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Differential formation and enhanced removal of specific cisplatin–DNA adducts in two cisplatin-selected resistant human testicular teratoma sublines

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Mechanisms of cisplatin resistance have been studied in two independently-selected sublines expressing clinically-relevant levels of resistance (3-fold) and established from a primary testicular teratoma obtained from previously untreated patients. Resistance was not associated with any significant modification in cellular uptake of cisplatin, in total glutathione levels or associated enzyme activities. However, immunochemical quantitation of specific platinum–DNA adduct formation and removal revealed that both resistant sublines were more proficient in repairing certain adducts than their generally repair deficient respective parental lines. SUSA/CP$^+$ cells were more efficient in removing the intrastrand adducts in the sequence Pt–AG and the bifunctional Pt–$(GMP)_2$ lesions, as well as DNA–DNA interstrand cross-links, whilst H12.1/DDP cells were highly proficient in removing the major Pt–GG intrastrand adducts.

Key words: Cisplatin resistance, platinum–DNA adducts, repair of DNA damage, testicular teratoma cell lines.

Introduction

Initial investigations with a human tumor subline (SUSA) derived from a primary testicular teratoma taken from a previously untreated patient[1] provided evidence linking the apparent hypersensitivity to cisplatin identified in this cell line with an inability to repair platinated DNA.[2] Subsequently we reported that *in vitro* exposure of these SUSA cells to fractionated X-irradiation resulted in the expression of some resistance to cisplatin accompanied by enhanced repair of the intrastrand adduct PtAG and interstrand cross-links and increased tolerance of drug-induced damage.[3] The aim of the present studies was to establish whether selection for resistance by *in vitro* exposure to cisplatin using this SUSA cell line also resulted in a subline expressing any modified repair capacity or increased DNA damage tolerance. In this way, we planned to examine further our original hypothesis[4,5] that different resistance mechanisms may operate or predominate depending on whether the selection agent used was X-irradiation or specific antitumor drugs. In addition, we carried out a parallel series of investigations as part of a collaborative study, using another human tumor cell line (H12.1) also derived from a primary testicular teratoma taken from a previously untreated patient.[6] Preliminary results[7] indicated that this parental cell line also was sensitive to cisplatin and deficient in removing platinum–DNA intrastrand cross-links.

To identify the mechanisms of cisplatin resistance operating in these cisplatin-selected resistant sublines, the following parameters were examined: cisplatin uptake; glutathione levels and associated enzyme activities; formation and removal of the four major platinum–DNA adducts and of interstrand cross-links.

Materials and methods

Derivation, maintenance and characterization of cell lines

The SUSA parental (P) cell line was established from a human testicular teratoma obtained from a previously untreated patient.[1] This line was maintained as a monolayer in RMPI 1640 medium supplemen-

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ted with 10% FCS at 37°C in a humidified atmosphere of 5% CO_2 in air. Media and sera were purchased from Gibco-Biocult (Renfrewshire, UK). The cisplatin resistant subline was established at the ICRF Laboratories from the parental line by mutagenesis with ethylmethane sulfonate (EMS) (35 μg/ml for 24 h) followed by exposure to 150 ng/ml cisplatin for 20 days before repeating the EMS mutagenesis (100 μg/ml for 24 h) and then adding cisplatin (300 ng/ml) for 5 days, prior to removing the drug and adding fresh medium. One of the few surviving colonies was isolated and expanded and the resultant culture was exposed to 250 ng/ml cisplatin for 2 weeks, followed by 1 week's growth in drug-free medium. The drug responses of this established subline to cisplatin were then established by colony forming assay. This subline designated SUSA/CP$^+$, was serially maintained by continuous exposure to cisplatin (250 ng/ml) for 2 weeks followed by 1 week's growth in the absence of drug. For experimentation, cells were used after this period of growth in drug-free medium. Routine culture conditions of this subline once established were identical to those used for the parental line, which was also maintained in culture concurrently during the period of development of the subline. Cell line H12.1 was established from an orchiectomy specimen of a 19 year old previously untreated patient. The primary histology showed seminoma, embryonal carcinoma, mature teratoma and chorioncarcinoma. This cell line was maintained as a confluent monolayer in RPMI 1640 medium supplemented with 10% FCS. The production of β-human-choriogonadotropin *in vitro* has been demonstrated.[6] The cisplatin resistant subline H12.1/DDP was selected at the University of Hannover Medical School by repeated pulsed exposures to cisplatin. Cells were allowed to grow to confluency and then exposed to cisplatin for 1 h prior to trypsinisation, washing and re-seeding into new flasks. The initial concentration of cisplatin was 0.1 μg/ml and this was increased gradually to 10 μg/ml over a period of 49 weeks. The resulting resistance has proved stable without further drug exposure for at least 3 months. Table 1 lists the population doubling times, colony-forming efficiencies and cell volumes of the two parental and cisplatin resistant sublines. There were no significant differences in any of these parameters between the two pairs of cell lines, nor any evidence that any of these properties changed significantly during a period of 3 months in continuous culture.

