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COMBINED DEPLETION OF O^6-ALKYLGUANINE-DNA ALKYLTRANSFERASE AND GLUTATHIONE TO MODULATE NITROSOUREA RESISTANCE IN BREAST CANCER

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Abstract—MCF-7 human breast cancer cells possess high levels of O^6-alkylguanine-DNA alkyltransferase and moderate levels of glutathione, and are more resistant to chloroethylnitrosoureas (CNUs) than cells with low levels of either molecule. The role of each as a component of CNU resistance was assessed using O^6-benzylguanine (O^6-bG) or O^6-methylguanine (O^6-mG) to deplete the alkyltransferase and L-buthionine sulfoxamine (L-BSO) to deplete glutathione. O^6-bG and O^6-mG potentiated 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) cytotoxicity, resulting in a dose modification factor of 5.4 and 2.3, respectively, which reflected the more potent inhibitory effect of O^6-bG. L-BSO alone had little effect on the survival of MCF-7 cells following BCNU exposure, but when combined with O^6-mG, BCNU cytotoxicity was additive, yielding a dose modification factor of 3.2. O^6-bG or O^6-mG and L-BSO acted independently, as neither class of inhibitor affected the other's mechanism of CNU resistance. Furthermore, MCF-7 cells overexpressing GSTμ were not more resistant to BCNU than the parent cell line in either the presence or absence of O^6-bG or L-BSO. These results indicate that on a relative basis in MCF-7 cells, the alkyltransferase is the cell's first line of defense against CNUs. This suggests that therapeutic trials based on O^6-bG-induced biochemical modulation of CNU resistance may increase the efficacy of these chemotherapeutic agents against human malignant cells and that L-BSO may have little additive effect when used with these agents.

Key words: O^6-alkylguanine-DNA alkyltransferase; O^6-benzylguanine; chloroethylnitrosoureas; breast cancer

One of the major mechanisms of nitrosourea resistance in tumor cells appears to be mediated by O^6-alkylguanine-DNA alkyltransferase [1, 2]. This protein repairs O^6-chloroethylguanine monoadducts and prevents DNA cross-link formation from the N^1,O^6-ethanoguanine intermediate [3]. Cells that lack this protein (mer$^-$) are significantly more sensitive to CNUs† than cells with high levels of alkyltransferase (mer$^+$) [4, 5]. In addition, inhibitors of the alkyltransferase—O^6-mG, O^6-bG and streptozotocin—sensitize mer$^+$ cells to CNUs [6–9]. On the other hand, there is a wide range in the CNU IC_{50} of mer$^-$ cell lines [4], and there is also variability in the extent to which mer$^+$ cells can be sensitized to CNUs following inactivation of the alkyltransferase [6–9]; in some tumors there appears to be no correlation between alkyltransferase activity and CNU resistance [10, 11]. This suggests that other drug-resistance mechanisms are operative in tumor cells.

Intracellular GSH has been proposed as a second mechanism of resistance to chloroethylating nitrogen mustard derivatives as well as CNUs [12, 13]. GSH protects cells from oxidative damage [14] and becomes conjugated to alkylating agents, reducing DNA interstrand cross-links and cytotoxicity [15]. GSH can quench the cross-link reaction of the CNU-induced monoadduct *in vitro* in the absence of GST [16]. In addition, denitrosation of BCNU occurs via the GSH-dependent μ-class GSH-*S* transferase [17], suggesting that there are two mechanisms of GSH-mediated resistance to BCNU—direct quenching and enzymatic denitrosation. A recent report raised the possibility that GSH and GSTμ may be a dominant mechanism of BCNU resistance [18]. L-BSO, a potent inhibitor of γ-glutamyl cysteine synthetase, depletes cellular pools of GSH and has been shown to sensitize cells to melphalan, BCNU and chlorambucil as well as to non-alkylators such as doxorubicin and *cis*-platinum [13, 14, 19, 20]. In the above-mentioned studies, the effect of L-BSO has been studied without attention to alkyltransferase levels, just as studies of alkyltransferase inhibitors have not measured GSH levels.

