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Carmustine-induced Toxicity, DNA Crosslinking and O^6-methylguanine-DNA Methyltransferase Activity in Two Human Lung Cancer Cell Lines

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O^6-methylguanine-DNA methyltransferase (O^6-MT) probably plays an important role in the repair of chloroethylnitrosourea-induced DNA damage. O^6-MT was studied as a possible drug resistance factor in two human lung cancer cell lines, one small cell lung cancer (U1690) and one non-small cell lung cancer (U1810), with different sensitivities to carmustine. The U1810 cell line was 3.4-fold more resistant to carmustine than U1690 cells, although the two cell lines were equally sensitive to mustine, melphalan and cisplatin. A 23-fold higher level of DNA interstrand crosslinks was observed following exposure of U1690 cells to carmustine compared with U1810 cells. The O^6-MT activity of U1810 cells was 11 times higher than that of U1690 cells. The O^6-MT activity in the U1810 cells showed a dose-dependent decrease after exposure to carmustine. These results show a correlation between increased O^6-MT activity, decreased drug induced DNA interstrand crosslinking and cellular resistance to carmustine.

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INTRODUCTION

HUMAN LUNG cancer can be separated into small cell lung cancer (SCLC) and non-SCLC (NSCLC), based on the clinical behaviour, the marker profile and the morphology of the tumour. SCLC is characterised by a frequent initial response to chemotherapy and radiotherapy, high levels of various neuroendocrine markers and growth in clusters *in vitro*. NSCLC responds poorly to chemotherapy and radiotherapy, expresses low neuroendocrine marker levels and grows in monolayer in culture [1–6]. The resistance of lung cancer cells to cytostatic drugs is an important clinical problem.

The nitrosourea carmustine undergoes aqueous decomposition in the blood plasma to yield a reactive chloroethyl diazonium hydroxide entity which initially reacts with the O^6-atom of guanine base residues, and then gives rise to bifunctional lesions, such as DNA interstrand crosslinks. The level of DNA interstrand crosslinking and cytotoxicity following exposure to chloroethylnitrosoureas such as carmustine depends in part on the activity of the DNA repair enzyme O^6-methylguanine-DNA methyltransferase (O^6-MT) [7, 8]. Cells with a deficient enzyme activity (mer^- or mex^- cells) show an increased sensitivity to chloroethylnitrosourea compounds [9]. The increased drug sensitivity has been correlated to increased DNA interstrand crosslinking in mer^- cells. The modification of the crosslinking level by O^6-MT is caused by enzymatic excision of drug-DNA monoadducts before a crosslink is formed. This is supported by the observation that methylating agents can increase DNA crosslinking levels induced by chloroethylnitrosoureas by a competitive inhibition of the O^6-MT activity [10]. More direct evidence for a relationship between the O^6-MT and sensitivity to chloroethylnitrosoureas was obtained by transfection of the *ada* gene from *Escherichia coli* (the gene coding for the inducible O^6-MT of *E. coli*) to mammalian cells. An increased enzyme expression was obtained in cells with an inherently low transferase activity, and the cells acquired resistance to chloroethylnitrosoureas [11].

In this paper we present results of studies of two human lung cancer cell lines, one SCLC and one NSCLC, which differ in sensitivity to carmustine. The aim of the work was to investigate the possible relationship between cytotoxicity and DNA interstrand crosslinking after exposure to carmustine, and to correlate this to the O^6-MT activity in the cell lines.

MATERIALS AND METHODS

Drugs and chemicals

Carmustine was a gift from Bristol Laboratories, Syracuse. Immediately before drug incubations, 3 mg carmustine was dissolved in 40 μl 99.5% ethanol and diluted in cell culture medium supplemented with 10% fetal calf serum (FCS) to the desired drug concentrations.