Cytotoxicity assays

Cytotoxicity assays on the SUSA lines were carried out using the soft agar clonogenic assay method of Courtenay *et al.*,[8] whilst the H12.1 lines were cloned directly into 0.16% agarose.[9] Cisplatin was purchased from Sigma Chemicals (Poole, UK).

Total glutathione (GSH) levels, and total glutathione S-transferase (GST), glutathione reductase (GR) and glutathione peroxidase (GP) activities

Logarithmically-growing cells were harvested 3 days after initial plating. The assays used were as follows: GSH content was measured by the GR method of Griffith;[10] GST according to the proce-

Table 1. Cell line characteristics

Cell line	Subline derivation (origin)	Cisplatin[a] IC_{50}(μg/ml)	PDT[b] (h)	Cell volume (μm^3)	CFE[c] (%)
SUSA/P	(primary teratoma)	0.40 ± 0.05	27 ± 2	2374 ± 166	5–15
SUSA/CP$^+$	mutagenesis by EMS and then selection in continuous cisplatin	1.20 ± 0.15	26 ± 1	2402 ± 120	5–15
H12.1/P	(primary teratoma —embryonal ca)	0.26 ± 0.03	38 ± 3	2491 ± 26	1–4
H12.1/DDP	pulsed 1 h exposures to increasing cisplatin concentrations	0.81 ± 0.10	35 ± 6	2543 ± 16	2–5

[a]Cells were exposed to drug for 1 h and results derived from full dose–response curves and expressed as IC_{50} values—the concentration required to reduce cell survival by 50% (± SEM).
[b]Population doubling times were established from logarithmically-growing cultures.
[c]Colony forming efficiencies determined by cloning the SUSA cell lines in soft agar or the H12.1 cell lines directly onto plastic.

dure of Habig and Jackoby,[11] using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate; GR by the method of Horn[12] for the SUSA cells or that of Smith *et al.*[13] for the H12.1 cells; and GP using cumene hydroperoxide according to the modified method of Paglia and Valentine[14] for the SUSA cells or the method of Gunzler *et al.*[15] for the H12.1 cells involving *t*-butyl-hydroperoxide. Enzyme values were normalized for cellular protein using the method described by Lowry *et al.*[16] The slightly different methodologies reflect the fact that experiments with the SUSA cell lines were carried out at the ICRF in London, whilst those on the H12.1 cell lines were conducted at the University of Hannover Medical School.

Drug uptake studies

For analyses of cisplatin uptake, cells (2×10^6) were exposed to 0, 10, 25 or 50 μg/ml cisplatin for 1 h. Then, according to the procedure described earlier,[17] cells were harvested and washed twice in ice-cold PBS. Total cell number per sample was calculated by hemocytometer counting. Cell pellets were dried at 65°C for 2 h prior to solubilization in concentrated nitric acid. Drug uptake was quantitated by atomic absorption spectophotometry (AAS) (Perkin Elmer model 4000) and results were expressed as pmol Pt/10^6 cells ±SE and then corrected for differences in cellular volume. Each experiment was repeated at least twice, using duplicate samples.

Quantitation of platinum–DNA adducts

Cells were treated for 1 h with 5 μg/ml (16.7 μM) cisplatin solubilized in 0.9% saline immediately prior to use and then washed with PBSA and harvested immediately or after an 18 h incubation in drug-free medium to permit 'repair' or adduct removal to occur. Cells were lysed in the presence of 100 mM ammonium bicarbonate to inactivate monofunctionally-bound drug and DNA was isolated using a phenol/chloroform procedure,[18] and enzymatically digested to nucleotides and platinated nucleotides. Separation of platinated products was carried out using an anion exchange chromatography column.[19] Details of the competitive enzyme-linked immunosorbent assay (ELISA) procedure using antiserum W101 to quantitate Pt–GG and $Pt(GMP)_2$ have been described in earlier publications.[2,20] Antisera 3/65 and 3/43 were used to detect Pt–AG and Pt–GMP adducts, respectively.[20]

Measurement of DNA–DNA interstrand cross-links

Interstrand cross-links were measured in the SUSA cell lines at 0, 14 and 24 h after a 1 h exposure to cisplatin using the alkaline elution technique of Kohn *et al.*,[21] with minor modifications detailed earlier.[22]

Results

Cisplatin cytotoxicity assays

Dose–response curves for the two parental testicular teratoma cell lines and their respective cisplatin resistant sublines are shown in Figure 1. IC_{50} values were interpolated and a comparison (see Table 1) shows that both the SUSA/CP^+ and the H12.1/DDP sublines proved 3-fold more resistant to cisplatin than their respective parental lines. This level of resistance has remained stable over a 3 month period of continuous culture. Exposure of SUSA cells to EMS treatment only did not significantly modify their responses to cisplatin.

