Relationship Between O^6-methylguanine-DNA Methyltransferase Levels and Clinical Response Induced by Chloroethylnitrosourea Therapy in Glioma Patients[1]

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ABSTRACT: ***Background:*** Adjuvant nitrosourea chemotherapy fails to prolong survival significantly as many tumors demonstrate resistance to these drugs. It has been documented in cell lines that O^6-methylguanine DNA methyltransferase (MGMT) plays an important role in chloroethylnitrosourea (CENU) drug resistance. ***Methods:*** We evaluated MGMT expression in 22 glioma specimens by using an immunofluorescence assay and compared the results with clinical responses of the patients to CENU-based chemotherapy. ***Results:*** Eight tumor samples had no detectable MGMT, whereas other samples had from 9,989 to 982,401 molecules/nucleus. In one group (12 patients), the tumor decreased in size or was stable (effective group), whereas in the other group (10 patients), the tumor demonstrated continuous growth during chemotherapy (progressive group). The Mer^- patients (MGMT < 60,000 molecules/nucleus) appeared to have more chance of stable disease or response to CENU therapy than the Mer^+ patients (MGMT > 60,000 molecules/nucleus) ($X^2 = 4.791$, $p = 0.0286$). In patients with glioblastomas multiforme (GBMs), the median time to progression (TTP) of Mer^+ patient was shorter than that of Mer^- patient ($t = 2.04$, $p = 0.049$). As a corollary, the MGMT levels were significantly higher in GBM tumors from the progressive group than those from the effective group ($t = 2.26$, $p = 0.029$). However, there was no significant correlation between MGMT levels and either the survival time ($r = 0.04$, $p = 0.8595$) or TTP ($r = 0.107$, $p = 0.6444$). ***Conclusion:*** This study suggests that being MGMT positive is indicative of a more aggressive disease that progresses more rapidly with CENU therapy. However, MGMT negative tumors are not always sensitive to CENU agents, suggesting that other factors are also important.

RÉSUMÉ: Relation entre les niveaux de o^6-méthylguanine-ADN méthyltransférase et la réponse clinique au traitement par la chloroéthyl-nitrosurée chez les patients atteints de gliome. ***Introduction:*** La chimiothérapie adjuvante à base de nitrosurées ne prolonge pas significativement la survie des patients atteints de gliome malin parce que plusieurs de ces tumeurs y sont résistantes. Il a été démontré qu'en culture cellulaire, la o^6-méthylguanine-ADN méthyltransférase (MGMT) joue un rôle important dans la résistance pharmacologique à la chloroéthyl-nitrosurée (CENU). ***Méthodes:*** Nous avons évalué l'expression de la MGMT dans 22 spécimens de gliome à l'aide d'un essai par immunofluorescence et avons comparé ces résultats avec la réponse clinique des malades traités par chimiothérapie à base de la CENU. ***Résultats:*** Huit spécimens n'avaient pas de MGMT détectable alors que les autres spécimens avaient de 9,989 à 982,401 molécules/noyau. Dans un groupe de 12 malades, la taille de la tumeur a diminué ou est restée stable (groupe répondeur), tandis que dans un autre groupe de 10 patients, la tumeur a continué de progresser durant la chimiothérapie (groupe avec progression). Le groupe de patients Mer- (MGMT < 60,000 molécules/noyau) a semblé avoir une plus grande probabilité que la maladie soit stable ou de présenter une réponse au traitement par la CENU que le groupe Mer+ (MGMT <60,000 molécules/noyau) ($X2 = 4.791$, $p = 0.0286$). Chez les malades porteurs de glioblastomes multiformes (GBM), le laps de temps moyen écoulé jusqu'à ce qu'on note une progression (TMP) était plus court pour le groupe Mer+ que pour le groupe Mer- ($t = 2.04$, $p = 0.049$). En corollaire, les niveaux de MGMT étaient significativement plus élevés dans les tumeurs des patients du groupe avec progression que dans celles des patients du groupe où le traitement était efficace ($t = 2.26$, $p = 0.029$). Cependant, il n'y avait pas de relation significative entre les niveaux de MGMT et le temps de survie ($r = 0.04$, $p = 0.8595$) ou le TMP ($r = 0.107$, $p = 0.644$). ***Conclusion:*** Cette étude suggère que la positivité pour la MGMT indique la présence d'une maladie plus agressive qui progresse plus rapidement sous traitement par la CENU. Cependant, les tumeurs négatives pour la MGMT ne sont pas toujours sensibles à la CENU, ce qui suggère que d'autres facteurs sont également importants dans la réponse à la chimiothérapie.

