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DNA repair gene polymorphisms affect cytotoxicity in the National Cancer Institute Human Tumor Cell Line Screening Panel

D. B. YAROSH, A. PEÑA, & D. A. BROWN

AGI Dermatics, Freeport, NY, USA

Abstract
Polymorphisms in DNA repair genes have been suggested to increase the risk of cancer and other diseases, but the epidemiological studies are often not consistent, and the results confusing. We have examined the effect of polymorphisms in base and nucleotide excision–repair genes, as well as regulatory and signalling genes, on cytotoxic sensitivity of tumour cell lines used for screening anticancer drugs by the National Cancer Institute. It was found that for the TP53 P72R and ERCC2 D312N polymorphisms, the heterozygous genotype was most sensitive, while for the OGG1 S326C and NOS3 g. −786T > C polymorphisms the homozygous-variant genotype was most sensitive. The biggest increase in sensitization was found with the XRCC1 R399Q homozygous dominant genotype. The sensitization was found across a broad range of drugs, indicating the importance of DNA repair responses. It was also found that while the other gene polymorphisms were in Hardy–Weinberg equilibrium, the TP53 P72R heterozygous genotype was relatively depleted. For the OGG1 polymorphism, the repair of 8-oxo-guainine from DNA was measured in three panel cell lines that differed in their OGG1 genotype. The cell line with the homozygous-variant genotype had a much poorer repair than the other genotypes, as predicted. The correlation of polymorphisms with cytotoxicity may be an approach to understanding their effects which may be difficult to reveal in epidemiological studies.

Keywords: *Polymorphisms, cytotoxicity, human DNA repair genes, 8-oxo-guanine, nitric oxide synthetase*

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Introduction

Polymorphisms in human genes may contribute to the risks of common diseases. Among the genes most likely to have a direct impact on health are those involved in DNA repair. These genes control the delay in cell division and the removal of lesions following DNA damage. In the TP53 regulatory gene that arrests cell division, mutations cause the cancers in Li–Fraumeni syndrome (Frebourg & Friend 1992), while mutations in any one of eight nucleotide excision–repair genes cause the cancer-prone disease xeroderma pigmentosum (XP) (Kondo et al. 1989).

Polymorphisms have been described in many DNA repair genes. In some cases, their link to cancer has been examined (Goode et al. 2002). There are only a few cases

Correspondence: D. B. Yarosh, AGI Dermatics, 205 Buffalo Avenue, Freeport, NY 11520, USA. E-mail: danyarosh@agiderm.com

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in which a preponderance of evidence indicates a relationship between a particular polymorphism and cancer risk. Most often, the studies are small and not well controlled, the effects are small and the reports are contradictory. In addition, often the effect of the polymorphism on gene expression or the activity of the gene product has not been demonstrated, and it is not known how the polymorphism results in increased cancer risk. Furthermore, the polymorphisms are not known to be linked to other potential effects, such as cell death or ageing.

The problem is illustrated in the human gene for 8-oxo-guanine glycosylase 1 (OGG1). It encodes a DNA glycosylase that initiates base excision–repair of oxidized guanine bases. The OGG1 polymorphism S326C has been associated with an increased risk of several types of cancer (Goode et al. 2002). However, three separate *in vitro* biochemical studies of the activity of the protein produced by the variant gene failed to identify any deficit in activity or reduced DNA repair of oxidatively damaged DNA (Kohno et al. 1988, Dherin et al. 1999, Janssen et al. 2001). It remains a mystery, therefore, whether or not the polymorphism in OGG1, or some linked and hidden allele, or something else, is responsible for the cancer risk.

Other molecules that contribute to the response to toxic agents are signalling molecules. The molecular signal molecule nitric oxide (NO) induces vasodilation, and is made by the enzyme nitric oxide synthetase (NOS) from L-arginine. Polymorphisms in the constitutive nitric oxide synthetase NOS3 gene have been described, one of which (g. −786T > C) in the promoter region reduces NOS3 gene expression, protein concentrations and enzyme activity (Song et al. 2003). The cc homozygous-variant genotype has been linked to diseases such as internal carotid artery stenosis (Ghilardi et al. 2002) and primary essential hypertension (Rossi et al. 2003). The homozygous-variant genotype was also linked to a reduction in breast cancer invasion, presumably because reduced NO production resulted in reduced vasodilation and therefore reduced opportunity for invasion (Ghilardi et al. 2003). NO induced after ionizing radiation has been reported to increase apoptosis and inhibit proliferation (Wang et al. 2003). On the other hand, ultraviolet light (UV)-induced NO has been reported to inhibit apoptosis and thereby reduce cytotoxicity (Fukunaga-Takenaka et al. 2003).