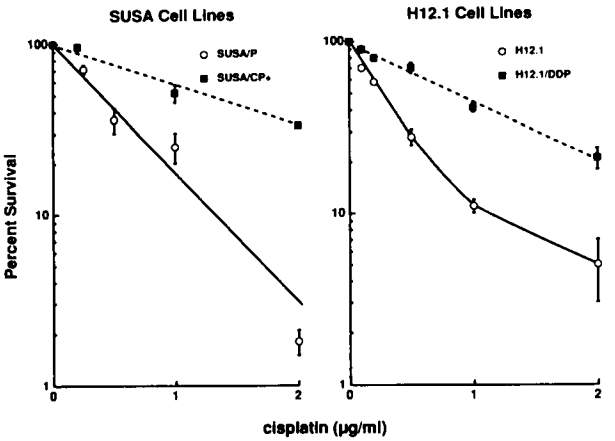

Figure 1. Survival curves for SUSA/P, SUSA/CP^+, H12.1 and H12.1/DDP cells exposed to a range of cisplatin concentrations for 1 h, as judged by colony forming assays. Points, mean of two to three experiments in which duplicate cultures were tested. Bars, SE

Table 2. Total glutathione levels[a] and associated enzyme activities[b]

Cell line	GSH (nmol/mg)	GST (units/mg)[c]	GR (units/mg)[d]	GP (units/mg)[d]
SUSA/P	18.8 ± 3.0	160.0 ± 4.0	23.3 ± 0.7	4.5 ± 0.7
SUSA/CP$^+$	20.6 ± 2.0	176.6 ± 9.4	18.5 ± 2.0	6.5 ± 1.2
H12.1/P	12.8 ± 1.6	750.3 ± 121.2	20.7 ± 4.8	32.8 ± 8.6
H12.1/DDP	13.6 ± 1.0	525.0 ± 24.3[e]	27.2 ± 3.2	18.5 ± 5.8[f]

[a]Mean values ± SE of duplicate assays of at least two experiments expressed per mg protein.
[b]Mean values ± SE of duplicate assays of two to four separate experiments.
[c]One unit is the amount of enzyme required to conjugate 1 nmol/min of CDNB at 25°C for SUSA or at 37°C for H12.1. Values are the mean ± SE of duplicate assays of two to four separate experiments.
[d]One unit is the amount of enzyme required to oxidize 1 nmole/min NADPH at 25°C for SUSA or at 37°C for H12.1. Values are the mean ± SE of duplicate assays of two to four separate experiments.
[e]$p = 0.04$.
[f]$p > 0.05$.

Cellular content of GSH, GST, GR and GP

Levels of total GSH and associated enzyme activities, normalized for cellular protein content, are listed in Table 2. In the SUSA/CP$^+$ subline none of these parameters were significantly altered from parental cell values. Similarly, GSH levels and activities of GR and GP were comparable amongst the two H12.1 cell lines, although GST activity proved significantly lower in the H12.1/DDP subline ($p = 0.04$). The considerably higher levels of activity of both GST and of GP recorded in the H12.1 lines versus the SUSA lines probably reflect the different assay methods used and the higher temperature employed in each assay (37 versus 25°C) by the two laboratories.

Cellular uptake of cisplatin

For each of the cell lines dose-dependent uptake of cisplatin was established (data not shown), with results of linear regression analyses indicating that the correlation coefficients ranged from 0.96 to 1.00. Drug uptake proved marginally lower in the SUSA/CP$^+$ cells versus SUSA/P cells (2.23±0.38 versus 3.05±0.38 pmol Pt/10^6 cells/μg Pt/unit cell volume). This 1.4-fold difference, however, was not statistically significant ($p > 0.05$). Similarly, no significant differences were noted between the H12.1 and H12.1/DDP cell lines with values of 2.79 ± 0.55 and 3.55 ± 0.91 pmol Pt/10^6 cells/μg Pt/unit cell volume ($p > 0.05$).