Melphalan was obtained from the Wellcome Foundation, London. Stock solutions of melphalan were prepared by dissolving 100 mg melphalan in 1.8 ml 92% ethanol with 2% HCl and diluting with 9 ml 60% propylene glycol with 1.2% dipotassium hydrogen phosphate. These solutions were immediately frozen at −70°C in aliquots of 1.85 mg melphalan. The stock solutions were renewed after 3 months. Immediately before each incubation, a stock solution was further diluted in cell culture medium to the desired drug concentrations.

Mustine was obtained from the Boots Company, Nottingham. Immediately before each incubation 10 mg mustine was dissolved in 10 ml of Eagle's minimal essential medium (MEM) with Earle's salts (Flow Laboratories, Rickmansworth) and

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further diluted in cell culture medium to the desired drug concentrations.

Cisplatin was a gift from Bristol Laboratories. Immediately before drug incubation 2 mg cisplatin was dissolved in 100 μl dimethyl sulphoxide and diluted to the appropriate concentrations in cell culture medium.

[Methyl-^{14}C]thymidine (52 mCi/mmol, 50 μCi/ml) and [methyl-^{3}H]thymidine (5 Ci/mmol, 1 mCi/ml) were obtained from the Radiochemical Centre, Amersham.

Cells

Two human lung cancer cell lines, U1690 (SCLC) and U1810 (NSCLC) were used [12]. The NSCLC cell line is characterised by expression of EGF receptors, cytokeratins, low levels of neuron specific enolase (NSE) and *in vitro* growth in monolayer. The SCLC cell line shows high levels of neuroendocrine markers, including NSE and neurofilaments and a tendency to grow in clusters *in vitro* [2, 3, 12]. Cells were grown in Eagle's MEM with Earle's salts supplemented with 2 mmol/l L-glutamine, 10% FCS, 125 I.U. benzylpenicillin and 125 μg/ml streptomycin. U1690 cells contained 42.6 (S.D. 3.9) chromosomes and U1810 cells 84.6 (8.7) chromosomes. The doubling time of U1690 was 22 h and of U1810 45 h. L1210 mouse leukaemia cells were grown in RPMI 1640 medium with Hepes buffer (Flow), supplemented with 2 mmol/l L-glutamine, 10% FCS, 125 I.U. benzylpenicillin and 125 μg/ml streptomycin.

Drug-induced cytotoxicity

Drug-induced cytotoxicity was measured as inhibition of colony formation [13]. Appropriate numbers of U1690 and U1810 cells suspended in Eagle's MEM supplemented with 2 mmol/l L-glutamine and 10% FCS were plated in 6 cm Petri dishes and left overnight to attach. The cells were either exposed to various concentrations of melphalan, mustine or cisplatin for 30 min in medium without FCS and L-glutamine, or to carmustine for 2 h in medium with 10% FCS and 2 mmol/l L-glutamine. The longer incubation time with carmustine and the presence of FCS were chosen since it is known that decomposition to the reactive chloroethyl diazonium hydroxide entity is a delayed process (half-life in phosphate buffer 50 min) which is accelerated in the presence of plasma (half-life in plasma 17 min) [14]. After the drug incubations, the cells were grown in fresh drug-free medium with 10% FCS and 2 mmol/l L-glutamine for 14 days. The dishes were rinsed with phosphate-buffered saline (PBS), fixed with 7.4% formaldehyde solution (diluted in PBS) and stained for 15 min with Giemsa solution (diluted 1:4 in distilled water) and the surviving fraction was calculated.