We have hypothesized that if DNA repair gene polymorphisms increase the risk for cancer and other diseases, then their effects should also be observed on the cellular level in the form of increased cytotoxicity. To test this hypothesis we have used the cytotoxicity data from the National Cancer Institute drug screening of 60 human tumour cell lines, which records growth inhibition produced by over 40 000 anticancer drugs (Monks et al. 1991). We analysed the response of these cell lines to a subset of 40 drugs, which were selected for different mechanisms of action. This variety of cytotoxic assault is a model of the range of environmental and endogenous toxins to which human cells may be exposed. The cytotoxic responses of these cell lines were correlated with polymorphisms in DNA repair genes selected for their potential to increase cancer risk. For one of the DNA repair genes (OGG1) we tested the functional significance of the difference predicted by the cytotoxic responses.

Materials and methods

Polymorphism analysis

Single nucleotide polymorphisms (SNPs) in the genes under investigation were detected by the Amplifour™ system (Marligen, Gaithersburg, MD, USA), which is a

PCR-based detection method using two different fluorescent primers for the dominant or variant alleles with fluorescence detection. The primers are labelled with either fluorescein (green) or sulforhodamine (red) and generate a fluorescence signal of the respective colour upon incorporation into a PCR product. Incorporation of only one primer indicates either homozygous dominant with one colour, or homozygous variant alleles with the other colour, while incorporation of both primers indicates the heterozygous genotype with both colours. The test does not distinguish gene copy number, so that homozygosity means the same gene in one, two or more copies, while heterozygosity means at least one copy of each genotype.

The National Cancer Institute (NCI) supplied DNA purified from 57 of the 60 cell lines in the screening panel, under a Materials Transfer Agreement (Table I). Table I also shows the chromosomal localization of each of the genes in this study, and the estimated ploidy of each cell line at that locus based on the karyotype. The DNA was analysed for SNPs at the alleles of interest, and the distribution of polymorphic genotypes is shown in Table II.

Growth inhibition

The NCI supplied the cytotoxicity data from screening tests for the 40 drugs (Table III) selected for study from their database of about 43 000 drugs (http://www.dtp.nci.nih.gov/docs/cancer/cancer_data.html). Drugs were selected from each of the mechanism-of-action groups. In the screening test, cell suspensions were added to a microtiter plate and incubated for 24 h at 37°C. The drugs were added at concentrations spanning five ten-fold dilutions, and incubated for 48 h. The cells were assayed by staining the cells with sulforhodamine B, and a plate reader was used to read the optical densities. The results are expressed as a GI_{50} value, which is the drug concentration producing 50% growth inhibition, with correction for the cell count at time zero. For drugs with multiple data sets from different dose ranges, the data set with the highest dose was used to ensure that the GI_{50} dose was included in the dose range.

In describing the response of the polymorphic genotypes to these drugs, relative sensitivity means that a particular genotype had a lower average GI_{50} than the other genotypes, and relative resistance means that a particular genotype had a higher average GI_{50} than the other genotypes.

Statistical analysis

The drug sensitivities for the polymorphs of each gene of interest were calculated in the following manner. For each drug, the average GI_{50} for each polymorphic genotype was calculated, e.g. the GI_{50} for the homozygous dominant, heterozygous and homozygous-variant genotypes. Then the GI_{50}s for all the drugs were analysed among genotypes by non-parametric Friedman repeated measures ANOVA. In this test the GI_{50} is only used for ranking among the genotypes for each drug, so errors due to sample size among genotypes are minimized. Statistical analysis of each pair of polymorphic genotypes was done by the Tukey–Kramer multiple comparison test. Analysis of relative sensitivity of the genotypes to an individual drug was by parametric ANOVA.

To determine the relative sensitivity of each pair of genotypes, the relative sensitivity to each drug was calculated by dividing the GI_{50} of one genotype by the GI_{50} of the

Table I. NCI screening panel tumour cell lines and estimated ploidy at DNA repair gene loci.