To compare these mechanisms for their relative impact on CNU resistance, we chose to study the MCF-7 human breast cancer cell line, which has

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† Abbreviations: CNU, chloroethylnitrosourea; O^6-mG, O^6-methylguanine; O^6-bG, O^6-benzylguanine; GSH, glutathione; L-BSO, L-buthionine-[*S*,*R*]-sulfoxamine; BCNU, 1,3-bis(2-chloroethyl)-1-nitrosourea; GST, glutathione-*S*-transferase.

high levels of alkyltransferase and moderately high levels of GSH. By depleting alkyltransferase, GSH or both, it was possible to identify a hierarchy of resistance mechanisms and to assess the need to consider the use of two classes of inhibitors to effectively modulate tumor drug resistance.

MATERIALS AND METHODS

Cell culture. Human breast cancer cells, MCF-7 and MCF-μ3 (the gift of K. Cowan, National Cancer Institute), were grown as monolayers in Dulbecco's Modified Eagle's Medium, pH 7.2, supplemented with 7% fetal bovine serum, 3% calf serum, 100 U/mL penicillin/streptomycin solution, 20 mM HEPES, 2 mM glutamine and 10 μg/mL bovine insulin (Sigma, St. Louis, MO). Cells were maintained at 37° under a humidified atmosphere of 95% air and 5% CO_2.

Glutathione measurement. Total glutathione levels (GSH/GSSG) in breast cancer cells were measured using a modification of the Tietze assay as described by Akerboom and Sies [21]. Briefly, the cells were treated with L-BSO, either O^6-bG or O^6-mG, or the combination, for 24 hr, trypsinized and washed twice with ice-cold PBS. Pelleted cells were resuspended in cold 1 N perchloric acid + 1 mM EDTA and extracted on ice for 1 hr at a density of 10^6 cells/mL. The mixture was neutralized with KOH and placed on ice to precipitate excess salt. Then 1.0 mL of the resulting supernatant was diluted into 0.1 M K_2HPO_4/1 mM EDTA (pH 7.0) to a total volume of 2.5 mL. NADPH and 5,5′-dithio-bis-2-nitrobenzoic acid (DTNB) were added to the cuvet, and the baseline absorbance at 412 nm was determined using a Varian spectrophotometer. Then GSH reductase was added, and the absorbance after 2 min of incubation at 25° was measured and compared with that of GSH standards. The protein content of cell extract precipitates was determined using the Biorad assay. GSH reductase, NADPH, and DTNB were obtained from the Sigma Chemical Co. All other chemicals were of reagent grade, and all were made fresh immediately prior to use.

Glutathione-S-transferase. Overall GST activity was measured using a modification of the method of Habig *et al.* [22]. Briefly, 50–200 μL of cell extract was added to a reaction cuvet containing 1 mM 1-chloro-2,4-dinitrobenzene (CDNB), 2.5 mM GSH, and 1 mM EDTA in 100 mM potassium phosphate buffer, pH 6.5. The rate of color development at 25° was monitored as the change in absorbance at 340 nm. Under these conditions, 1 mU of GST is defined as the amount that catalyses the conjugation of 1 nmol of CDNB to GSH per min.

Alkyltransferase assay. Alkyltransferase measurements were determined as described and are reported as femtomoles of O^6-mG removed from [^{3}H] methylated DNA per μg DNA in the cell extract [6].

Clonogenic assay. Cells were treated as adherent monolayers 24 hr after plating, at which time they were in log phase growth. Cells received fresh medium, 0.5 mM O^6-mG, 0.1 mM L-BSO or the combination for 24 hr at 37°/5% CO_2 or 25 μM O^6-bG for 1 hr alone or 23 hr after addition of 0.1 mM L-BSO. Following pretreatment, cells were harvested with 0.025% trypsin/EDTA (Gibco, Grand Island, NY), and plated in triplicate tissue culture plates at a density of 5000 cells/plate in serum-free medium. Cells were allowed to adhere for 3 hr at 37°. Then control and treated groups were exposed to various concentrations of BCNU for 2 hr, followed by replacement with serum-containing medium and incubation at 37° and 5% CO_2. Cultures exposed to O^6-mG were replenished with medium containing 0.1 mM O^6-mG, whereas those exposed to O^6-bG were replenished with 8 μM O^6-bG for the length of the incubation period to maintain alkyltransferase depletion, as previously described [23]. After 14 days of growth, colonies were stained with 1% methylene blue and plates were scored for colony survival. Colonies of greater than 30 cells were scored. Growth studies indicated that concentrations of O^6-bG, O^6-mG, L-BSO and the combination did not alter significantly the cloning efficiency of the MCF-7 cell line.

