0026-895X/99/020390-06$3.00/0

MOLECULAR PHARMACOLOGY, **56:**390–395 (1999).

Effect of Loss of DNA Mismatch Repair on Development of Topotecan-, Gemcitabine-, and Paclitaxel-Resistant Variants after Exposure to Cisplatin

XINJIAN LIN and STEPHEN B. HOWELL

Department of Medicine and the Cancer Center, University of California at San Diego, La Jolla, California

Received February 3, 1999; accepted May 7, 1999

This paper is available online at http://www.molpharm.org

ABSTRACT

Loss of DNA mismatch repair (MMR) causes genomic instability by markedly increasing the frequency of sporadic mutations in both coding and noncoding sequences. Little is known about how loss of MMR affects sensitivity to the mutagenic effect of chemotherapeutic agents. We wanted to determine how loss of MMR affects the ability of cisplatin, a known mutagen, to generate human tumor cell variants resistant to other drugs with which cisplatin is commonly combined in treatment regimens. We compared the ability of cisplatin to produce variants resistant to topotecan, gemcitabine, and paclitaxel in two pairs of MMR-proficient and -deficient cells that included sublines of the human colon carcinoma cell line HCT-116 and sublines of the human endometrial adenocarcinoma cell line HEC59. Cells were exposed to increasing concentrations of cisplatin for 1 h, and the surviving population was tested for the frequency of variants resistant to these single molecular target drugs 10 days later. The frequency of variants increased linearly with cisplatin concentration for all three drugs. Cisplatin was 2.6 ± 0.3- (S.D.), 3.6 ± 0.9-, and 2.3 ± 0.1-fold more potent at producing topotecan-, gemcitabine-, and paclitaxel-resistant variants in the MMR-deficient than in the MMR-proficient HCT116 cells ($P < .05$ for all). Cisplatin was 1.4 ± 0.3- and 1.4 ± 0.4-fold more potent at generating topotecan- and gemcitabine-resistant variants in MMR-deficient HEC59 cells than in MMR-proficient HEC59+ch2 cells. Cisplatin was not more potent in generating paclitaxel-resistant variants in the MMR-deficient HEC59 cells. Spontaneous rates of generation of cells resistant to these three drugs were also measured in the HCT116 sublines. MMR-deficient HCT116 cells exhibited rates of generation of resistant variants that were 1.94- and 1.51-fold higher ($P < .05$) than those in the MMR-proficient cells for topotecan and gemcitabine, respectively; loss of MMR had no effect on the rate of generation of variants resistant to paclitaxel. We conclude that the loss of MMR increases the ability of cisplatin to generate variants resistant to topotecan, gemcitabine, and possibly paclitaxel and that MMR also plays a role in controlling the spontaneous rate of generation of variants resistant to topotecan and gemcitabine.

Mismatch repair (MMR) is a postreplicative DNA repair process that corrects single-base mismatches and small mismatched loops in DNA by removing such errors from the newly synthesized strand (Parson et al., 1993). Loss of MMR results in genomic instability that is associated with a marked increase in the mutation rate throughout the genome. For example, loss of MMR increases the rate of mutation in the hypoxanthine guanine phosphoribosyl transferase (Bhattacharyya et al., 1994), *APRT* (Hess et al., 1994), *APC* (Huang et al., 1996), transforming growth factor receptor-βII subunit (Markowitz et al., 1995), *BAX* (Rampino et al., 1997), BRCA1, and c-*myc* genes (Wooster et al., 1994) and at microsatellite sequences in a myriad of locations. The elevated mutation rate provides a pool of mutants on which selection can act during tumor development (Umar and Kunkel, 1996). Loss of MMR due to mutation of *MSH2* or *MLH1* underlies the majority of cases of hereditary nonpolyposis colon cancer identified to date (Fishel et al., 1993; Leach et al., 1993; Papadopoulos et al., 1994). Although a single functional copy of these genes is sufficient to sustain normal MMR activity, at some point during the oncogenic process the remaining wild-type allele is somatically mutated so that the cells lose all MMR function (Leach et al., 1993; Hemminki et al., 1994; Liu et al., 1995). The resulting genomic instability likely predisposes to additional genetic changes that are required to create a fully malignant colon cancer, such as the activation of oncogenes or the inactivation of tumor suppressor genes (Liu et al., 1995). Loss of MMR function is also common in a variety of sporadic cancers, including endometrial, ovarian, breast, prostate, lung, and pancreatic cancer (see review in Fishel and Kolodner, 1995).

This work was supported in part by a fellowship award from the China Scholarship Council in the People's Republic of China (to X.L.). This work was conducted in part by the Clayton Foundation for Research–California Division. S.B.H. is a Clayton Foundation Investigator.

ABBREVIATION: MMR, DNA mismatch repair.