DNA repair gene:		TP53	OGG1	ERCC2	XRCC1	NOS3
Chromosome:		17p13.1	3p26.2	19q13.3	19q13.2	7q36
Cell line	Organ					
NCI-H23	lung	3	3	1	1	4
NCI-H522	lung	3	2	2	2	3
A549/ATCC	lung	4	2	2	2	3
EKVX	lung	2	2	2	2	3
NCI-H226	lung	3	2	2	2	8
NCI-H322M	lung	2	2	1	1	1
NCI-H460	lung	3	1	2	2	4
HOP-62	lung	4	4	2	2	6
HOP-92	lung	n.a.	n.a.	n.a.	n.a.	n.a.
HT29	colon	2	3	2	2	3
HCC-2998	colon	2	2	2	2	3
HCT-116	colon	3	2	2	2	2
SW-620	colon	2	3	2	2	5
COLO 205	colon	5	2	2	2	3
HCT-15	colon	2	2	2	2	2
KM12	colon	2	2	2	2	2
MCF7	breast	n.a.	n.a.	n.a.	n.a.	n.a.
NCI/ADR-RES	breast	n.a.	n.a.	n.a.	n.a.	n.a.
MDA-MB-231	breast	n.a.	n.a.	n.a.	n.a.	n.a.
HS 578T	breast	1	4	2	2	3
MDA-MB-435	breast	3	3	2	2	3
BT-549	breast	3	2	3	3	2
OVCAR-3	ovarian	0	3	3	3	2
OVCAR-4	ovarian	2	4	9	9	1
OVCAR-5	ovarian	3	2	3	3	1
OVCAR-8	ovarian	4	3	0	0	5
IGROV1	ovarian	4	3	4	4	3
SK-OV-3	ovarian	3	3	3	3	3
CCRF-CEM	leukaemia	2	2	2	2	2
K-562	leukaemia	n.a.	n.a.	n.a.	n.a.	n.a.
MOLT-4	leukaemia	4	4	4	4	4
HL-60(TB)	leukaemia	1	2	2	2	1
RPMI-8226	leukaemia	3	3	3	3	4
SR	leukaemia	2	2	2	2	3
UO-31	kidney	2	3	2	2	3
SN12C	kidney	4	4	2	2	3
A498	kidney	4	2	4	4	6
CAKI-1	kidney	3	2	3	3	4
786-0	kidney	4	3	4	4	5
ACHN	kidney	3	2	2	2	4
TK-10	kidney	5	2	2	2	4
LOX IMVI	melanoma	2	3	2	2	3
MALME-3M	melanoma	n.a.	n.a.	n.a.	n.a.	n.a.
SK-MEL-2	melanoma	4	7	5	5	4
SK-MEL-5	melanoma	n.a.	n.a.	n.a.	n.a.	n.a.
SK-MEL-28	melanoma	n.a.	n.a.	n.a.	n.a.	n.a.
M14	melanoma	n.a.	n.a.	n.a.	n.a.	n.a.
UACC-62	melanoma	n.a.	n.a.	n.a.	n.a.	n.a.
UACC-257	melanoma	n.a.	n.a.	n.a.	n.a.	n.a.
PC-3	prostate	5	5	4	4	6
DU-145	prostate	3	2	2	2	3

Table I (*Continued*)

DNA repair gene: Chromosome: Cell line	Organ	TP53 17p13.1	OGG1 3p26.2	ERCC2 19q13.3	XRCC1 19q13.2	NOS3 7q36
SNB-19	CNS	2	3	2	2	4
SNB-75	CNS	2	3	3	3	3
U251	CNS	5	2	3	3	5
SF-268	CNS	n.a.	n.a.	n.a.	n.a.	n.a.
SF-295	CNS	n.a.	n.a.	n.a.	n.a.	n.a.
SF-539	CNS	n.a.	n.a.	n.a.	n.a.	n.a.

Ploidy is estimated from the karyotypes of the cells at http://home.ncifcrf.gov/CCR/60SKY/new/demo1.asp. n.a., Ploidy at this site not available.

other. The relative sensitivities for all the drugs were then averaged. For each pair of genotypes, we also calculated the per cent of drugs in which one genotype was more sensitive than the other. These two measurements, average relative sensitivity and per cent of drug sensitivity, are measures of the depth and breadth, respectively, of the difference between two genotypes. The detailed results of the statistical analysis are presented in Table V, and a summary of the relative sensitivities and range of sensitivities is found in Table VI. No method to compensate for extra gene copies was used in the results.

OGG1 S326C polymorphisms and 8-oxo-guanine measurements

To correlate cytotoxicity with DNA repair activity among the polymorphic genotypes, we selected three cell lines arising from breast tumours that differed in the OGG1 S326C polymorphism. We chose these three lines because they were available from a cell bank, differed in the OGG1 gene, and were the same tissue type. We did not find any other set of three cell lines that fulfilled the criteria from those studied here. The BT-549 cell line is homozygous dominant, the MDA-MB231 cell line is heterozygous, and the MCF-7 cell line is homozygous variant. The cells were purchased from the

Table II. Distribution of polymorphic genotypes.

Gene polymorphism	Homozygous dominant	Heterozygous	Homozygous variant	Deviation from Hardy–Weinberg (χ^2)
TP53 P72R				
All	35 (61%)	8 (14%)	14 (25%)	yes (0.003)
wt-TP53	13 (62%)	5 (24%)	3 (14%)	
m-TP53	22 (61%)	3 (8%)	11 (31%)	
OGG1 S326C	32 (56%)	18 (32%)	7 (12%)	no (0.59)
ERCC2 D312N	33 (58%)	14 (25%)	10 (18%)	no (0.074)
XRCC1 R194W	52 (91%)	5 (9%)	0 (0%)	no (1.0)
XRCC1 R399Q	28 (49%)	17 (30%)	12 (21%)	no (0.13)
NOS3 g. −786T > C	26 (46%)	18 (32%)	13 (23%)	no (0.16)

Number of cell lines with each genotype and the per cent of all cell lines are in parentheses.

Table III. Drugs with NSC number and mechanism of action class.

