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Hong Bing Deng · Mahesha Adikari
Hemant K. Parekh · Henry Simpkins

Ubiquitous induction of resistance to platinum drugs in human ovarian, cervical, germ-cell and lung carcinoma tumor cells overexpressing isoforms 1 and 2 of dihydrodiol dehydrogenase

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Abstract We have recently demonstrated that overexpression of dihydrodiol dehydrogenase (DDH) in human ovarian carcinoma cells (2008/C13*) is associated with cisplatin and carboplatin resistance. Furthermore, we have also elucidated that transfection of parental human ovarian carcinoma cells with a full-length DDH1 cDNA leads to induction of resistance to the platinum drugs. The development of cisplatin resistance in the transfected cells is associated with an increase in DDH enzyme activity. Previous studies have identified several different mechanisms for development of cisplatin resistance, including altered DNA repair capacity, increased GSH-based detoxification, and increased metallothionein content. However, none of these mechanisms has been found to be universally associated with the development of cisplatin resistance in tumor cells from different tissue sources. The present study was undertaken to assess whether overexpression of DDH1 or DDH2 (in human ovarian, cervical, lung and germ-cell tumor cell lines) could specifically induce resistance to the platinum drugs in these cell lines. We demonstrated a ubiquitous association of increased expression of DDH1 or DDH2 (as judged by increased enzyme activity in transfected clones) with development of resistance to cisplatin and carboplatin. Moreover, we also found a lack of cross-resistance to anticancer drugs that have a different mode of action including paclitaxel, vincristine, doxorubicin hydrochloride, and melphalan. Although at present it is not clear how DDH is involved in platinum drug resistance, the identification of this gene as a causal factor in a series of cell lines derived from different tumors with different intracellular compositions indicates the importance of deciphering this hitherto undefined pathway which can produce resistance to platinum drugs.

Keywords Human tumor cells · Ectopic expression · Dihydrodiol dehydrogenase isoform 1 · Dihydrodiol dehydrogenase isoform 2 · Platinum drug resistance

Abbreviations ARE: Antioxidant response element · Cisplatin: *cis*-Diamminedichloroplatinum · DDH: Dihydrodiol dehydrogenase · GST: Glutathione S-transferase · MRP: Multiple drug resistance-associated protein

Introduction

Cisplatin, one of the most widely used anticancer drugs, has been found to be effective against a wide variety of cancers [17]. The basis of its cytotoxic effects has been postulated to be its reactivity with the N7-position of guanosine in nucleic acids, particularly DNA, leading to the formation of intrastrand and interstrand crosslinks [28]. Unfortunately, the efficacy of cisplatin is restricted due to the existence of intrinsic tumor cell resistance or to the acquisition of tumor cell resistance subsequent to drug treatment [32].

In order to decipher the mechanisms of cisplatin resistance, in our laboratory we have utilized the parental human ovarian carcinoma cell line (2008) and its ninefold cisplatin-resistant variant (2008/C13*). Several biochemical alterations thought to be associated with cisplatin resistance have been identified in the 2008/C13* cells. Previous work in our laboratory and that from other laboratories has demonstrated that the 2008/C13* cells exhibit a decreased intracellular accumulation of cisplatin [2], increased replicative bypass of cisplatin-DNA adducts [19], reduced expression of membrane-

H. B. Deng · M. Adikari · H. K. Parekh · H. Simpkins
Department of Pathology and Laboratory Medicine, Temple University School of Medicine, 3400 N. Broad Street, Philadelphia, PA 19140, USA

H. Simpkins (✉)
Fels Institute of Cancer Research and Molecular Biology, Temple University School of Medicine, Room 206, OMS, 3400 N. Broad Street, Philadelphia, PA 19140, USA
E-mail: simpkih@tuhs.temple.edu
Tel.: +1-215-7074353
Fax: +1-215-7072781

associated β-tubulin [6], and decreased expression of the intermediate filament, cytokeratin 18 [26]. Moreover, the mitochondria in the 2008/C13* appear morphologically aberrant and these cells are hypersensitive to lipophilic cations as compared to the parental cells [1]. Variations in the activation of protein kinase C activity [13] and in the cAMP signal transduction pathway [20] have also been observed in 2008/C13* cells.