In addition to being involved in carcinogenesis, loss of MMR is of concern with respect to the use of chemotherapeutic agents to treat established tumors. Loss of MMR produces drug resistance directly by impairing the ability of the cell to detect adducts in its DNA that mimic base mismatches. For example, loss of MMR causes high-level resistance to the antimetabolite 6-thioguanine (Griffin et al., 1994), moderate levels of resistance to the methylating *N*-methyl-*N*′-nitro-*N*-nitrosoguanidin (Kat et al., 1995), and low-level resistance to the platinum-containing drugs cisplatin and carboplatin (Fink et al., 1997) in cell lines cultured in vitro. These agents cause cell death via apoptosis. The triggering of the apoptotic response requires the cell to be able to recognize the presence of the damage in DNA produced by these anticancer drugs. The current hypothesis is that when MMR is disabled, the cell cannot sense DNA lesions and cannot generate signals that eventually result in apoptosis. Thus, such cells may survive on the basis that they are tolerant of adducts in their DNA (Fishel et al., 1993).

Cisplatin is a highly effective chemotherapeutic agent with activity in a wide spectrum of tumors, but it is a mutagen in bacterial (Yarema et al., 1994, 1995) and mammalian (Turnbull et al., 1979; Wiencke et al., 1979; Johnson et al., 1980; Cariello et al., 1992) cells. The most abundant lesions produced in DNA are intrastrand cross-links between the N7 atoms of adjacent purines; 65% of adducts are in GpG sequences, 25% are in ApG sequences, and 6% are in GpNpG sequences (Eastman, 1986). Such adducts halt the progress of DNA polymerases when they are present in the lagging strand, but mutagenic translesional bypass occurs at a significant level on the leading strand and results in the misincorporation of nucleotides opposite the intrastrand adducts (Hoffmann et al., 1996). Intrastrand guanine-guanine adducts themselves and compound lesions consisting of the adduct and a misincorporated base on the opposite strand are recognized and processed by the MMR system (Duckett et al., 1996; Yamada et al., 1997). In the current study, we sought to determine whether loss of MMR enhances the ability of cisplatin to generate clones resistant to other drugs with a known single molecular target that are commonly used in combination with cisplatin. We report here that MMR-deficient cells are more sensitive than MMR-proficient cells to the ability of cisplatin to generate variants that are resistant to topotecan, gemcitabine, and paclitaxel.

Materials and Methods

Reagents. Cisplatin and paclitaxel were gifts from Bristol-Myers Squibb (Princeton, NJ). A stock solution of 1 mM cisplatin in 0.9% NaCl was stored in the dark at room temperature. Paclitaxel was dissolved in dimethyl sulfoxide, diluted with saline to form a stock solution of 5 μM, and stored at −20°C. Topotecan was purchased from SmithKline Beecham Pharmaceuticals (King of Prussia, PA), dissolved in deionized water, and stored as a 10 μM stock solution at −20°C. The clinical formulation of gemcitabine was purchased from Eli Lilly and Co. (Indianapolis, IN) and was diluted directly in tissue culture medium.

Cell Lines. Clones of the human colorectal adenocarcinoma cell line HCT116 that had undergone chromosome 3 transfer (clone HCT116/3–6, identified here as HCT116+ch3) and chromosome 2 transfer (clone HCT116/2–1, identified here as HCT116+ch2) were obtained from Drs. C. R. Boland, M. Koi, and T. A. Kunkel (Koi et al., 1994). The human endometrial adenocarcinoma cell line HEC59 and its subline complemented with chromosome 2 (HEC59+ch2) were also obtained from Drs. C. R. Boland, M. Koi, and T. A. Kunkel (Umar et al., 1997). These cell lines were maintained in Iscove's modified Dulbecco's medium (Irvine Scientific Co., Inc., Irvine, CA) containing 10% FBS and 400 μg/ml geneticin (Life Technologies, Inc., Grand Island, NY) for HCT116+ch2 and HCT116+ch3 and 600 μg/ml for HEC59+ch2. The absence and presence of human MLH1 protein in HCT116+ch2 and HCT116+ch3 cells, as well as of MSH2 in HEC59 and in HEC59+ch2, were verified by immunoblot analysis (data not shown). All cell lines tested negative for contamination with *Mycoplasma* spp.