Induction and repair of platinum–DNA adducts

Levels of total DNA-platination immediately after a 1 h exposure to 5 μg/ml cisplatin (zero time) and following an 18 h post-treatment incubation are listed in Table 3. These data confirm our original observation[2] that the SUSA/P cells appear deficient in removal of adducts during the 18 h post-teatment incubation period and extend this observation to include another teratoma cell line H12.1, also established from a previously untreated patient. However, the total platination levels are about 2-fold higher in the H12.1/P versus the SUSA/P cells, consistent with their inherent 1.5-fold higher sensitivity to the cytotoxic effects of cisplatin (see Table 1). The major adduct formed in all lines is Pt–GG, but whereas this comprises 60–67% of the total platination immediately following drug treatment in both parental lines and in the H12.1/DDP cells, consistent with the pattern reported previously in other malignant teratoma[2,23], bladder[2] and ovarian[17,18,24] cell lines, in the SUSA/CP$^+$ cells this figure is only 40%. This reduction is accounted for by a rise in the proportion of Pt–AG adducts formed from 11% in the SUSA/P cells to approximately 30% of the total platination after treatment of these resistant cells.

Table 3. Total platination of DNA, determined as the total of the four platinum–nucleotides measured by competitive ELISA immediately and 18 h following exposure to 5 μg/ml cisplatin for 1 h

Cell line	Total platination level[a] (fmol Pt/μg DNA)		Percent removal (%)
	0 h	18 h	
SUSA/P	16.4 ± 2.3	15.7 ± 1.0	4
SUSA/CP$^+$	11.4 ± 0.6	10.6 ± 0.9	7
H12.1/P	28.7 ± 1.1	33.0 ± 2.2	0
H12.1/DDP	11.6 ± 1.0	9.1 ± 0.7	22

[a]Total platination was calculated by adding together the amount of each of the four individual adducts. Values are the mean ± range of two different ELISAs utilizing two samples independently-derived, each performed on four dilutions in dublicate wells.

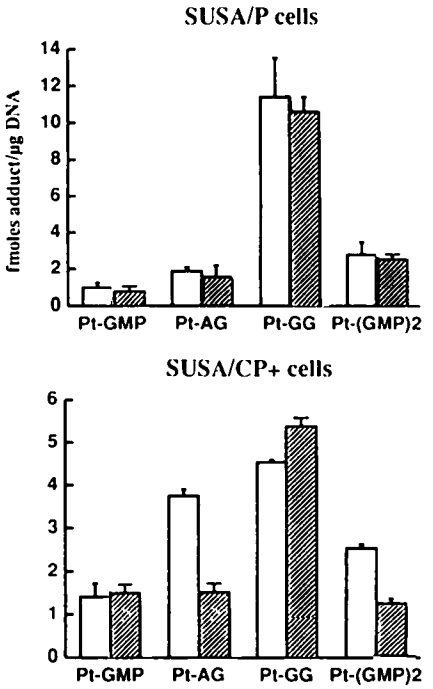

Figure 2. Induction and removal of Pt–GMP, Pt–AG, Pt–GG and Pt–$(GMP)_2$ in the human testicular teratoma cell lines SUSA/P and SUSA/CP^+. The number of adducts was determined immediately after a 1 h exposure to 5 μg/ml cisplatin (open boxes) and following an 18 h post-treatment incubation period (hatched boxes). Columns (± range) represent the mean (normalized for DNA content) of two different competitive ELISAs, each performed in four dilutions on duplicate cells.

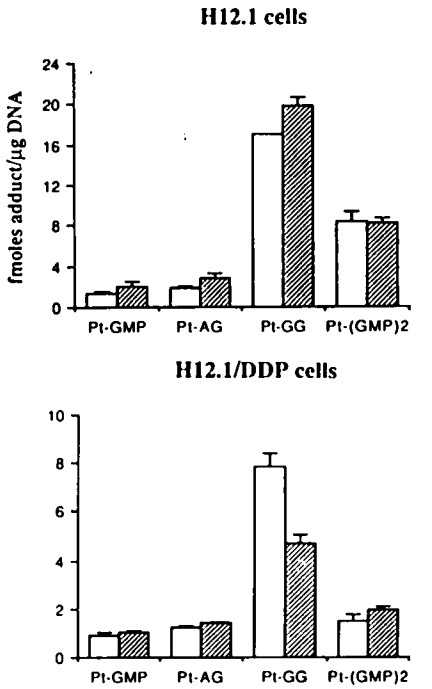

Figure 3. Induction and removal of Pt–GMP, Pt–AG, Pt–GG and Pt–$(GMP)_2$ in the human testicular teratoma cell lines H12.1 and H12.1/DDP. The number of adducts was determined immediately after a 1 h exposure to 5 μg/ml cisplatin (open boxes) and following an 18 h post-treatment incubation period (hatched boxes). Columns (± range) represent the mean (normalized for DNA content) of two different competitive ELISAs, each performed in four dilutions on duplicate cells.