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Chloroethylnitrosoureas (CENUs) have long been used as front-line drugs for malignant gliomas. However, adjuvant nitrosourea chemotherapy only has a minimal effect on survival time because many tumors are resistant to these drugs.

Chloroethylnitrosoureas act by releasing a chloroethyldiazonium ion that alkylates several sites in DNA, the most important of which is at the O^6-position of deoxyguanosine. This

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chloroethyl adduct undergoes an intramolecular circularization and then crosslinks the DNA, producing a lethal lesion.[1,2] O^6-methylguanine-DNA methyltransferase (MGMT) can transfer the alkyladduct from O^6-guanine to a cysteine residue at position 145 of the alkyltransferase peptide chain, thereby inactivating the alkyladduct.[3,4] By repair of O^6-alkylguanine DNA adducts formed by CENUs, MGMT prevents further formation of DNA crosslinks thereby decreasing the cytotoxicity of CENUs.[5] This stoichiometric "suicide" method of repair is limited by the number of alkyltransferase molecules available. It has been documented, in tumor cell lines and in xenografts, that MGMT plays an important role in nitrosourea drug resistance.[5-10] Free base inhibitors such as O^6-benzylguanine (O^6-BG) inactivate the alkyltransferase molecules in the cells and therefore reverse CENU drug resistance.[7,11,12] Clinical trials are currently underway to determine its efficacy;[13] however, there have been only a few clinical investigations examining *MGMT* expression in brain tumor samples in relation to patient survival and/or clinical response to chemotherapy.[14-17]

Traditionally, MGMT levels have been determined by bioenzymatic assay in which fresh tissue is used.[5] The characterization of the *MGMT* gene has made it possible to determine MGMT expression by RNA and DNA blot analysis as well as by reverse transcription-polymerase chain reaction (RT-PCR) techniques.[13,18,19] However, determining MGMT expression with cellular extracts may be misleading because they may contain normal tissues that influence the results. Immunofluorescence assays have the advantage of allowing quantification of MGMT activity only in the malignant cells. Furthermore, one can use paraffin embedded specimens.[20,21] In the present study, we evaluated MGMT expression in human glioma specimens by an immunofluorescence assay and compared these results with the responses of the patients to CENU-based chemotherapy.

Materials and Methods

Clinical data

There were 22 patients with gliomas (13 men and nine women) involved in this study ranging in age from 32 to 71 years (mean 50 years). Diagnosis was confirmed by routine pathological examination. Paraffin-embedded slides were used for MGMT detection (details following). All patients underwent radiotherapy after resective surgery. Following radiotherapy, chemotherapy was begun when there was evidence of progression as defined on computerized tomography (CT) or magnetic resonance (MR) imaging. Either BCNU (200 mg/m^2) or PCV (CCNU 110 mg/m^2 at day 1; procarbazine 60 mg/m^2 days 8-21; and vincristine 1.4 mg/m^2 days 8 and 29) was given every 6 to 8 weeks (Table 1). The CT and/or MR images were obtained before and after chemotherapy to measure changes in tumor size. A reduction in the tumor mass greater than 25% but less than 50% was considered as a minor response, whereas a reduction greater than or equal to 50% was considered a partial response. An increase in tumor size of more than 25%, was considered progression. The tumor was considered stable if it neither increased nor decreased by no more than 25%. Patients received one to five cycles of treatment and were followed for 2 to 43 months (Table 1). Patient response to chemotherapy was also evaluated by the time to progression (TTP), which was the time in months ftom the date of first treatment with CENU until the date that the patient developed progressive disease. Survival time was determined from the date of initial chemotherapy.