Assay for Frequency of Resistant Variants. Two million cells were seeded into 10 ml of medium in 75-cm^2 flasks and allowed to grow exponentially for 2 days. Cells were then exposed for 1 h to increasing concentrations of cisplatin. Thereafter, the cells were washed twice and recultured in regular medium for 10 to 14 days, during which the cultures were split 2:1 as needed to keep them from becoming confluent. All the cells were then trypsinized and seeded into each of five 100-mm tissue culture dishes at 100,000 cells/dish. A concentration of drug resulting in a cloning efficiency of approximately 0.0002% was added. These concentrations for the HCT116+ch2 and HCT116+ch3 cells were 12.5 and 10 nM topotecan, 30 and 10 nM gemcitabine, and 30 and 10 nM paclitaxel, respectively; for the HEC59 and HEC59+ch2 cells, they were 8.0 and 6.0 nM topotecan, 15 nM gemcitabine, and 6.0 and 3.0 nM paclitaxel, respectively. At the same time, either 250 (in the case of the HCT116+ch2 and HCT116+ch3 cells) or 300 (in the case of the HEC59 and HEC59+ch2 cells) were seeded into each of three 60-mm dishes in drug-free medium for determination of cloning efficiency. After 14 days, colonies were counted after staining with 0.1% crystal violet. The resistant frequency was calculated from the equation: resistant frequency = $a/[b(5 \times 10^5)]$, where a is the number of colonies present in the five drug-treated dishes, and b is the cloning efficiency. Each experiment was performed a minimum of three times, and the data are presented as mean ± S.D.

Measurement of Rate of Generation of Resistant Variants. The rate of spontaneous generation of resistant variants was measured using a refined version of the "maximum likelihood estimation" technique (Glaab and Tindall, 1997). Briefly, populations of HCT116+ch2 and HCT116+ch3 cells were grown exponentially in 25-cm^2 flasks. The frequencies of resistant variants in the cultures were measured as described above, and 1×10^5 cells were subcultured in a 25-cm^2 flask in 5 ml of regular medium and allowed to proliferate for 4 days. The frequency of resistant variants was then measured again, and the process repeated for a total of five iterations. Total cell numbers were determined at each step, and plating efficiencies from the previous selection, along with the exact number of cells subcultured, were used to calculate population doubling according to the following equation: population doubling = (ln[total number of cells] − ln[number of cells plated × plating efficiency])/ln2. The rate of generation of resistant variants was then obtained by plotting the observed resistant variant frequency as a function of population doubling and calculating the slope by linear regression. The slope of the curve yields the rate of generation of resistant variants (resistant variants/cell/generation).

Statistical Analysis. Frequency and rate data were analyzed by use of a two-sided paired Student's t test with the assumption of unequal variances.

Results

The concentration-survival curves for cisplatin for the MMR-proficient HCT116+ch3 and MMR-deficient HCT116+ch2 cells are shown in Fig. 1A, and those for the MMR-deficient HEC59 and MMR-proficient HEC59+ch2 cells are shown in Fig. 1B. Figure 2 summarizes the number of resistant colonies/10^6 clonogenic cells for all three drugs as a function of cisplatin con-

centration for the MMR-proficient HCT116+ch3 and MMR-deficient HCT116+ch2 cells. As shown in Fig. 1A, for both types of cells there was a nearly linear increase in the number of topotecan-resistant colonies/10^6 clonogenic cells as a function of intensity of cisplatin exposure up to a concentration of 75 μM. Based on extrapolation of the curve presented in Fig. 2A, this corresponded to an $IC_{99.9}$ exposure. The magnitude of the difference between the cell lines was similar across the whole cisplatin concentration range tested. Based on the ratio of the slopes of the curves, the MMR-deficient cells were 2.6 ± 0.3 (S.D.)-fold more sensitive to the ability of cisplatin to generate resistant variants (P = .003, n = 3). Figure 2B shows the number of gemcitabine-resistant colonies as a function of cisplatin concentration. After exposure to cisplatin, the MMR-deficient cells yielded 3.6 ± 0.9 (S.D.)-fold more colonies resistant to gemcitabine than did the MMR-proficient cells (P = .004, n = 3). The same thing was observed for paclitaxel. As shown in Fig. 2C, cisplatin produced 2.3 ± 0.1 (S.D.)-fold more paclitaxel-resistant colonies from the population of MMR-deficient cells than did the MMR-proficient cells (P = .006, n = 3). As for topotecan, there was a relatively linear increase in number of colonies/10^6 clonogenic cells that were resistant to gemcitabine and paclitaxel as a function of cisplatin concentration.

Among the three drug-resistant phenotypes scored and based on the slope of the curves presented in Fig. 2, cisplatin was most potent in producing topotecan-resistant variants in both the MMR-proficient and -deficient cells. The relative effectiveness in producing resistant variants was 1:0.62:0.24 for topotecan/gemcitabine/paclitaxel in the MMR-proficient cells and 1:0.83:0.22 for the MMR-deficient cells for the same cisplatin exposure.

