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Preclinical report

DNA repair protein levels vis-à-vis anticancer drug resistance in the human tumor cell lines of the National Cancer Institute drug screening program

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Nucleotide excision repair (NER) is a multi-enzyme DNA repair pathway in eukaryotes. Several NER genes in this pathway including XPB, XPD, XPA and ERCC-1 have been implicated in anticancer drug resistance in human tumor cells. In this study, we assessed the levels of the above-mentioned proteins in the NCI panel of 60 human tumor cell lines in relation to the cytotoxicity patterns of 170 compounds that constitute the standard agent (SA) database. The database consists of drugs used in the clinic for which a mechanism of action has been at least partially defined. The ERCC-1, XPD and XPB protein expression patterns yielded significant negative Pearson correlations with 13, 32 and 17 out of the 170 compounds, respectively (using $p < 0.05$). XPA produced a random assortment of negative and positive correlations, and did not appear to confer an overall resistance or sensitivity to these drugs. Protein expression was also compared with a pre-defined categorization of the standard agents into six mechanism-of-action groups resulting in an inverse association between XPD and alkylating agent sensitivity. Our present data demonstrate that XPD protein levels correlate with resistance to alkylating agents in human tumor cell lines suggesting that XPD is implicated in the development of this resistance. NER activity, using the *in vitro* cell-free system repair assay, revealed no correlation between NER activity and the level of XPD protein in four cell lines with widely varying XPD protein levels. This lack of correlation may be due to the contribution of XPD to other functions including interactions with the Rad51 repair pathway. [© 2002 Lippincott Williams & Wilkins.]

Key words: Bifunctional alkylating agent, DNA repair, drug resistance, endogenous protein, National Cancer Institute human cell line panel, nucleotide excision repair, xeroderma pigmentosum complementary group D.

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Introduction

Drug resistance to chemotherapeutic agents presents a major obstacle to the successful treatment of cancer. Several mechanisms and gene products have been identified that contribute to drug resistance in human cancers.[1–6] Most of these mechanisms are associated with specific agents and/or tumor types, suggesting that drug resistance is multifactorial.[1–4,6–10]

Exposure of tumor cells to DNA-damaging agents such as radiation, anticancer drugs or other toxic agents leads to different cellular responses depending on the degree and type of DNA damage.[11] DNA repair is an important system that allows tumors to cope with DNA damage and survive genotoxic stress.[12] Repair of DNA lesions induced by anticancer drugs has been implicated in acquired drug resistance. Nucleotide excision repair (NER) is a major DNA repair system implicated in the repair of DNA lesions induced by UV light and by some chemotherapeutic agents.[12–14] Recent studies have suggested

that several NER genes including xeroderma pigmentosum (XP) A, XPB, XPD and excision repair cross-complementing (ERCC)-1 may be involved in anticancer drug resistance/sensitivity.[1,3,8,10,15–18] The NER mechanism comprises a four-step model with more than 20 gene products being involved in this complicated process.[12,17] ERRC-1 mutant cell lines have been shown to be hypersensitive to alkylating agents.[18,19] The expression of XPB and ERCC-1 is increased in cisplatin drug resistance, especially in ovarian cancer.[8,17]

XPD and XPB DNA helicases are components of the transcription factor TFIIH complex that is involved in transcription and NER.[20] In a recent study, we investigated XPD expression in a panel of 14 human tumor cell lines that had not been pre-selected for chloroethylnitrosourea (CENU) resistance, and found a significant correlation between XPD protein levels and 1,3-bis-(2-chloroethyl)-1-nitrosourea (BCNU) cytotoxicity.[3] These results suggest that XPD may be involved in alkylating agent resistance in human tumor cell lines.

In the present study, we assess the protein levels of ERCC-1, XPD, XPB and XPA, a DNA damage binding protein,[13] in a panel of 60 human tumor cell lines used by the National Cancer Institute (NCI) for anticancer drug screening. These protein levels were then correlated with the cytotoxicity patterns of 170 compounds tested in this cell line panel as a means of assessing whether these proteins play a role in anticancer drug resistance.