L-BSO was obtained from the Sigma Chemical Co. and was prepared immediately before use. O^6-mG was obtained from the NCI Drug Synthesis Branch, solubilized at 10 mM in 0.1 N HCl, and diluted into (fresh) medium. O^6-bG was provided by Dr. Moschel, Frederick Cancer Research Center, and dissolved in DMSO. BCNU was obtained from the NCI Drug Synthesis Branch, was dissolved in absolute ethanol immediately prior to use and was diluted with appropriate medium.

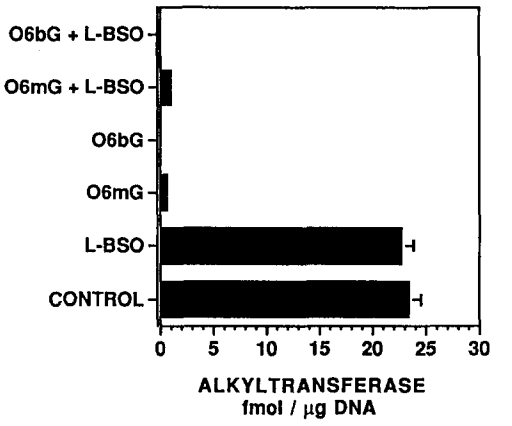

Fig. 1. Depletion of alkyltransferase. MCF-7 cells were exposed to drug-free medium, to 0.1 mM L-BSO, 0.5 mM O^6-mG or the combination for 24 hr, or to 25 μM O^6-bG for 1 hr or a combination of L-BSO for 24 hr with O^6-bG for the final hour and assayed for alkyltransferase activity. Data represent the means ± SD of duplicate determinations from three experiments.

RESULTS

Inactivation of alkyltransferase by O^6-mG and O^6-bG but not L-BSO. MCF-7 cells were found to have high levels of alkyltransferase activity, 23.4 ± 5.2 fmol/μg DNA, as high or higher than other CNU-resistant cell lines that we have studied,

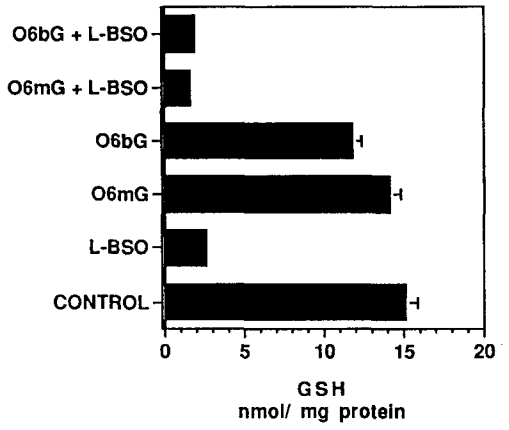

Fig. 2. Depletion of glutathione. MCF-7 cells were exposed to drug-free medium, 0.1 mM L-BSO, 0.5 mM O^6-mG, 25 μM O^6-bG, or the combination of L-BSO and either O^6-mG or O^6-bG for 24 hr and then assayed for glutathione levels [21]. Values are the means ± SD of duplicate determinations from three to six different experiments.

such as VACO6, HL-60, and HT-29 [6, 9]. O^6-mG caused a concentration- and time-dependent decline in alkyltransferase activity (Fig. 1) to 0.64 ± 0.1 fmol/μg DNA, 2.8% of control 24 hr following exposure to 0.5 mM O^6-mG. O^6-bG depleted alkyltransferase at concentrations of 25 μM to undetectable levels (< 0.1 fmol/μg DNA) after a 1-hr exposure. In contrast, a 24-hr exposure to 0.1 mM L-BSO had no effect on alkyltransferase activity alone and the combination of 0.1 mM L-BSO and 0.5 mM O^6-mG did not enhance the depletion of alkyltransferase activity over that of O^6-mG alone.