HCT-116 cells are MMR deficient due to mutations in both alleles of the MLH1 gene (Hemminki et al., 1994). To determine whether the enhanced sensitivity to the ability of cisplatin to generate resistant variants was limited to this particular pair of cells or was unique to loss of MLH1, the sensitivity of another pair of MMR-proficient and -deficient cells was examined. HEC59 cells have mutations in both alleles of MSH2 that disable MMR, whereas the HEC59+ch2 cells, in which a wild-type copy of MSH2 has been transferred on chromosome 2, are MMR proficient (Umar et al., 1997). Figure 3 shows that cisplatin was 1.35 ± 0.31 (P = .018, n = 6)- and 1.42 ± 0.39 (P = .037, n = 6)-fold more potent at generating topotecan- and gemcitabine-resistant variants, respectively, in the MMR-deficient HEC59 cells than in the MMR-proficient HEC59+ch2 cells. The ratio for paclitaxel was 1.14 ± 0.56 (P = .702, n = 6), indicating that cisplatin was not more potent at generating paclitaxel-resistant variants in the MMR-deficient cells. In this model, the ratio of topotecan, gemcitabine, and paclitaxel variants generated was 1:0.95:0.56 for the MMR-deficient cells and 1:0.96:0.70 for the MMR-proficient cells.

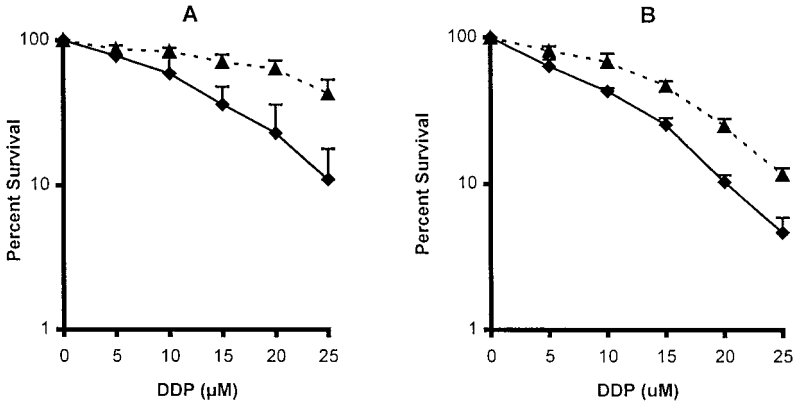

Fig. 1. Cisplatin concentration-survival curves. A, MMR-deficient HCT116+ch2 (▲) and MMR-proficient HCT116+ch3 (♦) cells. B, MMR-deficient HEC59 (▲) and MMR-proficient HEC59+ch2 (♦) cells. Each point represents the mean of three to five experiments performed with triplicate cultures. Bars, S.D. (Data reproduced from Fink et al., 1996.)

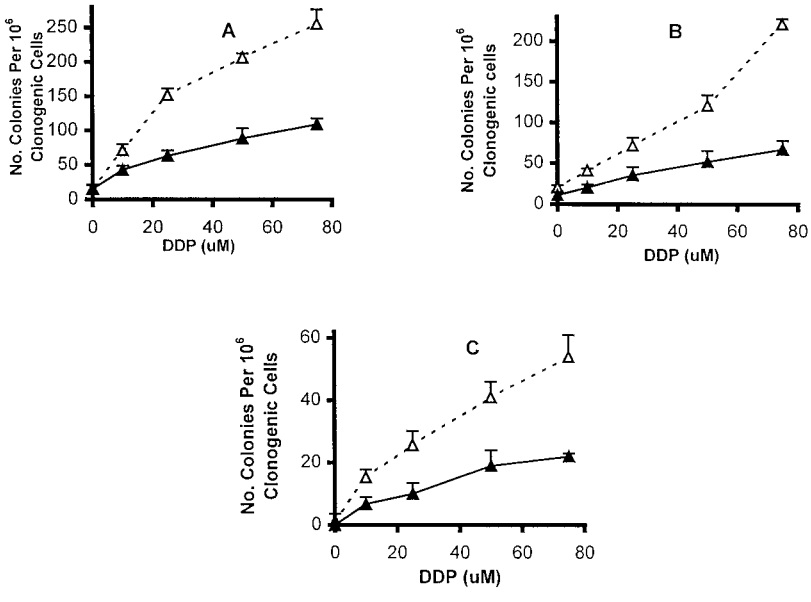

Fig. 2. Number of topotecan (A)-, gemcitabine (B)-, and paclitaxel (C)-resistant colonies/10^6 clonogenic cells as a function of cisplatin concentration for the MMR-deficient HCT116+ch2 (△) and MMR-proficient HCT116+ch3 (▲) cells. Each data point is the mean (±S.D.) of three experiments for each concentration of cisplatin.