An increased level of expression of the oncogene c-*fos* in the 2008/C13* cells has recently been reported, and partial reversal of the cisplatin resistance phenotype has been achieved by treatment of the 2008/C13* cells with an antisense oligonucleotide directed against c-*fos* [25]. Examination of the basal levels of the drug-detoxifying enzyme GST and the drug transport pump (MRP, involved in the transport of drugs conjugated to glucuronides) has revealed no significant difference between the parental 2008 and the cisplatin-resistant 2008/C13* cells (unpublished observations; [27]). All these data indicate that there are multiple mechanisms interconnected in a very complex way responsible for cisplatin resistance in the 2008/C13* cell line, some of which may have clinical relevance.

Recently, utilizing the cDNA microarray technique and the 2008 and 2008/C13*, cells lines, we have demonstrated a causal relationship between the overexpression of dihydrodiol dehydrogenase (DDH; also termed AKR1C) and the development of resistance to cisplatin and carboplatin [11]. DDH belongs to a superfamily of monomeric, cytosolic NADP(H)-dependent oxidoreductases that catalyze the metabolic reduction and either activation or inactivation of several xenobiotics [29–31]. In fact, increased expression of a carbonyl reductase has been demonstrated in a doxorubicin-resistant tumor cell line [3]. Specifically, increased expression of DDH has been shown in an ethacrynic acid-resistant colon carcinoma cell line [8]. The aforementioned drugs require metabolic conversion to either an active moiety (in the case of doxorubicin) or an inactive moiety (in the case of ethacrynic acid). However, metabolic activation/inactivation of cisplatin by DDH has not been previously demonstrated, especially in the development of the drug-resistant phenotype. In humans, at least four isoforms of DDH have been identified and characterized [4], viz. DDH1 (also called AKR1C1), DDH2 (AKR1C2), DDH3 (AKR1C3) and DDH4 (AKR1C4). Indeed, increased expression of DDH has been found to be a poor prognostic factor in patients with non-small-cell lung cancer [12].

The present study was undertaken to address several important issues regarding the role of DDH in the development of tumor cell resistance to platinum drugs. Considering that platinum drugs are utilized as first-line treatment in patients with cervical carcinomas, germ-cell tumors as well as lung adenocarcinomas, it was of interest to determine if overexpression of DDH could be causally associated with development of cisplatin and carboplatin resistance in a variety of tumor cell lines from different tissue sources or whether its overexpression induces resistance only in human ovarian carcinomas. Furthermore, due to significant similarity between the two major isoforms of DDH (viz. DDH1 and DDH2), and lack of information regarding their tissue-specific expression, it was also important to decipher the role (if any) of these individual isoforms in the development of tumor cell resistance to platinum drugs. Finally, we also wanted to examine if increased expression of DDH can induce a multidrug-resistant phenotype that is distinct from that induced by overexpression of the drug efflux pumps, P-glycoprotein and MRP.

Experimental procedures

Materials. Cisplatin was purchased from Aldrich (Milwaukee, Wis.). Carboplatin, vincristine, doxorubicin hydrochloride, melphalan, 1-acenaphthenol, and $NADP^+$ were obtained from Sigma Chemical Co. (St. Louis, Mo.). Paclitaxel was generously provided by the Drug Synthesis and Chemistry Branch, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, NIH. Cell culture reagents and gentamicin were obtained from Cellgro (Herndon, Va.).

