Theranostic proteomic profiling of cyclins, cyclin dependent kinases and Ras in human cancer cell lines is dependent on p53 mutational status

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Abstract. Despite major advances in the molecular biology of the cancer cell over the past two decades, the great majority of patients are still treated by conventional cytotoxic drugs. The chemotherapy regimens employed frequently include platinating agents, taxanes, intercalating agents and topoisomerase inhibitors. Attempts to predict the therapeutic efficacy of such drugs by molecular profiling (theranostics) have up to the present time had limited success. Genes responsible for the control of cell division, senescence and apoptosis whose normal functions become corrupted during carcinogenesis, might potentially play a part in determining chemotherapeutic response. Here we have examined the relationships between the chemoresponsiveness of 18 human *in vitro* cancer cell lines and proteomic expression of Ras, cyclins B1 and D1 and cyclin-dependent kinases Cdk1 and Cdk4. When all 18 cell lines were examined as a single group, proteomic expression did not provide any helpful theranostic predictors. Clear relationships between proteomic expression and drug efficacy emerged, however, when Ras, cyclin B1, cyclin D1, Cdk1 and Cdk4 were examined separately in p53 wild-type and p53 mutant cell subsets. We suggest that the theranostic relationships we have detected *in vitro* may have potential relevance *in vivo* and should prompt clinical theranostic studies which take account of p53 mutational status.

Introduction

The rapid pace of development in the molecular cell biology of cancer is increasingly moving the emphasis in the management of this disease towards theranostic technology (1-3), where molecular profiles of cancers can be used to predict the most effective drugs for a particular tumour (4-6). The application of theranostic molecular profiling in this manner would not only benefit patients undergoing standard chemotherapy, by selecting the most effective drugs and avoiding unnecessary toxicity from ineffective agents, but also aid the stratification of patients into groups most likely to respond to new therapeutic agents in phase I/II clinical studies.

Continued progress in theranostics requires the demonstration of clear relationships between genomic/proteomic molecular profiles and therapeutic responsiveness to specific anticancer drugs/drug combinations in the tumours of individual patients. In typical solid tumours in the clinic, however, responses to chemotherapy are frequently not marked and may thus provide insufficiently-distinct endpoints for comparison with the complex, multiparameter, genomic and proteomic profiles now available. Impaired access of drugs to clinical tumours (7) may also add further uncertainties to comparisons of theranostic profiles and therapeutic responsiveness in the *in vivo* situation.

Human *in vitro* cancer cell lines provide a model system in which relative therapeutic responsiveness can be accurately evaluated to several decades of logarithmic cell killing (8-10). Collections of human *in vitro* cancer cell lines have been effectively used as a primary screen to detect relative anticancer activity in a wide range of compounds (11) and in studies of intrinsic and acquired drug resistance (12,13). Additionally, drugs have direct access to cells in such systems, providing consistent levels for intercomparisons, without the problems of the uncertain drug availability found with *in vivo* tumours.

Using such an *in vitro* human cancer cell model system we have previously demonstrated a relationship between resistance to cis-diamminedichloroplatinum (II) (cisplatin CDDP) and high levels of proteomic expression of cyclin D1 (14). Subsequently, over-expression of cyclin D1 has been reported to confer resistance to CDDP-mediated apoptosis in an elastase-myc, transgene-expressing pancreatic tumour cell line (15) and siRNA-directed suppression of cyclin D1 expression has resulted in enhanced susceptibility to CDDP-mediated apoptosis. In addition, cyclin D1 anti-sense has been shown to increase sensitivity to CDDP in human

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Abbreviations: CDDP, cis-diamminedichloroplatinum (II); HIFCS, heat inactivated foetal calf serum; PBS, phosphate-buffered saline; Cdk, cyclin dependant kinase; DMEM, Dulbecco's modified minimum essential medium

Key words: theranostic, proteomic, cyclins, Cdks, Ras, clonogenic cell survival, p53 mutational status

pancreatic cancer cells (16), head and neck cancer cells (17) and gastric cancer cells (18). In 14 primary human neuroblastoma cell lines, however, no relationship could be demonstrated between cyclin D levels and CDDP resistance (19). There is thus a continued need for an improved understanding of the relationship between cyclin D expression and CDDP cytotoxicity.

Sensitivity to cis-diamminedichloroplatinum has also been shown to correlate with Ras expression (20) and with the functionality of the p53 tumour suppressor protein (TP53) in ovarian cancer (21,22), non-small cell lung cancer (23) and malignant melanoma (24). Moreover, TP53 has been shown to have a role in the sensitivity of human cancer cells to etoposide (24,25), doxorubicin (26,27) and paclitaxel (28,29).

In general, theranostic relationships of CDDP, etopside, doxorubicin and paclitaxel to positive and negative cell signalling factors have been investigated in isolation. Such an approach has not so far met with success (30). We have previously reported, however, that as few as two gene products, examined in combination, in a range of human cancer cell lines, can provide a theranostic test for radiosensitivity of greater significance than that of either gene product alone (31). For this reason, in a search for further potential theranostic relationships in human cancer cells, we chose to examine the relationship of cyclin D, cyclin B, Cdk4, Cdk1 and the Ras oncogene to sensitivity to cis-diammine dichloroplatinum, paclitaxel, doxorubicin and etoposide in the context of p53 wild-type as compared to p53 mutant cells.

Materials and methods

The origins and *in vitro* growth characteristics of the 18 human cell lines described here along with procedures for drug exposure, clonogenic cell survival assay, Western blotting and DNA sequencing have already been reported (10,14). Human cell lines were routinely maintained in DMEM (Dulbecco's modified minimal essential medium) except RT112 and H322 which grow most successfully in RPMI (Roswell Park Memorial Institute) medium and MGHU-1 which are grown in Ham's F12 medium. All media were supplemented with 10% heat-inactivated foetal calf serum (HIFCS).

Cell growth, contamination checks, batching and storage. The cell lines studied, with their histological classification, are listed in Table I. All are well established, many having been growing *in vitro* for several years. Cell lines were either donations or purchased by our laboratories. On receipt, all were grown for 5 passages to provide sufficient cells for batch storage in liquid nitrogen. During this period microbial contamination was excluded by at least one passage in antibiotic-free medium and mycoplasma testing was carried out on all lines. To minimise the effects of potential genetic drift throughout the duration of the experiments described here, clonogenic assays and Western blotting were all carried out on cells grown up from the same passage (as close as possible in cell doublings to the original cell batches which had entered the laboratories). To obtain data for comparing therapeutic responsiveness and gene expression, each cell line from the earliest batched passage was taken from liquid nitrogen and grown up through 4-6 passages to provide sufficient cells for clonogenic assays and cell lysates for Western blotting.

Cytotoxic drug clonogenic cell survival curves. Freshly harvested, exponentially-growing, pre-confluent cells (>95% viable) were resuspended in Ham's F12 + 10% HIFCS containing the appropriate drug concentration prepared by dilution from 1 mg/ml light protected stock solutions at cellular concentrations of 10^2, $2x10^2$, $5x10^2$, 10^3 and 10^4 cells per 2 ml of Ham's F12 medium + 10% HIFCS. Aliquots (2 ml) of these solutions were seeded in replicate 60 mm wells of 6-well tissue culture dishes. Each assay was incubated for 14 days in the dark at 37˚C in humidified 95% air/5% CO_2. Drug was left in for the duration of the experiments. After a 14-day incubation, the medium was removed, wells were washed with PBS, fixed with 70% ethanol, stained with giemsa and colonies of >200 cells scored as positive clones.