The spontaneous rate of generation of variants resistant to topotecan, gemcitabine, and paclitaxel was determined by repeatedly measuring the frequency of resistant variants in expanding populations of the HCT116+ch2 and HCT116+ch3 cells, and the results are shown in Fig. 4 and Table 1. MMR-deficient HCT116 cells exhibited a 1.95-fold increase in spontaneous rate of generation of variants resistant to topotecan compared with MMR-proficient HCT116+ch3 cells ($P = .026$, $n = 3$). The MMR-deficient cells also had a 1.51-fold higher rate of generation of variants resistant to gemcitabine ($P = .025$, $n = 3$). However, the rate of 5.5×10^{-7} for the generation of variants resistant to paclitaxel in the HCT116+ch2 cells was similar to the rate of 5.8×10^{-7} observed in the HCT116+ch3 cells ($P = .789$).

Discussion

The major finding that emerges from these experiments is that in two independent model systems, loss of MMR increases the ability of cisplatin to generate human tumor cell variants resistant to the topoisomerase I inhibitor topotecan, the antimetabolite gemcitabine, and the tubulin depolymerization inhibitor paclitaxel. Topotecan, gemcitabine, and paclitaxel are representative of three different classes of agents that are commonly used, either in combination or sequentially, with cisplatin during the treatment of human cancer. Although the specific mechanisms by which cisplatin generates these resistant variants are not yet understood, in the case of topotecan and gemcitabine, it has already been established that resistance can result from mutations in either topoisomerase I (Benedetti et al., 1993) or deoxycytidine kinase (Owens et al., 1992) genes, respectively. In the case of paclitaxel, whose target is the β tubulins, the role of single mutations in mediating resistance has not been established. However, previous studies have documented that paclitaxel-resistant variants arise at a rate as high as that for etoposide, another drug with a single molecular target in the cell (Dumontet et al., 1996). Thus, it is reasonable to speculate that the resistance in some of the surviving clones reflects mutations produced by cisplatin in the topoisomerase I, deoxycytidine kinase, and β tubulin genes.

It is important to point out that the experimental approach used in these studies measures what is commonly referred to as the "mutant frequency," but it is not known whether the surviving colonies are in fact true genetic mutants or whether they are phenotypically stable. Because the drug-resistant phenotype was not scored until 10 days after the cisplatin exposure and because it persisted during at least the five cell divisions required to form a colony, the resistant state is unlikely to be due to a transient epigenetic effect. In addition, from the point of view of the cancer chemotherapist, what is important is whether at 10 days after a cisplatin exposure, the population of tumor cells contains within it a larger number of cells that will survive exposure to topotecan, gemcitabine, or paclitaxel. Such cells can contribute significantly to clinical failure regardless of whether they are true genetic mutants or have a stable phenotype. Because topotecan, gemcitabine, and paclitaxel are so often used in combination with or sequentially after cisplatin, the ability of cisplatin to generate resistant variants has the potential of limiting the effectiveness of such combinations. One would predict that resistance would emerge more rapidly in tumors containing larger number of MMR-deficient cells.

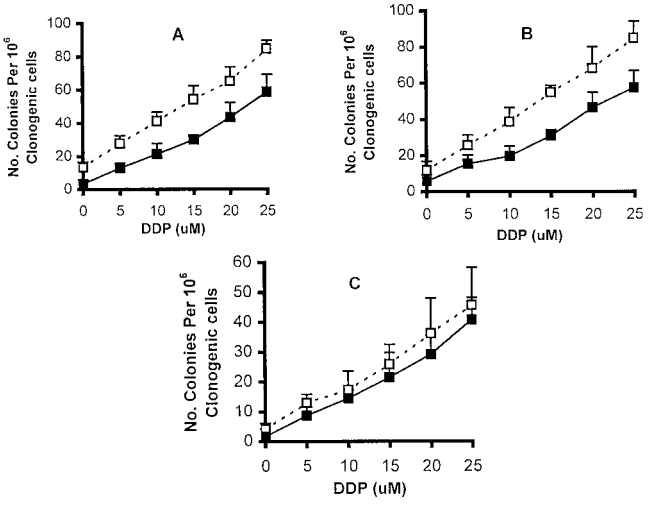

Fig. 3. Number of topotecan (A)-, gemcitabine (B)-, and paclitaxel (C)-resistant colonies/10^6 clonogenic cells as a function of cisplatin concentration for the MMR-deficient HEC59 (□) and MMR-proficient HEC59+ch2 (■) cells. Each data point is the mean (±S.D.) of six experiments for each concentration of cisplatin.