Cell Lines. The human ovarian carcinoma cells A2780 and SKOV-3 as well as the cisplatin-sensitive and cisplatin-resistant 2008 and 2008/C13*, respectively, were grown in RPMI 1640 medium supplemented with 10% fetal bovine serum and gentamicin at a final concentration of 10 μg/ml as described previously. The A431 (human cervical carcinoma) cells, the Tera-2 (human germ-cell tumor) cells and the Calu-6 (human lung adenocarcinoma cells) cells were obtained from ATCC (Manassas, Va.) and maintained as instructed by the supplier.

Anticancer drug sensitivity of parental cells constitutively overexpressing either DDH1 or DDH2. In order to correlate the function of each of the isoforms of DDH (DDH1 and DDH2) with the cisplatin resistance phenotype, it was necessary to evaluate the effects of forced overexpression of a recombinant protein of each of these genes on anticancer drug cytotoxicity. Thus, primer pairs from the mRNA sequence of each of the candidate genes (using the full-length sequence data from the sequence deposited in the GenBank database; see reference 13) were designed to enable us to generate a full-length cDNA. These were then cloned into the eukaryotic expression vector pCR3.1 (Invitrogen, San Diego, Calif.). Orientation of the full-length cDNA as well as its sequence were determined by restriction enzyme digestion and automated DNA sequencing, respectively. The expression vector with the insert in the right orientation were then transfected [26] into subconfluent parental human tumor cell lines using the lipofectamine reagent

(GIBCO-Life Sciences). The transfected cells were propagated in a medium containing 500–1000 μg/ml geneticin (G418 sulfate) for 3–5 weeks. Individual G418-resistant colonies were picked (20 colonies for each transfection experiment), grown and screened for the expression of the recombinant protein using the enzyme activity assays described below.

Utilizing immunohistochemical technique and enzyme activity assay, we have previously demonstrated [11] a positive correlation between increased expression of the DDH gene product (endogenous as well as the recombinant protein) and the enzyme activity (using 1-acenaphthenol as substrate). Thus, in this study we utilized enzyme activity assays as a surrogate for assessing the intracellular levels of the ectopically expressed DDH1 and DDH2 proteins since the antibodies recognize primarily DDH1. It is also important to note that the suffix "D" or the suffix "DD" following the name of the cell line indicates whether the clones were transfected with DDH1 or DDH2, respectively; this suffix is followed by the number of the clone utilized in this study. The clones that expressed a high level of the recombinant message were then subjected to growth inhibition assays in the presence of different concentrations of various anticancer drugs. The cytotoxic effects of these drugs were assessed using a tetrazolium dye as described previously [26].

Dihydrodiol dehydrogenase enzyme activity. The DDH enzyme activity was measured in the cytosol fraction of the parental and DDH1 or DDH2 transfected human tumor cell lines as described previously [8]. The rate of formation of NADPH at 340 nm (extinction coefficient, 6,500 M^{-1} cm^{-1}) was assessed. For isolation of the cytosolic fraction, cells were plated at density of 2×10^6 cell/dish and allowed to adhere overnight. The cells were then washed (3×) with chilled phosphate-buffered saline, and collected by scraping in a buffer containing 10 mM sodium phosphate, pH 7.4, and 150 mM KCl and 0.5 mM EDTA (Buffer A). After a brief centrifugation, the cells were resuspended in Buffer A containing a protease inhibitor cocktail and homogenized with a glass Dounce homogenizer with a tight fitting pestle. The lysed homogenate was centrifuged at 14,000*g* for 20 min. The supernatant fraction thus obtained was further centrifuged at 100,000*g* for 60 min to separate the cytosol fraction (supernatant) from the microsomal fraction (pellet). Aliquots of the cytosolic fraction were immediately stored at −70°C. The protein concentration of the cytosol fraction was determined by the Coomassie blue dye-binding assay using commercially available Bio-Rad protein assay reagent and bovine serum albumin as standard.