Detection of MGMT in tumor specimens

A quantitative immunofluorescence assay for MGMT was performed as previously described.[16,20] Briefly, two slides from each tumor sample were stained in parallel: one with antitransferase monoclonal antibody 3B8, which was raised against recombinant human MGMT[3] (test sample), and one without this antibody (background control). Quantum Simple Cellular Microbeads, used in calibrating the digitized fluorescence intensity, were spotted onto slides and fixed in parallel. The tissue samples and beads were treated with 0.1% Triton X-100 in phosphate-buffered saline (PBS), blocked with 5% nonfat milk/PBS, and stained with the first antibody at 37 µg/ml and the second goat anti-mouse immunoglobulin G antibody linked to fluorescein isothiocyanate (FITC) at 15 µg/ml. Nuclei were stained with 0.2 µg/ml 4'6-diamidino-2-phenylindole-2HC1 (DAPI) for 3 minutes, and the slides were rinsed and mounted with 50% glycerol and Slowfade antifade reagent.

Images from the slides of the tissues and beads were captured by epifluorescence microscopy for which a Nikon Diaphot microscope equipped with Fluorite lenses and with green and blue filter sets was used (for FITC and DAPI fluorescence, respectively). The images were digitized using a Star I CCD Camera. The images were analyzed with the Optimas image analysis system, by using a macroinstruction that automates data collection and performs all calculations. A value for MGMT (in molecules/nucleus) for each nucleus was derived from measurements of nuclear areas and densitometric fluorescent intensities. Intensity was quantified following comparison to a calibration curve derived from positive control standards. For each tumor specimen, a distribution was determined from densitometric intensity measurements of approximately 100 nuclei in the test and background control sections. The MGMT level was scored as zero if the mean value on the test section did not differ significantly from the mean value on the background control section. The value in molecules/nucleus for each tumor specimen was defined as the difference between the means of the two distributions for the test and background control slide for each given tumor sample. Calculations were performed in duplicate for each sample and results were averaged.

Statistical analysis

The relationship between MGMT expression and survival time or TTP of the patients were evaluated using linear regression analysis. In addition, the patients were divided into several groups for comparisons using Students' t-test and contingency analysis. The MGMT-rich (Mer$^+$) group had MGMT level higher than 60,000 molecules/nucleus, whereas a tumor with a MGMT level below 60,000 molecules/nucleus was considered as MGMT-poor (Mer$^-$). The patients were placed in the progression group if the tumor mass increased in size during chemotherapy, whereas if the tumor remained stable or decreased in its volume during treatment, the patients were assigned to the effective group. The patients were also separated into groups by histological tumor type: a glioblastoma multiforme (GBM) tumor group and an anaplastic astrocytoma (AA) tumor group.

Results

Twenty-two patients were involved in this study. Thirteen patients had GBMs and nine patients had AAs. The expression of

Table 1: Clinical data of 22 glioma patients.

Case No.	Sex	His[5]	Age (yrs)	MGMT level[6]	/	phenotype[7]	Response[8]	cycle	TTP[9]	ST[10]
1	M	GBM	71	0	/	Mer^-	minor response	4	6	12
2	F	GBM	57	0	/	Mer^-	partial response	3	9	11
3	M	GBM	64	98136	/	Mer^+	partial response	2	>2	6
4	M	GBM	63	0	/	Mer^-	stable	2	4	4
5[4]	M	GBM	43	9989	/	Mer^-	stable	5	9	11
6	M	GBM	60	92685	/	Mer^+	progressive	3	2	5
7	F	GBM	61	0	/	Mer^-	progressive	2	2	3
8[4]	M	GBM	42	63430	/	Mer^+	progressive	1	1.5	6
9[4]	M	GBM	49	261940	/	Mer^+	progressive	2	1	7
10	F	GBM	55	519000	/	Mer^+	progressive	2	3	5
11	F	GBM	52	496000	/	Mer^+	progressive	3	2	8
12	F	GBM	65	0	/	Mer^-	progressive	1	1	3
13[1]	M	GBM	62	127000	/	Mer^+	progressive	2	4	5
14	M	AA	39	387066	/	Mer^+	progressive	4	5	>34
15[3]	F	AA	44	83234	/	Mer^+	progressive	4	5	>42
16[1]	F	AA	32	0	/	Mer^-	partial response	5	>43	>43
17	M	AA	32	0	/	Mer^-	stable	4	6	10
18[4]	F	AA	36	0	/	Mer^-	stable	4	13	19
19[4]	M	AA	41	826073	/	Mer^+	partial response	5	9	12
20[4]	M	AA	44	326845	/	Mer^+	minor response	4	10	12
21	F	AA	51	982401	/	Mer^+	stable	2	9	12
22[2,4]	M	AA	43	53112	/	Mer^-	minor response	1	NE[11]	14