Depletion of GSH. MCF-7 cells were found to have GSH levels of 15.1 ± 3.8 nmol/mg protein, a level associated with resistance to cross-linking agents [19]. L-BSO at nontoxic concentrations (0.1 mM) depleted GSH levels to 17 ± 7% of control by 24 hr (Fig. 2), and no further decline was noted at 48 hr (data not shown). Toxic (0.2 mM) concentrations of L-BSO induced similar levels of GSH depletion (data not shown). A slight decrease in GSH levels was caused by 25 μM O^6-bG but not 0.5 mM O^6-mG. When combined with L-BSO, both O^6-mG and O^6-bG caused a slightly greater reduction in GSH over that seen with L-BSO alone. Thus, O^6-bG and O^6-mG are selective inhibitors of alkyltransferase and L-BSO selectively depletes glutathione levels.

Glutathione-S-transferase. The total GST in untreated MCF-7 cells was 17.6 ± 3.6 mU/mg protein, similar to that previously reported [24], but less than that observed in cells selected for drug resistance.

Cytotoxicity of O^6-bG, O^6-mG, L-BSO and the combination. The effect of the three drug-resistance modulators, O^6-bG, O^6-mG and L-BSO, was studied in cytotoxicity studies of clonogenic survival of MCF-7 after BCNU exposure. MCF-7 was markedly resistant to BCNU with an IC_{90} of 83 μM (Fig. 3). This is higher than the IC_{90} reported for more than 90% of human tumor cell lines [4]. L-BSO by itself had only a small effect on BCNU resistance, decreasing the IC_{90} to 63 μM (Fig. 3A). O^6-mG sensitized MCF-7 cells reducing the IC_{90} to 36 μM, and the combination of O^6-mG and L-BSO further sensitized MCF-7 cells to BCNU resulting in a decrease in the IC_{90} to 26 μM. This suggests that the effects of O^6-mG and L-BSO are additive. In contrast, O^6-bG was a better modulator of resistance than either O^6-mG or L-BSO, decreasing the IC_{90} to 15 μM BCNU (Fig. 3B). Analysis of dose-modification factors (Table 1) calculated for the IC_{50} and IC_{90} of BCNU indicated that modulator potency was O^6-bG > O^6-mG >> L-BSO. While the combination of L-BSO and O^6-mG increased the cytotoxic effect of BCNU over that seen with either modulator alone, O^6-bG alone was more potent, and the combination of O^6-bG plus L-BSO did not result in additive toxicity.

Effect of GSTμ overexpression in BCNU resistance. GSTμ is not normally expressed in MCF-7 but has been shown to detoxify CNUs [25]. Recently, the human GSTμ cDNA was transfected into MCF-7 cells (yielding the MCF-7 μ3 clone), yet resistance to BCNU did not increase [25]. To determine whether the protective role of GSTμ in CNU detoxification was masked by the high level of alkyltransferase, MCF-7 cells were treated with vehicle, O^6-bG, L-BSO, or the combination prior to exposure to BCNU. Figure 4 shows the concentration-response curve of these drug combinations. The IC_{50} values for BCNU alone (25 μM) and BCNU plus O^6-bG (2.8 μM) are very similar to those noted for the parental MCF-7 cell line (Fig. 3 and Table 1). A small effect was noted using L-BSO alone, but no additive effect was noted with the combination of L-BSO and O^6-bG.

Thus, although it might be argued that it has been difficult to observe a protective role for GSTμ in MCF-7 because it has a higher IC_{50} for BCNU than many other cell lines, our results suggest that when alkyltransferase is depleted by O^6-bG, there is no increase in the IC_{50} of BCNU over that seen in the parent MCF-7 cell line also treated with O^6-bG. Instead, the same degree of sensitization has been observed in the two cell lines.

DISCUSSION

Tumor drug-resistance is clearly a multifactorial process. Nitrosoureas, as opposed to other chemotherapeutic agents, are not actively pumped from the cell by P-glycoprotein 170 and thus are not part of the multidrug-resistance phenotype [26]. These agents are also not affected by the variant multidrug-resistance pathway mediated by altered topoisomerase II [27]. Nitrosoureas do not induce the same resistance mechanisms seen in nitrogen mustard resistant cells and acquired cross-resistance between the mustards and nitrosoureas is not common [28].