The cytosolic fractions were then assayed for DDH activity essentially as described previously [8]. Briefly, the assay mixture consisted of 4 m*M* $NADP^+$, 100 m*M* potassium phosphate, the indicated concentrations of the substrate and the cytosolic fraction. The reaction was started by addition of substrate, and the disappearance of NADPH at 25°C was monitored with the aid of a Beckman DU-70 recording spectrophotometer. An assay mixture containing all of the components except the substrate served as the blank. Initial rates of NADPH disappearance were determined in duplicate.

Results

Effect of constitutive overexpression of DDH1 or DDH2 on the cisplatin and carboplatin sensitivity of the parental human ovarian carcinoma cells. Whilst the 2008/C13* cells were found to be ninefold resistant to cisplatin compared to the parental 2008 cells, overexpression of DDH1 in 2008 cells increased the cisplatin IC_{50} value against 2008/D2 and 2008/D12 by eightfold and sevenfold, respectively (Table 1), when compared with the parental 2008 cells (IC_{50} 2 ± 0.4 μ*M*). Also of note was that overexpression of DDH1 induced resistance to the cisplatin analogue carboplatin, commonly used in the treatment of advanced ovarian carcinomas (Table 1). The 2008/C13* cells were found to be 16-fold resistant to the cytotoxic effects of carboplatin compared to the 2008 cells (IC_{50} 36 ± 11 μ*M*). In addition, the DDH1-transfected clones (2008/D2 and the 2008/D12) were threefold resistant to carboplatin compared to the 2008 cells (Table 1). In order to extend these initial studies [11] to include the other common isoform of DDH (DDH2), we also transfected DDH2 in 2008 cells. Constitutive overexpression of DDH2 in 2008 cells was found to induce a threefold resistance to cisplatin and a 2.4-fold resistance to carboplatin in the 2008/DD21 clone (Table 1). As shown in Table 1, the induction of resistance to platinum drugs correlated very well with the increased DDH enzyme activity observed in the transfected clones. The parental cells demonstrated an activity of 6.7 nmol/min/mg protein when 1-acenaphthenol was utilized as a substrate in the presence of $NADP^+$ (4 m*M*). Comparatively, the DDH activity in the 2008/C13*, 2008/D2, 2008/D12 and the 2008/DD21 cells was fivefold, fourfold, fourfold and threefold higher, respectively (Table 1). Thus, the induction of resistance to platinum drugs in the transfected clones (as well as the 2008/C13* cells) was functionally associated with an increase in the DDH enzyme activity suggesting a direct correlation between the two phenotypes.

Effect of constitutive overexpression of DDH1 or DDH2 on the cisplatin and carboplatin sensitivity of the parental human ovarian carcinoma cell lines, A2780 and SKOV-3 cells. Unequivocal evidence has been presented that demonstrates a causal link between upregulation of DDH1 or DDH2 and development of cisplatin and carboplatin resistance in 2008 cells. In an attempt to understand whether such a phenomenon was cell line-specific or not, we transfected two additional human

Table 1 Overexpression of DDH1 or DDH2 in human ovarian carcinoma cell lines (as judged by enzyme activity) and its association with cisplatin and carboplatin sensitivity of parental human ovarian carcinoma cell lines and the DDH1- or DDH2-transfected clones. The IC_{50} values were determined by the MTT assay as described in "Experimental procedures". The values presented are the means ± SD from at least three independent experiments, each performed in triplicate. The degree of resistance was calculated as the ratio of anticancer drug IC_{50} value in individual transfected clonal cell lines to the anticancer drug IC_{50} value in the parental cells. The results of the enzyme activity assay are presented as means ± SD of two independent experiments each performed in duplicate. The DDH activity in the mock-transfected (empty vector-tranfected) cells using 1-acenaphthenol as substrate was essentially similar to that observed in the parental cells; thus the enzyme activity utilizing only the parental cells is presented. It is important to note that the suffix "D" or the suffix "DD" following the name of the cell line indicates whether the clones were transfected with DDH1 or DDH2, respectively; this suffix is followed by the number of the clone utilized in this study