[1] Only these 2 patients had received BCNU with radiotherapy as initial treatment and on relapse were treated with BCNU alone.

[2] Refused further chemotherapy after 1 dose.

[3] Received PCV x 2 cycles, then BCNU x 2 cycles.

[4] Received concomitant high dose tamoxifen therapy, but had progressed on tamoxifen therapy before starting BCNU therapy.

[5] GBM, glioblastoma multiforme; AA, anaplastic astrocytoma.

[6] MGMT levels were presented as Molecules/nucleus determined by immunofluorescence assay.

[7] Mer^+ consisted of MGMT levels higher than 60,000 Molecules /nucleus in the tumor sample; Mer^- consisted of MGMT levels below 60,000 Molecules /nucleus in the tumor cells.

[8] Minor response means that the tumor mass was reduced in size by > 25% but < 50%; Partial response means that the tumor mass reduction is ≥ 50%; Progressive is that the patient's tumor increased by > 25%; Stable is that the tumor mass did not decrease down to 25% or did not increase up to 25%.

[9] TTP, time to progression in months after initial chemotherapy.

[10] Survival time is the time in months after initial chemotherapy until the patient died.

[11] NE, could not be evaluated.

MGMT in tumor samples varied widely. Eight samples had no detectable MGMT; the remaining tumors had MGMT expression ranging from 9,989 to 982,401 molecules/nucleus. The response to chemotherapy was modest; only four patients had a partial response (tumor volume decrease ≥ 50%). Three patients showed minor response (tumor volume decrease > 25% but < 50%) and five patients remained stable during CENU based chemotherapy. With almost half of the patients (10 of 22), the tumor continuously grew during chemotherapy. The average TTP in patients was 6.7 months and the average survival was 13 months (range 2 - 43 months) (Table 1). By linear regression analysis, there was no significant correlation between MGMT levels in tumor cells and either the patient survival time ($r = 0.04$, $p = 0.8595$) or TTP ($r = 0.107$, $p = 0.6444$). However, the age of the patients, as expected, was negatively correlated with TTP ($r = 0.507$, $p = 0.019$) or with survival time ($r = 0.573$, $p = 0.0053$). Mer^- patients were less likely to be in the progressive group (two of 10) than Mer^+ patients (eight of 12), ($x^2 = 4.791$, $p = 0.0286$). However, there was no significant difference between the Mer^+ and Mer^- group for survival time ($t = 0.094$, $p = 0.4633$) or TTP ($t = 1.156$, $p = 0.1428$). Furthermore, there was no significant difference in MGMT levels between the progressive and effective group ($t = 0.098$, $p = 0.461$).

Because GBMs are much more aggressive than AAs, the survival time was significantly shorter in patients with GBM tumors (6.9 ± 0.8 months) compared to those with AAs (22 ± 4.6 months). The TTP of the patients was also shorter in the GBM group than in the AA group. Patients in the GBM group were much older than those in the AA group (mean 57 compared with 40 years, respectively). However, the MGMT levels between these two groups were not significantly different ($t = -1.24$, $p = 0.122$) (Table 2).

Specific to the GBM group alone, the TTP of Mer^+ patients (2.2 ± 0.4 months) was shorter than that of Mer^- patients (5.2 ± 1.4 months), but there was no significant difference in survival time between these two groups ($t = 0.889$, $p = 0.207$) (Table 2).