Drug-induced DNA interstrand crosslinking

The alkaline elution technique developed by Kohn and coworkers [15] was used with minor modifications [16]. U1690 and U1810 cells were labelled overnight with ^{14}C-thymidine, exposed to carmustine for 2 h in medium with 10% FCS, and incubated in drug-free medium with 10% FCS for 6 h. The cells were resuspended in ice-cold medium with 4% FCS and irradiated with 6 Gy. Cells were collected on a polycarbonate filter (pore size 2 μm, diameter 25 mm, Nucleopore). To each filter was added an internal standard consisting of 0.12×10^6 L1210 leukaemia cells, which were labelled with ^{3}H-thymidine and irradiated with 3 Gy. The cells were then washed twice with 10 ml ice-cold PBS and lysed with 5 ml sarcosyl solution (2% sarcosyl, 0.1 mol/l glycine, 0.025 mol/l Na_2EDTA and adjusted to pH 10.0 with 5 mol/l NaOH). Another 2 ml of sarcosyl solution with 0.5 g/ml proteinase K was added, and allowed to remain in contact with the filters for 1 h. The DNA was then slowly eluted from the filters during 16 h with a tetraethyl-ammoniumhydroxide (TEAH) solution (0.02 mol/l EDTA, 64 ml/l TEAH, 0.1% sarcosyl, pH 12.1), and collected in 8 fractions. The ^{3}H and ^{14}C activities in the fractions were counted by liquid scintillation. DNA remaining on the filters at the end of the elution was removed by hydrolysis in 0.4 ml 1 mol/l HCl at 60°C for 1 h, followed by treatment with 2.5 ml 0.4 mol/l NaOH at room temperature for 1 h. The radioactivity released from the filters was measured by liquid scintillation counting. DNA remaining in the funnels, filter holders and tubes was removed by pumping 2.5 ml of 0.4 mol/l NaOH through the system. The radioactivity thus obtained was added to the radioactivity released from the filters. The fraction of ^{14}C-labelled DNA remaining on the filter at the time when 25% of ^{3}H-labelled DNA of internal standard cells remains on the filter was determined for each sample. The amount of DNA crosslinks was calculated according to the formula developed by Kohn *et al.* [15].

O^6-MT-assay

Cell extracts were prepared by lysing 10×10^6 cells of each cell line in 100 μl extraction buffer consisting of 50 mmol/l Tris-HCl (pH 7.5), 1 mmol/l EDTA, 10 mmol/l dithiothreitol (DTT) and 0.2% Triton X-100. Debris was removed by centrifugation for 5 min at 14 000 rpm. Protein concentrations were estimated by the method of Lowry *et al.* [17]. O^6-MT activity in the cell extracts was measured by the removal of O^6-[^{3}H]-methylguanine from methylated *Micrococcus luteus* DNA [18]. The *M. luteus* DNA was alkylated with ^{3}H-methylnitrosourea (MNU) (specific activity 29 Ci/mmol) followed by heat treatment to remove N-alkylated purines [18]. The substrate was dissolved in a buffer consisting of 70 mmol/l Hepes KOH (pH 7.8), 10 mmol/l DTT and 1 mmol/l EDTA to give $1–2 \times 10^3$ counts per 100 μl (substrate mixture). Assays were performed by mixing 100 μl substrate mixture with up to 35 μg crude cell extract and incubating for 1 h at 37°C. The mixtures were then chilled on ice, 20 μg heat-denatured carrier DNA was added and the DNA was precipitated with 120 μl cold 0.8 mol/l TCA. The samples were mixed and left on ice for 5 min and then centrifuged for 10 min at 10 000 rpm in an Eppendorf table top centrifuge. The supernatants were removed and the pellets hydrolysed in 200 μl 0.1 mol/l HCl at 70°C for 30 min to release all purines. The samples were then chilled and centrifuged for 10 min at 10 000 rpm. The radioactivity in 180 μl of the resulting supernatant was counted to estimate the relative content of O^6-[^{3}H]-methylguanine compared to control DNA (obtained from incubations consisting only of substrate mixture without cell extract).

Inactivation of O^6-MT by treatment with carmustine

U1810 and U1690 cells were plated in 10 cm Petri dishes, allowed to grow until subconfluence, and then incubated with 0, 200 or 400 μmol/l carmustine for 2 h at 37°C. The O^6-MT activity was measured directly after exposure to carmustine, as described above.