Cell lysates for Western blotting. For Western blotting, 10^7 cells were grown in 162-cm^2 tissue culture flasks (Costar Ltd., High Wycombe, Bucks) until they were pre-confluent but still growing exponentially. Cells were then removed by trypsinisation, resuspended in complete medium plus 10% FCS and washed 3 times by serial centrifugation and resuspension in ice-cold PBS (phosphate-buffered saline) without serum. Viable ($1-3x10^8$) cells were then pelletted by centrifugation and resuspended at $3x10^7$ cells per ml of lysis buffer [(1X stock solution: 10% (w/v) SDS 10 ml, 0.5 M Tris, pH 6.8, glycerol 10 ml, double distilled water 62 ml; to 10 ml of stock solution were added 100 μl of 10 μM leupeptin plus 10 μl 100 μM PMSF)]. Protein estimations were performed and the final concentration of the lysates adjusted to 300 μg total cellular protein per 100 μl of lysis buffer.

Western blotting. Two independent Western blottings with lysates for each cell line loaded in pairs on each gel were carried out. Total cellular protein (150 μg) in 50 μl of lysate buffer was added per lane to 7.5-12% Laemmli separating gels depending on the molecular mass of the protein being assayed. Electrophoresis was carried out at 16˚C using 60 V over 16 h and a constant current of 500 mA. Proteins were transferred to nitrocellulose at 22˚C over 16 h using a semi-dry blotting apparatus (Bio-Rad, Richmond, CA) and blocked with Tris base pH 4.7 (100 mM; 0.5% Tween-20, 100 mM NaCl, 5% HIFCS, 2% non-fat milk) at room temperature, in darkness for 1 h. The membrane was then incubated with the relevant polyclonal or monoclonal antibody appropriately directed against the protein under investigation, followed by goat or rabbit anti-mouse conjugated antibodies (Dako, UK) at 1/1000 and developed in alkaline phosphatase buffer containing nitroblue tetrazolium 75 mg/ml in 70% (v/v) DMF and 5-Bromo-4-Chloro-3-Indoyl Phosphate (Sigma, Poole, Dorset, UK) (50 mg/ml in dimethylformamide). Primary antibodies used were: Cdk1, mouse monoclonal sc-54 at 1/250 (Santa Cruz Biotechnology); Cdk4, rabbit polyclonal sc-260 at 1/250 (Santa Cruz Biotechnology); cyclin

Table I. Histological type, p53 mutational status and therapeutic sensitivity (D0.1 values) of 18 human *in vitro* cancer cell lines.

			Therapeutic sensitivity							
			CDDP		Paclitaxel		Etoposide		Doxorubicin	
Cell line	Histology	p53 status	D0.1		D0.1		D0.1		D0.1	
2780	Ovarian carcinoma	Wild-type	0.897	r=0.998, p=0.0001	0.006	r=-0.619, p=0.075	0.110	r=-0.974	0.013	r=-0.915
A431	Squamous carcinoma vulva	Mutant	1.024	r=-0.984, p=0.0001	0.001	r=-0.913, p=0.011	0.303	r=-0.835	0.033	r=-0.976
A549	Adenocarcinoma lung	Wild-type	2.701	r=-0.990, p=0.0001	0.001	r=-0.921, p=0.003	0.246	r=-0.939	0.03	r=-0.981
COR L23	Large cell lung carcinoma	Wild-type	1.127	r=-0.832, p=0.0001	0.0006	r=0.821, p=0.045	0.399	r=-0.952	0.028	r=-0.940
G361	Melanoma	Wild-type	0.613	r=-0.972, p= 0.001	0.002	r=-0.850, p=0.0001	0.169	r=-0.949	0.018	r=-0.949
H322	Small cell carcinoma lung	Mutant	1.532	r=-0.995, p=0.0001	0.004	r=-0.817, p=0.004	0.306	r=-0.983	0.026	r=-0.957
HEP2	Squamous carcinoma larynx	Wild-type	0.923	r=-0.949, p=0.0001	0.002	r=-0.746, p=0.0001	0.233	r=-0.969	0.021	r=-0.957
HRT18	Adenocarcinoma rectum	Wild-type	3.422	r=-0.994, p=0.0001	0.013	r=-0.990, p=0.0001	0.539	r=-0.993	0.062	r=0.840
HT29/5	Adenocarcinoma colon clone 5	Mutant	2.306	r=-0.979, p=0.0001	0.001	r=-0.979, p=0.0001	0.672	r=-0.958	0.058	r=-0.946
HX142	Neuroblastoma	Wild-type	0.818	r=-0.997, p= 0.0001	0.018	r=-0.910, p=0.0001	0.107	r=-0.958	0.016	r=-0.981
KB	Oral epidermoid carcinoma	Wild-type	1.262	r=-0.909, p=0.0001	0.001	r=-0.892, p=0.0001	0.114	r=-0.950	0.011	r=0.987
SK-MEL-2	Melanoma	Mutant	1.391	r=-0.977, p=0.0001	0.001	r=-0.973, p=0.005	0.098	r=-0.971	0.012	r=-0.939
SK-MEL-3	Melanoma	Mutant	0.746	r=-0.962, p=0.0001	0.001	r=-0.735, p=0.0001	0.198	r=-0.996	0.028	r=-0.928
MGHU-1	Transit cell carcinoma bladder	Wild-type	1.892	r=-0.992, p=0.0001	0.002	r=-0.752, p=0.032	0.264	r=-0.846	0.022	r=-0.832
MOR	Adenocarcinoma lung	Mutant	2.282	r=-0.957, p=0.0001	0.001	r=-0.979, p=0.004	0.322	r=-0.968	0.022	r=-0.962
OAW42	Ovarian carcinoma	Wild-type	0.367	r=-0.993, p=0.0001	0.018	r=-0.873, p=0.0001	0.302	r=-0.926	0.038	r=-0.949
RPMI-7951	Melanoma	Mutant	0.713	r=-0.976, p=0.0009	0.002	r=-0.957, p=0.0001	0.153	r=-0.878	ND	ND
RT112	Transit cell carcinoma bladder	Mutant	0.794	r=-0.981, p=0.0001	0.003	r=-0.848, p=0.004	0.231	r=-0.961	0.024	r=-0.902

B1, mouse monoclonal 14561C at 1/1000 (PharMingen); cyclin D1, mouse monoclonal 14841C at 1/125 (PharMingen). Ras, mouse monoclonal OP22, recognising both mutant and wild-type Ras, at 1/200 (Oncogene Science Ltd., New York, USA). Quantitation of each protein was carried out by measurement of either, optical density on a Shimadzu scanning densitometer with tungsten light and expressed as O.D. units per 150 μg of total cellular protein or by enhanced chemiluminescence (ECL). Band images produced by either modality were read on 'Phoretix' analysis software and expressed as peak intensities per 150 μg of total cellular protein. Titration curves obtained by loading different amounts of total cellular protein have previously shown that linear relationships for optical density (O.D.) could be obtained over the range found for cyclin D1 and cyclin B1 across the cell lines (14). In addition, there was strong linear correlation between cell numbers and total cellular protein when compared by Western blotting. Each Western blot was run with a range of different cell lines. In order to compare different levels of protein between the cell lines, the mean