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Table 2: Comparison of age, TTP, survival time and MGMT levels between groups.

Group	n	Age	TTP	Survival time	MGMT levels[3]	Response (Eff: Prog)
Whole Patients						
Mer+,[1]	12	50.3 ± 2.5	4.5 ± 4.3	12.9 ± 13.6		4:8
Mer−		10	50.3 ± 4.7	10.3 ± 4.3	13.2 ± 3.6	8:2
		t = 0.01	t = –1.34	t = –0.06		x^2 = 4.791
		p = 0.498	p = 0.108	p = 0.478		p = 0.0286
Eff[2]	12	48.1 ± 3.8	10 ± 3.2	13.8 ± 2.9	191 ± 100	
Prog	10	52.9 ± 2.9	2.7 ± 0.5	12.1 ± 4.4	203 ± 63	
		t = 1.01	t = –2.45	t = –0.33	t = 0.098	
		p = 0.162	p = 0.017	p = 0.372	p = 0.461	
GBM	13	57.2 ± 2.4	3.6 ± 0.8	6.9 ± 0.8	128 ± 51	5:8
AA	9	40.2 ± 2.0	12.5 ± 4.5	22 ± 4.6	295 ± 125	7:2
		t = 5.35	t = –1.97	t = –3.27	t = –1.24	x^2 = 3.316
		p = 0.00002	p = 0.045	p = 0.006	p = 0.122	p = 0.0686
GBM						
Mer+	7	54.9 ± 3	2.2 ± 0.4	6.1 ± 0.4		1:6
Mer−	6	60 + 4	5.2 ± 1.4	7.7 ± 1.7		4:2
		t = 1. 05	t = 2.04	t = 0.89		X^2= 3. 745
		p = 0.16	p = 0.049	p = 0.207		p = 0.053
Eff	5	59.6 ± 4.7	6 ± 1.4	8.8 ± 1.6	21 ± 19	
Prog	8	55.6 ± 2.7	2.1 ± 0.4	5.6 ± 0.5	195 ± 74	
		t = 0.71	t = 3.03	t = 1.67	t = –2.26	
		p = 0.253	p = 0.019	p = 0.077	p = 0.029	

[1]MGMT phenotype (Mer+, Mer−), TTP, survival time were defined as in Table 1 legend. The value is expressed as mean ± standard error.
[2]Eff, effective patients were those in which the tumor mass was reduced in size or remained stable, with chemotherapy; Prog, were the patients where the tumor increased in size during chemotherapy.
[3] MGMT levels were Molecules/nucleus x 10^3.

When we compared the GBM patients between the effective and progressive groups, the MGMT levels were much higher (195,007 ± 74,223 molecules/nucleus) in the progressive patients than in the effective patients (21,625 ± 19,225 molecules/nucleus). The TTP and survival times in the effective group were also longer than those in the progressive group (Table 2). However, there was no linear correlation between MGMT levels and TTP (r = 0.338, p = 0.259) or survival time (r = 0.119, p = 0.698) in these patients. Again in GBM patients, those who were Mer− were less likely to have progressive disease (two of six) than those who were Mer+ (six of seven), (x^2 = 3.745, p = 0.053). There were only nine patients with AAs and only two of these were in the progression group. However, it is clear that progression in AA patients with high MGMT levels is not necessary ominous because both of these patients are alive at 34 months or more.

Discussion

It has been shown that MGMT can repair O^6-alkylguanine adducts, such as the chloroethyl adduct formed by CENUs, preventing the formation of cytotoxic interstrand crosslinks.[5] Accumulating evidence indicates that MGMT-positive tumor cells are more resistant to CENUs than that MGMT-negative tumor cells.[5,6,8-10] It has also been confirmed by many investigations that MGMT inhibitors such as O^6-BG can sensitize tumor cells to CENUs via inhibition of MGMT.[7,10-12] However, MGMT expression does not always correlate linearly with drug resistance, and some MGMT negative tumors also show resistance to these agents.[18] We previously investigated MGMT protein levels and MGMT activity in 14 human tumor cell lines and compared these results with BCNU cytotoxicity. Although there was no significant linear correlation between MGMT expression and CENU resistance, cell lines with high MGMT expression were more resistant to BCNU than MGMT poor cell lines. In addition, BCNU-sensitive cell lines were all MGMT-poor, indicating that MGMT plays a major role in BCNU drug resistance.[18]