NSC	Drug	Class
750	Busulfan	alkylating
762	Nitrogen mustard	alkylating
3088	Chlorambucil	alkylating
6396	Thiotepa	alkylating
8806	Melphalan	alkylating
26980	Mitomycin C	alkylating
34462	Uracil N mustard	alkylating
79037	CCNU	alkylating
95441	MeCCNU	alkylating
95466	PCNU	alkylating
119875	cisPt	alkylating
172112	Spirohydantoin mustard*	alkylating
178248	Chlorozotocin	alkylating
256927	CHIP	alkylating
271674	Carboxyppt	alkylating
338947	Clomesone	alkylating
348948	Cyclodisone	alkylating
353451	Mitozolamide	alkylating
363812	Tetraplatin*	alkylating
409962	BCNU	alkylating
757	Colchicine*	anti-mitotic
49842	Vinblasine	anti-mitotic
67574	Vincristine	anti-mitotic
125973	Taxol	anti-mitotic
94600	Camptothecin	topo I inhibitor
122819	VM-26	topo II inhibitor
123127	Doxorubicin*	topo II inhibitor
141540	VP-16	topo II inhibitor
249992	*m*-AMSA	topo II inhibitor
267469	d-Doxorubicin	topo II inhibitor
740	Methotrexate	DNA/RNA anti-metabolite
19893	5-Fluorouracil	DNA/RNA anti-metabolite
102816	5-AzaC	DNA/RNA anti-metabolite
264880	5,6-d5azaC	DNA/RNA anti-metabolite
752	Thioguanine	DNA anti-metabolite
755	Thiopurine	DNA anti-metabolite
27640	2′d5FU	DNA anti-metabolite
32065	Hydroxyurea	DNA anti-metabolite
63878	Ara-C	DNA anti-metabolite
303812	Aphidicolin	DNA anti-metabolite

*Not all cell lines were tested with these drugs.

American Type Culture Collection (Manassas, VA, USA) and grown in the recommended media. MCF-7 cells were grown in RPMI-1640 media containing 10% foetal bovine serum (Cambrex, Walkersville, MD, USA) and 1% penicillin-streptomycin (Invitrogen, Carlsbad, CA, USA). MDA-MB231 cells were grown in RPMI-1640 containing 5% foetal bovine serum and 1% penicillin-streptomycin. BT-549 cells were grown in RPMI-1640 containing 10% foetal bovine serum.

Cells were grown in 100-mm cell culture plates (Corning, Corning, NY, USA) at a plating density of 5×10^5. Before cell treatment, $FeSO_4$ (Sigma, St Louis, MO, USA) and $CuSO_4$ (Sigma) were added at 0.1 mM to the media. After 10 min, H_2O_2 was

added to the media at a final concentration of 0.5 mM and the cells incubated for another 10 min before the media was changed. Cells were then either taken immediately or incubated at 37°C in 5% CO_2 for 2 or 6 h.

DNA was purified from the cells and the amount was calculated by the OD_{260}. The frequency of 8-oxo-guanine (8oG) in DNA was measured by the endonuclease-sensitive site assay (Cadet & Weingled 1993) , using purified OGG1 enzyme to cut the DNA at sites of 8oG. The size distribution of treated DNA was determined on 0.4% alkaline agarose gels stained with ethidium bromide, and the number average molecular weight calculated using the size distribution and λ-Hind III DNA fragments as calibration standards. The reciprocal of the number average molecular weight in this assay is the number of 8oG per DNA base.

Results

Mutations in TP53

The TP53 (p53) gene codes for a protein that regulates cellular response to DNA damage, and mutations in this gene increase cancer risk. All of the tumour cell lines in the panel expressed TP53 mRNAs, and they have been sequenced (O'Connor et al. 1997). Most cell lines had a mutation in the TP53 gene mRNA, which was a missense mutation. One cell line had both a mutant and wild-type mRNA sequence, and this was scored as a mutant cell line because tetramers of mutant and wild-type TP53 proteins cannot activate transcriptional targets (Vogelstein & Kinzler 1992). The mutant TP53 genotype was a statistically significant variable for 17 of the 40 drugs tested and in all or nearly all of the polymorphic gene backgrounds (Table IV). However, the mutant TP53 status was not statistically correlated with the polymorphic genotypes in any of the genes examined (χ^2, $p \geq 0.15$). This suggests that the effects of the DNA repair genes can be examined independently of the TP53 status in the cell line panel.

The mutant TP53 genotype was associated with sensitivity to many types of drugs, including alkylating agents, topoisomerase inhibitors, and DNA/RNA anti-metabolites (Table IV) (O'Connor et al. 1997). Therefore, we cannot assume a priori that a DNA repair gene is only correlated with reduced survival from one class of DNA damage.

TP53 P72R polymorphism

The TP53 P72R polymorphism changes an amino acid, but does not inactivate the TP53 protein. The distribution of TP53 P72R genotypes among the cell lines did not follow the Hardy–Weinberg distribution (Table II), reflecting a loss of heterozygosity (LOH) (χ^2, $p = 0.003$). LOH was more striking among the mutant TP53 cell lines.