RESULTS

Drug-induced cytotoxicity

Colony formation after exposure of U1690 and U1810 cells to different concentrations of carmustine is shown in Fig. 1a.

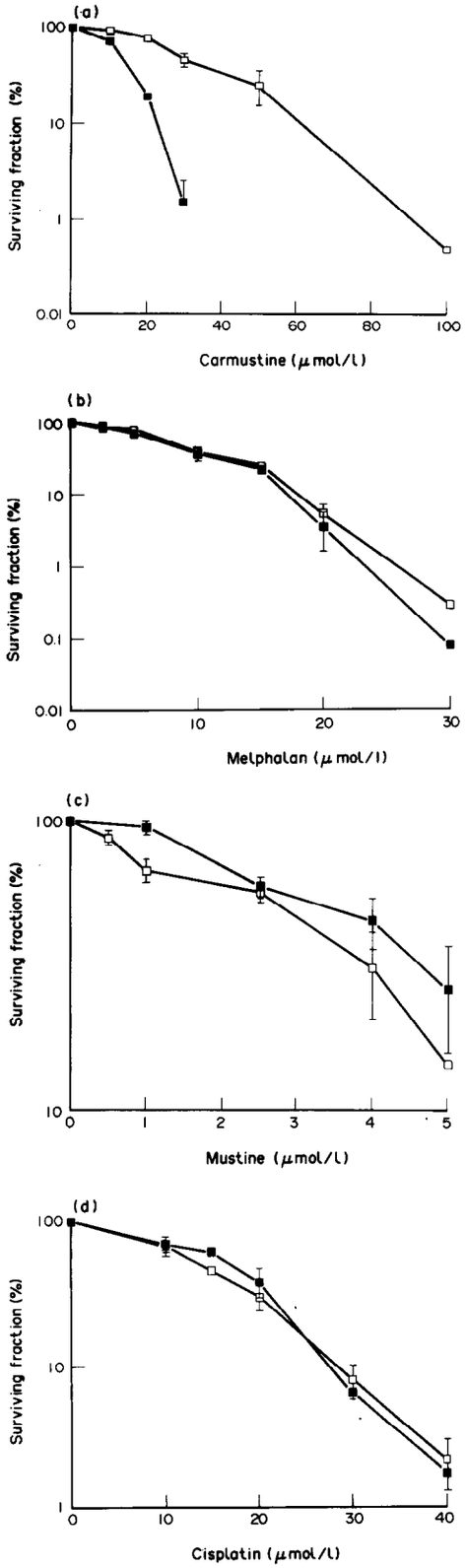

Fig. 1. Colony forming ability of U1690 (■) and U1810 (□) lung cancer cells after exposure to different concentrations of carmustine for 2 h (a), or to melphalan (b), mustine (c) and cisplatin for 30 min (d). Mean of 2–5 experiments; bars indicate S.E.

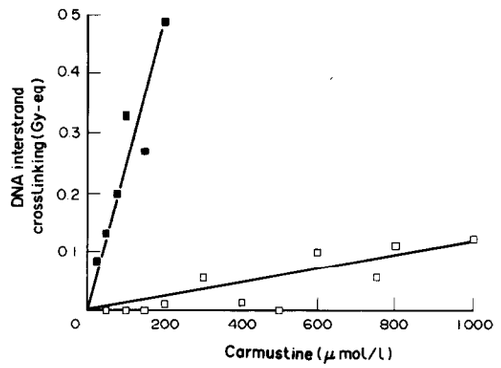

Fig. 2. DNA interstrand crosslinking in U1690 (■) and U1810 (□) cells after a 2-h exposure to different concentrations of carmustine followed by a drug-free incubation for a further 6 h. DNA interstrand crosslinking is shown as Gy-equivalents [15]. Lines indicate regression analyses with zero intercept.

U1810 cells were 3.4-fold more resistant than U1690 cells. When colony formation was studied after exposure of the cells to melphalan, mustine and cisplatin, no differences in sensitivity between the cell lines were observed (Figs 1b–d). Thus, the difference in sensitivity is specific for carmustine, and does not extend to other classes of DNA crosslinking drugs.