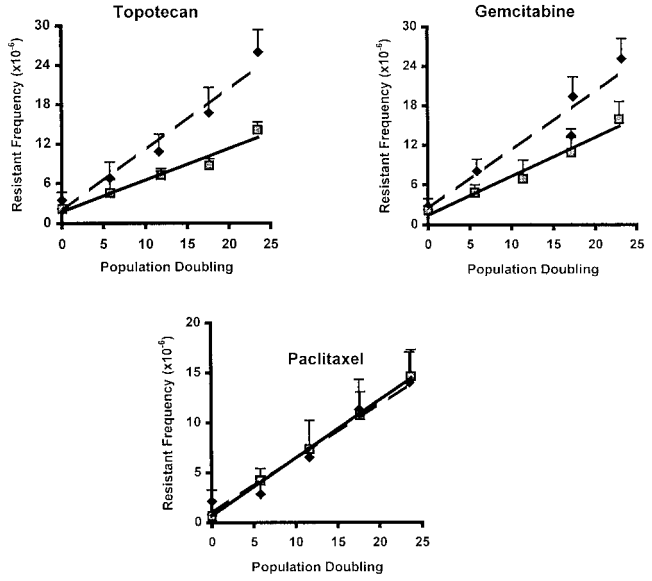

Fig. 4. Effect of loss of MMR on the spontaneous rate of generation of variants resistant to topotecan, gemcitabine, and paclitaxel in the HCT116+ch2 (dashed line) and HCT116+ch3 (solid line) cells. Each curve shows the change in frequency of resistant variants with increasing numbers of population doublings. The rate of generation of resistant variants is given by the slope of the linear regression line. Each data point is the mean (±S.D.) of three experiments.

TABLE 1

Spontaneous rates of generation of variants resistant to topotecan, gemcitabine, and paclitaxel in MMR-proficient and -deficient HCT-116 cells

	Spontaneous Rate[a]		P
	HCT-116 + ch3	HCT-116 + ch2	
Topotecan	4.8×10^{-7}	9.3×10^{-7}	.026
Gemcitabine	5.0×10^{-7}	8.9×10^{-7}	.025
Paclitaxel	5.8×10^{-7}	5.5×10^{-7}	.789

[a] Resistant variants/clonogenic cell/generation.

At the present time, no truly isogenic pairs of human tumor cells are available that differ solely by virtue of mutations in one or another of the genes that are required for MMR. Thus, the HCT116 and HEC59 sublines were used as surrogates with the recognition that the MMR-deficient and -proficient sublines differ in the expression of other genes as well. However, the observation that the MMR-deficient HCT116+ch2 cells are hypersensitive to the ability of cisplatin to generate resistant variants was reproduced in the HEC59 cell system as well, and the other phenotypic differences thus far identified between these pairs have been linked to loss of MMR (Koi et al., 1994; Umar et al., 1997).

Based on the slopes of the curves of the number of resistant variants/10^6 clonogenic cells as a function of cisplatin concentration, cisplatin was 4.4- and 1.6-fold, respectively, more effective in generating topotecan-resistant variants in the HCT116 and HEC59 cell systems and 3.2- and 1.5-fold, respectively, more effective in generating gemcitabine-resistant variants than in producing paclitaxel-resistant variants. The magnitude of the effect of MMR loss on the ability of cisplatin to generate resistant variants was generally greater in the HCT116 model than in the HEC59 model. Whether this reflects differences in residual MMR function due to mutations in MLH1 versus MSH2 awaits further investigation with true knockout cell pairs. It is possible that in hMLH1-deficient cells, the MSH2/MSH6 heterodimer still recognizes and processes the cisplatin adduct in a manner that contributes to a mutagenic propensity that is even greater than that resulting from the simple failure to recognize the cisplatin adduct that appears to accompany MSH2 loss.

The HCT116+ch2 cells have previously been shown to have a higher rate of mutation to 6-thioguanine due to mutations in hypoxanthine guanine phosphoribosyl transferase (Bhattacharyya et al., 1994; Aquilina et al., 1995). The results of the current study add topotecan and gemcitabine, two other drugs for which resistance can arise as a result of mutations in single gene, to the list of drugs for which the spontaneous rate of generation of resistant variants is increased in MMR-deficient HCT116+ch2 cells. These cells did not, however, demonstrate an increased rate of generation of variants resistant to paclitaxel. This observation, and the fact that cisplatin was less effective in producing paclitaxel-resistant variants than topotecan- or gemcitibine-resistant variants, may be related to the fact that paclitaxel works by binding to the β subunit of tubulin, of which there are six distinct isotypes in mammalian cells, some of which may be functionally redundant.

The ability of cisplatin to simultaneously select for the overgrowth of MMR-deficient cells, and to more readily generate variants resistant to drugs commonly used in combination with it, suggests that the presence of such cells in a tumor may predispose to the rapid emergence of drug resistance and treatment failure.

Acknowledgments

We thank Sibylle Nebel, Daniel Fink, Doreen Hom, and Dennis Heath for their technical assistance and Bristol-Myers Squibb for generously providing the cisplatin and paclitaxel used in these studies. We also thank Drs. C. R. Boland, M. Koi, and T. A. Kunkel for kindly providing the HCT116+ch2 and HCT116+ch3 cell lines.

References

Aquilina G, Hess P, Fiumicino S, Ceccotti S and Bignami M (1994) A mutator phenotype characterizes one of two complementation groups in human cells tolerant to methylation damage. *Cancer Res* **55:**2569–2575.