Drug resistance differed among the TP53 genotypes (Table V; Friedman ANOVA, $p < 0.001$), in the order homozygous variant > homozygous dominant > heterozygous genotype. The difference between heterozygous and either the homozygous dominant or homozygous-variant genotype was significant ($p < 0.001$, Tukey–Kramer test), but the difference between the homozygous dominant and homozygous-variant genotype was not ($p > 0.05$, Tukey–Kramer test). On average, the heterozygous genotype had 73% of the resistance of the homozygous dominant genotype, and was more sensitive to 85% of the drugs tested. The heterozygous genotype had 48% of the resistance of the homozygous-variant genotype, and was more sensitive in 87% of the cases.

Table IV. Significance (p) for drugs in which mutant TP53 status was a significant* co-variate with a DNA repair gene polymorphism.

Drug	Drug class†	TP53 P72R	OGG1 S326C	ERCC2 D312N	XRCC1 R194W	XRCC1 R399Q	NOS3 g. −786T >C
Busulfan	1	0.008	0.006	0.005	0.005	0.008	0.005
Nitrogen mustard	1	0.025	0.014	0.018	0.012	0.018	0.016
Chlorambucil	1		0.028	0.036	0.032	0.026	0.031
Thiotepa	1	0.043	0.016	0.020	0.020	0.019	0.018
Melphalan	1		0.039	0.048	0.040	0.031	0.045
Mitomycin C	1		0.041	0.041	0.039		0.034
Uracil N-mustard	1		0.042			0.040	
cis-Platin	1	0.017	0.009	0.014	0.012	0.008	0.007
Chlorozotocin	1	0.024	0.011	0.016	0.015	0.018	0.013
CHIP	1	0.050					
Carboxy-platin	1	0.012	0.003	0.006	0.005	0.004	0.005
VM-26	2		0.046	0.055	0.050		0.047
m-AMSA	2		0.044		0.050		0.045
5-FU	3	0.001	<0.001	<0.001	<0.001	<0.001	<0.001
5,6-d5azaC	3	0.024	0.005	0.009	0.007		0.007
Ara-C	3		0.029	0.033	0.032	0.025	0.036
Aphidicolin	3	0.007	0.004	0.006	0.005	0.003	0.005

*Only those correlations in which $p < 0.05$ by ANOVA are included.
†Drug class: (1) alkylating agents, (2) topoisomerase II inhibitors and (3) DNA/RNA anti-metabolites.

OGG1 polymorphism S326C

The OGG1 gene codes for the 8-oxo-guanine glycosylase 1, which incises DNA at the site of 8oG and related oxidized bases. The distribution of the OGG1 polymorphic genotypes in the cell line panel followed Hardy–Weinberg distribution (Table II; χ^2, $p = 0.59$), indicating that there was no selective LOH.

Drug resistance differed among the genotypes (Table V; $p = 0.0005$, Friedman ANOVA) in the order heterozygous >homozygous dominant >homozygous variant. The homozygous-variant genotype was significantly different than either the

Table V. Detailed correlation of genotypes with resistance to toxicity.

Gene locus	Relative resistance (% sensitive)*			ANOVA (p)**
	Variant/dominant	Variant/hetero	Hetero/dominant	
TP53 P72R	1.31 (43%)	2.10 (13%)‡	0.73 (85%)‡	<0.001
OGG1 S326C	0.86 (60%)	0.76 (73%)‡	1.20 (25%)†	0.0005
ERCC2 D312N	0.91† (70%)	1.04 (45%)	0.90 (83%)‡	0.0003
XRCC1 R194W	(no variant alleles)	(no variant alleles)	1.08 (53%)	0.669***
XRCC1 R399Q	1.53 (18%)‡	1.40 (35%)	1.17 (28%)†	<0.0001
NOS3 g. −786T >C	0.78 (80%)‡	0.84 (70%)‡	0.99 (68%)	<0.001

*GI_{50} of the numerator is divided by GI_{50} of the denominator; a value of <1 indicates the greater sensitivity of the numerator. Shown in parentheses are the percentage of drugs in which the numerator genotype was more sensitive than the denominator genotype.
**Friedman non-parametric ANOVA; post-tests †$p < 0.05$, ‡$p < 0.01$.
***Wilcoxon matched-pairs signed-ranks test.

Table VI. Summary of correlations of genotypes with cytotoxicity.

Gene allele	Most sensitive	Intermediate	Most resistant	Range of sensitivity (%)*	ANOVA
$TP53_{P72R}$	hetero PR	dominant PP	variant RR	27	<0.001
$OGG1_{S326C}$	variant CC	dominant SS	hetero SC	24	0.005
$ERCC2_{D312N}$	hetero DN	variant NN	dominant DD	10	0.003
$XRCC1_{R194W}$	dominant RR	(no variants)	hetero RW	8	0.669
$XRCC1_{R399Q}$	dominant RR	hetero RQ	variant QQ	53	<0.0001
$NOS3_{g.-786T>C}$	variant cc	hetero tc	dominant tt	22	<0.001

*Difference between the most resistant and the most sensitive as a per cent of the most sensitive GI_{50}.

homozygous dominant or heterozygous genotype ($p < 0.01$, Tukey–Kramer test). In this test the heterozygous and homozygous dominant were not statistically significantly different, although the q-statistic was borderline significant. By the non-parametric Dunn's multiple comparison test, the heterozygous genotype was statistically significantly more resistant than the homozygous dominant genotype.