A

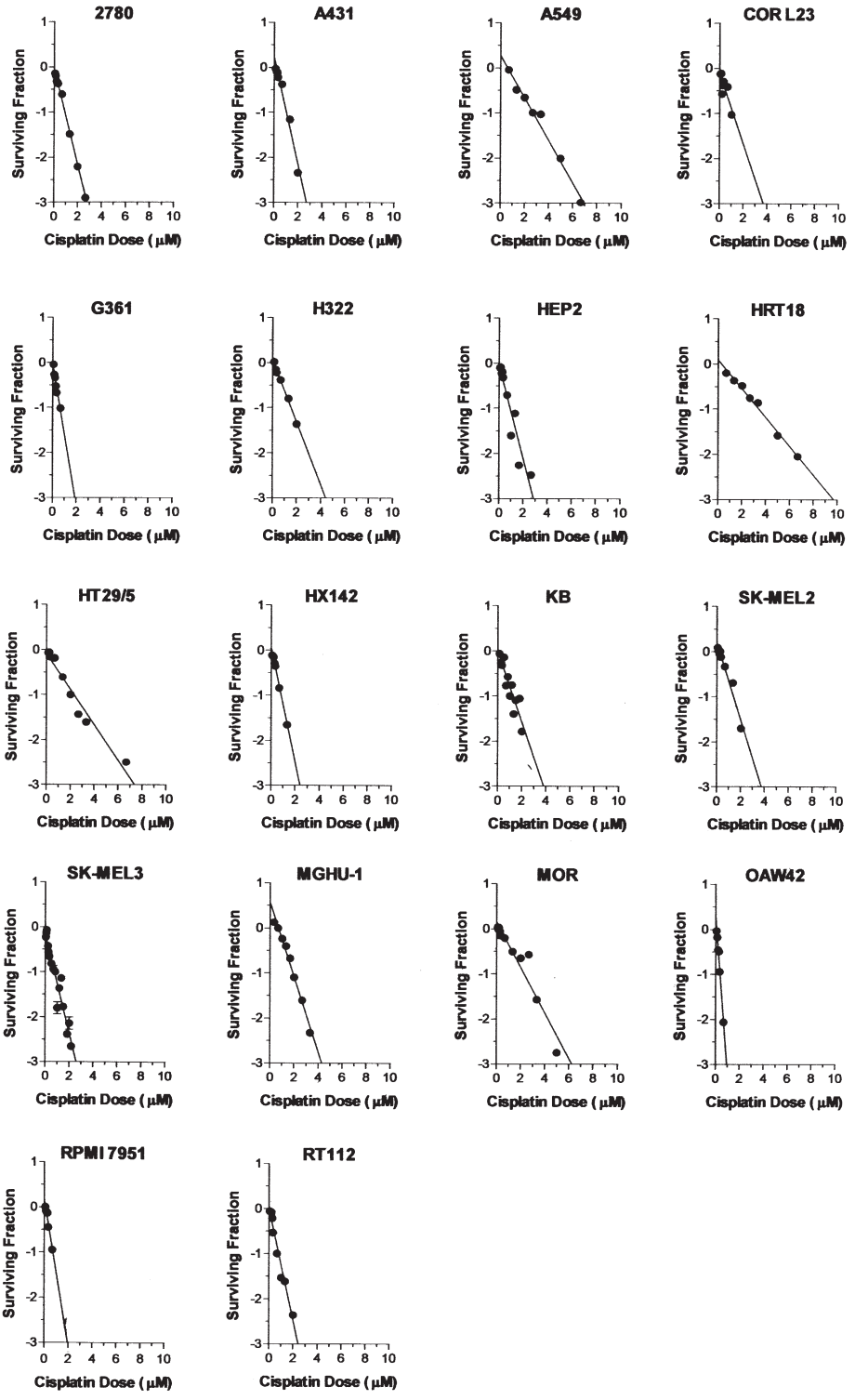


Figure 1. Cell survival curves obtained by clonogenic cell survival assay following exposure to (A) CDDP, (B) paclitaxel, (C) doxorubicin and (D) etoposide. The data points from all assays were pooled to provide means and SEMs. Where no error bars are visible the SEM lay within the magnitude of the relevant symbol. Repeat clonogenic assays were carried out over the appropriate range of drug concentrations to yield data which could be closely fitted by linear/curvilinear regression analysis (Table I) (GraphPad Software, San Diego, CA, USA).

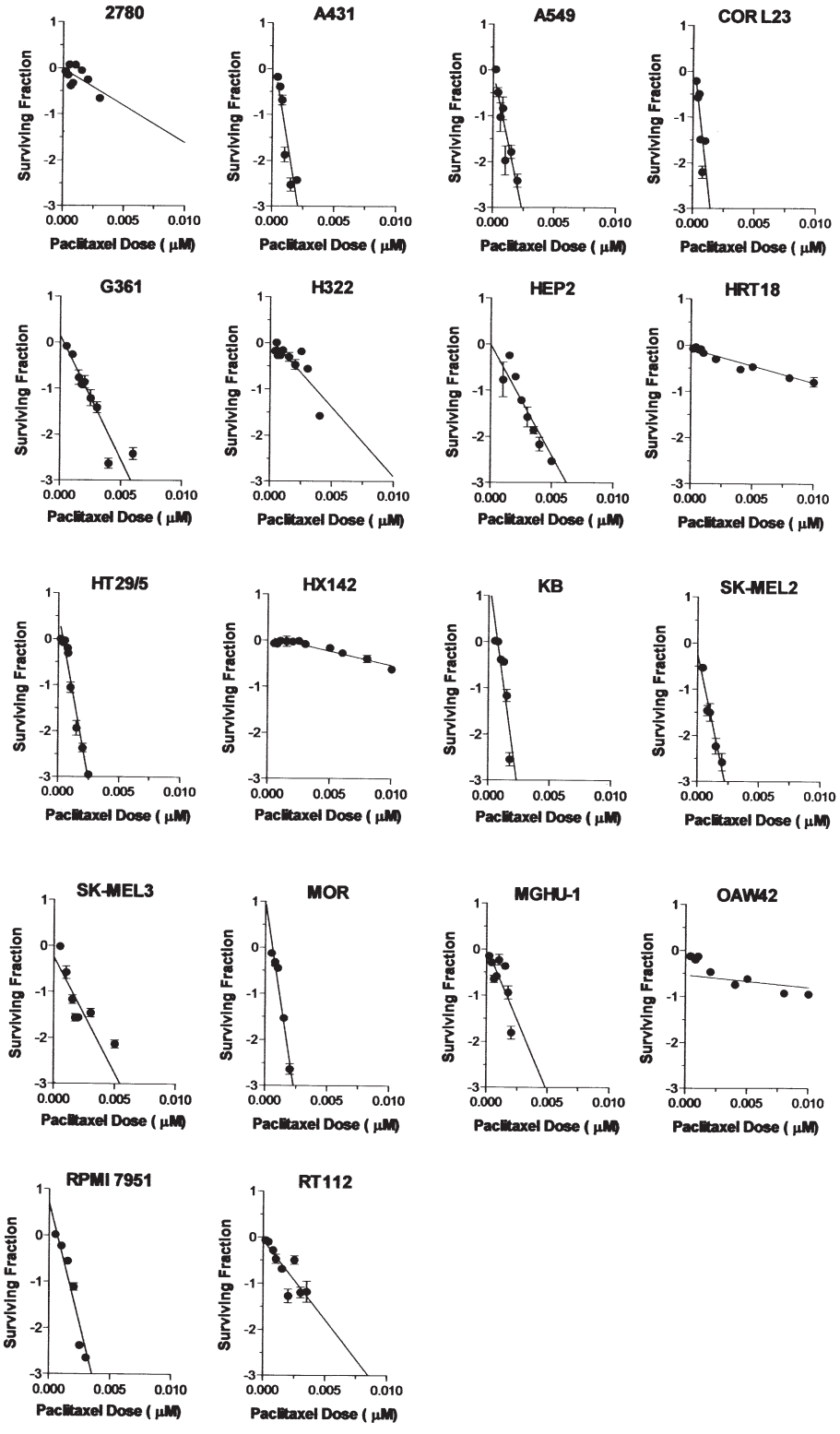
O.D. value for all the lines was calculated and the relative O.D. for protein in each individual cell line was normalised to the mean O.D. and multiplied by an arbitrary value of 5.0.

