[CANCER RESEARCH 50, 6119-6129, October 1, 1990]

Perspectives in Cancer Research

Mammalian O^6-Alkylguanine-DNA Alkyltransferase: Regulation and Importance in Response to Alkylating Carcinogenic and Therapeutic Agents[1]

Anthony E. Pegg

Departments of Cellular and Molecular Physiology and Pharmacology, Milton S. Hershey Medical Center, The Pennsylvania State University College of Medicine, Hershey, Pennsylvania 17033

Repair of O^6-Alkylguanine: Saturation Kinetics

Alkylating agents and compounds converted metabolically to alkylating agents form an important class of carcinogens and mutagens that includes a number of environmentally important *N*-nitroso- compounds. A number of alkylating agents are also useful antitumor drugs. There are many factors determining the sensitivity to the toxic, mutagenic, and carcinogenic potential of these compounds (1-4).[2] One of these is the capacity to repair alkylated DNA. Even simple alkylating agents such as *N*-methyl-*N*-nitrosourea and related compounds form a complex spectrum of adducts in DNA and all of these may have some pathophysiological significance. However, there is a substantial body of experimental evidence which indicates that the formation and persistence of O^6-alkylguanine and *O*-alkylpyrimidines may be of major importance in mutagenesis and in the initiation of tumors (3-5). The formation of adducts at the O^6-position of guanine also contributes to the cytotoxicity of both monofunctional and bifunctional alkylating agents (6-8).

Repair of O^6-alkylguanine occurs via an alkyltransferase reaction, as described below. The unusual features of this transfer, which occurs stoichiometrically without regeneration of the alkyl acceptor site, account for the observed repair kinetics in mammalian tissues and in cultured cells. These kinetics indicate a rapid rate of repair for O^6-alkylguanine but a saturation of the repair process. The level of alkylation at which the repair becomes saturated is highly dependent on the cell type. This finding is readily explained by the observations that the cellular content of alkyltransferase differs widely from one cell type to another. This article reviews recent progress in understanding the significance, mechanism of action, specificity, and regulation of alkyltransferase, with particular emphasis on studies which evaluate the potential role of this protein in carcinogenesis and in response to chemotherapeutic agents. Much of our present knowledge of the mammalian alkyltransferase has been obtained with the aid of pioneering observations made using bacteria. Some of this work is described in comparisons to the mammalian protein but a more detailed description can be obtained from a number of recent articles describing the prokaryotic alkyltransferases and references in these articles (9-15).

Alkyltransferase Mechanism and Structure

O^6-Methylguanine adducts in DNA are repaired by the action of a protein [EC 2.1.1.63] which transfers the methyl group to an internal cysteine residue (1, 6, 9, 10, 11). This reaction is stoichiometric, forming *S*-methylcysteine in the protein and guanine within the substrate DNA. No other protein is involved in this transfer; the same protein acts as a methyltransferase and as a methyl acceptor protein. This unusual reaction has led to some controversy concerning the correct nomenclature for the protein, which is not strictly an enzyme since it acts only once. It is also not solely an acceptor protein, since the protein itself mediates the transfer of the methyl group from the DNA. Although this has been referred to as a suicide reaction (9), this terminology should probably be avoided, since it invites inaccurate comparison with an important class of enzyme inhibitors also described as mechanism-based enzyme-activated irreversible inhibitors. The reaction involved is analogous to the first half of a ping-pong enzyme mechanism, in which a group is transferred from a substrate to a site on the enzyme. In the absence of the second substrate, the group remains covalently attached to the enzyme protein. However, in the case of the alkyltransferase, it appears that no acceptor substrate exists and the methyl group is not transferred away from the cysteine site. The number of O^6-methylguanine adducts that can be repaired is limited to the number of molecules of the protein available. The complete absence of regeneration of the acceptor site *in vivo* has not been proven unequivocally but there is no restoration of activity of the methylated protein after incubation under a variety of conditions *in vitro* and regeneration *in vivo* is so slow that it cannot be distinguished from *de novo* synthesis of the protein.

Although first identified from its action on methylated DNA substrates, the repair protein is able to remove a variety of other adducts from the O^6-position of guanine (see below) and, therefore, is better described as an alkyltransferase. The first such alkyltransferase to be isolated and characterized was that derived from the *ada* gene of *Escherichia coli*. Although the original preparations of this protein had a molecular weight of about 19,000, it is now clear that the gene product is actually a M_r 39,000 protein which contains two domains separated by a hinge region which is very sensitive to proteases (10, 11, 16, 17). The intact *ada* protein, which has now been purified in large amounts (16), contains two alkyltransferase functions, one that repairs the *S*-stereoisomer of methylphosphotriesters and the other that can act to repair either O^6-alkylguanine or O^4-alkylthymine. The protein is readily split by a proteolytic cleavage between lysine-178 and glutamine-179 (10, 11). The M_r 19,000 product which repairs O^6-alkylguanine represents the carboxyl terminal fragment from this cleavage, and the cysteine acceptor site is residue 321. Site-directed mutagenesis of this residue, converting it to alanine (18) or histidine (19), abolishes the repair activity. The capacity to repair methylphosphotriesters resides in the amino-terminal domain, and the methyl group is transferred to cysteine 69. This methylation converts the *ada* protein into a strong activator of the transcription of the *ada* gene, presumably by causing a configurational change in the protein which enhances its binding to the pro-

Received 5/3/90; accepted 6/26/90.