In this study, three known components of CNU resistance were compared to determine their relative contribution to drug resistance in the human breast cancer cell line, MCF-7. We found that L-BSO-

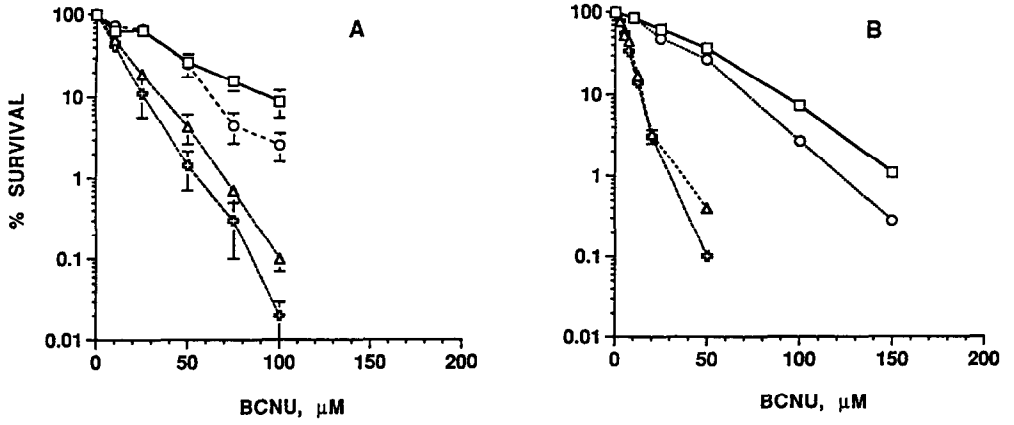

Fig. 3. Modulation of BCNU cytotoxicity. (A) Effects of L-BSO and O^6-mG. MCF-7 cells were exposed to 0.1 mM L-BSO, 0.5 mM O^6-mG, neither, or the combination for 24 hr prior to replating and exposure to BCNU for 2 hr. Cultures were then grown as in panel B, but as separate experiments. Symbols: (□) no pretreatment; (○) L-BSO; (△) O^6-mG; and (+) L-BSO plus O^6-mG. (B) Effects of L-BSO and O^6-bG. MCF-7 cells were exposed to 0.1 mM L-BSO for 24 hr, 25 μM/mL O^6-bG for 1 hr, the combination, or drug-free medium prior to exposure to BCNU for 2 hr. Cultures were then grown for 10 days and colonies were counted. Symbols: (□) no pretreatment; (○) L-BSO; (△) O^6-bG; and (+) L-BSO plus O^6-bG. Each point represents the mean ± SEM of triplicate determinations of five independent experiments for each condition. The error bars for Fig. 3B were less than 3% and are obscured by the symbols.

Table 1. Dose modification factor for O^6-mG, O^6-bG and/or L-BSO plus BCNU compared with BCNU alone

	IC_{50}		IC_{90}	
Modulator	μM BCNU	DMF_{50}*	μM BCNU	DMF_{90}*
None	28	—	83	—
L-BSO	28	1.0	63	1.3
O^6-mG	10	2.8	36	2.3
L-BSO and O^6-mG	8.0	3.5	26	3.2
O^6-bG	2.7	10.4	15	5.4
L-BSO and O^6-bG	2.6	10.8	12	6.5

MCF-7 cells were pretreated with medium alone, L-BSO, O^6-mG or O^6-bG, or the combination of L-BSO and either O^6-mG or O^6-bG, as described in Materials and Methods, and then exposed to BCNU to assess clonal survival.