P53 mutational status. The p53 mutational status of the cell lines studied here has been previously described (31-36). In contrast to the p53 wild-type cell lines, none of the p53 mutants produced detectable p21WAF1/CIP1.

Results

Therapeutic sensitivity. Fig. 1 shows clonogenic cell survival data for cis-diamminedichloroplatinum, paclitaxel, doxorubicin and etoposide. A minimum of 4-6 separate clonogenic assays were usually necessary for close fitting by linear regression to a high degree of significance for each

B

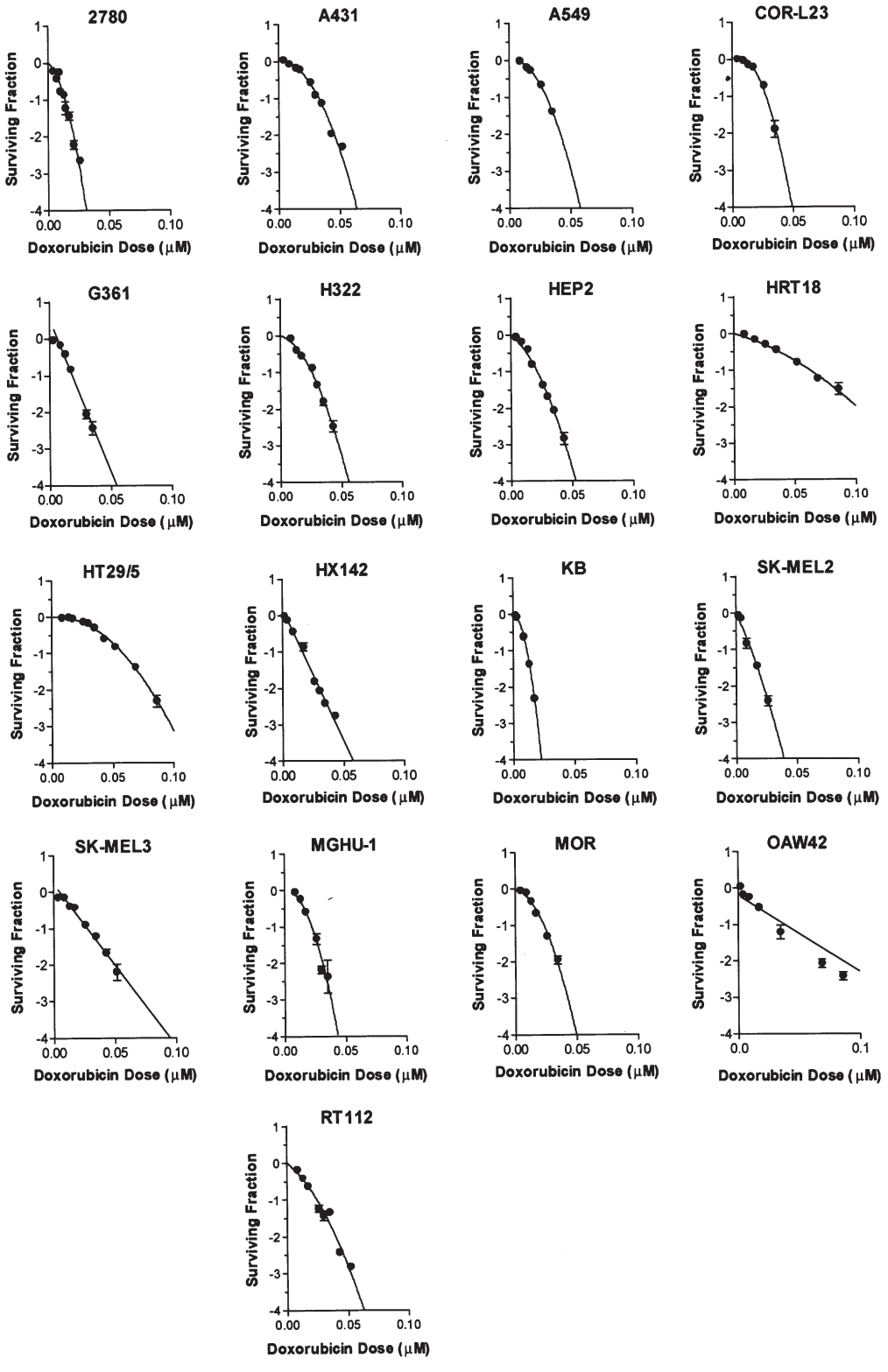


cell line (Table I). Whilst cis-diamminedichloroplatinum (14) and paclitaxel (Fig. 1A and B) could be fitted by straight linear regression, cell survival data for etoposide and doxorubicin required curvilinear fitting (Fig. 1C and D).

Effect of p53 mutational status on therapeutic sensitivity. Relative sensitivity to the four drugs was examined independently in p53 wild-type and p53 mutant cell lines by comparison of therapeutic iso-effect D0.1 values (Table I) obtained by interpolation of the cell survival curves shown in Fig. 1. No p53 mutational status differences were apparent in relative sensitivity to CDDP, etoposide or doxorubicin (Fig. 2A, C, D). With regard to paclitaxel, however, 4/10 wild-type p53 cell lines were relatively resistant, whereas all p53 mutant cells were equally sensitive (Fig. 2B).

The effect of p53 status on cross sensitivity between the four drugs was also examined. A high degree of cross sensitivity between etoposide and doxorubcin was seen in both wild-type and mutant p53 cell lines (Fig. 3A and C) which would