Materials and methods

Western blot analysis

ERCC-1, XPD, XPB and XPA protein levels in the 60 human tumor cell lines of the NCI were determined by Western blot as described.[3] Briefly, total cellular extracts were separated by SDS–PAGE, electroblotted onto nitrocellulose membrane (BioRad, Hercules, CA), followed by blocking in 5% non-fat milk overnight at 4°C and hybridization with primary antibody overnight at 4°C. The ERCC-1 antibody (a kind gift from Dr Nicolas GJ Jaspers, Department of Cell Biology and Genetics, Erasmus University, Rotterdam, The Netherlands), a rabbit polyclonal anti-ERCC-1 antiserum, was used at 1:2000 dilution. The XPD polyclonal antibody, MER-2, raised against the whole recombinant XPD protein in mouse (a kind gift from Dr Larry Thompson, Lawrence Livermore National Laboratory, Livermore, CA) was used at 1:5000 dilution. Anti-XPB monoclonal antibody, 1G12 (a kind gift from Dr Jean-Marc Egly, Institut de Génétique et de Biologie Moléculaire et Cellulaire, Strasbourg, France), was used at 1:1000 dilution. The XPA antibody (rabbit polyclonal IgG FL-273; Santa Cruz Biotechnology, Santa Cruz CA) was used at a concentration of 1 μg/ml. Following several washes, the blot was incubated with secondary antibody (sheep anti-mouse Ig at 1:1000 or donkey anti-rabbit Ig at 1:500) (Amersham Pharmacia Biotech, Piscataway, NJ) for 1 h at 4°C. Specific binding was visualized with the electrochemiluminescence (ECL) system (Amersham Pharmacia Biotech), followed by subsequent exposure of the blot to film (Eastman Kodak, Rochester, NY). Band intensities were quantitated by densitometry readings using the Scion Image program with an HP ScanJet 5100C Scanner (Hewlett Packard, Greeley, CO). Values were standardized with α-tubulin in order to correct for protein-loading differences. The α-tubulin expression was detected with mouse monoclonal IgG primary antibody at 1:1000 dilution (Amersham Pharmacia Biotech) and secondary antibody at 1:1000 dilution. Normalized gene expression was obtained by dividing the densitometry reading of each gene by the densitometry reading of α-tubulin, for each cell line. The results represent the mean of three separate experiments.

NCI anticancer drug screening database

The drug screening data was accessed from the existing anticancer screening database generated as described.[21] The sulforhodamine B assay[22] was used to determine the cytotoxicity or growth inhibition of the 60 human tumor cell lines of the NCI panel. Drug potency (and thus cell line sensitivity) was measured as the negative log ($base_{10}$) of the molar concentration required for 50% growth inhibition relative to a time-zero absorbance-corrected control (pGI50). Each of the 170 compounds comprising the standard agent (SA) database was tested in the panel of 60 cell lines in 10 independent passes.

In vitro repair assay and NER activity

The 2959-bp plasmid pbluescript (pSK; Stratagene, La Jolla, CA) was prepared by alkaline lysis method (Qiagen, Ontario, Canada). The different forms of DNA obtained after plasmid preparation were separated on two successive sucrose gradients, and the fractions containing the supercoiled DNA was isolated and purified (linear, circular and supercoiled). The pSK plasmid was then treated with cisplatin

(0.5 μg Pt/100 μg DNA) as described previously.[23] Nuclear extracts were prepared as described by Aboussekhra *et al.*[24] Each reaction mixture contained 300 ng of damaged or untreated pSk plasmid, 40 μg of cell extract in reaction buffer containing 45 mM HEPES–KOH (pH 7.8), 7.4 mM $MgCl_2$, 0.9 mM DTT, 0.4 mM EDTA, 2 mM ATP, 20 μM each of dGTP, dTTP and dCTP, 4 μM dATP, 40 mM phosphocreatine, 2.5 μg of creatine phosphokinase, 3.4% glycerol, 18 μg of bovine serum albumin, and 4 μCi [α-^{32}P]ATP.[25] Reactions were carried out at 30°C for 3 h. The plasmid DNA was then purified and linearized with *Eco*RI, and electrophoresed overnight on a 1% agarose gel containing 0.5 μg/ml ethidium bromide. The gel was then fixed for 15 min in 15% methanol and 10% acetic acid, dried on Whatman filter paper, and exposed for autoradiography. For data presentation, autoradiographs were scanned and processed with Adobe Photoshop. The experiments were repeated 3 times and the mean $\pm$ SE determined.

Correlation analysis

The similarity pattern search program COMPARE[26] was used to find the compounds or agents whose potency appeared to depend on the molecular target levels measured in the 60 NCI cell lines. In this 'molecular target' application of COMPARE[27–29] the seed entered for the correlation is constructed from the values for each of the NER-related gene products, ERCC-1, XPD, XPB and XPA, measured in the 60 cell lines. Negative Pearson correlation coefficients (PCCs) indicate that cell lines with *more* of the target are *less* sensitive to the agent, consistent with the anticipated role of the targets as *resistance factors*. The significance of the correlations found must take into account one or all of the following: knowledge of the target and its relationship to known drug mechanisms of action, PCCs with their associated *p* values, and, most importantly, laboratory confirmation of a direct interaction.

Mechanism of action (MOA) analysis

A meta-analysis of the correlations between the molecular target and the standard agents was conducted to detect trends that might exist for groups of compounds associated with a particular type of cytotoxic or cytostatic activity. This method (TG Myers, unpublished data) uses SAS software Proc ANOVA that was performed for each of the molecular targets. Out of 170 agents, 122 were pre-assigned to six groups based on MOA.[30] With MOA as the classification variable and (linear-transformed) PCC as the independent variable, 95% confidence intervals were generated. An empirical evaluation of the performance of this analysis using molecular target correlations that have been independently validated has suggested that confidence intervals that do NOT overlap with the near-zero interval −0.10 to 0.10 indicate significant trends (TG Myers, unpublished data). As with the molecular target COMPARE, a significant trend in positive correlations, revealed by the meta-analysis, is evidence that the molecular