Total platination immediately following drug treatment is marginally, but not significantly, reduced (1.4-fold) in the SUSA/CP^+ cells compared to SUSA/P cells and following an 18 h post-treatment period there is no significant reduction in total overall platination levels in either cell type. However, examination of specific adduct formation and removal (see Figure 2) shows that whilst SUSA/P and SUSA/CP^+ cells are both deficient in the removal of the major Pt–GG adducts, SUSA/CP^+ cells, unlike the parental cells, show definite proficiency in removing Pt–AG adducts and the bifunctional $Pt(GMP)_2$ lesions. The H12.1/DDP cells have a 2.5-fold lower level of total platination immediately following drug treatment than their parental cells (Table 3) reflecting, at least in part, their 3-fold level of resistance. These H12.1/DDP cells also showed some ability to remove PtDNA adducts during the 18 h post-treatment incubation period. Examination of specific adduct formation and removal in these H12.1 cell lines (see Figure 3) shows that it is the major Pt–GG lesion only which is repaired under these conditions by the resistant cells.

The technique of alkaline elution was used to quantitate interstrand cross-links in the SUSA cell lines. The results listed in Table 4 show some dose dependency for interstrand cross-links formation in both lines and the levels at 14 h appear comparable. However, whilst as reported earlier[2] the number of interstrand cross-links in the SUSA/P cells continued to increase over the 24 h period, in contrast in the SUSA/CP^+ cells there was a major reduction in the number of cross-links measured between 14 and 24 h, indicative of substantial repair of interstrand cross-links by this resistant subline. The higher ISC levels in the SUSA/P cells at 24 h may explain, at least in part, their 3-fold higher sensitivity to cisplatin.

Discussion

Relatively few *in vitro* studies have specifically addressed the problem of cisplatin resistance using testicular tumor cell lines,[2,24,25,26] although this is clearly a research area of definite clinical interest.

Table 4. DNA–DNA interstrand cross-linking in SUSA-P and SUSA-CP$^+$ cells, after a 1 h exposure to cisplatin

Cell line	Cisplatin (μg/ml)	Interstrand cross-links		Percent removal[b]
		14 h	24 h	
SUSA/P	5	7.4 ± 3.9	10.9 ± 4.5	0
	10	18.7 ± 4.9	20.8 ± 13.0	0
SUSA/CP$^+$	5	12.1 ± 9.4	1.6 ± 2.7	87
	10	19.4 ± 4.2	9.1 ± 12.7	53

[a]Values expressed in rad-equivalents ± SE of at least two experiments in which duplicate or triplicate filters were evaluated.
[b]Percentage decrease in ISC at 24 h compared with values at 14 h.

This publication describes two cisplatin-resistant sublines which have been derived from testicular tumor cell lines established form samples from previously untreated patients. These parental cell lines were cisplatin sensitive and had been shown to be deficient in the removal of the major DNA intrastrand cross-links.[2,7] One of the aims of this study, therefore, was to determine whether this repair capacity was modified in drug-selected resistant sublines. We have previously reported that exposure of one of these parental lines (SUSA) to fractionated X-irradiation *in vitro*, generated a subline expressing 3-fold resistance to cisplatin and proving more proficient in repairing the specific intrastrand adduct Pt–AG and DNA–DNA interstrand cross-links than the parental cells. In addition, therefore, we aimed to investigate whether different resistance mechanisms might be 'switched-on' in sublines selected for resistance to cisplatin by exposure either to the drug itself or to fractionated X-irradiation.

Both these newly derived testicular tumor sublines, established independently in two different laboratories, expressed a 3-fold order of resistance to cisplatin which has proved stable over 3 months in culture with the one line (SUSA/CP$^+$) being maintained in the intermittent presence of cisplatin, whilst the other line (H12.1/DDP) has not been further exposed to drug. Recently, we have shown that over a 6 month period growth of SUSA/CP$^+$ cells in the total absence of drug generated a line designated SUSA/CP$^-$ which had a comparable sensitivity to cisplatin as the original SUSA/CP$^+$ cell line. Walker *et al.*[26] reported a similar stability in a 4-fold cisplatin resistant SUSA subline established by continuous exposure to the drug over 11 months. These findings that cisplatin resistance once established appears stable are in general agreement with literature reports both from our laboratory and from other groups.[7,22,25,27–29]