* Dose modification factor (DMF) is the ratio of BCNU concentration required to achieve either an IC_{50} or IC_{90} in the clonogenic assay in the absence versus the presence of inhibitors. Data represent mean values from five separate experiments for each modulator in combination with BCNU.

mediated depletion of glutathione had only a small effect on MCF-7 resistance to CNUs, whereas O^6-bG-mediated depletion of alkyltransferase overwhelmed any effect of L-BSO. Likewise, overexpression of GSTμ had no effect on BCNU resistance. For many tumor cell lines, there is a linear correlation between alkyltransferase activity and BCNU resistance. The relation we previously reported [9] for colon cancer cell lines was alkyltransferase [fmol/μg DNA] = 0.83 [IC_{50}(BCNU)] − 2.0. If we were to apply this equation to an alkyltransferase activity of 23.4 fmol/μg DNA for MCF-7, the expected IC_{50} for BCNU would be 30 μM, compared with the observed value of 28 μM. Thus, the relationship between alkyltransferase activity and BCNU resistance is similar in MCF-7 to that seen in colon cancer cell lines. Taken together, these results indicate that in the MCF-7 cell line, which has high levels of both alkyltransferase and glutathione, the alkyltransferase is much more important than glutathione to the defense of the cell against CNUs. Under conditions of incomplete alkyltransferase depletion with O^6-mG, the addition of L-BSO resulted in additive toxicity, whereas with complete alkyltransferase depletion with O^6-bG, L-BSO had no additional effect.

The glutathione-*S*-transferase (GSTμ) is capable

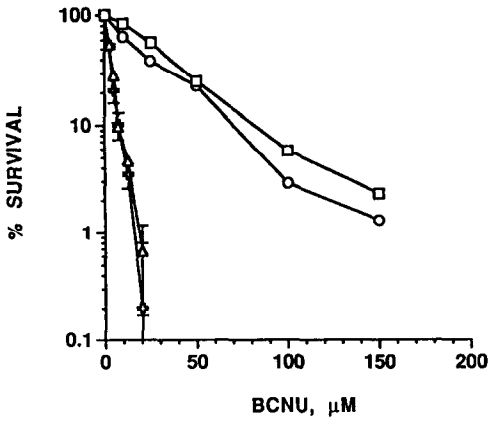

Fig. 4. Modulation of BCNU cytotoxicity in MCF-7 cells overexpressing GSTμ. MCF-7 μ3 cells were treated with L-BSO, O^6-bG and BCNU either alone or in combination, as described in the legend of Fig. 3B. Symbols are as described in the legend to Fig. 3B.

of detoxifying the chloroethylnitrosoureas and may also serve as a drug-resistance mechanism [18]. However, using the MCF-7 μ3 cell line, which overexpresses human GSTμ [25], we found that the cells did not have increased BCNU resistance compared with MCF-7 cells and that when alkyltransferase was depleted by O^6-bG there was still no effect of GSTμ overexpression. This suggests that in MCF-7 cells GSTμ-catalysed denitrosation (or perhaps more generally—detoxification) of BCNU is not a major contributor to nitrosourea resistance in either the presence or absence of alkyltransferase activity.

Our results clearly establish the importance of the alkyltransferase in BCNU resistance and suggest that glutathione and GSTμ, although proposed recently [18] as important mechanisms of CNU resistance, play a much lesser role in this process in MCF-7 cells. This apparent hierarchy in drug-resistance mechanisms may be dependent on the degree of modulation of each mechanism that can be achieved. We previously noted that if alkyltransferase inactivation by either O^6-mG or O^6-bG was maintained for only a brief period of time after BCNU exposure, there was a much smaller dose modification factor than if regeneration was prevented for at least 12–18 hr [6, 9, 21]. This could be accomplished by prolonged exposure to low-dose O^6-mG (0.1 mM) or low-dose O^6-bG [23] after addition of BCNU. Because persistent CNU-induced adducts are slowly converted to interstrand cross-links over an 8–12 hr period [29], it is important to block alkyltransferase regeneration for at least this period of time.

Other factors, such as excision repair pathways [30], polyamines [31], topoisomerase II [32], and poly(adenosine diphosphate-ribose) polymerase [33], may also play a role in CNU resistance although dose modification factors reported by modulators of these pathways are less than that reported here for alkyltransferase depletion. By evaluating various inhibitor combinations, a comprehensive understanding of the interaction among CNU-resistance mechanisms can be established and their modulation individualized for use against particular tumor types.