Benedetti P, Fiorani P, Capuani L and Wang JC (1993) Camptothecin resistance from a single mutation changing glycine 363 of human DNA topoisomerase I to cysteine. *Cancer Res* **53:**4343–4348.

Bhattacharyya NP, Skandalis A, Ganesh A, Groden J and Meuth M (1994) Mutator phenotypes in human colorectal carcinoma cell lines. *Proc Natl Acad Sci USA* **91:**6319–6323.

Cariello NF, Swenberg JA and Skopek TR (1992) In vitro mutational specificity of cisplatin in the human hypoxanthine guanine phosphoribosyltransferase gene. *Cancer Res* **52:**2866–2873.

Duckett DR, Drummond JT, Murchie AIH, Reardon JT, Sancar A, Lilley DMJ and Modrich P (1996) Human mutSa recognizes damaged DNA base pairs containing O^6-methylguanine, O^4-methylthymine, or the cisplatin-d(GpG) adduct. *Proc Natl Acad Sci USA* **56:**4881–4886.

Dumontet C, Duran GE, Steger KA, Beketic-Oreskovic L and Sikic BI (1996) Resistance mechanism in human sarcoma mutants derived by single-step exposure to paclitaxel (Taxol). *Cancer Res* **56:**1091–1097.

Eastman A (1986) Reevaluation of interaction of cis-dichloro(ethylenediamine)platinum (II) with DNA. *Biochemistry* **25:**3912–3915.

Fishel R and Kolodner RD (1995) Identification of mismatch repair genes and their role in the development of cancer. *Curr Opin Genet Dev* **5:**382–395.

Fishel R, Lescoe MK, Rao MR, Copeland NG, Jenkins NA, Garber J, Kane M and Kolodner R (1993) The human mutator gene homolog MSH2 and its association with hereditary nonpolyposis colon cancer. *Cell* **75:**1027–1038.

Fink D, Nebel S, Aebi S, Nehme A and Howell SB (1997) Loss of DNA mismatch repair due to knockout of MSH2 or PMS2 results in resistance to cisplatin and carboplatin. *Intl J Oncol* **11:**539–542.

Fink D, Nebel S, Aebi S, Zheng H, Cenni B, Nehme A, Christen R and Howell SB (1996) The role of DNA mismatch repair in platinum drug resistance. *Cancer Res* **56:**4881–4886.

Glaab WE and Tindall K (1997) Mutation rate at the *hprt* locus in human cancer cell lines with specific mismatch repair-gene defects. *Carcinogenesis* **18:**1–8.

Griffin S, Branch P, Xu YZ and Karran P (1994) DNA mismatch binding and incision at modified guanine bases by extracts of mammalian cells: Implications for tolerance to DNA methylation damage. *Biochemistry* **33:**4787–4793.

Hemminki A, Peltomaki P, Mecklin JP, Jarvinen H, Salovaara R, Nystrom-Lahti M, de la Chapelle A and Aaltonen LA (1994) Loss of the wild type MLH1 gene is a feature of hereditary nonpolyposis colorectal cancer. *Nat Genet* **8:**405–410.

Hess P, Aquilina G, Dogliotti E and Bignami M (1994) Spontaneous mutations at *aprt* locus in a mammalian cell line defective in mismatch recognition. *Somat Cell Mol Genet* **20:**409–421.

Hoffmann JS, Pillaire MJ, Garcia-Estefania D, Lapalus S and Villani G (1996) In vitro bypass replication of the cisplatin-d (GpG) lesion by calf thymus DNA polymerase beta and human immunodeficiency virus type I reverse transcriptase is highly mutagenic. *J Biol Chem* **271:**15386–15392.

Hoffmann JS, Pillaire MJ, Lesca C, Burnouf D, Fuchs RP, Defais M and Villani G (1997) Fork-like DNA templates support bypass replication of lesions that block DNA synthesis on single-stranded templates. *Proc Natl Acad Sci USA* **93:**13766–13769.

Huang J, Papadopoulos N, McKinley AJ, Farrington SM, Curtis LJ, Wyllie AH, Zheng S, Willson JK, Markowitz SD, Morin P, Kinzler KW, Vogelstein B and Dunlop MG (1996) APC mutations in colorectal tumors with mismatch repair deficiency. *Proc Natl Acad Sci USA* **93:**9049–9054.

Johnson NP, Hoeschele JD, Rahn RO, O'Neill JP and Hsie AW (1980) Mutagenicity, cytotoxicity, and DNA binding of platinum (IIII)-chloroammines in Chinese hamster ovary cells. *Cancer Res* **40:**1463–1468.