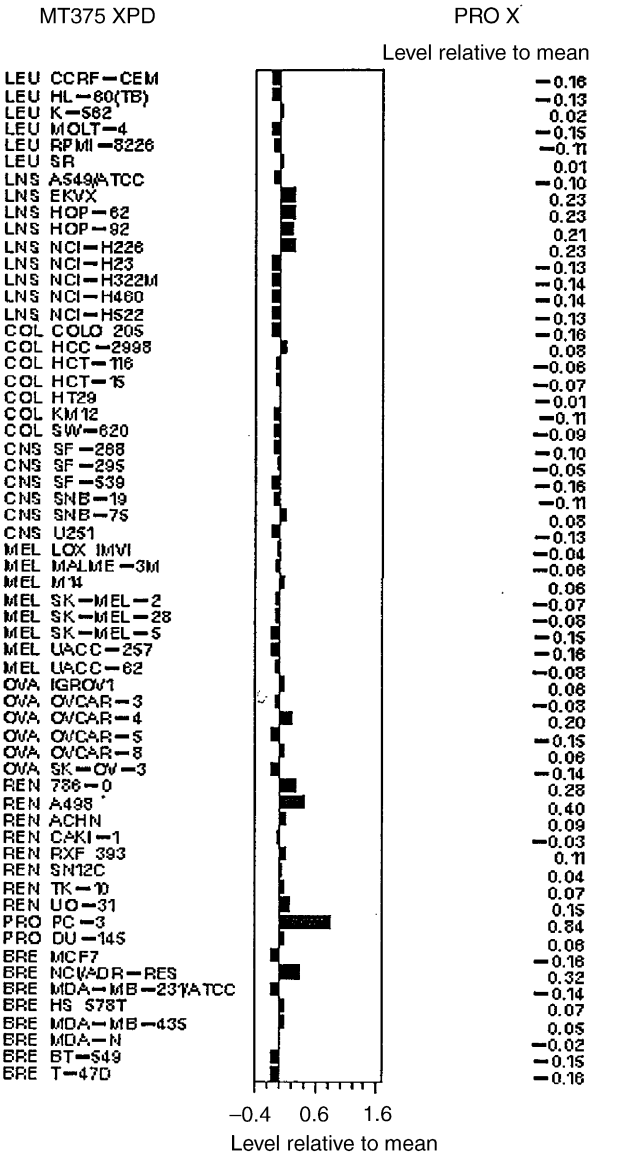

Figure 1. Mean XPD protein levels in the NCI panel of 60 cell lines.

Table 1. ERCC-1, XPD, XPB and XPA protein levels in the NCI panel of 60 cell lines

