved that cDNA microarray analysis could be useful to evaluate the keeping or overcoming of cisplatin resistance in human cancer cell lines.

The prediction of the cisplatin resistance and sensitivity of tumors is an extremely important criteria to use anti-cancer drug for cancer chemotherapy. One approach to solve the limitation of anti-cancer drug based chemotherapy is to elucidate the mechanisms of drug resistance and then develop ways to overcome resistance effectively or to prevent its occurrence. As for clinical application, microarray can be used to compare the gene expression profiles directly from patient samples to differentiate between chemotherapy sensitive group and the resistant group. It will really help to decide the treatment modalities. Thus, microarray analysis will be applied for personalization of chemotherapy such as selection of effective chemotherapy protocol and prediction of therapeutic efficacy in near future.

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