Carmustine-induced DNA interstrand crosslinking

Chloroethylnitrosoureas induce several types of DNA lesions. DNA interstrand crosslinks have been shown to correlate with cytotoxicity [10, 19], and were therefore studied in the two lung cancer cell lines after exposure to carmustine. In both cell lines the maximum number of DNA interstrand crosslinks was obtained 12–18 h after drug exposure (data not shown), but the difference in crosslinking at 6 h was representative of the peak concentration. There was a pronounced dose dependent increase in DNA interstrand crosslinking in the U1690 cells, while the crosslinking effect of carmustine was slight in the U1810 cells (Fig. 2). The difference in DNA crosslinking was 23-fold as estimated from the slopes of regression lines.

Measurement of the O^6-MT activity

The O^6-MT activity differed in the two cell lines (Fig. 3). The U1810 cells, which showed the lowest level of DNA interstrand crosslinking after carmustine treatment, showed the

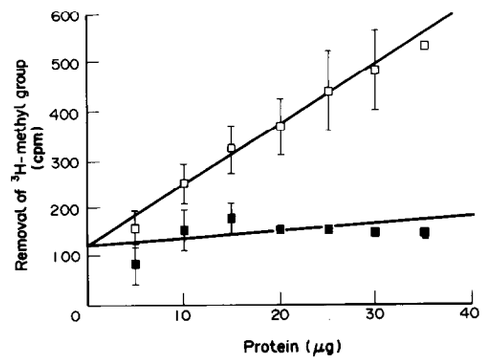

Fig. 3. Removal of ^{3}H-methyl groups from the O^6 positions in guanine residues in DNA after incubation with extracts from U1690 (■) and U1810 (□) cells for 1 hour. Mean of 2–5 experiments; bars indicate S.E.

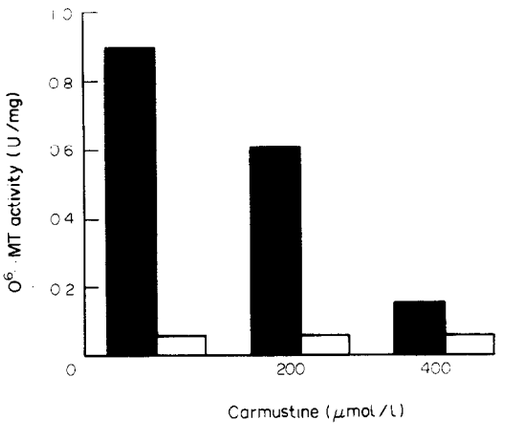

Fig. 4. Inactivation of O^6-MT by exposure to carmustine. U1810 (□) and U1690 (■) cells were incubated with 0, 200 or 400 μmol/l carmustine for 2 h at 37°C, and O^6-MT activities were measured directly after drug treatment.

highest enzyme activity. The difference in O^6-MT activity was 11-fold as estimated from the slopes of regression lines. Studies in our laboratory with northern blotting showed O^6-MT mRNA to be detectable in U1810 but not in U1690 cells (unpublished results), in accordance with the lack of O^6-MT activity in these measurements. The U1690 cells can therefore be regarded as mer$^-$.

Measurement of the O^6-MT activity after exposure to carmustine

The O^6-MT activity in the two cell lines after exposure to carmustine is shown in Fig. 4. There was a dose-dependent inactivation of the O^6-MT in the carmustine-resistant U1810 cells after treatment with carmustine while U1690 cells were not affected by treatment with carmustine. Following 400 μmol/l carmustine the O^6-MT activity of U1810 cells approaches that obtained with the mer$^-$ U1690 cells.