In our present clinical observation, Mer+ patients appeared more likely to have progressive disease with CENU-based chemotherapy than the Mer− patients. However, we did not find a statistically different TTP between Mer+ and Mer− groups in the entire series of patients, possibly due to the small number of cases. However, the TTP of the Mer+ group was shorter than the Mer− group (4.5 compared with 10.3 months, respectively). Because GBM and AA behave differently, we considered them separately. Even failing to respond to chemotherapy, patients with AA survive much longer than patients with GBM. In our series, this is indicated by two AA patients with high MGMT levels and no response to CENU therapy, who have survived more than 34

and 42 months, respectively, after initial CENU therapy. This survival time was much longer than that for any GBM patient. The MGMT levels were similar between GBM and AA patients, whereas the survival times and TTP of AA patients were longer than GBM patients. The AA patients were much younger than the GBM patients, which is also a factor affecting survival time.

In the GBM patients, those who demonstrated progressive disease with CENU therapy had higher MGMT expression than the patients in the effective group. Thus, the TTP in patients in the effective group was longer compared to patients in the progressive group, suggesting that MGMT contributes to CENU resistance in GBM. In the previously reported large multicenter series,[16] patients whose gliomas showed high MGMT levels had a shorter time to treatment failure and shorter survival time, with a mortality rate 1.7 times greater than patients whose gliomas showed low MGMT levels. In this series, although we did not find a significant difference in survival time between patients harboring MGMT-rich and MGMT-poor GBMs, possibly due to the small sample size, patients with MGMT-positive tumors were more likely to have progressive disease with CENU therapy. Nonetheless, the results are complicated by the fact that some patients with low MGMT levels in their gliomas failed to respond to CENU based chemotherapy. This is in agreement with our previous finding with human tumor cell lines demonstrating that some MGMT poor cell lines are also resistant to CENU.[18]

The mechanism of drug resistance in brain tumors is multifactorial.[24] There are at least three possible sites of drug resistance concerning the tumor cell: drug inactivation, such as glutathione-S-transferase (GST)-mediated metabolism of nitrosoureas;[25,26] transportation of the drug out of the tumor cells, such as mediated by multidrug resistance gene[27,28] and repair of the damaged DNA.[5,8,18,29-31] One of the best recognized mechanisms of nitrosourea resistance in human tumors relating to DNA repair is MGMT. Nucleotide excision repair (NER), a multienzyme complex, is responsible for repairing a wide variety of DNA lesions,[32] including lesions induced by ultraviolet (UV) light or by some chemotherapeutic agents.[33,34] Authors of several studies have suggested that NER may also be implicated in anticancer drug resistance.[18,29-31,35] Recently, using RT-PCR, we examined *ERCC2* gene expression in 10 human brain tumor cell lines and found a significant correlation between *ERCC2* expression and the cytotoxicity of BCNU or SarCNU, the latter being a novel CENU analog with enhanced antitumor activity against human gliomas.[23,31] We also examined four samples in this series and found ERCC2-positive tumors in two patients with progressive disease, one of which was MGMT negative, whereas two patients whose treatment was considered effective had ERCC2-negative tumors (data not shown). Previously we determined that ERCC2 protein levels were significantly correlated with UV sensitivity and BCNU resistance. Moreover, UV sensitivity was correlated to BCNU cytotoxicity, suggesting that NER, specifically ERCC2, is involved in nitrosourea resistance in human tumor cell lines.[18]

Conclusions

Our present clinical observation confirms that MGMT plays an important role in CENU drug resistance in human gliomas. Patients with MGMT-negative gliomas are less likely to progress on CENU chemotherapy than patients with MGMT-positive gliomas. This notwithstanding, MGMT-positive tumors are usually, but not always, resistant to CENUs, whereas MGMT-negative tumors are not always sensitive to CENUs, suggesting that other factors may also affect a patient's response to chemotherapy.