The homozygous-variant genotype had only 76% the resistance of the heterozygous genotype, and was more sensitive in 73% of the drugs. The homozygous dominant genotype had only 83% of the resistance of the heterozygous genotype, and was more sensitive in 75% of the cases.

ERCC2 polymorphism D312N

The ERCC2 (XPD) gene codes for a subunit of the transcription factor TFIIH, which is involved in DNA unwinding during nucleotide excision–repair and also initiation of basal transcription. Mutations in this gene cause the genetic disease XP complementation group D. The distribution of the polymorphic genotypes did not significantly deviate from the Hardy–Weinberg distribution (Table II; χ^2, p = 0.074).

The sensitivities of the genotypes to all the drugs differed (Table V, $p = 0.0003$, Friedman ANOVA), with the order of resistance homozygous dominant > homozygous variant = heterozygous. In post-test analysis using the Tukey–Kramer test, the homozygous dominant genotype was significantly different than either the heterozygous ($p < 0.01$) or homozygous-variant genotypes ($p < 0.05$), but the heterozygous and homozygous-variant genotypes were not statistically significantly different.

Although the degree of resistance of the homozygous dominant group was statistically significant it was small (Table V). The homozygous-variant genotype had 91% the resistance of the homozygous dominant genotype, and was more sensitive in 70% of the drugs. The heterozygous genotype had 90% of the resistance of the homozygous dominant genotype, and was more sensitive in 83% of the cases. This suggests that the homozygous dominant population is about 10% more resistant to cytotoxic damage than the other genotypes.

XRCC1 polymorphisms R194W and R399Q

The XRCC1 gene encodes a DNA base excision–repair protein that serves as a scaffolding protein to coordinate the activity of other DNA enzymes to repair single-stranded breaks. No homozygous-variant genotypes were found in the cell line panel, and the ratio of homozygous dominant to heterozygous genotypes followed

the Hardy–Weinberg distribution (Table II; χ^2 p = 1.0). The sensitivity of the homozygous dominant and heterozygous genotypes to the toxic agents did not differ ($p = 0.669$, Wilcoxon matched-pairs signed-rank test), and the heterozygous genotype was sensitive to only 3% more drugs than the homozygous dominant, but this difference was not statistically significant (Table V).

The R399Q polymorphic genotypes also followed the Hardy–Weinberg distribution (Table II; $\chi^2 = 0.13$). The differences among the groups in sensitivity to all the drugs were significant (Table V; $p < 0.0001$, Friedman ANOVA), and the order of resistance was homozygous variant > heterozygous > homozygous dominant. The homozygous variant group was statistically significantly more resistant than either of the other genotypes ($p < 0.01$, Tukey–Kramer test). The difference was also significant in analysis of sensitivity to vinblastine ($p = 0.025$, ANOVA), and in post-tests of this drug the homozygous-variant genotype was significantly more resistant than the homozygous dominant genotype ($p = 0.045$, Bonferroni adjusted multiple comparison test). This finding is unexpected since vinblastine is an anti-mitotic and not normally associated with single-strand breaks.

The homozygous-variant genotype was correlated with increased drug resistance by 35% relative to the homozygous dominant genotype, and the homozygous-variant genotype was correlated with greater resistance than the homozygous dominant genotype to 82% of the drugs (Table V). The homozygous-variant genotype showed increased drug resistance of 29% relative to the heterozygous genotype, and the homozygous-variant genotype was more resistant than the heterozygous genotype to 65% of the drugs. The difference between homozygous variant and homozygous dominant genotypes was significant, but the difference with the heterozygous genotype was not. The difference between the heterozygous and homozygous dominant genotype was also significant.

NOS3 polymorphism g. −786T > C

The NOS3 gene codes for endothelial nitric oxide synthetase, which is a key enzyme in the production of NO to control vasodilation. The g. −786T > C polymorphism in the promoter sequence has been related to reduced transcription and reduced expression of NOS3. The polymorphic genotypes followed the Hardy–Weinberg distribution ($\chi^2 = 0.16$).

The genotypes differed in their relative sensitivities (Table V, $p < 0.05$, Friedman ANOVA), and the homozygous-variant genotype was more sensitive than the homozygous dominant or heterozygous genotype to all the drugs ($p < 0.001$, Tukey–Kramer test). The homozygous dominant and heterozygous genotypes did not differ in sensitivity. In the particular case of the drug thioguanine, the homozygous-variant genotype reduced survival to 68% of the homozygous dominant genotype and 35% of the heterozygous genotype, and the difference among the genotypes was statistically significant ($p = 0.018$, ANOVA). Overall, the homozygous-variant genotype survival was 78% of the homozygous dominant, and 84% of the heterozygous genotype. The homozygous-variant genotype was more sensitive to 80% of the drugs than the homozygous dominant and 70% of the drugs than the heterozygous genotype.