Cells	IC_{50} (μM)		DDH activity (nmol/min/mg protein)
	Cisplatin	Carboplatin	
2008 (parental)	2.1 ± 0.4	35.8 ± 11.1	6.7 ± 2.0
2008/C13*	18.6 ± 1.8 (8.9)	578 ± 119 (16.1)	32.8 ± 8.4 (4.9)
2008/D2	16.1 ± 2.0 (7.7)	97.4 ± 20.9 (2.7)	26.3 ± 5.5 (3.9)
2008/D12	13.8 ± 3.0 (6.6)	101 ± 18.4 (2.8)	25.4 ± 8.2 (3.8)
2008/DD21	6.2 ± 1.2 (3.0)	87.1 ± 6.1 (2.4)	21.3 ± 9.0 (3.2)
A2780 (parental)	0.3 ± 0.1	4.1 ± 0.7	None detected
A2780/D5	1.2 ± 0.1 (4.0)	18.3 ± 4.1 (4.5)	11.4 ± 2.1
SKOV3 (parental)	5.4 ± 1.4	73.5 ± 21.6	47.3 ± 3.0
SKOV/D6	16.5 ± 0.4 (3.0)	219 ± 68 (3.0)	58.1 ± 2.7 (1.2)

ovarian tumor cell lines (A2780 and SKOV-3). The DDH enzyme activities and the cisplatin and carboplatin sensitivity of the parental and the DDH-transfected clones are presented in Table 1. The DDH enzyme activity was below the limit of detection in the A2780 cells, whereas the SKOV-3 cells displayed an activity equivalent to 47.3 nmol/min/mg protein. The cisplatin IC_{50} against the parental A2780 and the SKOV-3 cells was 0.3 ± 0.1 μM and 5 ± 1 μM, respectively. Forced, constitutive overexpression of DDH1 resulted in a fourfold increase in the cisplatin and carboplatin resistance of the A2780-transfected clones and this correlated well with a significant increase in the DDH enzyme activity observed in the A2780/D5 clone (Table 1). Similarly, the SKOV-3-transfected clones displayed a threefold resistance to cisplatin and carboplatin as compared to the parental cells, although the basal enzyme activity in the parental cells was relatively high (Table 1).

Effect of constitutive overexpression of DDH1 or DDH2 on the cisplatin and carboplatin sensitivity of the parental human cervical, germ-cell tumor and lung carcinoma. In addition to their efficacy in the treatment of human ovarian carcinomas, cisplatin and/or carboplatin have been shown to have a high therapeutic index against cervical carcinomas, germ-cell tumors and lung cancers. Importantly, increased expression of DDH in non-small-cell lung cancer has been demonstrated as an indicator of a poor prognosis. Thus, we assessed the effects of increased expression of either DDH1 or DDH2 in tumor cell lines from these tissue sources on their cisplatin and carboplatin sensitivity. Utilizing the assay conditions outlined in "Experimental procedures" and 1-acenaphthenol as subtrate, basal DDH activity was undetectable in all of the cell lines utilized (A431, Tera-2 and Calu-6). Based on our MTT assay data (Table 2), the Tera-2 cells (germ-cell tumor) were found to be highly sensitive to the cytotoxic effects of cisplatin and carboplatin. Overexpression of DDH1 in the Tera-2 cells (as judged by increase in the enzyme activity) conferred a 46-fold and 35-fold resistance to the cytotoxic effects of cisplatin and carboplatin, respectively (Table 2).

In addition, forced expression of DDH1 or DDH2 in the cervical carcinoma cells (A431) induced a twofold cisplatin resistance (Table 2) and in the case of DDH1, induced a 2.5-fold resistance against carboplatin (Table 2).