DISCUSSION

In this paper we have studied two human lung cancer cell lines, U1810 (NSCLC) and U1690 (SCLC) with different sensitivities to carmustine. The U1690 cells were 3.4-fold more sensitive to carmustine than the U1810 cells. No differences in sensitivity to melphalan, mustine or cisplatin were observed. The difference in sensitivity to carmustine is in agreement with the clinical response of the two types of lung cancer to therapy with this drug [20].

The U1810 cell line, which has a high O^6-MT activity, shows a low sensitivity to carmustine and low levels of DNA interstrand crosslinking after treatment with the drug. This is in agreement with previous observations pointing to a role for the O^6-MT activity as a resistance factor against the chloroethylnitrosourea compounds [8, 19, 21]. The carmustine-sensitive cell line U1690 is mer$^-$, i.e it is lacking in O^6-MT activity. mer$^-$ cells have been found among tumour cell lines of different histogenetic origin; for instance melanoma, astrocytoma, lung carcinoma and ovarian carcinoma lines [22, 23]. It is therefore likely that a deficient O^6-MT activity may have clinical implications in several types of cancer. Little is, however, known about the quantitative relationship between the O^6-MT activity of human tumour cells and the sensitivity of tumours to clinical chemotherapy with chloroethylnitrosourea compounds. We are at present studying the expression of O^6-MT in biopsy specimens from human tumours, to establish the incidence of mer$^-$ tumours. We are also comparing the O^6-MT expression to response to chemotherapy.

Treatment of U1810 cells with carmustine resulted in inactivation of O^6-MT. Following 400 μmol/l carmustine O^6-MT activity was almost abolished. Similar results have been presented by Gerson *et al.*, who also studied the effect of O^6-methylguanine on carmustine cytotoxicity in two human leukaemic cell lines, HL-60 and K562 [21]. The carmustine-resistant HL-60 approached the sensitive K562 in sensitivity after pretreatment and post-treatment with O^6-methylguanine. It is of interest to investigate the possibility to sensitise resistant tumours with high O^6-MT activities by inactivating the enzyme and thereby achieve more efficient chemotherapy.

The difference between the two cell lines in carmustine-induced cytotoxicity was smaller than the difference in DNA interstrand crosslinking (Figs 1a, 2). U1690 cells thus tolerate a higher level of DNA interstrand crosslinking than U1810 cells. One reason for this could be a difference in cellular capacity to repair DNA interstrand crosslinks. This is the subject of further studies. It should also be remembered that the alkaline elution technique employed in this study measures the average level of DNA crosslinks in the entire genome. Differences in the distribution of DNA lesions within the genome, which cannot be detected with this technique, may be of importance for cytotoxicity. Further, drug-induced lesions other than DNA interstrand crosslinks, such as DNA intrastrand crosslinks may also contribute to the cytotoxicity.

There was a difference in the DNA content between the two cell lines with 84.6 (S.D. 8.7) chromosomes in U1810 cells and 42.6 (3.9) chromosomes in the U1690 cells. In cell culture the doubling time was inversely correlated to the DNA content. The higher content of DNA in U1810 cells does not make this cell line more resistant to DNA crosslinking agents in general, since the U1810 cells show similar sensitivity as U1690 cells, to mustine, melphalan and cisplatin (Figs 1b–d).

Shapiro [24] observed that glioma cells which acquired resistance to nitrosourea compounds showed an over-representation of chromosome 22. Interestingly, this increase was correlated to an increased expression of platelet-derived growth factor (PDGF). Our cell lines represent one further example of a correlation between PDGF expression and carmustine resistance. Thus, U1810 cells express both peptide chains of PDGF, while no measurable expression of the two chains was seen in the U1690 cells [25]. The genes for the A and B chains of PDGF and O^6-MT are located on different chromosomes. The A chain gene is situated on chromosome 7 while the B chain gene is situated on chromosome 22 [25]. The O^6-MT gene has recently been cloned and mapped to chromosome 10 [26, 27]. Although the genes for PDGF and O^6-MT are located on different chromosomes, some common factor(s) may possibly be involved in the regulation of the expression of both PDGF and O^6-MT, or PDGF may directly be involved in the regulation of O^6-MT expression.