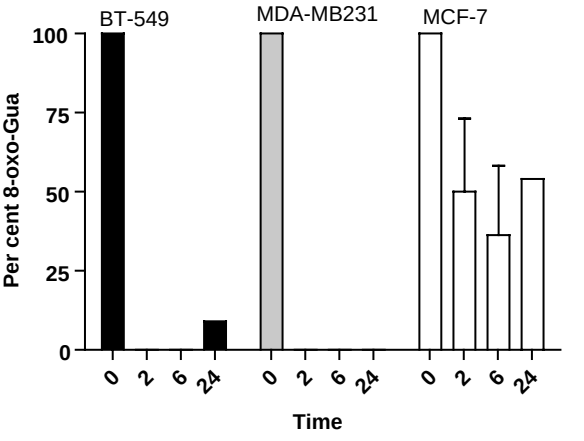

Figure 1. Repair of 8-oxo-guanine from DNA of cell lines differing in the OGG1 S326C polymorphic allele. Cell line BT-549 (solid bars) is homozygous dominant (SS); cell line MDA-MB231 (grey bars) is heterozygous (SC); and cell line MCF-7 (open bars) is homozygous variant (CC). The amount of 8-oxo-guanine is expressed as a per cent of the time zero value. No 8-oxo-guanine was found in BT-549 cells at 2 or 6 h, and none was found in MDA-MB231 at 2, 6 or 24 h. Experiments were repeated two (BT-549, MDA-MB231) or four (MCF-7) times. Error bars are the standard error of the mean.

Correlation of OGG1 genotype with cellular DNA repair activity

We tested the prediction for the OGG1 S326C polymorphic genotypes that the homozygous-variant genotype would be defective in DNA repair relative to the other genotypes. We chose this gene polymorphism to test because the enzyme activity of the gene product was well-known and readily assayed. We chose three breast cancer cell lines from the panel (BT-549, homozygous dominant; MDA-MG231, heterozygous; and MCF-7, homozygous variant) and we measured the ability of each cell line to repair 8oG in DNA following treatment with hydrogen peroxide (Figure 1). The homozygous dominant and heterozygous cell lines showed little or no detectable 8oG in their DNA at 2 h post-treatment and at later times, and this supported the finding of little or no difference between these genotypes in drug sensitivity. Also consistent with the prediction from the cytotoxicity data of the panel, the homozygous variant cell line showed 8oG remaining in DNA up to 24 h after treatment, indicating a deficiency in repair that may explain the increased drug sensitivity.

Discussion

The NCI drug-screening panel cell lines respond to cytotoxic challenge by thousands of drugs in a coherent pattern that reflects the mechanisms of drug action and drug resistance (Weinstein et al. 1997). These tumour cell lines do show karyotypic complexity, including ploidy, numerical change and structural rearrangements (Table I) (Roschke et al. 2003). Nevertheless, these cell lines show correlations between drug resistance and expression of over 8000 genes (Roschke et al. 2003) or DNA repair protein levels (Xu et al. 2002). We have examined the panel for the effect of polymorphisms in selected DNA repair genes on cytotoxic response. The polymorphic genotypes of the genes OGG1, ERCC2/XPD, XRCC1 and NOS3 were all in Hardy–Weinberg equilibrium, demonstrating that there was no significant loss of these genes

from the panel caused by LOH. Variations in polymorphic gene dosages due to numerical changes in chromosomes among the lines would tend to obscure the effects of a particular polymorphic allele, and so the statistical significance of a correlation between a polymorphic genotype and drug sensitivity is in fact a conservative result.

GI_{50} measures the cytotoxic or cytostatic response interpolated from a broad range of doses. Our analysis is not dependent on the precision of the GI_{50} value, or on averaging them for a genotype, because in the Friedman non-parametric ANOVA a significant difference is detected only when one genotype consistently ranks higher or lower than the other genotypes in drug sensitivity as each of 40 drugs are examined. We have analysed the GI_{50} responses to all 40 drugs because we have not made the a priori assumption that for any one drug only one DNA repair pathway controls the cytotoxic response. Rather, we have let the data inform us by examining the breadth of the effect of a particular polymorphic genotype across a range of toxic challenges.

Most of the panel lines contained intact TP53 with missense mutations, and GI_{50} depended on TP53 mutant status in all mechanism-of-action drug classes except anti-mitotics (Table IV) (O'Connor et al. 1997). However, the TP53 mutations were not linked to any of the DNA repair gene polymorphic alleles, and thus segregation of the mutant TP53 genes with one or the other of the polymorphic genotypes could not account for these results. In a similar manner, the organ source of the tumours also was not correlated with any of the polymorphic genotype status (data not shown).