The comparable levels of glutathione and related enzyme activities in these pairs of cell lines suggest that there is no differential inactivation of cisplatin via these pathways in the resistant sublines. The one exception, namely a reduction in GST activity in the H12.1/DDP cells is also consistent with this conclusion. Kelland *et al.*[25] also reported unchanged GSH levels in a 3.8-fold cisplatin resistant stable variant of the original GCT27 cell line. However, elevated GSH levels and significant elevations in the activities of certain GSH-associated enzymes, including GST, have been associated with cisplatin resistance in certain cell lines,[22,27–29] but this has not been an invariable finding.[3,27,29]

Resistance to cisplatin in drug-selected sublines of human origin has been associated with significantly decreased cisplatin accumulation,[17,29–32] but this is by no means a universal finding.[22,27,33,34] Uptake of drug was reduced marginally in the SUSA/CP$^+$ cells (1.4-fold) and increased slightly (1.3-fold) in the H12.1/DDP cells relative to their respective parental cells. Neither of these differences though were statistically significant. This tends to contrast with the significant reduction which was observed in the SUSA-DXR$_{10}$ subline[3] and the 1.6-fold reduction described in the GCT27cisR subline.[25] This marginal reduction in uptake in the SUSA/CP$^+$ cells was reflected in a reduced level (1.4-fold) of total adduct formation measured immunochemically. However, a more marked reduction (2.5-fold) in total adduct formation was apparent in the H12.1/DDP cells, which actually exhibited a slight increase in cisplatin uptake. Indeed, published reports indicate that reduced drug uptake may not be reflected in reduced total platination levels[4,24,35,36] and there are even reports of higher total platination levels of the DNA being quantitated in resistant cells despite their reduced cisplatin uptake.[3,17] This latter observation has been interpreted as indicative of increased tolerance to DNA damage.

Quantitation of the specific adducts formed and removed during a 18 h post-treatment incubation period showed that both cisplatin resistant testicular tumor sublines proved more proficient in repairing certain adducts than their respective parental cells. Thus SUSA/CP$^+$ cells were more efficient in removing the bifunctional Pt–(GMP)$_2$ and Pt–AG adducts formed, as well as interstrand cross-links and in these latter two respects were very similar to the irradiated cisplatin resistant SUSA-DXR$_{10}$ subline reported earlier.[3] However, with the H12.1/DDP cells proficient removal of the major PtGG adducts was identified. The marked increase in re-

pair of the major Pt–GG adducts was also reported recently in a cisplatin resistant subline established from a human ovarian tumor cell line (SK-OV-3), which was inherently repair deficient, by exposure to fractionated X-irradiation.[17] These results therefore suggest that irrespective of whether cisplatin resistance is selected for by exposure to the drug itself or to X-irradiation, removal of cisplatin-induced DNA damage can be modified in cells inherently repair deficient either by enhancing removal of the two major intrastrand cross-links Pt–GG or Pt–AG and/or of interstrand cross-links. However, it remains to be established whether these relatively modest reductions in platination of specific adducts are responsible for the differential cytotoxicity of cisplatin in these sublines. Although, it is interesting in this respect that it has been shown that repair synthesis by human cell extracts in cisplatin-damaged DNA is preferentially determined by minor adducts.[37] It is also very apparent that all these cells, both parental lines and resistant sublines, still retain and so appear to tolerate a very high level of total platination, even after an 18 h 'repair' period. Several studies have highlighted the fact that a substantial fraction of cisplatin adducts are never removed from DNA.[35,38,39] In addition, since the methods used here only measure DNA damage and its repair in the overall genome, heterogeneity of drug-induced DNA and its repair in specific genes, as reported in relation to cisplatin[39,40] cannot be ruled out. Future studies will investigate gene specific repair in these sublines and aim to monitor repair of specific platinum–DNA adducts introduced into a circular DNA duplex using cellular extracts prepared according to the procedures described earlier by Wood and his colleagues.[41,42]

In summary, we have described the derivation of two new cisplatin resistant sublines from human testicular tumors by cisplatin selection, which appear to express modified abilities to repair specific platinum–DNA lesions. These sublines should prove valuable in studies aimed at identifying the lesions responsible for repair synthesis induced by cisplatin damage.