[1] Research on this topic in the author's laboratory is supported by Grant CA-18137 from the National Cancer Institute.

[2] In most cases only the most recent primary manuscripts have been cited. Full citations for earlier work are contained in these references and in the review articles cited.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/50/19/6119/2440997/cr0500196119.pdf by guest on 24 August 2022

moter region for the *ada* and *alkA* genes (11, 18). Such activation is the basis of the adaptive response which activates genes needed to repair alkylation damage. Direct methylation of cysteine-69 in the *ada* protein by reaction with methylating agents such as methyl iodide or methyl methanesulfonate also activates its transcriptional regulation capability (20). The normal physiological stimulus that activates the adaptive response is likely to be alkylation of DNA, and this system may exist to protect bacteria against *N*-nitroso- compounds produced by endogenous nitrosation processes (21).

E. coli also contains another gene, described as *ogt*, which also codes for an alkyltransferase able to repair O^6-alkylguanine or O^4-alkylthymine (12, 13). The product of this gene is a protein of M_r 19,000 which is distinct from the carboxyl terminal domain of the *ada* gene product but has a significant (29%) degree of homology to this protein. In contrast to the *ada* gene product, which, as described above, is highly inducible in response to alkylating agents, the *ogt* gene is not up-regulated and the content of this protein is very low. However, in uninduced cells the *ogt* product provides the majority of the alkyltransferase activity (13, 22). Significant amounts of the protein have now been obtained and purified after subcloning the gene into a multicopy expression vector (13). Similar alkyltransferase proteins have been detected in extracts of a variety of other bacteria (10, 23), including *Salmonella typhimurium* (22) and *Bacillus subtilis* (14, 15). The latter gene has been cloned and sequenced and the protein shows substantial homology to the *ogt* and COOH-terminal *ada* proteins (14, 15). Several groups have reported the lack of alkyltransferase activity in yeast (*e.g.*, Ref. 24), but a protein similar to the mammalian alkyltransferase has now been detected in extracts from *Saccharomyces cerevisiae* (25).

Although the mammalian alkyltransferase has not yet been purified to homogeneity in amounts sufficient for detailed biochemical examination, several peptide sequences have been obtained from the human protein (26–28), and a few fragments from the rat (29) and the bovine protein (30) have been partially characterized. Recently, a cDNA[3] for the human alkyltransferase has been cloned in the laboratory of S. Mitra (31). This important advance was accomplished by constructing a HeLa cell cDNA library in an *E. coli* expression vector and selecting for the conversion of *E. coli* ada^- mutants to cells resistant to methylating agents. One such plasmid which conferred resistance to MNNG was found to produce a significant increase in alkyltransferase activity. The sequence of the protein derived from this nucleotide sequence indicates that the human alkyltransferase has a molecular weight of about 22,000, consisting of 207 amino acids. The sequence includes peptides corresponding to those obtained by direct sequencing of the human alkyl transferase (26–28), confirming the identity. A region of the protein from residues 106 to 169 shows substantial similarity to the regions of the *ogt* product (residues 103 to 163) and the *ada* protein (residues 285 to 345) which contain the cysteine acceptor site. In fact, the sequence -PCHRV- containing this residue is identical in these three proteins and in the *B. subtilis* alkyltransferase, suggesting very strongly that cysteine-145 is the acceptor in the human protein (31). Despite the similarity in this region, the remaining parts of the protein show little resemblance to the *E. coli* proteins (31), and antibodies to the *ada* protein do not cross-react with the mammalian enzyme (23).

The alkyltransferases all bind quite tightly to double-stranded DNA (a property which has been exploited in their purification). The region of the protein responsible for this binding has not been defined, although there are several sequences rich in basic amino acids. Little is known about the mechanism of the transfer reaction. A substantial energy barrier must be overcome to remove the alkyl group from the O^6 position of guanine. A plausible mechanism for this transfer is that a basic residue on the alkyltransferase interacts with the cysteine and serves as a proton acceptor, generating a thiolate anion from it. This could then attack the alkyl group. The proline preceding the cysteine acceptor site may serve to bend the protein chain in such a way as to position the cysteine correctly. It has been pointed out that the sequence of a hydrophobic region followed by the -PCH- motif at the acceptor site is also present in the active site of thymidylate synthase, an enzyme in which this cysteine is known to act as a nucleophile to attack the carbon at position 6 of dUMP (10, 11). Further mechanistic studies are obviously needed. Low molecular weight substrates (see below) may be of considerable value in elaborating this mechanism. The M_r 19,000 carboxyl domain of the *ada* gene product has been crystallized (32) and large amounts of the intact M_r 39,000 *ada* protein have been isolated and subjected to physical chemical analysis (16). This should allow the three-dimensional structure of the protein to be determined and the part of the structure responsible for the affinity for double-stranded DNA to be investigated.