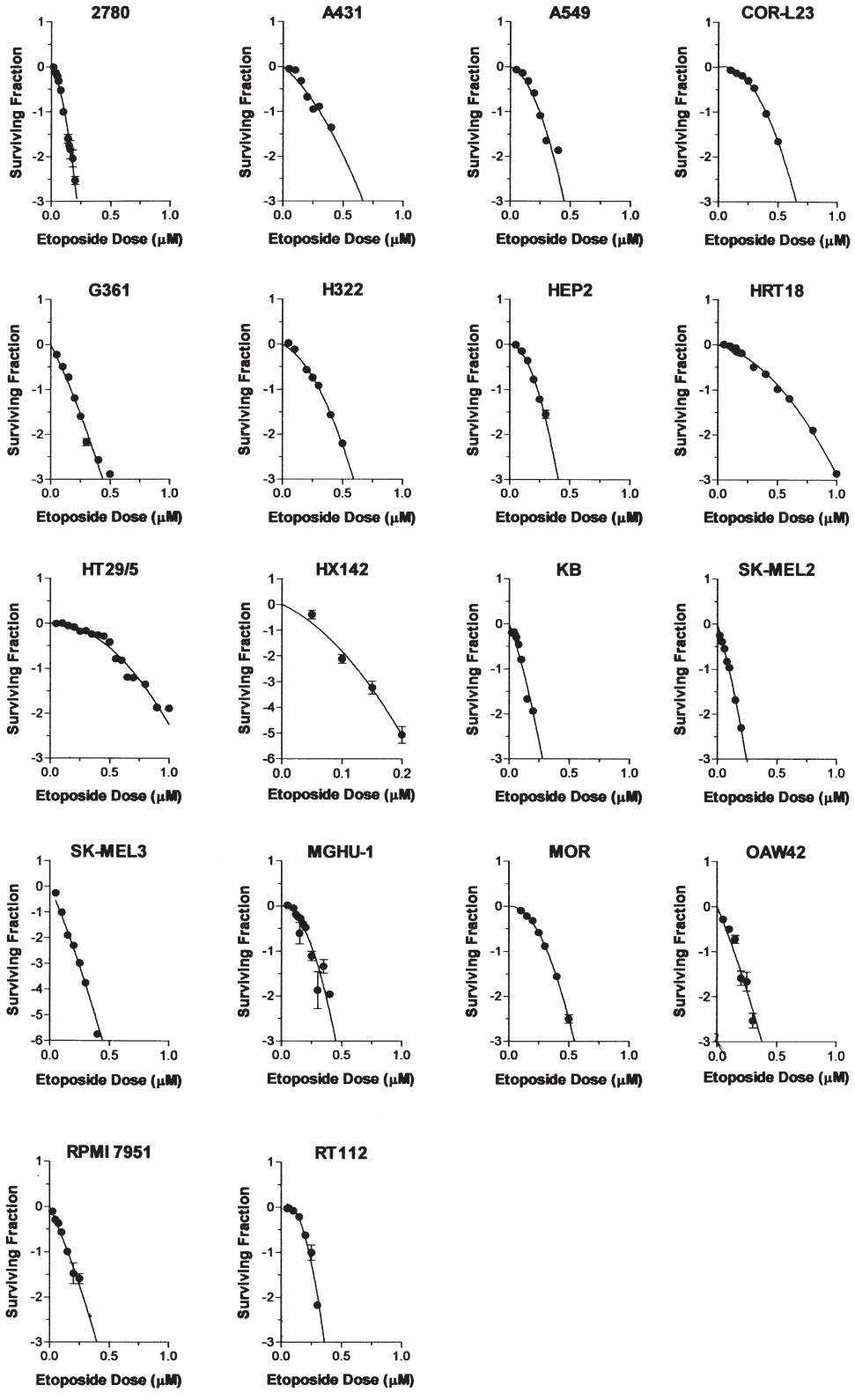
C



be consistent with both drugs causing impairment of topoisomerase function. A weaker cross sensitivity between CDDP and doxorubicin seen in the wild-type p53 lines, however, did not occur in p53 mutant cells (Fig. 3B and C). Otherwise no cross sensitivity was found between the four drugs. In particular no cross-sensitivity between CDDP and paclitaxel was detected.

Proteomic expression of cyclins, cyclin dependent kinases and Ras. Fig. 4 shows histograms of the relative expression of Ras, cyclins D1, B1, Cdk1 and Cdk4 across the human *in vitro* cell lines. The patterns of expression for Cdk1 and Cdk4 are closely similar, in keeping with a recent report (37). Ras, cyclin B1 and cyclin D1 show more variable patterns.

D



Relationship of proteomic expression of cyclins and cyclin-dependent kinases to therapeutic effect in p53 wild-type vs. p53 mutant human cancer cells. The D0.1 values were compared to the relative expression of cyclin D1, cyclin B1, Cdk1, Cdk4 and Ras (Table II). When all 18 cell lines were examined, weak theranostic relationships between CDDP D0.1 levels and Cdk1 and Cdk4 expression were found (Table II; r=-0.520, p=0.027 and r=-0.517, p=0.028 respectively). These indicated that increased CDDP sensitivitivity was found in cell lines expressing higher levels of Cdk1 or Cdk4. The previously reported (14) significant relationship between elevated cyclin D1 expression and CDDP resistance that we had noted in 16 human *in vitro* cancer cell lines was not apparent in the 18 cell lines examined here. When these relationships were examined independently in p53 mutant and p53 wild-type cell lines, however, CDDP resistance was found to be related to elevation of both cyclin D1 and cyclin B1 in p53 mutant cell

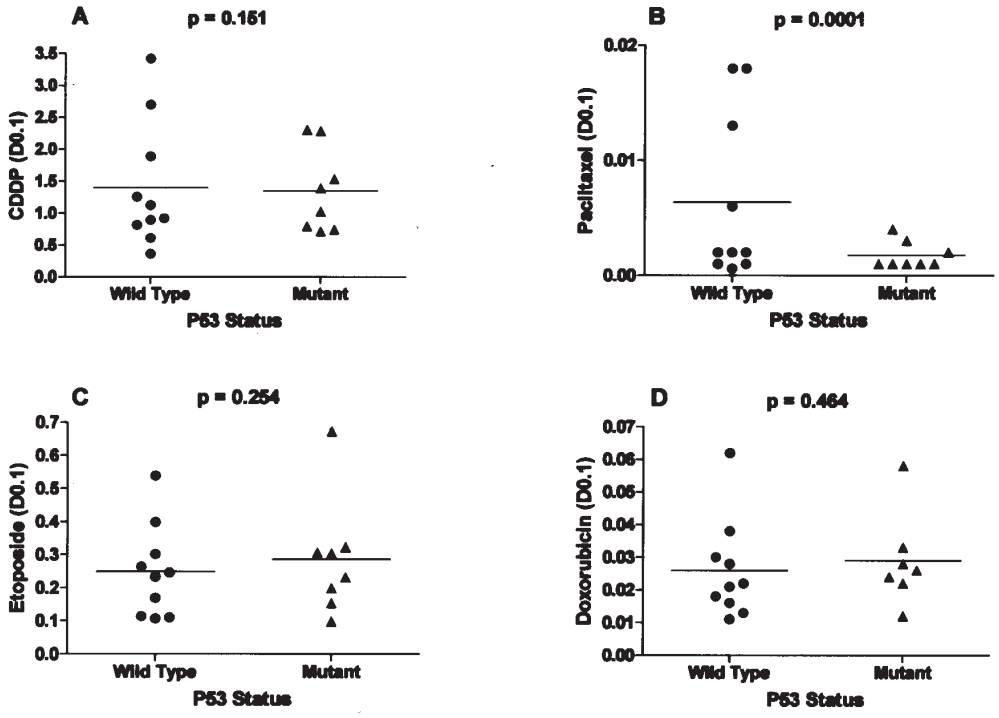


Figure 2. Comparison of D0.1 values for (A) CDDP, (B) paclitaxel, (C) etoposide, (D) doxorubicin in 10 wild-type and 8 mutant p53 cell lines. D0.1 values (Table I) were obtained by interpolation of the clonogenic cell survival curves depicted in Fig. 1. (Spearman two-tailed analysis, GraphPad Software).