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References

1. Hogan B, Fellows M, Avner P, *et al.* Isolation of a human teratoma cell line which expresses F9 antigen. *Nature* 1977; **270**: 51–58.
2. Bedford P, Fichtinger-Schepman AMJ, Shellard SA, *et al.* Differential repair of platinum–DNA adducts in human bladder and testicular tumor continuous cell lines. *Cancer Res* 1988; **48**: 3019–24.
3. Hill BT, Shellard SA, Hosking LK, *et al.* Enhanced DNA repair and tolerance of DNA damage associated with resistance to *cis*-diammine-dichloroplatinum(II) after *in vitro* exposure of a human teratoma cell line to fractionated X-irradiation. *Int J Rad Oncol Biol Phys* 1990; **19**: 75–83.
4. Hill BT, *In vitro* drug-radiation interactions using fractionated X-irradiation regimens. In: Hill BT, Bellamy AS, eds. *Antitumor drug–radiation interactions.* Boca Raton, FL: CRC Press 1990: 207–22.
5. Hill BT, Whelan RDH, Hosking LK, *et al.* Interactions between antitumor drugs and radiation in mammalian tumor cell lines: differential drug responses and mechanisms of resistance following fractionated X-irradiation or continuous drug exposure *in vitro*. *NCI Monogr* 1988; **6**: 177–81.
6. Casper J, Schmoll JH, Schmidt V, *et al.* Cell lines of human germinal cancer. *Int J Androl* 1987; **10**: 105–13.
7. Hill BT, Shellard SA, Whelan RDH, *et al.* Deficient removal of the major platinum–DNA intrastrand cross-links in the sequences pGpG and pApG appears characteristic of testicular teratoma cell lines derived from tumours from untreated patients and may account for their sensitivity to cisplatin. *Proc Am Soc Clin Oncol* 1992; **11**: 100.
8. Courtenay VD, Selby PJ, Smith IE. Growth of human tumour cell colonies from biopsies using two soft-agar techniques. *Br J Cancer* 1978; **38**: 77–81.
9. Whelan RDH, Hill BT. The influence of agarose concentration on the cloning efficiency of a series of established human cell lines. *Cell Biol Int Rep* 1981; **5**: 1137–42.
10. Griffith OW. Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinyl pyridine. *Anal Biochem* 1980; **106**: 207–12.
11. Habig WH, Jackoby WB. Assays for differentiation of glutathione S-transferases. *Methods Enzymol* 1981; **77**: 398–405.
12. Horn HD. Glutathione reductase. In: Bergmeyer HU, ed. *Methods in enzyme analysis.* New York: Academic Press 1965: 875–9.
13. Smith IK, Vierheller TL, Thorne CA. Assay of glutathione reductase in crude tissue homogenates using 5,5′-dithiobis(2-nitrobenzoic acid). *Anal Biochem* 1988; **175**: 408–13.
14. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med* 1976; **70**: 158–69.
15. Gunzler WA, Kremers H, Floke L. An improved coupled test procedure for glutathione peroxidase (E.C. 1.11.1.9) in blood. *Z Clin Chem Clin Biochem* 1974; **12**: 444–8.
16. Lowry OH, Rosebrough NJ, Farr AL, *et al.* Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951; **193**: 265–75.
17. Dempke WCM, Shellard SA, Hosking LK. Mechanisms associated with the expression of cisplatin resistance in a

human ovarian tumor cell line following exposure to fractionated X-irradiation *in vitro*. *Carcinogenesis* 1992; **13**: 1209–15.