Other cellular resistance factors may also be active against carmustine. Glutathione transferases may play an important role in protecting cells against DNA-reactive drugs including carmustine. Among the different subclasses of glutathione transferases, the Mu class may be of special importance for protection against nitrosourea compounds [28]. The possible role of glutathione transferases as resistance factors in the two lung cancer cells are under investigation.

We have demonstrated differences in O^6-MT levels between

a SCLC and a NSCLC cell line. The different enzyme activities may partly explain the different sensitivity to carmustine exposure *in vitro*. It will be of interest in the future to study the stability of O^6-MT expression in human lung cancer cell lines of different histogenetic types. It should be of special interest to study O^6-MT expression in SCLC of "variant type", or SCLC cell lines which have been transformed to NSCLC by the transfection of oncogenes or growth factors [29, 30].

1. Minna JD, Higgins GA, Glatstein EJ. Cancer of the lung. In: de Vita VT Jr, Hellman S, Rosenberg SA, eds. *Cancer. Principles and Practice of Oncology*. Philadelphia, JB Lippincott, 1985, 507–597.
2. Bergh J, Nilsson K, Dahl D, Andersson L, Virtanen I, Lehto V-P. Expression of intermediate filaments in established human lung cancer cell lines: an indicator of differentiation and dervation. *Lab Invest* 1984, **51**, 307–316.
3. Carney DN, Gazdar AF, Bepler G, *et al.* Establishment and identification of small cell cancer cell lines having classic and variant features. *Cancer Res* 1985, **45**, 2913–2923.
4. Pattengill OS, Sorenson GD. Tissue culture and *in vitro* characteristics. In: Greco FA, Oldham RK, Bunn PA Jr, eds. *Small Cell Lung Cancer*. New York, Grune & Stratton, 1981, 51–78.
5. Baillie-Johnson H, Twentyman PR, Fox NE, Reeve JG, Bleehan NM. Establishment and characterization of cell lines from patients with lung cancer (predominantly small cell carcinoma). *Br J Cancer* 1985, **52**, 495–504.
6. Bepler G, Jaques G, Neumann K, Aumüller G, Gropp C, Havemann K. Establishment, growth properties, and morphological characteristics of permanent human small cell lung cancer cell lines. *J Cancer Res Clin Oncol* 1987, **113**, 31–40.
7. Erickson LC, Bradley MO, Ducore JM, Kohn KW. DNA cross-linking and cytotoxicity in normal and transformed human cells treated with antitumor nitrosoureas. *Proc Natl Acad Sci USA* 1980, **77**, 467–471.
8. Scudiero DA, Meyer SA, Clatterbuck BE, Mattern MR, Ziolkowski CHJ, 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* 1984, **44**, 2467–2474.
9. Karran P, Lindahl T. Cellular defence mechanisms against alkylating agents. *Cancer Surv* 1985, **4**, 583–599.
10. Zlotogorski C, Erickson LC. Pretreatment of normal human fibroblasts and human colon carcinoma cells with MNNG allows chloroethylnitrosourea to produce DNA interstrand cross-links not observed in cells treated with chloroethylnitrosourea alone. *Carcinogenesis* 1983, **4**, 759–763.
11. Dumenco LL, Warman B, Hatzoglou M, Lim IK, Abboud SL, Gerson SL. Increase in nitrosourea resistance in mammalian cells by retrovirally mediated gene transfer of bacterial O^6-alkylguanine-DNA alkyltransferase. *Cancer Res* 1989, **49**, 6044–6051.
12. Bergh J, Nilsson K, Ekman R, Giovanella B. Establishment and characterization of cell lines from human small and large cell carcinomas of the lung. *Acta Path Microbiol Immun Scand* 1985, **93**, 133–147.