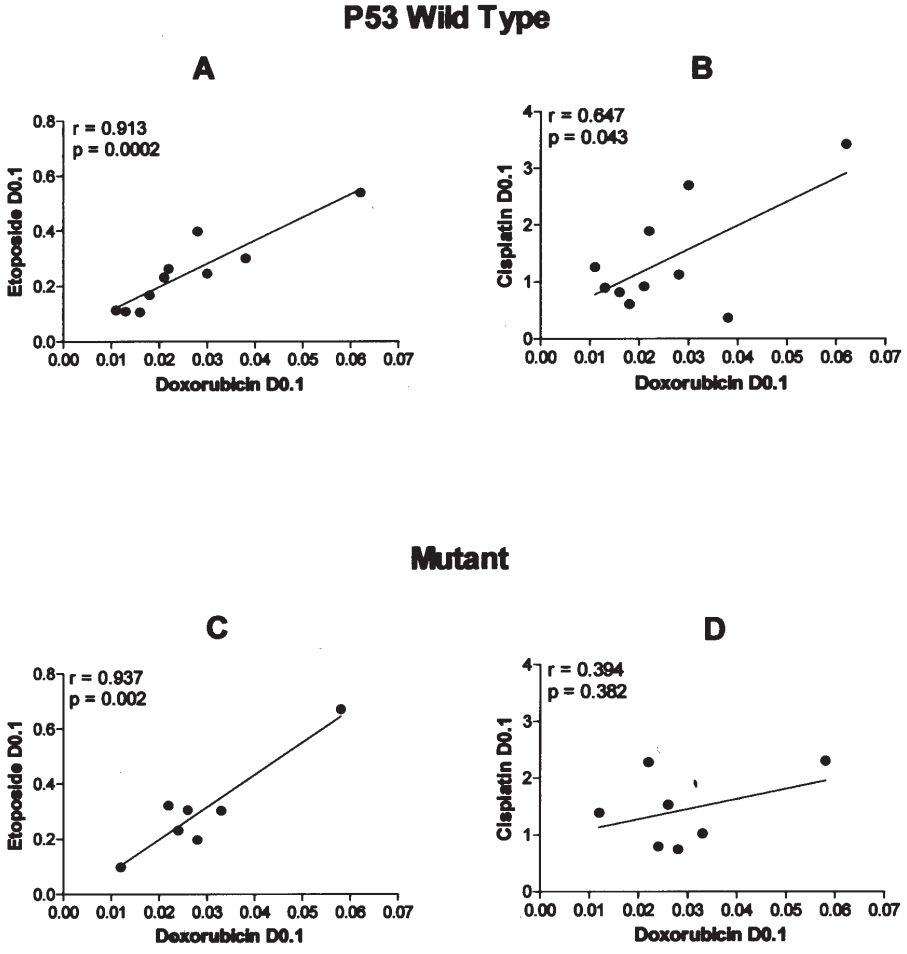


Figure 3. Cross sensitivity between etoposide (A) and doxorubicin (C) in both p53 wild-type and p53 mutant cell lines and cisplatin and doxorubicin in p53 wild-type but not p53 mutant cell lines. Comparison of D0.1 values listed in Table I by linear regression analysis.

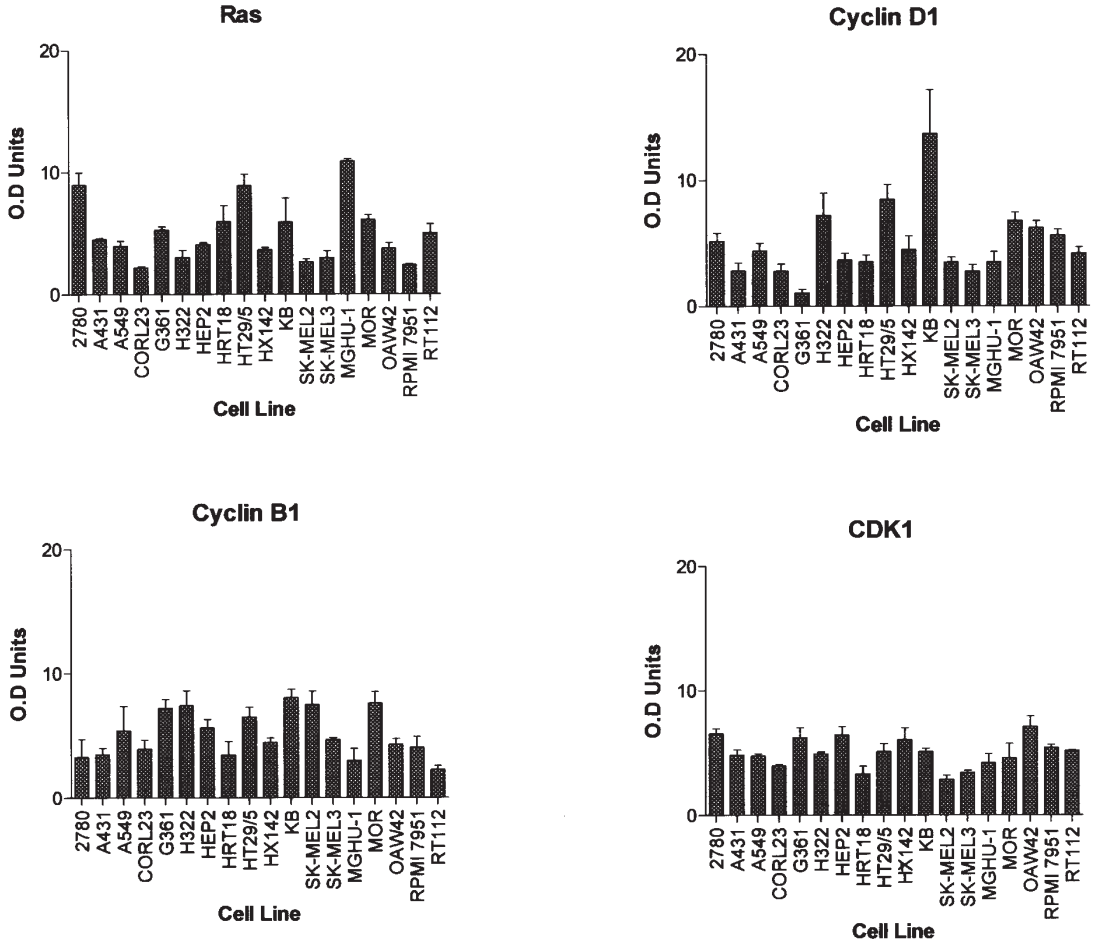


Figure 4. Histograms showing relative proteomic expression of Ras, cyclin B1, cyclin D1, Cdk1 and Cdk4 (y-axis) across 18 cancer cell lines (x-axis). Each bar represents mean ± 1 SEM.

lines (r=0.750, p=0.032 and r=0.756, p=0.030 respectively, Fig. 5B and D; Table II) but not in p53 wild-type cell lines (r=0.081, p=0.824 and r=0.258, p=0.472 respectively) (Fig. 5A and C). In addition wild-type p53 cell lines expressing increased Cdk1 were markedly more sensitive to CDDP (r=-0.805, p=0.005; Fig. 5E and Table II) and to a lower degree of significance, sensitive to etoposide (r=-0.649, p=0.042; Table II). In p53 mutant cell lines, no relationship was apparent between Cdk1 expression and CDDP sensitivity (r=0.068, p=0.874; Fig. 5F).