Transfection of either DDH1 or DDH2 in the human lung carcinoma cells (Calu-6) led to isolation of clones expressing enzyme activity of 29.1 or 26.2 nmol/min/mg protein, respectively (the parental cells have undetectable levels of DDH activity; Table 2). Increased expression of DDH1 in Calu-6 cells conferred a sixfold and twofold resistance to the cytotoxic effects of cisplatin and carboplatin, respectively, (Table 2). Similarly, increased expressed of DDH2 in the Calu-6 cells led to a twofold resistance against the platinum drugs (Table 2).

Of particular significance to the present study was our observation of a high level of endogenous DDH activity (104.9 nmol/min/mg protein) in the human lung adenocarcinoma cell line, A549; Table 2). Of all the cell lines utilized in this study (Tables 1 and 2), the parental A549 cells were least sensitive to cisplatin (IC_{50} 23.2 ± 2.1 μM) and carboplatin (IC_{50} 206 ± 11.3 μM) (Table 2). Thus, the increased DDH activity observed in these cells correlates well with the intrinsic platinum drug resistance of these cells.

Cross-resistance profile of DDH1-expressing human tumor cells. We have provided extensive evidence that confirms the role of DDH1 and DDH2 in the development of cisplatin and carboplatin resistance in a variety

Table 2 Overexpression of DDH1 or DDH2 in human tumor cell lines (as judged by enzyme activity) and its association with cisplatin and carboplatin sensitivity of parental human tumor cell lines and the DDH1 or DDH2 transfected clones. The IC_{50} values were determined by the MTT assay as described in "Experimental procedures". The values presented are means ± SD from at least three independent experiments, each performed in triplicate. The degree of resistance was calculated as the ratio of the anticancer drug IC_{50} value of individual transfected clonal cell lines to the anticancer drug IC_{50} value of the parental cells. The results of the enzyme activity assay are presented as means ± SD of two independent experiments each performed in duplicate. The DDH activity in the mock-transfected (empty vector-transfected) cells using 1-acenaphthenol as substrate was essentially similar to that observed in the parental cells; thus the enzyme activity utilizing only the parental cells is presented. It is important to note that the suffix "D" or the suffix "DD" following the name of the cell line indicates whether the clones were transfected with DDH1 or DDH2, respectively; this suffix is followed by the number of the clone utilized in this study

Cells	IC_{50} (μ*M*)		DDH activity (nmol/min/mg protein)
	Cisplatin	Carboplatin	
Human cervical carcinoma cells			
A431 (parental)	4.9 ± 1.1	37.3 ± 2.0	None detected
A431/D9	10.6 ± 1.1 (2.2)	93.6 ± 3.2 (2.5)	26.3 ± 2.2
A431/DD9	9.6 + 0.8 (1.9)	ND	22.3 ± 5.4
Human germ-cell tumor			
Tera-2 (parental)	0.5 ± 0.02	0.8 ± 0.1	None detected
Tera/D9	23.0 ± 0.4 (46)	27.7 ± 8.9 (34.6)	32.5 ± 7.8
Human lung carcinoma cells			
Calu-6 (parental)	1.5 ± 0.8	24.7 ± 9.6	None detected
Calu/D7	9.4 ± 1.3 (6.3)	51.6 ± 6.9 (2.1)	29.1 ± 2.5
Calu/DD13	3.7 ± 0.04 (2.5)	42.9 ± 10.3 (1.7)	26.2 + 5.1
A549 (parental)	23.2 ± 2.1	206 ± 11.3	104.9 ± 0.9

of tumor cell lines. In an effort to determine if DDH expression alters the sensitivity of tumor cells to other structurally and functionally different anticancer drugs, we performed growth inhibition assays utilizing the parental and DDH1-transfected clones listed in Tables 1 and 2. As shown in Table 3, except for the Tera/D9 cells, none of the DDH1-transfected clones was found to be cross-resistant to paclitaxel, vincristine, doxorubicin hydrochloride or melphalan. In Tera/D9 cells, a 17-fold and 7-fold cross-resistance against the cytotoxic effects of vincristine and doxorubicin hydrochloride, respectively, was evident. The reason(s) for this anomalous behavior of the DDH1-overexpressing Tera-2 cells is not clear at present. Considering that increased expression of carbonyl reductase has been previously associated with doxorubicin resistance, it is possible that metabolic inactivation of doxorubicin hydrochloride in the Tera/D9 cells could account for the observed resistance. Furthermore, considering the similarity between the DDH1 and DDH2 isoforms, we assume (although we