Cell lines	Protein levels			
	XPD	XPA[a]	ERCC-1	ERCC-3
Leukemia[b]				
K-562	0.18 ± 0.01	0.69 ± 0.22	0.59 ± 0.20	0.97 ± 0.13
MOLT-4	0.00 ± 0.01	0.65 ± 0.18	0.66 ± 0.09	0.61 ± 0.04
CCRF-CEM	0.00 ± 0.00	0.68 ± 0.24	1.03 ± 0.36	0.59 ± 0.14
RPMI-8226	0.05 ± 0.04	0.65 ± 0.19	0.70 ± 0.24	0.88 ± 0.14
HL-60(TB)	0.03 ± 0.02	0.67 ± 0.2	0.51 ± 0.19	0.27 ± 0.00
SR	0.17 ± 0.05	0.74 ± 0.36	0.78 ± 0.37	1.00 ± 0.00
Central nervous system				
SF-268	0.05 ± 0.02	0.41 ± 0.19	0.83 ± 0.21	1.69 ± 0.60
SF-295	0.10 ± 0.06	0.47 ± 0.24	1.01 ± 0.29	0.78 ± 0.43
SF-539	0.00 ± 0.00	0.00 ± 0.00	1.88 ± 2.09	3.03 ± 1.33
SNB-19	0.04 ± 0.02	0.24 ± 0.15	1.30 ± 0.47	1.76 ± 1.03
SNB-75	0.23 ± 0.07	0.23 ± 0.11	0.73 ± 0.31	0.52 ± 0.18
U251	0.03 ± 0.02	0.48 ± 0.19	1.04 ± 0.53	0.83 ± 0.16
Breast				
BT-549	0.00 ± 0.01	0.53 ± 0.10	0.91 ± 0.39	0.98 ± 0.07
HS 578T	0.23 ± 0.13	0.07 ± 0.01	0.74 ± 0.25	1.32 ± 0.53
MCF7	0.00 ± 0.00	0.92 ± 0.37	1.20 ± 0.49	1.46 ± 0.25
MCF7/ADR-RES	0.47 ± 0.27	0.21 ± 0.12	1.07 ± 0.28	1.68 ± 0.72
MDA-MB-231	0.02 ± 0.03	0.36 ± 0.18	0.11 ± 0.05	0.79 ± 0.19
MDA-MB 435	0.21 ± 0.13	0.70 ± 0.15	0.94 ± 0.13	1.15 ± 0.64
MDA-N	0.14 ± 0.04	0.86 ± 0.09	1.13 ± 0.50	0.91 ± 0.47
T-47D	0.00 ± 0.00	0.78 ± 0.35	1.48 ± 0.85	0.47 ± 0.07
Colon				
COLO 205	0.00 ± 0.00	1.43 ± 0.22	1.02 ± 0.26	1.41 ± 0.72
HCC-2998	0.24 ± 0.05	1.39 ± 0.21	1.26 ± 0.45	2.05 ± 0.85
HCT-116	0.09 ± 0.02	0.95 ± 0.23	0.95 ± 0.41	1.22 ± 0.54
HCT-15	0.09 ± 0.04	0.52 ± 0.31	0.86 ± 0.36	0.75 ± 0.26
HT29	0.14 ± 0.04	1.02 ± 0.27	1.01 ± 0.49	1.01 ± 0.27
KM12	0.05 ± 0.04	1.46 ± 0.15	0.85 ± 0.2	0.87 ± 0.53
SW-620	0.06 ± 0.07	1.19 ± 0.11	1.27 ± 0.51	1.1 ± 0.65
Lung				
A549/ATCC	0.06 ± 0.06	1.46 ± 0.62	1.32 ± 0.43	1.34 ± 0.66
EKVX	0.38 ± 0.16	0.80 ± 0.15	0.99 ± 0.35	1.35 ± 0.58
HOP-62	0.39 ± 0.10	0.48 ± 0.11	0.75 ± 0.15	1.08 ± 0.54
HOP-92	0.37 ± 0.15	0.40 ± 0.22	0.64 ± 0.20	1.8 ± 0.30
NCI-H322M	0.01 ± 0.02	0.95 ± 0.17	1.46 ± 0.58	1.74 ± 1.05
NCI-H226	0.39 ± 0.09	0.27 ± 0.08	0.80 ± 0.11	1.324 ± 0.17
NCI-H23	0.03 ± 0.01	0.56 ± 0.09	0.87 ± 0.19	1.22 ± 0.34
NCI-H460	0.02 ± 0.01	0.58 ± 0.01	0.90 ± 0.10	0.60 ± 0.40
NCI-H522	0.028 ± 0.01	0.440 ± 0.04	0.464 ± 0.077	0.417 ± 0.04
Melanoma				
LOX IMVI	0.12 ± 0.07	1.03 ± 0.53	1.00 ± 0.50	3.40 ± 0.61
M14	0.22 ± 0.16	0.82 ± 0.30	0.75 ± 0.421	1.13 ± 0.35
MALME-3M	0.09 ± 0.08	1.01 ± 0.45	0.92 ± 0.38	0.96 ± 0.32
SK-MEL-2	0.08 ± 0.10	0.57 ± 0.19	0.70 ± 0.15	1.70 ± 0.59
SK-MEL-5	0.00 ± 0.01	0.48 ± 0.18	1.04 ± 0.15	0.97 ± 0.24
SK-MEL-28	0.07 ± 0.05	0.70 ± 0.31	1.12 ± 0.13	1.24 ± 0.09
UACC-62	0.07 ± 0.05	0.37 ± 0.18	0.94 ± 0.08	1.14 ± 0.60
UACC-257	0.00 ± 0.00	0.79 ± 0.30	0.99 ± 0.24	2.50 ± 0.14
Ovarian				
IGROV-1	0.22 ± 0.15	0.73 ± 0.30	0.68 ± 0.04	1.01 ± 0.30
OVCAR-3	0.08 ± 0.05	0.29 ± 0.11	0.92 ± 0.59	2.15 ± 0.92
OVCAR-4	0.36 ± 0.08	0.14 ± 0.01	1.48 ± 0.37	1.28 ± 0.53
OVCAR-5	0.01 ± 0.02	1.04 ± 0.38	0.88 ± 0.27	0.76 ± 0.30
OVCAR-8	0.22 ± 0.09	0.36 ± 0.19	0.82 ± 0.20	0.93 ± 0.29
SK-OV-3	0.02 ± 0.03	0.62 ± 0.34	1.48 ± 0.46	1.22 ± 0.24

Table 1. (*Continued*)

Cell lines	Protein levels			
	XPD	XPA[a]	ERCC-1	ERCC-3
Prostate				
DU-145	0.21 ± 0.09	0.81 ± 0.14	1.01 ± 0.19	0.84 ± 0.229
PC-3	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.70 ± 0.700
Renal				
786-O	0.43 ± 0.16	0.595 ± 0.11	0.62 ± 0.11	1.07 ± 0.396
A498	0.56 ± 0.18	0.66 ± 0.12	0.68 ± 0.13	0.993 ± 0.33
ACHN	0.25 ± 0.10	0.809 ± 0.15	0.692 ± 0.24	0.739 ± 0.04
CAKI-1	0.13 ± 0.04	0.375 ± 0.02	0.39 ± 0.11	0.96 ± 0.412
RXF-393	0.26 ± 0.07	0.28 ± 0.07	0.43 ± 0.09	1.04 ± 0.442
SN12C	0.19 ± 0.07	0.52 ± 0.18	0.78 ± 0.21	1.25 ± 0.54
TK-10	0.23 ± 0.06	0.51 ± 0.17	0.73 ± 0.29	1.04 ± 0.30
UO-31	0.31 ± 0.16	0.44 ± 0.16	0.69 ± 0.21	0.81 ± 0.22

[a]Protein levels for XPA, ERCC-1, XPD and XPB are expressed as the mean value of at least three separate experiments. The protein level for XPA, ERCC-1 and XPD for each cell line was divided by the corresponding protein level for PC-3 cells, whereas the XPB protein level for each cell line was divided by the XPB protein level for SR cells, for each individual experiment.
[b]Tissue origin of the tumor cell line.