Kat A, Thilly WG, Fang W, Longley MJ, Li G and Modrich P (1995) An alkylation-toleration, mutator human cell line is deficient in strand-specific mismatch repair. *Proc Natl Acad Sci USA* **90:**6424–6428.

Koi M, Umar A, Chaudan DP, Cherian SP, Carethers JM, Kunkel TA and Boland CR (1994) Human chromosome 3 corrects mismatch repair deficiency and microsatellite instability and reduces *N*-methyl-*N*′-nitro-*N*-nitroguanidine tolerance in colon tumor cells with homozygous hMLH1 mutation. *Cancer Res* **54:**4308–4312.

Leach FS, Nicolaides NC, Papadopoulos N, Liu B, Jen J, Parsons R, Peltomäki P, Sistonen, P, Aaltonen LA and Nyström-Lahti M (1993) Mutations of a mutS homolog in hereditary nonpolyposis colorectal cancer. *Cell* **75:**1215–1225.

Liu B, Nicolaides NC, Markowitz S, Willson JKV, Parsons RE, Jen J, Papadopoulos N, Peltomäki P, de la Chapelle A and Hamilton SR (1995) Mismatch repair gene defects in sporadic colorectal cancers with microsatellite instability. *Nat Genet* **9:**48–55.

Markowitz S, Wang J, Myeroff L, Parsons R, Sun L, Lutterbaugh J, Fan RS, Zborowska E, Kinzler KW and Vogelstein B (1995) Inactivation of the type II TGF-β receptor in colon cancer cells with microsatellite instability. *Science (Wash DC)* **268:**1336–1338.

Owens JK, Shewach DS, Ullman B and Mitchell BS (1992) Resistance to 1-β-D-arabinofuranosylcytosine in human T-lymphoblasts mediated by mutation with the deoxycytidine kinase gene. *Cancer Res* **52:**2389–2393.

Papadopoulos N, Nicolaides NC, Wei YF, Ruben SM, Carter KC, Rosen CA, Haseltine WA, Fleischmann RD, Fraser CM and Adams MD (1994) Mutation of a mutL homolog in hereditary colon cancer. *Science (Wash DC)* **263:**1625–1629.

Parsons R, Li GM, Longley MJ, Fang WH, Papadopoulos N, Jen J, de la chapelle A, Kinzler KW, Vogelstein B and Modrich P (1993) Hypermutability and mismatch repair deficiency in RER$^+$ tumor cells. *Cell* **75:**1227–1236.

Rampino N, Yamamoto H, Inonv Y, Li Y, Sawai H, Reed JC and Perucho M (1997) Somatic frameshift mutations in the BAX gene in colon cancers of the microsatellite mutator phenotype. *Science (Wash DC)* **275:**967–969.

Turnbull NC, Popescu JA, Dipaolo JA and Myhr BC (1979) Cisplatinum (IIII) diammine dichloride causes mutation, transformation, and sister-chromatid exchanges in cultured mammalian cells. *Mutat Res* **66:**267–275.
Umar A, Koi M, Risinger JI, Glaab WE, Tindall KR, Kolodner RD, Boland CR, Barrett JC and Kunkel TA (1997) Correction of hypermutability, *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine resistance, and defective DNA mismatch repair by introducing chromosome 2 into human tumor cells with mutations in MSH2 and MSH6. *Cancer Res* **57:**3948–3955.
Umar A and Kunkel TA (1996) DNA-replication fidelity, mismatch repair and genome instability in cancer cells. *Eur J Biochem* **238:**297–307.
Wiencke JK, Cervenka J and Paulus H (1979) Mutagenic activity of anticancer agent cis-dichlorodiammine platinum-IIII. *Mutat Res* **68:**69–77.
Wooster R, Cleton-Jansen AM, Collins N, Mangion J, Cornelis RS, Cooper CS, Gusterson BA, Ponder BA, Von Deimling A and Wiestler OD (1994) Instability of short tandem repeats (microsatellites) in human cancers. *Nat Genet* **6:**152–156.
Yamada M, O'Regan E, Brown R and Karran P (1997) Selective recognition of a cisplatin-DNA adduct by human mismatch repair proteins. *Nucleic Acids Res* **25:**491–495.
Yarema KJ, Lippard SJ and Essigmann JM (1995) Mutagenic and genotoxic effects of DNA adducts formed by the anticancer drug cis-diamminedichloroplatinum(II). *Nucleic Acids Res* **23:**4066–4072.
Yarema KJ, Wilson JM, Lippard SJ and Essigmann JM (1994) Effects of DNA adduct structure and distribution on the mutagenicity and genotoxicity of two platinum anticancer drugs. *J Mol Biol* **236:**1034–1048.

Send reprint requests to: Dr. Stephen B. Howell, Department of Medicine 0058, UCSD Cancer Center University of California, San Diego, 9500 Gilman Dr., La Jolla, CA 92093. E-mail: showell@ucsd.edu