Table 3 Cross-resistance profile of DDH1-overexpressing human tumor cells. The IC_{50} values were determined by the MTT assay as described "Experimental procedures". The values presented are as means ± SD and are from at least three independent experiments, each performed in triplicate. The degree of resistance was calculated as the ratio of the anticancer drug IC_{50} value in the individual transfected, clonal cell line to the anticancer drug IC_{50} value in the parental cells

Cells	IC_{50}			
	Paclitaxel (n*M*)	Vincristine (n*M*)	doxorubicin hydrochloride (n*M*)	Melphalan (μ*M*)
Human ovarian carcinoma cells				
2008	39.3 ± 0.9	21.0 ± 0.5	706 ± 87.5	9.0 ± 2.9
2008/C13*	10.1 ± 6.6	16.0 ± 5.2	377 ± 56.5	8.8 ± 0.9
2008/D2	28.3 ± 3.1	13.6 ± 2.0	375 ± 50.2	5.7 ± 1.3
A2780	12.9 ± 4.5	2.6 ± 1.4	0.1 ± 0.03	1.0 ± 0.1
A2780/D5	12.0 ± 2.3	2.9 ± 0.5	0.1 ± 0.007	0.5 ± 0.3
SKOV-3	188 ± 33.2	1.4 ± 0.1	2.2 ± 0.1	8.7 ± 1.8
SKOV/D6	193 ± 15.5	1.3 ± 0.2	1.6 ± 0.3	7.7 ± 1.0
Human cervical carcinoma cells				
A431	121 ± 80	1.6 ± 0.3	252 ± 72.0	> 25.0[a]
A431/D9	94.5 ± 70.2	1.4 ± 0.3	206 ± 7.4	> 25.0
Human germ cell tumor				
Tera-2	220 ± 101	0.2 ± 0.09	52.7 ± 8.9	> 25.0
Tera/D9	187 ± 43.9	3.0 ± 0.6 (17)	373 ± 123 (7)	> 25.0
Human lung carcinoma cells				
Calu-6	169 ± 53	17.1 ± 11.4	752 ± 331	> 25.0
Calu/D7	101 ± 15.8	8.6 ± 2.9	536 ± 64.5	> 25.0

[a]Precise values cannot be determined because higher concentrations of melphalan (solubilized in DMSO at it highest solubility levels) cannot be utilized

have not performed this analysis) that increased expression of DDH2 in these cell types would not induce resistance to paclitaxel, vincristine, doxorubicin hydrochloride or melphalan.

Discussion

The present report details the extensive analysis of the effects of increased expression of DDH1 or DDH2 on the cisplatin and carboplatin sensitivity of human ovarian carcinoma, cervical carcinoma, germ-cell tumor and lung adenocarcinoma cells. The results presented clearly indicate the universality of the association of increased expression of DDH with the development of tumor cell resistance against platinum drugs. Furthermore, our study also demonstrates that both DDH1 and DDH2 have the ability to confer platinum drug resistance in all the human tumor cell line studies. These observations have very important implications for the utility of the platinum drugs in relation to their efficacy in the chemotherapeutic treatment of cervical carcinomas, germ-cell tumors and/or lung cancers.