Table 2. COMPARE-generated Pearson correlations between XPD protein levels and standard drug cytotoxicity in the NCI panel of 60 cell lines

Rank order	Chemical	PCC
1	chip[a]	−0.438**
2	dihydro-lenperone	0.401*
3	paclitaxel	−0.378**
4	pibenzimol HCl	−0.374**
5	macbecin II	−0.368**
6	homoharringtonine	−0.365**
7	bis-pyridocarbazolium DMS	−0.360*
8	flavone acetic acid ester	0.351*
9	largomycin	−0.310*
10	actinomycin D	−0.309*
11	asaley	−0.303*
12	phyllanthoside	−0.296*
13	dihydro-5-azacytidine	−0.293*
14	bruceantin	−0.290*
15	mitramycin	−0.284*
16	chromomycin A3	−0.283*
17	bactobolin	−0.281*
18	didemnin B	−0.316*
19	vinblastine sulfate	−0.279*
20	echinomycin	−0.275*
21	*S*-trityl-L-cysteine	−0.272*
22	PCNU[a]	−0.269*
23	tetrocarcin A-sodium SAL	−0.266*
24	diglycoaldehyde[a]	−0.266*
25	Adriamycin	−0.265*
26	O^6-methylguanine	0.265*
27	CCNU[a]	−0.264*
28	mitomycin C[a]	−0.262*
29	VM-26	−0.259*
30	deoxydoxorubicin	−0.258*
31	triciribine phosphate	0.255*
32	ICRF-187	−0.255*

*$p < 0.05$; **$p < 0.01$.
[a]Alkylating agent.

Table 3. Relationship between NER protein levels and six major clinical MOA

MOA[a]	*N*[b]	Protein	Lower 95% confidence limit	Upper 95% confidence limit
Anti-DNA	16	ERCC-1	−0.152	−0.014
		XPD	−0.124	0.027
		XPB	−0.162	−0.033
		XPA	−0.023	0.141
Anti-NUC	19	ERCC-1	−0.023	0.104
		XPD	−0.161	−0.023
		XPB	−0.101	0.017
		XPA	0.156	0.307
T1	23	ERCC-1	−0.022	0.093
		XPD	−0.159	−0.033
		XPB	−0.170	−0.063
		XPA	−0.174	−0.036
T2	16	ERCC-1	−0.141	−0.003
		XPD	−0.220	−0.069
		XPB	−0.225	−0.096
		XPA	−0.001	0.164
TU	13	ERCC-1	−0.124	0.029
		XPD	−0.276	−0.108
		XPB	−0.047	0.095
		XPA	0.022	0.204
ALK	35	ERCC-1	−0.130	−0.036
		XPD	−0.196	−0.145
		XPB	−0.170	−0.083
		XPA	−0.120	−0.009

[a]Anti-DNA, anti-DNA agent; ALK, alkylating agent; T1, topoisomerase I inhibitor; T2, topoisomerase II inhibitor; TU, tubulin-active anti-mitotic agent; Anti-NUC, nucleotide synthesis inhibitor.
[b]Number of compounds in the MOA group.

target is a sensitizing factor. Conversely, a significant trend in negative correlations is evidence that the molecular target is a resistance factor.

Results

ERCC-1, XPA and XPB proteins were detected in all of the 60 cell lines, with a 25-fold or less range of protein expression. However, XPD protein levels varied to a much greater extent (200-fold), with four cell lines having barely detectable XPD protein levels (Figure 1 and Table 1). Only one cell line had negligible XPA protein levels.

ERCC-1, XPD and XPB protein levels were compared to the NCI standard SA database and resulted in a preponderance of highly negative correlations indicating that these targets tend to confer resistance to these clinical agents. However, the PCCs were generally modest or poor. Comparisons of ERCC-1 and XPB protein levels were statistically significant ($p<0.05$) with the cytotoxicity of 13 and 17 out of 170 agents, respectively (data not shown). However, XPD protein levels correlated significantly with resistance to 32 out of 170 agents (Table 2). Two out of 13 agents for ERCC-1, and two out of 17 agents for XPB (data not shown) and five out of 32 agents for XPD turned out to be alkylating agents (Table 2). The XPA target produced a random assortment of positive and negative correlations, and does not appear to confer an overall resistance or sensitivity to these drugs.

A meta-analysis of the possible correlations of gene expression and the six major clinical MOA was performed. This analysis involves grouping drugs into known MOA, and comparing the PCC for each protein with each drug in the group in order to determine if there is a significant correlation between the protein levels and specific MOA drug resistance. No association between ERCC-1, XPB or XPA protein levels and MOA was found. However, a significant association between XPD protein levels and alkylating agent resistance was observed (Table 3).