Relationships between proteomic expression of Ras and therapeutic sensitivity are only seen in p53 mutant cell lines. Theranostic proteomic profiles of Ras were also found to be influenced by p53 mutational status. No relationship between Ras expression and chemosensitivity to any of the four drugs could be identified when all the cell lines were examined as a single group or in the p53 wild-type subset of cells. In p53 mutant cell lines, however, a highly significant relationship between Ras expression and etoposide resistance was identified (r=0.904, p=0.002) (Fig. 6F) and lesser, but still statistically significant, relationships were found between Ras expression and resistance to doxorubicin (r=0.792, p=0.034) (Table II and Fig. 6D) and resistance to CDDP (r=0.722, p=0.043) (Table II and Fig. 6B).

Discussion

P53 mutational status can exert a strong influence on theranostic predictors. In the cell lines described here, p53 mutational status had a strong influence on the theranostic relationships of cyclins D1 and B1, cyclin dependent kinases Cdk1 and Cdk4 and the Ras proto-oncogene. In the whole group of 18 human *in vitro* cell lines the only potential theranostic parameters were weak relationships between Cdk1, Cdk4 and CDDP sensitivity. To our knowledge, these relationships have not been previously reported. Cell cycle retardation by CDDP in F9 mouse teratocarcinoma cells

Table II. Linear regression analyses relating expression of Ras, cyclin B1, cyclin D1, Cdk1 and Cdk4 to drug sensitivity.

	Ras	Cyclin D	Cyclin B	Cdk1	Cdk4
All cell lines					
CDDP	r=0.348	r=0.125	r=0.125	**r=-0.520**	**r=-0.517**
	p=0.157	p=0.623	p=0.622	**p=0.027**	**p=0.028**
Paclitaxel	r=-0.065	r=-0.018	r=-0.299	r=0.382	r=0.475
	p=0.799	p=0.944	p=0.228	p=0.118	p=0.046
Doxorubicin	r=0.140	r=-0.030	r=-0.238	r=-0.193	r=-0.141
	p=0.592	p=0.909	p=0.358	p=0.459	p=0.590
Etoposide	r=0.265	r=0.056	r=-0.070	r=-0.224	r=-0.269
	p=0.288	p=0.825	p=0.782	p=0.372	p=0.281
Wild-type p53					
CDDP	r=0.189	r=-0.081	r=-0.258	**r=-0.805**	r=-0.617
	p=0.601	p=0.824	p=0.472	**p=0.005**	p=0.058
Paclitaxel	r=-0.183	r=-0.014	r=-0.372	r=0.289	r=0.410
	p=0.613	p=0.969	p=0.289	p=0.418	p=0.239
Doxorubicin	r=-0.174	r=-0.280	r=-0.417	r=-0.482	r=-0.202
	p=0.630	p=0.434	p=0.231	p=0.159	p=0.575
Etoposide	r=-0.183	r=-0.354	r=-0.453	**r=-0.649**	r=-0.344
	p=0.613	p=0.315	p=0.188	**p=0.042**	p=0.331
Mutant p53					
CDDP	**r=0.722**	**r=0.750**	**r=0.756**	r=0.068	r=-0.527
	p=0.043	**p=0.032**	**p=0.030**	p=0.874	p=0.180
Paclitaxel	r=-0.289	r=0.259	r=-0.101	r=0.435	r=-0.093
	p=0.488	p=0.536	p=0.811	p=0.281	p=0.827
Doxorubicin	**r=0.792**	r=0.525	r=-0.089	r=0.552	r=0.162
	p=0.034	p=0.226	p=0.850	p=0.199	p=0.728
Etoposide	**r=0.904**	r=0.700	r=0.210	r=0.448	r=-0.104
	p=0.002	p=0.053	p=0.618	p=0.266	p=0.807

has, however, been linked to a marked reduction in Cdk1 (p34 cdc2) half-life, with a resulting decrease in Cdk1 protein following exposure to the drug (38).

Strong relationships were found, however, between Cdk1 and CDDP sensitivity in wild-type p53 cell lines and between Ras and etoposide resistance in mutant p53 cell lines, which we also believe are novel findings. Otherwise a moderate relationships between Cdk1 proteomic expression and etoposide sensitivity was found in p53 wild-type cell lines and Ras expression was related to CDDP and doxorubicin resistance in mutant p53 cell lines.

Proteomic theranostic relationships with drug sensitivity may not reflect cross sensitivity between the drugs themselves. Although p53 mutational status influenced the theranostic relationships of the cyclins, Cdks and Ras, it was not, *per se*, related to chemosensitivity in the case of CDDP, doxorubicin, or etoposide. Moreover, strong cross sensitivity between CDDP and doxorubicin therapeutic sensitivity occurred independently of p53 mutational status. These results conflict with the findings of Pestell *et al*, that loss of functional p53 can increase CDDP cytotoxicity in the A2780 ovarian cancer cell line (21), and with the study of Reles *et al*, who reported a correlation between p53 mutation and resistance to platinum-based chemotherapy accompanied by shortened survival in ovarian cancer (22). This most likely reflects the multifactorial nature of cancer cell drug sensitivity which could be expected to pose similar problems in the clinical context.

P53 mutational status alone, did, however, affect paclitaxel sensitivity, in that mutant p53 cell lines were equally sensitive to this drug, whereas p53 wild-type cell lines exhibited a range of sensitivities. This observation, although restricted to a wide range of human cell lines of different