The mechanisms underlying cisplatin resistance are thought to be multifactorial and include decreased drug accumulation [2, 18], enhanced cellular detoxification due to increased levels of reduced glutathione [23] and intracellular metallothioneins [14, 15] as well as increased DNA repair [16, 22]. In addition, alterations in oncogene expression and signal transduction pathways have also been suggested to be associated with cisplatin resistance [10]. Thus, the altered expression of c-fos, H-ras, c-myc, Her-2/neu, Bcl-2, or Bcl-X_L have been found to confer cisplatin resistance after they are artificially overexpressed, but precisely how these lead to cisplatin resistance is poorly defined [5, 21, 24]. Moreover, none of these changes have been associated with the development of cisplatin resistance in multiple tumor cell lines. This suggests that mechanisms responsible for the development of cisplatin resistance in an experimental setting may not be ubiquitous in conferring such resistance in other tumor types (not surprising since the cellular milieu is likely to be markedly different). Furthermore, the disparate biochemical functions of the so-called ''cisplatin-resistance associated genes'' have made it difficult to define whether these genes play a primary or secondary role in the development of cisplatin resistance. The notion that a single, universal factor is not responsible for mediating cisplatin resistance under both clinical as well as experimental settings has hampered the efforts aimed at development of therapies that would aid in circumventing either the intrinsic or the acquired drug resistance phenotype.

We had previously demonstrated that overexpression of DDH1 could induce the cisplatin-resistant phenotype observed in three different human ovarian carcinoma cells [11]. In the present study we show that ectopic expression of DDH2 in human ovarian carcinoma cells conferred cisplatin and carboplatin resistance in these cells, providing evidence for the first time of the role of both these enzymes in the development of platinum drug resistance. In addition, we also showed that overexpression of DDH1 or DDH2 induced cisplatin and carboplatin resistance in human cervical, germ-cell tumor cells as well as lung carcinoma cells. Of critical importance is the observation that increased expression of either DDH does not induce cross-resistance to other commonly utilized anticancer drugs (except in the case of Tera/D9 cells, see Table 3).

The involvement of the oxidoreductase family of enzymes in drug resistance has been previously documented. Thus, up-regulation of a carbonyl reductase has been reported to induce the development of doxorubicin resistance in tumor cells [3]. Increased expression of DDH has also been observed in an ethacrynic acid-resistant human colon carcinoma cell line [8] and was thought to contribute to the drug-resistant phenotype of these cells, although increased expression of GST was later postulated to be the causative factor. The overexpression of DDH has been thought to be associated with an increased binding of trans-activating factors (transcription factors) to an antioxidant response element (ARE) 5′ to the DDH gene transcription start site [9]. Ciaccio et al. have demonstrated indirectly, the existence of an ARE-like element in the 5′ flanking region of the DDH gene that is required for transcriptional activation by ethacrynic acid [9]. To date three proteins have been identified in an ARE binding complex, viz. jun-D, c-fos and jun-B. These proteins in different combinations make up the complex of transcription factor termed AP-1. Our preliminary results indicate that the AP-1 binding activity is threefold higher in the nuclear extracts of the 2008/C13* cells as compared to that observed in the nuclear extracts of the 2008 cells (unpublished observation).

Whilst the foregoing discussion sheds some light on the factors involved in the transcriptional up-regulation of the DDH gene, what is not known at present is the mechanism whereby overexpression of DDH would lead to development of cisplatin resistance. Not surprisingly, cisplatin is not a substrate for the DDH enzyme and neither does it inhibit the enzyme activity in vitro, when 1-acetonaphthol is used as a substrate (unpublished observations). These observations suggest that DDH is acting via a hitherto unidentified mechanism to induce cisplatin resistance. Detoxification of the free radicals generated by cisplatin [11] by the enzyme is a possibility. In this regard, increased expression of peroxiredoxin II, a cytosolic enzyme with peroxidase activity, has been shown to confer cisplatin resistance in a gastric carcinoma cell line [7]. Thus, it is plausible that an increase in the activity of DDH in the various human tumor cells would be sufficient to repair the biochemical lesions induced by cisplatin or carboplatin (due to generation of free radicals), thus leading to development of drug resistance. Detailed investigations in this regard are underway in our laboratory.

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