Four cell lines expressing a wide range of XPD protein levels (MCF-7, 0.0 ± 0.0; PC-3, 1.0 ± 0.0; Molt-4, 0.005 ± 0.00; and A498, 0.559 ± 0.18) were examined for NER activity. There was no correlation ($r=0.104$) between XPD levels and NER activity

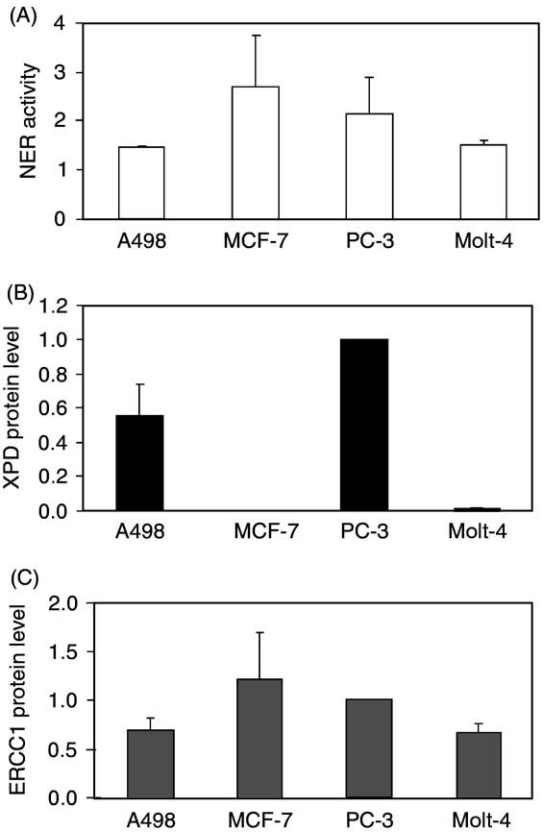

Figure 2. *In vitro* NER activity correlates with ERCC-1 but is independent of XPD endogenous protein levels. The NER activity was measured in 40 μg of protein from A498, MCF-7, PC-3 and Molt-4 nuclear extracts utilizing a cisplatin-damaged PSK plasmid (A). XPD (B) and ERCC-1 (C) protein levels relative to tubulin were determined in the same nuclear extracts as in (A) by Western blot analysis. The values represent the mean of three independent experiments $\pm$ SE.

(Figure 2). We further correlated ERCC-1, XPA and XPB protein levels with NER activity in the same cell lines. While ERCC-1 protein levels strongly correlate (r=0.994, p=0.0051) with NER activity (Figure 2), XPA and XPB protein levels did not (r=0.8389, p=0.161 and r=0.764, p=0.235, respectively).

Discussion

Resistance to chemotherapy is a major impediment in the successful treatment of human cancers. Alkylating agents comprise a multitude of chemotherapeutic drugs that alkylate various cellular components including DNA, RNA and proteins. Repair of toxic lesions is paramount to cell survival. NER is involved in the repair of a wide variety of DNA lesions,[12] including lesions induced by some chemotherapeutic agents and ultraviolet (UV) light.[13,14,31] XPD, one component of the NER system, functions as a helicase and may also be involved in DNA damage recognition.[32,33] XPD can correct the NER defect in the UV-sensitive Chinese hamster ovary cell line, UV5, and in cell lines derived from XPD patients.[34]

Here, we examined four NER proteins previously implicated in drug resistance/sensitivity in a panel of 60 human tumor cell lines and compared them with the drug toxicity patterns of 170 compounds used for anticancer drug screening by the NCI. Our results show that XPD protein levels correlate with drug cytotoxicity in 32 out of the 170 compounds. In particular, XPD is associated with alkylating agent resistance. Other investigators have demonstrated that ERCC-1 and XPB are also implicated in drug resistance in some tumor cell lines.[1,8,17] However, in the present study, although ERCC-1 and XPB protein levels show significant correlation with some of the 170 agents, there was no significant association with any particular type of drug as defined by a particular MOA. It is possible that ERCC-1 is associated with cisplatin drug resistance in a particular tumor type such as ovarian cancer, as previously described.[17] Furthermore, while XPA did not show a significant correlation with alkylating agents in the NCI cell line panel, low XPA levels in testicular cancer cell lines correlate with cisplatin sensitivity.[35] There are no testicular cancer cell lines in the NCI cell line panel. We hypothesize that XPD might modulate or being involved in a DNA repair mechanisms other than NER. For example, it has been proposed that NER components along with recombination repair are necessary for the removal of DNA cross-links in bacteria and *Saccharomyces cerevisiae*.[36] ERCC-1 and XPF complement CHO cell line NER mutants groups 1 and 4, respectively. These mutant cell lines are very hypersensitive to bifunctional alkylating agents.[18,19] The ERCC-1/XPF complex functions as an endonuclease that is probably implicated in the initial steps of inter-strand cross-link repair followed by recombinational repair via the Rad51 complex.[36]

XPD may be implicated in the mechanism of resistance to alkylating agents, since functional XPD is required for the preferential removal of *N*-ethylpurines induced by ethylnitrosourea, in the actively transcribed DNA.[37]

The relationship of increased XPD protein levels to nucleotide excision repair activity was examined in four cell lines of varying XPD protein levels. There was no correlation of XPD protein levels with NER activity. This result is consistent with recent reports

suggesting that XPD is not an NER rate-limiting enzyme[38] (R Aloyz and L Panasci, unpublished result). ERCC-1 protein levels in the four cell lines strongly correlated with NER repair activity. Consistent with this results, ERCC-1 mRNA levels have been reported to correlate with NER repair activity in human lymphocytes and, moreover, complementation of ovarian cancer cell line extracts with ERCC-1/XPF protein resulted in an increase in NER repair activity.[38,39]