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REFERENCES

1. Brent TP, Houghton PJ and 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* **82**: 2985–2989, 1985.
2. Pegg AE, Mammalian O^6-alkylguanine-DNA alkyltransferase: Regulation and importance in response to alkylating carcinogenic and therapeutic agents. *Cancer Res* **50**: 6119–6129, 1990.
3. Gonzaga PE and Brent TP, Affinity purification and characterization of human O^6-alkylguanine-DNA alkyltransferase complexed with BCNU-treated, synthetic oligonucleotide. *Nucleic Acids Res* **17**: 6581–6590, 1989.
4. Scudiero DA, Meyer SA, Clatterbuck BE, Mattern MR, Ziolkowski CHJ and Day RS III, Sensitivity of human cell strains having different abilities to repair O^6-methylguanine in DNA to inactivation by alkylating agents including chloroethylnitrosoureas. *Cancer Res* **44**: 2467–2474, 1984.
5. Yarosh DB, Foote RS, Mitra S and Day RS III, Repair of O^6-methylguanine in DNA by demethylation is lacking in *Mer*$^-$ human tumor cell strains. *Carcinogenesis* **4**: 199–205, 1983.
6. Gerson SL, Trey JE and Miller K, Potentiation of nitrosourea toxicity by chronic inactivation of O^6-alkylguanine-DNA alkyltransferase. *Cancer Res* **48**: 1521–1527, 1988.
7. Dolan ME, Stine L, Mitchell RB, Moschel RC and Pegg AE, Modulation of mammalian O^6-alkylguanine-DNA alkyltransferase *in vivo* by O^6-benzylguanine and its effect on the sensitivity of a human glioma tumor to 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea. *Cancer Commun* **2**: 371–377, 1990.
8. Gibson NW, Hartley JA, Barnes D and Erickson LC, Combined effects of streptozotocin and mitozolomide against four human cell lines of the Mer$^+$ phenotype. *Cancer Res* **46**: 4995–4998, 1986.
9. Gerson SL, Berger NA, Arce C, Petzold S and Willson JKV, Modulation of nitrosourea resistance in human colon cancer by O^6-methylguanine. *Biochem Pharmacol* **43**: 1101–1107, 1992.
10. Walker MC, Masters JRW and Margison GP, O^6-Alkylguanine-DNA alkyltransferase activity and nitrosourea sensitivity in human cancer cell lines. *Br J Cancer* **66**: 840–843, 1992.
11. Silber JR, Bobola MS, Ewers TG, Muramoto M and Berger MS, O^6-Alkylguanine-DNA alkyltransferase is not a major determinant of sensitivity to 1,3-bis(2-chloroethyl)-1-nitrosourea in four medulloblastoma cell lines. *Oncol Res* **4**: 1–8, 1992.
12. Evans CG, Bodell WJ, Tokuda K, Doane-Setzer P and Smith MT, Glutathione and related enzymes in rat brain tumor cell resistance to 1,3-bis(2-chloroethyl)-1-