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References

1. Kohn KW, Erickson LC, Laurent G, et al. DNA crosslinking and the origin of sensitivity to chloroethylnitrosourea. *In*: Prestayko AW, Crooke ST, Baker LH, Carter SK, Schein PS eds. Nitrosoureas. New York: Academic Press 1981; 69-83.
2. Tong WP, Kirk MC, Ludlum DB. Formation of the cross-link 1-[N3-deoxycytidyl), 2[N1-deoxyguanosinyl] ethane in DNA treated with N, N'-bis (2-chloroethyl) 1-N-nitrosourea. Cancer Res 1982; 42: 3102-3105.
3. Yarosh, DB. The role of O^6-methylguanine-DNA methyltransferase in cell survival, mutagenesis and carcinogenesis. Mutat Res 1985; 145: 1-16.
4. Pegg AE. Mammalian O^6-alkylguanine-DNA alkyltransferase: regulation and importance in response to alkylating carcinogenic an agents. Cancer Res 1990; 50: 6119-6129.
5. Brent TP, Houghton PJ, Houghton JA. O^6-alkylguanine-DNA alkyltransferase activity correlates with the therapeutic response of human rhabdomyosarcoma xenografts to CCNU. Proc Natl Acad Sci USA 1985; 82: 2985-2989.
6. Gonzaga PE, Potter PM, Niu TQ, et al. Identification of the cross-link between human O^6-methylguanine-DNA methyltransferase and chloroethylnitrosourea-treated DNA. Cancer Res 1992; 52: 6052-6058.
7. Schold SC Jr, Kokkinakis DM, Rudy JL, et al. Treatment of human brain tumor xenografts with O^6-benzyl-2'-deoxyguanosine and BCNU. Cancer Res 1996; 56: 2076-2081.
8. Erickson LC, Laurent G, Sharkey NA, et al. DNA crosslinking and monoadduct repair nitrosourea-treated human tumor cells. Nature (Lond) 1980; 288: 727-729.
9. Scuderio DA, Meyers SA, Clatterbuck BE, et al. Ziolkowski Day RS. Sensitivity of human cell strains having different abilities to repair O^6-methylguanine in DNA to inactivation by alkylating agents including chloroethylnitrosoureas. Cancer Res 1984; 44: 2467-2474.
10. Dolan ME, Mitchell RB, Mummert C, et al. Effect of O^6-benzylguanine analogues on sensitivity of human tumor cells to the cytotoxic effects of alkylating agents. Cancer Res 1991; 51: 3367-3372.
11. Bobola MS, Berger MS, Silber JR. Contribution of O^6-methylguanine-DNA methyltranferase to resistance to 1,3-(2-chloroethyl-1-nitrosourea in human brain tumor-derived cell lines. Mol Carcinog 1995; 13: 81-88.
12. Phillips WP Jr, Willson JKV, Markowitz SD, et al. O^6 methylguanine-DNA methyltransferase (MGMT) transfectants of a 1,3-(2-chloroethyl-1-nitrosourea (BCNU)-sensitive colon cancer cell line selectively repopulate heterogeneous MGMT+/MGMT- xenografts after BCNU and O^6-benzylguanine plus BCNU. Cancer Res 97; 57: 4817-4823.
13. Koc ON, Phillips WP Jr, Lee K, et al. Role of DNA repair in resistance to drugs that alkylate O^6 of guanine. Cancer Treat Res 1996; 87: 123-146.
14. Mineura K, Watanabe K, Yanagisawa T, et al. Quantification of O^6-methylguanine-DNA methyltransferase mRNA in human brain tumors. Biochim Biophys Acta 1996; 1289: 105-109.
15. Nutt CL, Costello, JF, Bambrick LL, et al. O^6-methylguanine-DNA methyltransferase in tumors and cells of the oligodendrocyte lineage. Can J Neurol Sci 1995; 22: 111-115.