13. Puck TT, Marcus PI. Action of X-rays on mammalian cells. *J Exp Med* 1956, **103**, 653–666.
14. Colvin M, Brundrett R. Chemical decomposition of chloroethylnitrosoureas. In: Prestayko AW, Crooke ST, Baker LH, Carter SK, Schein PhS, eds. *Nitrosoureas. Current Status and New Developments*. New York, Academic Press, 1981, 43–49.
15. Kohn KW, Ewig RAG, Erickson LC, Zwelling LA. Measurement of strand breaks and cross-links in DNA by alkaline elution. In: Friedberg EC and Hanawalt PhC, eds. *DNA Repair. A Laboratory Manual of Research Procedures*, vol. 1. New York, Marcel Dekker, 1981, 379–401.
16. Hansson J, Lewensohn R, Ringborg U, Nilsson B. Formation and removal of DNA cross-links induced by melphalan and nitrogen mustard in relation to drug-induced cytotoxicity in human melanoma cells. *Cancer Res* 1987, **47**, 2631–2637.
17. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951, **193**, 265–275.
18. Karran P, Lindahl T, Griffin BE. Adaptive response to alkylating agents involves alteration *in situ* of O^6-methylguanine residues in DNA. *Nature* 1979, **280**, 76–77.
19. Erickson LC, Laurent G, Sharkey NA, Kohn KW. DNA crosslinking and monoadduct repair in nitrosourea-treated human tumour cells. *Nature* 1980, **288**, 727–729.
20. Figlin RA, Holmes EC, Petrovich Z, Sarna GP. Lung cancer. In: Haskell CM, eds. *Cancer Treatment*. Philadelphia, WB Saunders, 1990, 165–188.
21. Gerson SL, Trey JE, Miller K. Potentiation of nitrosourea cytotoxicity in human leukemic cells by inactivation of O^6-alkylguanine-DNA alkyltransferase. *Cancer Res* 1988, **48**, 1521–1527.
22. Day RS III, Ziolkowski CHJ, Scudiero DA, *et al.* Defective repair of alkylated DNA by human tumor and SV40-transformed human cell strains. *Nature* 1980, **288**, 724–727.
23. Day RS III, Ziolkowski CHJ, Scudiero DA, Meyer SA, Mattern M. Human tumor cell strains defective in the repair of alkylation damage. *Carcinogenesis* 1980, **1**, 21–32.
24. Shapiro JR. Biology of gliomas; heterogeneity, oncogenes, growth factors. *Semin Oncol* 1986, **13**, 4–15.
25. Söderdahl G, Betsholtz C, Johansson A, Nilsson K, Bergh J. Differential expression of platelet-derived growth factor and transforming growth factor genes in small- and non-small-cell human lung carcinoma lines. *Int J Cancer* 1988, **41**, 636–641.
26. Tano K, Shiota S, Collier J, Foote RS, Mitra S. 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.
27. Rydberg B, Spurr N, Karran P. cDNA cloning and chromosomal assignment of the human O^6-methylguanine-DNA methyltransferase. cDNA expression in *Escherichia coli* and gene expression in human cells. *J Biol Chem* 1990, **16**, 9563–9569.
28. Smith MT, Evans CG, Doane-Selzer P, Castro VM, Tahir MK, Mannervik B. Denitrosation of 1,3-bis(2-chloroethyl)-1-nitrosurea by class Mu glutathione transferases and its role in cellular resistance in rat brain tumor cells. *Cancer Res* 1989, **49**, 2621–2625.
29. Mabry M, Nakagawa T, Nelkin BD, *et al.* v-Ha-*ras* oncogene insertion: a model for tumor progression of small cell lung cancer. *Proc Natl Acad Sci USA* 1988, **85**, 6523–6527.
30. Falco JP, Baylin SB, Lupu R, *et al.* v-*ras*-Ha induces non-small cell phenotype, with associated growth factors and receptors, in a small cell lung cancer cell line. *J Clin Invest* 1990, **85**, 1740–1745.

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