nitrosourea and nitrogen mustard. *Cancer Res* **47**: 2525–2530, 1987.
13. Hansson J, Edgren M, Ehrsson H, Ringborg U and Nilsson B, Effect of *d,l*-buthionine-*S,R*-sulfoximine on cytotoxicity and DNA cross-linking induced by bifunctional DNA-reactive cytostatic drugs in human melanoma cells. *Cancer Res* **48**: 19–26, 1988.
14. Clark EP, Epp ER, Biaglow JE, Morse-Gaudio M and Zachgo E, Glutathione depletion, radiosensitization, and misonidazole potentiation in hypoxic Chinese hamster ovary cells by buthionine sulfoximine. *Radiat Res* **98**: 370–380, 1984.
15. Ali-Osman F, Caughlan J and Gray GS, Decreased DNA interstrand cross-linking and cytotoxicity induced in human brain tumor cells by 1,3-bis(2-chloroethyl)-1-nitrosourea after *in vitro* reaction with glutathione. *Cancer Res* **49**: 5954–5958, 1989.
16. Ali-Osman F, Quenching of DNA cross-link precursors of chloroethylnitrosoureas and attenuation of DNA interstrand cross-linking by glutathione. *Cancer Res* **49**: 5258–5261, 1989.
17. Smith MT, Evans CG, Doane-Setzer P, Castro VM, Tahir MK and Mannervik B, Denitrosation of 1,3-bis(2-chloroethyl)-1-nitrosourea by class mu glutathione transferases and its role in cellular resistance in rat brain tumor cells. *Cancer Res* **49**: 2621–2625, 1989.
18. Berhane K, Hao X-Y, Egyházi S, Hansson J, Ringborg U and Mannervik B, Contribution of glutathione transferase M3-3 to 1,3-bis(2-chloroethyl)-1-nitrosourea resistance in a human non-small cell lung cancer cell line. *Cancer Res* **53**: 4257–4261, 1993.
19. Dusre L, Mimnaugh EG, Myers CE and Sinha BK, Potentiation of doxorubicin cytotoxicity by buthionine sulfoximine in multidrug-resistant human breast tumor cells. *Cancer Res* **49**: 511–515, 1989.
20. Friedman HS, Colvin OM, Griffith OW, Lippitz B, Elion GB, Schold SC Jr, Hilton J and Bigner DD, Increased melphalan activity in intracranial human medulloblastoma and glioma xenografts following buthionine sulfoximine-mediated glutathione depletion. *J Natl Cancer Inst* **81**: 524–527, 1989.
21. Akerboom TPM and Sies H, Assay of glutathione, glutathione disulfide and glutathione mixed disulfides in biological samples. *Methods Enzymol* **77**: 373–382, 1981.
22. Habig WH, Pabst MJ and Jacoby WB, Glutathione-*S*-transferases: The first enzymatic step in mercapturic acid formation. *J Biol Chem* **249**: 7130–7139, 1974.
23. Gerson SL, Zborowska E, Norton K, Gordon N and Willson JKV, Synergistic efficacy of O^6-benzylguanine and BCNU in human colon cancer xenografts completely resistant to BCNU alone. *Biochem Pharmacol* **45**: 483–491, 1993.
24. Whelan RDH, Hosking LK, Townsend AJ, Cowan KH and Hill BT, Differential increases in glutathione *S*-transferase activities in a range of multidrug-resistant human tumor cell lines. *Cancer Commun* **1**: 359–365, 1989.
25. Townsend AJ, Tu CPD and Cowan KH, Expression of human μ or α class glutathione *S*-transferases in stably transfected human MCF-7 breast cancer cells: Effect on cellular sensitivity to cytotoxic agents. *Mol Pharmacol* **41**: 230–236, 1991.
26. Moscow JA and Cowan KH, Multidrug resistance. *J Natl Cancer Inst* **80**: 14–20, 1988.
27. Danks MK, Yalowich JC and Beck WT, Atypical multiple drug resistance in a human leukemic cell line selected for resistance to teniposide (VM-26). *Cancer Res* **47**: 1297–1301, 1987.
28. Teicher BA, Cucchi CA, Lee JB, Flatow JL, Rosowsky A and Frei E III, Alkylating agents: *in vitro* studies of cross-resistance patterns in human cell lines. *Cancer Res* **46**: 4379–4383, 1986.
29. Brent TP, Lestrud SO, Smith DG and Remack JS, Formation of DNA interstrand cross-links by the novel chloroethylating agent 2-chloroethyl(methylsulfonyl) methanesulfonate: Suppression by O^6-alkylguanine-DNA alkyltransferase purified from human leukemic lymphoblasts. *Cancer Res* **47**: 3384–3387, 1987.
30. Bronstein SM, Skopek TR and Swenberg JA, Efficient repair of O^6-ethylguanine, but not O^4-ethylthymine or O^2-ethylthymine, is dependent upon O^6-alkylguanine-DNA alkyltransferase and nucleotide excision repair activities in human cells. *Cancer Res* **52**: 2008–2011, 1992.
31. Seidenfeld J and Sprague WS, Comparisons between sensitive and resistant human tumor cell lines regarding effects of polyamine depletion on chloroethylnitrosourea efficacy. *Cancer Res* **50**: 521–526, 1990.
32. Eder JP, Teicher BA, Holden SA, Cathcart KNS and Schnipper LE, Novobiocin enhances alkylating agent cytotoxicity and DNA interstrand crosslinks in a murine model. *J Clin Invest* **79**: 1524–1528, 1987.
33. Chatterjee S, Cheng MF, Berger SJ and Berger NA, Alkylating agent hypersensitivity in poly(adenosine diphosphate-ribose) polymerase deficient cell lines. *Cancer Commun* **3**: 71–75, 1991.