Conclusion

In summary, XPD protein levels correlate with alkylating agent drug resistance and it appears that this is not related to increased NER repair activity. We have evidence that XPD may modulate drug resistance and homologous recombinational repair. Overexpression of XPD in SKMG-4 does result in alkylating agent drug resistance associated with an increase in the density of drug-induced Rad51 foci, and co-immunoprecipitation of Rad51 and XPD.[40] Thus, it is possible that the correlation between XPD and alkylating agent drug resistance found in this study is related to an XPD interaction with the Rad51 recombinational repair pathway.

References

1. Andersson BS, Sadeghi T, Siciliano MJ, Legerski R, Murray D. Nucleotide excision repair genes as determinants of cellular sensitivity to cyclophosphamide analogs. *Cancer Chemother Pharmacol* 1996; **38**: 406–16.
2. Brent TP, Houghton PJ, Houghton JA. O^6-Alkylguanine-DNA alkyltransferase activity correlates with the therapeutic response of human rhabdomyosarcoma xenografts to 1-(2-chloroethyl)-3-(*trans*-4-methylcyclohexyl)-1-nitrosourea. *Proc Natl Acad Sci USA* 1985; **82**: 2985–9.
3. Chen ZP, Malapetsa A, McQuillan A, *et al*. Evidence for nucleotide excision repair as a modifying factor of O^6-methylguanine-DNA methyltransferase-mediated innate chloroethylnitrosourea resistance in human tumor cell lines. *Mol Pharmacol* 1997; **52**: 815–20.
4. Kaye SB. Clinical drug resistance: the role of factors other than P-glycoprotein. *Am J Med* 1995; **99**: 40S–4S.
5. Nishikawa K, Rosenblum MG, Newman RA, Pandita TK, Hittelman WN, Donato NJ. Resistance of human cervical carcinoma cells to tumor necrosis factor correlates with their increased sensitivity to cisplatin: evidence of a role for DNA repair and epidermal growth factor receptor. *Cancer Res.* 1992; **52**: 4758–65.
6. Zamble DB, Lippard SJ. Cisplatin and DNA repair in cancer chemotherapy. *Trends Biochem Sci* 1995; **20**: 435–9.
7. Chresta CM, Riola EL, Hickman JA. Apoptosis and cancer chemotherapy. *Behring Institute Mitteilungen* 1996; **97**: 232–40.
8. Dabholkar M, Thornton K, Vionnet J, Bostick-Bruton F, Yu JJ, Reed E. Increased mRNA levels of xeroderma pigmentosum complementation group B (XPB) and Cockayne's syndrome complementation group B (CBS) without increased mRNA levels of multidrug-resistance gene (MDRI) or metallothionein-II (MT-II) in platinum-resistant human ovarian cancer tissues. *Biochem Pharmacol* 2000; **60**: 1611–9.
9. Dolan ME, Pegg AE, Dumenco LL, Moschel RC, Gerson SL. Comparison of the inactivation of mammalian and bacterial O^6-alkylguanine-DNA alkyltransferases by O^6-benzylguanine and O^6-methylguanine. *Carcinogenesis* 1991; **12**: 2305–9.
10. Phillips PC. Antineoplastic drug resistance in brain tumors. *Neurol Clin* 1991; **9**: 383–404.
11. Smith ML, Fornace AJ Jr. Mammalian DNA damage-inducible genes associated with growth arrest and apoptosis. *Mutat Res* 1996; **340**: 109–24.
12. Ma L, Hoeijmakers JH, van der Eb AJ. Mammalian nucleotide excision repair. *Biochim Biophys Acta* 1995; **1242**: 137–63.
13. Aboussekhra A, Wood RD. Repair of UV-damaged DNA by mammalian cells and Saccharomyces cerevisiae. *Curr Opin Genet Dev* 1994; **4**: 212–20.
14. Chu G. Cellular responses to cisplatin: The roles of DNA-binding proteins and DNA repair. *J Biol Chem* 1994; **269**: 787–90.
15. Chen ZP, Malapetsa A, Marcantonio D, Mohr G, Brien S, Panasci LC. Correlation of chloroethylnitrosourea resistance with ERCC-2 expression in human tumor cell lines as determined by quantitative competitive polymerase chain reaction. *Cancer Res* 1996; **56**: 2475–8.
16. Chen ZP, McQuillan A, Mohr G, Panasci LC. Excision repair cross-complementing rodent repair deficiency gene 2 expression and chloroethylnitrosourea resistance in human glioma cell lines. *Neurosurgery* 1998; **42**: 1112–9.
17. Dabholkar M, Bostick-Bruton F, Weber C, Bohr VA, Egwuagu C, Reed E. ERCC-1 and ERCC-2 expression in malignant tissues from ovarian cancer patients. *Natl J Cancer Inst* 1992; **84**: 1512–7.
18. Damia G, Imperatori L, Stefanini M, D'Incalci M. Sensitivity of CHO mutant cell lines with specific defects in nucleotide excision repair to different anticancer agents. *Int J Cancer* 1996; **66**: 779–83.
19. Hoy CA, Thompson LH, Mooney CL, Salazar EP. Defective DNA cross-link removal in Chinese hamster cell mutants hypersensitive to bifunctional alkylating agents. *Cancer Res* 1985; **45**: 1737–43.
20. Drapkin R, Reardon JT, Ansari A, *et al*. Dual role of TFIIH in DNA excision repair and in transcription by RNA polymerase II. *Nature* 1994; **368**: 769–72.
21. Monks D, Scudiero P, Skehan P, *et al*. Feasibility of a high-flux anticancer drug screen using a diverse panel of cultured human tumor cell lines. *J Natl Cancer Inst* 1991; **83**: 757–66.