16. Belanich M, Pastor M, Randall T, et al. Retrospective study of the correlation between the DNA repair protein alkyltransferase and survival of brain tumor patients treated with carmustine. Cancer Res 1996; 56: 783-778.
17. Jaeckle KA, Eyre HJ, Townsend JJ, et al. Correlation of tumor O^6-methylguanine-DNA methyltransferase levels with survival of malignant astrocytoma patients treated with bis-chloroethylnitrosourea: a Southwest Oncology Group study. J Clin Oncol 1998; 16: 3310-3315.
18. Chen Z-P, Malapetsa A, McQuillan A, et al. Evidence for nucleotide excision repair as a modifying factor of MGMT mediated innate chloroethylnitrosourea resistance in human tumor cell lines. Mol Pharmacol 1997; 52: 815-820.
19. Tano K, Shiota S, Collier J, et al. Isolation and structural characterization of a cDNA clone encoding the human DNA repair protein for O^6-alkylguanine. Proc Natl Acad Sci USA 1990; 87: 686-690.
20. Belanich M, Ayi TC, Li BF, et al. Analysis of O^6-methylguanine-DNA methyltransferase in individual human cells by quantitative immunofluorescence microscopy. Oncol Res 1994; 6: 129-137.
21. Citron M, White A, Decker R, et al. O^6-methylguanine-DNA methyltransferase in human brain tumors detected by activity assay and monoclonal antibodies. Oncol Res 1995; 7: 49-55.
22. Ayi TC, Loh KC, Ali RB, et al. Intracellular localization of human DNA repair enzyme methylguanine-DNA methyltransferase by antibodies and its importance. Cancer Res 1992; 52: 6423-6430.
23. Marcantonio D, Hollingshead MG, Alley MC, et al. SarCNU, a novel chloroethylnitrosourea analogue with enhanced antitumor activity against human glioma xenografts. Cancer Res 1997; 57: 3895-3898.
24. Phillips PC. Antineoplastic drug resistance in brain tumors. Neurol Clin 1991; 9: 383-404.
25. Hayes JD, Pulford DJ. The glutathione s-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. Critical Rev Biochem Mol Biol 1995; 30: 445-600.
26. Tew KD, Monks A, Barone L, et al. Glutathione-associated enzymes in the human cell lines of the National Cancer Institute drug screening program. Mol Pharmacol 1996; 50: 149-159.
27. Matsumoto T, Tani E, Yamaura I, et al. Effects of protein kinase C modulators on multidrug resistance in human glioma cells. Neurosurg 1995; 36: 565-571.
28. Nabors MW, Griffin CA, Zehnbauer BA, et al. Multidrug resistance gene (MDR1) expression in human brain tumors. J Neurosurg 1991; 75: 941-946.
29. Anderson BS, Sadeghi T, Siciliano MJ, et al. Nucleotide excision repair genes as determinants of cellular sensitivity to cyclophosphamide analogs. Cancer Chemother Pharmacol 1996; 38: 406-416.
30. Zeng-Rong N, Paterson J, Alpert L, et al. Elevated DNA repair capacity is associated with intrinsic resistance of lung cancer to chemotherapy. Cancer Res 1995; 55: 4760-4764.
31. Chen Z-P, McQuillan A, Mohr G, et al. ERCC2 gene expression and chloroethylnitrosourea resistance in human glioma cell lines. Neurosurgery 1998; 42: 1112-1119.
32. Ma L, Hoeijmakers JHJ, van der Eb AJ. Mammalian nucleotide excision repair. Biochim Biophys Acta 1995; 1242: 137-164.
33. Aboussekhra A, Wood RD. Repair of UV damaged DNA by mammalian cells and Saccharomyces cerevisiae. Curr Opin Genet Dev 1994; 4: 212-220.
34. Chu G. Cellular responses to cisplatin. The roles of DNA-binding proteins and DNA repair. J Biol Chem-1 994; 269: 787-790.
35. Dabholkar M, Bostick-Bruton F, Weber C, et al. ERCC-1 and ERCC-2 expression in malignant tissues from ovarian cancer patients. J Natl Cancer Inst 1992; 84: 1512-1517.

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