18. Shellard SA, Hosking LK, Hill BT. An anomalous relationship between cisplatin sensitivity and the formation and removal of platinum–DNA adducts in two human ovarian carcinoma cell lines *in vitro*. *Cancer Res* 1991; **51**: 4557–64.
19. Fichtinger-Schepman AMJ, Van der Veer JL, Den Hartog JHJ, *et al.* Adducts of the antitumour drug *cis*-diamminedichloroplatinum(II) with DNA: formation, identification and quantitation. *Biochemistry* 1985; **24**: 707–13.
20. Fichtinger-Schepman AMJ, Van Oosteram AT, Lohman PHM, *et al. cis*-Diamminedichloroplatinum (II)-induced DNA adducts in peripheral leukocytes from seven cancer patients: quantitative immunochemical detection of the adduct induction and removal after a single dose of *cis*-diamminedichloroplatinum (II). *Cancer Res* 1987; **47**: 3000–4.
21. Kohn KW, Ewig RAG, Erickson LC, *et al.* Measurement of strand breaks and cross-links by alkaline elution. In: Friedberg EC, Hanawalt PC, eds. *DNA repair: a laboratory manual of research procedures*, New York: Marcel Dekker 1981: 379–401.
22. Bedford P, Shellard SA, Walker C, *et al.* Differential expression of collateral sensitivity or resistance to cisplatin in human bladder carcinoma cell lines pre-exposed *in vitro* to either X-irradiation or cisplatin. *Int J Cancer* 1987; **40**: 681–6.
23. Hill BT, Scanlon KJ, Hansson J, *et al.* Deficient repair of cisplatin–DNA adducts identified in human testicular teratoma cell lines established from tumours from untreated patients. *Eur J Cancer* 1994, in press.
24. Hill BT, Shellard SA, Hosking LK, *et al.* Characterization of a cisplatin-resistant human ovarian carcinoma cell line expressing cross-resistance to 5-fluorouracil but collateral sensitivity to methotrexate. *Cancer Res* 1992; **52**: 3110–8.
25. Kelland LR, Mistry P, Abel G, *et al.* Establishment and characterization of an *in vitro* model of acquired resistance to cisplatin in a human testicular nonseminomatous germ cell line. *Cancer Res* 1992; **52**: 1710–6.
26. Walker MC, Povey S, Parrington JM, *et al.* Development and characterization of cisplatin-resistant human testicular and bladder tumour cell lines. *Eur J Cancer* 1990; **26**: 742–7.
27. Andrews PA, Murphy MP, Howell SB. Characterization of cisplatin-resistant COLO 316 human ovarian carcinoma cells. *Eur J Cancer Clin Oncol* 1989; **25**: 619–25.
28. Behrens BC, Hamilton TC, Masuda H, *et al.* Characterization of a *cis*-diamminedichloroplatinum(II)-resistant human ovarian cancer cell line and its use in evaluation of platinum analogues. *Cancer Res* 1987; **47**: 414–8.
29. Teicher BA, Holden SA, Kelly MJ, *et al.* Characterization of a human squamous carcinoma cell line resistant to *cis*-diamminedichloroplatinum(II). *Cancer Res* 1987; **47**: 388–93.
30. Bungo M, Fujiwara Y, Kasahara K, *et al.* Decreased accumulation as a mechanism of resistance to *cis*-diamminedichloroplatinum(II) in human non-small cell lung cancer cell lines: relation to DNA damage and repair. *Cancer Res* 1990; **50**: 2549–53.
31. Kashani-Sabet M, Wang W, Scanlon KJ. Cyclosporin A suppresses cisplatin-induced c-*fos* gene expression in ovarian carcinoma cells. *J Biol Chem* 1990; **265**: 11285–8.
32. Loh SY, Mistry P, Kelland LR, *et al.* Reduced drug accumulation as a major mechanism of acquired resistance to cisplatin in a human ovarian carcinoma cell line: circumvention studies using novel platinum (II) and (IV) ammine/amine complexes. *Br J Cancer* 1992; **66**: 1109–15.
33. Andrews PA, Murphy MP, Howell SP. Metallothionein-mediated cisplatin resistance in human ovarian carcinoma cells. *Cancer Chemother Pharmacol* 1987; **19**: 149–54.
34. Hospers GAP, Mulder NH, De Jong B, *et al.* Characterization of a human small cell lung carcinoma cell line with acquired resistance to *cis*-diamminedichloroplatinum(II) *in vitro*. *Cancer Res* 1988; **48**: 6803–7.
35. Eastman A, Schulte N. Enhanced DNA repair as a mechanism of resistance to *cis*-diamminedichloroplatinum(II). *Biochemistry* 1988; **27**: 4730–4.
36. Rawlings CJ, Roberts JJ. Walker rat carcinoma cells are exceptionally sensitive to *cis*-diamminedichloroplatinum(II) (cisplatin) and other difunctional agents but not defective in the removal of platinum–DNA adducts. *Mutat Res* 1986; **166**: 157–68.
37. Calsou P, Frit P, Salles B. Repair synthesis by human cell extracts in cisplatin-damaged DNA is preferentially determined by minor adducts. *Nucleic Acids Res* 1992; **20**: 6363–8.
38. Gibbons GR, Page JD, Mauldin SK, *et al.* Role of carrier ligand in platinum resistance in L1210 cells. *Cancer Res* 1990; **50**: 6497–501.
39. Jones JC, Zhen W, Reed E, *et al.* Gene-specific formation and repair of cisplatin intrastrand adducts and interstrand cross-links in Chinese hamster ovary cells. *J Biol Chem* 1991; **266**: 7101–7.
40. Zhen W, Link CJ, O'Connor PM, *et al.* Increased gene-specific repair of cisplatin interstrand cross-lines in cisplatin-resistant human ovarian cancer cell lines. *Mol Cell Biol* 1992; **12**: 3689–98.
41. Szymkpwski DE, Yarema K, Essigmann JM, *et al.* An intrastrand d(GpG) platinum crosslink in duplex M13 DNA is refractory to repair by human cell extracts. *Proc Natl Acad Sci USA* 1992; **89**: 10772–6.
42. Wood RD, Robins P, Lindahl T. Complementation of the xeroderma pigmentosum DNA repair defect in cell-free extracts. *Cell* 1988; **53**: 97–106.

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