22. Skehan P, Storeng R, Scudiero D, *et al*. New colorimetric cytotoxicity assay for anticancer-drug screening. *J Natl Cancer Inst* 1990; **82**: 1107–12.
23. Marinoni JC, Rossignol M, Egly JM. Purification of the transcription/repair factor TFIIH and evaluation of its associated activities *in vitro*. *Methods* 1997; **12**: 235–53.
24. Aboussekhra A, Biggerstaff M, Shivji MK, *et al*. Mammalian DNA nucleotide excision repair reconstituted with purified protein components. *Cell* 1995; **80**: 859–68.
25. Wood RD, Biggerstaff M, Shivji MKK. Detection and measurement of nucleotide excision repair synthesis by mammalian cells extracts *in vitro*. *Methods: Methods Enzymol* 1995; **7**: 163–75.
26. Paull KD, Shoemaker RH, Hodes L, *et al*. Display and analysis of patterns of differential activity of drugs against human tumor cell lines: development of mean graph and COMPARE algorithm. *J Natl Cancer Inst* 1989; **8**: 1088–92.
27. Alvarez M, Paull K, Monks A, *et al*. Generation of a drug resistance profile by quantitation of mdr-1/P-glycoprotein in the cell lines of the National Cancer Institute Anticancer Drug Screen. *J Clin Invest* 1995; **95**: 2205–14.
28. Lee JS, Paull K, Alvarez M, *et al*. Rhodamine efflux patterns predict P-glycoprotein substrates in the National Cancer Institute drug screen. *Mol Pharmacol* 1994; **46**: 627–38.
29. Paull KD, Hamel E, Malspeis L. Prediction of biochemical mechanisms of action from the *in vitro* antitumor screen of the National Cancer Institute. In: Foye W, ed. *Cancer chemotherapeutic agents*. Washington, DC: ACS 1995.
30. van Osdol WW, Myers TG, Paull KD, Kohn KW, Weinstein JN. Use of the Kohonen self-organizing map to study the mechanisms of action of chemotherapeutic agents. *J Natl Cancer Inst* 1994; **86**: 1853–9.
31. Xue Y, Nicholson WL. The two major spore DNA repair pathways, nucleotide excision repair and spore photoproduct lyase, are sufficient for the resistance of *Bacillus subtilis* spores to artificial UV-C and UV-B but not to solar radiation. *Appl Environ Microbiol* 1996; **62**: 2221–7.
32. Friedberg EC. Xeroderma pigmentosum, Cockayne's syndrome, helicases, and DNA repair: what's the relationship? *Cell* 1992; **71**: 887–9.
33. Sung P, Watkins JF, Prakash L, Prakash S. Negative superhelicity promotes ATP-dependent binding of yeast RAD3 protein to ultraviolet-damaged DNA. *J Biol Chem* 1994; **269**: 8303–8.
34. Weber CA, Salazar EP, Stewart SA, Thompson LH. Molecular cloning and biological characterization of a human gene, ERCC-2, that corrects the nucleotide excision repair defect in CHO UV5 cells. *Mol Cell Biol* 1998; **8**: 1137–46.
35. Koberle B, Masters JR, Hartley JA, Wood RD. Defective repair of cisplatin-induced DNA damage caused by reduced XPA protein in testicular germ cell tumours. *Curr Biol* 1999; **9**: 273–6.
36. Thompson LH, Schild D. The contribution of homologous recombination in preserving genomic integrity in mammalian cells. *Biochimie* 1999; **81**: 87–105.
37. Sitaram A, Plitas G, Wang W, Scicchitano DA. Functional nucleotide excision repair is required for the preferential removal of *N*-ethylpurines from the transcribed strand of the dihydrofolate reductase gene of Chinese hamster ovary cells. *Mol Cell Biol* 1997; **17**: 564–70.
38. Vogel U, Dybdahl M, Frentz G, Nexø BA. DNA repair capacity: inconsistency between effect of over-expression of five NER genes and the correlation of mRNA levels in primary lymphocytes. *Mutat Res* 2000; **461**: 197–210.
39. Ferry K, Hamilton TC, Johnson SW. Increased nucleotide excision repair in cisplatin-resistant ovarian cancer cells. *Biochem Pharmacol* 2000; **60**: 1305–13.
40. Panasci LC, Xu Z. XPD might play an important role in DNA repair. *Proc Am Ass Cancer Res* 2001; **42**: 1094.

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