The TP53 P72R allele did show Hardy–Weinberg disequilibrium with LOH, as expected when one tumour suppressor TP53 gene is mutated and the other gene is lost. The heterozygous genotype was significantly more sensitive than the homozygous or hemizygous forms, consistent with reports that TP53 P72R heterozygous genotype is selected against in human tumours in favour of the variant polymorphism (Marin et al. 2000, Papadakis et al. 2002).

The relative sensitivity of the homozygous variant OGG1 genotype is consistent with the observed increased risk of the variant allele for prostate cancer (Chen et al. 2003), nasopharyngeal cancer (Cho et al. 2003), and oesophageal, lung and stomach cancer (Goode et al. 2002). In the case of prostate or stomach cancer, the proximal cause of the cancer is not well known, while in non-smokers the cause of the other cancers is also not well understood.

The prediction of repair deficiency for the OGG1 S326C variant genotype from the cytotoxicity data was tested in three breast tumour cell lines that differed in their genotypes at this locus. Consistent with the ranking of sensitivity, the homozygous dominant MCF-7 cells were much less efficient in removal of 8oG than the homozygous dominant or heterozygous cells. There was no detectable difference in repair by the later two lines. These three cell lines no doubt differ in many other genes, and these data are suggestive but not proof that the OGG1 polymorphism was responsible for the difference in 8oG repair. The important point is that data from activity assays are consistent with the predictions from cytotoxicity in the cell line panel and the observations of cancer rates in people. This all lends strong credibility to the correlation between the variant polymorphism and increased risk of cancer, especially when a contributing aetiological agent is oxidative DNA damage.

The association of increased resistance to cell killing and the ERCC2 D312N homozygous-dominant genotype has not been previously described. Contradictory findings have been reported for the relationship of the homozygous dominant genotype to lung cancer (Goode et al. 2002). The variant allele was not related to basal cell

carcinoma risk overall, but only in those with a family history of it (Goode et al. 2002). The variant allele has also been related to the risk of prostate cancer (Rybicki et al. 2004). The variant allele was not found to be related to the risk for breast cancer (Tang et al. 2002). The variant allele was also not found to be related to the levels of polycyclic-aromatic hydrocarbon adducts to DNA in normal or benign breast tissue, although it was related to the levels in tumour tissue (Tang et al. 2002). Others have found that the variant allele is associated with chromosome changes after exposure to some toxic agents, like UV, but not others, like X-rays (Au et al. 2003). These chromosome changes are predominantly associated with cancer risk, and not cell survival.

The results for the XRCC2 R399Q polymorphism are consistent with the findings that the variant allele is associated with a decreased risk for non-melanoma skin cancer, oesophageal cancer and bladder cancer (Goode et al. 2002). For those with fewer than three sunburns, the homozygous-variant genotype was protective for non-melanoma skin cancer, but carried an increased risk for those with more than three. Other studies have given conflicting results in squamous cell carcinoma of the head and neck and lung cancer (Goode et al. 2002). For nasopharyngeal cancer, a null association has been reported (Cho et al. 2003).

Cellular or biochemical assays of the polymorphic forms of the XRCC2 gene have given conflicting results. The homozygous-variant genotype did not affect transversion mutations in the TP53 gene, nor repair of UV damage, nor strand break repair, nor cell survival after alkylation damage (Hou et al. 2003). Unexpectedly, higher levels of vinyl chlorine adducts were found in the homozygous-variant genotype than the others (Li et al. 2003), and more breaks per cell were found after bleomycin treatment in homozygous-variant genotypes than the others, although there was no difference after BPDE treatment (Wang et al. 2003).

The most sensitive polymorphic genotype was associated with increased cytotoxicity of 50%, while three gene polymorphisms were related to increased sensitivity rate of about 25%. Although these differences were modest, they stretched across a broad range of drugs, in some cases related to increased sensitivity to about 80% of the drugs tested. We should therefore reject the 'one drug-one DNA repair pathway' hypothesis of cytotoxic response. For example, in the case of the OGG1 DNA repair glycosylase, the proximal effect of the variant genotype may be a deficiency in repair of 8oG. Some environmental insults may directly produce this damage in DNA by oxidation. Other toxins (which may or may not be mutagens) may trigger stress responses such as oxidative bursts, mitochondrial breakdown or release of inflammatory mediators that indirectly result in 8oG in DNA, and cells deficient in 8oG repair would therefore appear unexpectedly sensitive to this toxic challenge.

This paper has examined the relationship of a single polymorphic genotype to cytotoxicity. Of course, each line contains polymorphisms at many different DNA repair genes, and more profound effects may yet be discovered in the future by examining combinations of polymorphisms in larger data sets, but these possibilities do not negate the findings shown here. The approach of correlating polymorphic genotypes with cytotoxicity in cultured cells may reveal effects of these polymorphisms in biological responses that may be more difficult or impossible to observe in purified proteins or in cancer epidemiology studies.

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