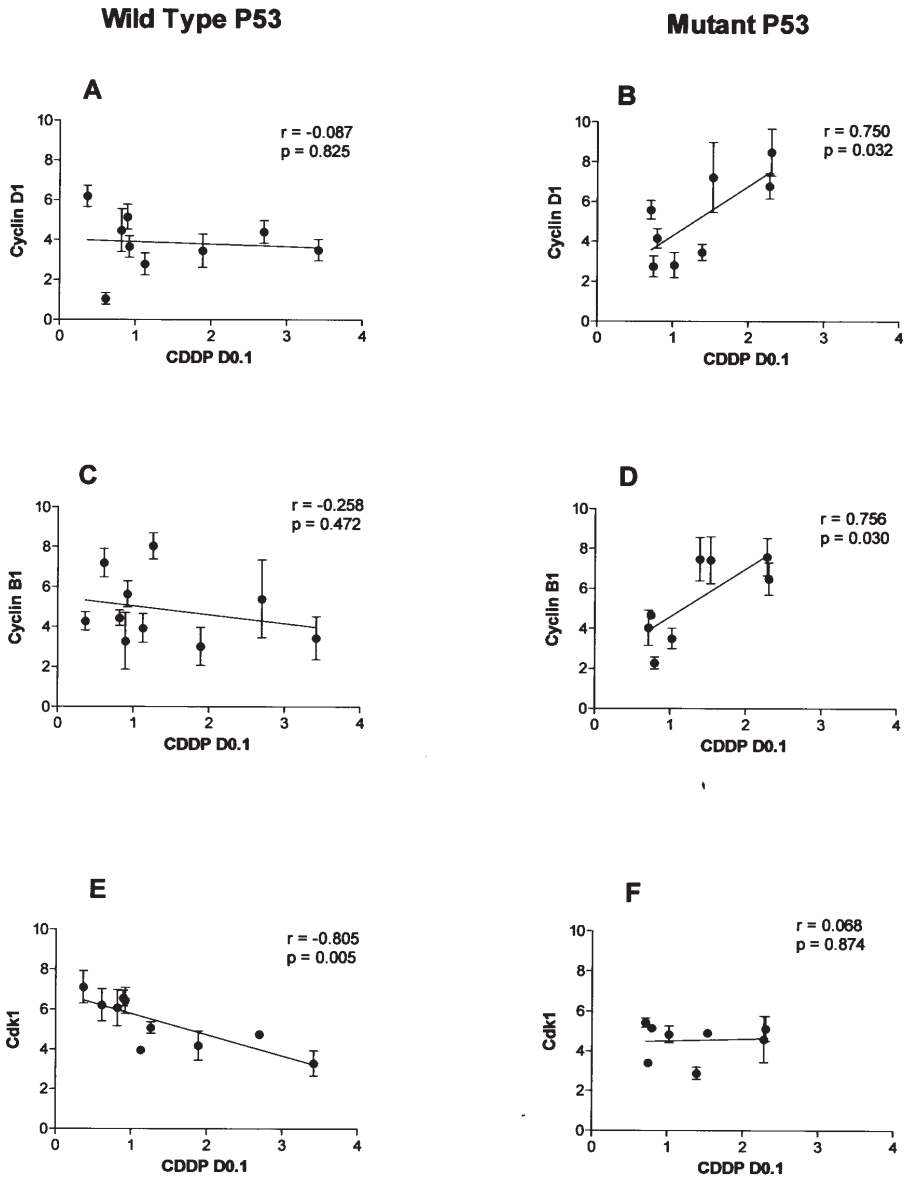


Figure 5. Linear regression analyses comparing sensitivity to CDDP (D0.1 values, Table I) with expression of cyclins B1 and D1 and cyclin dependent kinase Cdk1, in p53 wild-type and p53 mutant cell lines.

histogenetic origin, is consistent with the finding of King *et al* (39) that in human clinical non-small cell lung cancer paclitaxel can bypass mutant p53 and should be considered as a component of treatment for patients with metastatic NSCLC whose tumours exhibit p53 mutations.

Potential theranostic implications of cyclin D1 overexpression in p53 mutant cells. The relationship we detected between CDDP resistance and cyclin D1 overexpression was apparent in p53 mutant but not p53 wild-type cells. This finding, along with the uniform sensitivity of these cells to paclitaxel, indicates that, if a similar situation pertained in the clinic, paclitaxel could potentially be an alternative rather than an additive to CDDP, in p53 mutant cells expressing high levels of cyclin D1. This approach could potentially apply to ovarian cancer for which the standard first line treatment policy is combined paclitaxel and CDDP (22). Analysis of cyclin D1 expression and p53 mutational status in this situation could potentially provide a theranostic test to identify tumours that would benefit as much from single agent paclitaxel as from combined paclitaxel/CDDP treatment, or benefit more from paclitaxel plus a non-platinating agent. Alternatively, p53 wild-type cells overexpressing Cdk1 would be expected to be particularly sensitive to CDDP. Our results also suggest the possibility that cyclin B1 and Ras overexpression in p53 mutant cells might provide theranostic predictors in the clinic for CDDP and etoposide, respectively.

The theranostic relationships identified here have been obtained using cell lines derived from a wide range of histologies. Because of the many cell phenotypes, the inevitable selectivity for cells most adaptable to growth in tissue culture and the long period many of these cell lines have been passaged *in vitro*, they are, understandably, not believed to be very representative models of the *in vivo* clinical situation. Nonetheless, the findings we report may give clues to developing clinical theranostic tests which could be relevant to determining the optimal treatment of clinical cancers; not least in indicating the potential importance of analysing over-

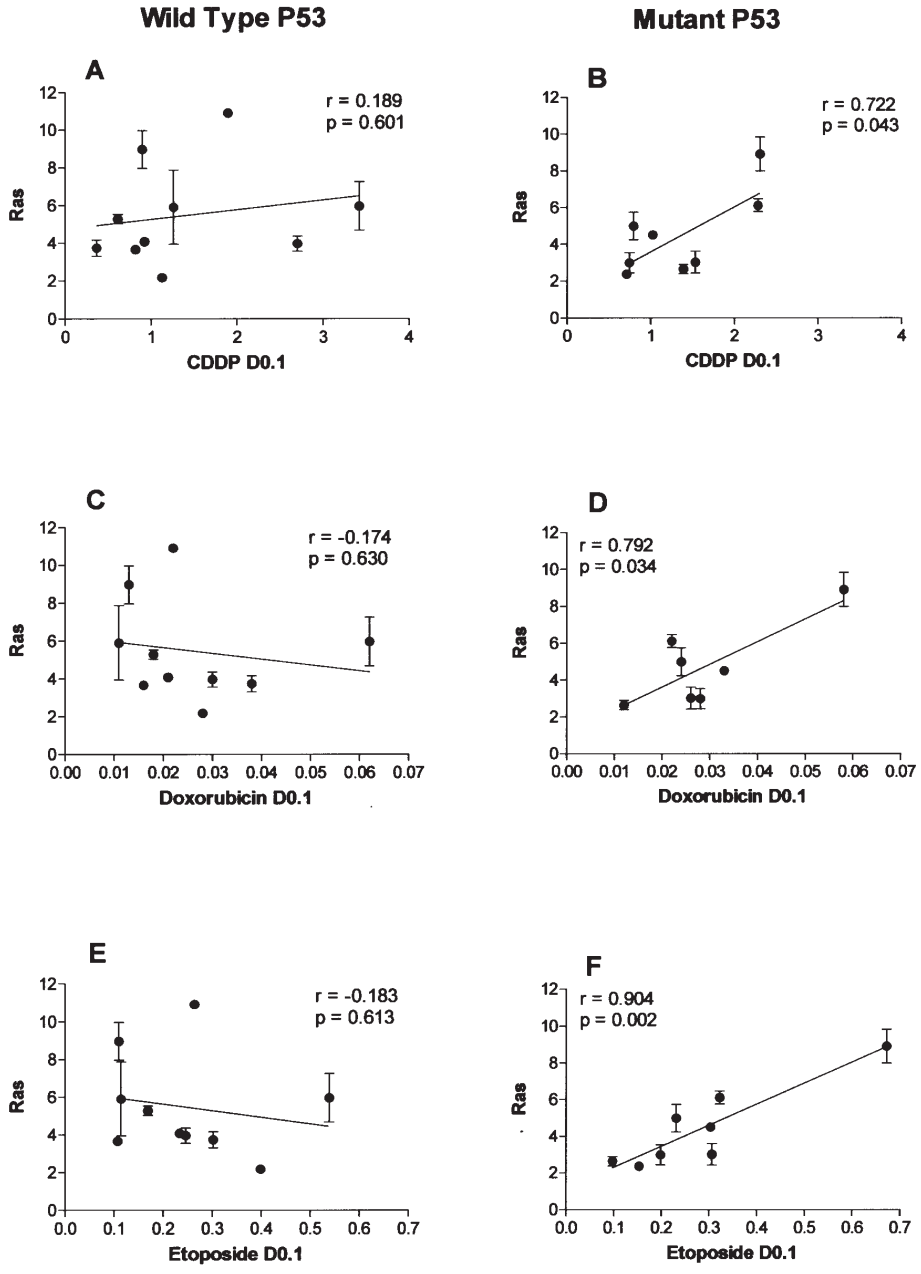


Figure 6. Linear regression analyses comparing Ras expression to therapeutic sensitivity to CDDP, doxorubicin and etoposide (D0.1 values, Table I) in p53 wild-type and p53 mutant cell lines.

expression of positive cell signalling proteins in the context of p53 mutational status.

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