The mammalian alkyltransferase requires no cofactors and has an optimal pH of about 7.8–8.5 (33, 34). The rate of repair is reduced by unmethylated DNA, probably because of the binding of the alkyltransferase to this DNA (1, 34). The alkyltransferase is strongly inhibited by certain metal ions such as Cd^{2+}, Cu^{2+}, Hg^{2+}, Zn^{2+}, Ag^{2+}, and Pb^{2+}. In most cases, this inactivation can be abolished by high concentrations of dithiothreitol, which is consistent with the reaction of the inactivating metal ion with the thiol group at the acceptor site (35, 36). The alkyltransferase is also inhibited by aldehydes (37) and by direct reaction with alkylating agents (33). These inactivations could also result from the reaction with the cysteine acceptor site and the extent to which they might actually occur in cells exposed to environmental toxic agents is not known.

Properties of Mammalian Alkyltransferase

The only reaction known to be catalyzed by the mammalian alkyltransferase is the removal of alkyl groups from the O^6-position of guanine. O^6-Methylguanine in double-stranded DNA is the preferred substrate but longer alkyl groups can also be removed. The rate of repair decreases with the size of the alkyl group along the series ethyl-, *n*-propyl-, and *n*-butyl-. The repair of the branched chain isopropyl- and isobutyl- groups was very much slower than that of the linear alkyl groups (38–40). This may have importance in the response of cells treated with agents such as *n*-propyl- and *n*-butyl- nitroso derivatives. Rearrangement of the alkylating species derived from these agents occurs because of the greater stability of the branched chain carbonium ion, and this leads to a mixture of DNA adducts at the O^6-position. The cellular alkyltransferase activity is likely to be exhausted removing the linear adduct and would leave behind a greater percentage of the branched chain derivative.

There is no doubt that most of the repair of O^6-ethylguanine

[3] The abbreviations used are: cDNA, complementary DNA; MNNG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; SCE, sister chromatid exchange; BCNU, 1,3-bis(2-chloroethyl)-1-nitrosourea; CHO, Chinese hamster ovary.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/50/19/6119/2440997/cr0500196119.pdf by guest on 24 August 2022

from DNA of mammalian cells treated with ethylating agents occurs via the alkyltransferase reaction, even though the rate of repair of ethyl adducts is at least 3 times slower than with methyl (38). The *E. coli ada* protein also removes longer adducts and the same order of rate of reaction applies, but the difference in the rates of repair between methyl and the longer adducts is much greater, amounting to 100- to 1000-fold (8, 9, 11, 13, 39, 41), and it is likely that the majority of the repair of longer adducts occurs via excision repair pathways (42). However, the *ogt* product was recently reported to repair O^6-ethylguanine at 17% of the rate of O^6-methylguanine, using comparable oligonucleotide substrates (13).

O^6-Benzylguanine in DNA is a good substrate for the mammalian alkyltransferase (43). Although, as described above, the rate of repair of DNA adducts by the alkyltransferase decreases with the size of the alkyl group, indicating that steric constraints limit the reaction, these considerations may be more than offset by the ability of the benzene ring to delocalize charge in the transition state for a displacement reaction. The mammalian alkyltransferase, therefore, is rapidly inactivated by reaction with O^6-benzylguanine (43). This does not appear to be the case for the *ada* gene product, which may be more sterically hindered.[4]

Another striking difference between the mammalian and the *E. coli* alkyltransferases is in the repair of O^4-methylthymine. Both the *ada* and the *ogt* gene products repair this adduct in DNA using the same cysteine acceptor site as used for O^6-methylguanine, although at rates much less (0.01% and 0.7%, respectively) than with O^6-methylguanine (10, 11, 13, 41). However, attempts to demonstrate a similar reaction using mammalian cell alkyltransferase preparations have failed and, if this reaction occurs at all, it does so at a rate which is vastly slower than the repair of O^6-methylguanine (5, 6, 37, 44–46). An interesting consequence of this difference is that O^4-alkylthymine accumulates in the DNA of cells treated with alkylating carcinogens under conditions where O^6-alkylguanine is rapidly removed by the alkyltransferase (5, 46–48). Therefore, in cell types, such as hepatocytes, that are relatively rich in alkyltransferase, the major predominating lesion may become O^4-alkylthymine (47).

Both the mammalian and the bacterial alkyltransferases are able to remove 2-hydroxyethyl- groups from DNA, although slowly, and can probably act on 2-chloroethyl- groups also (see below). However, they do not appear to act on O^6-ethylthioethylguanine (49) or S^6-thiomethylguanine, which is readily formed by growing cells in the presence of 6-thioguanine and then treatment with dimethylsulfate (50).

Investigations of the polynucleotide requirement for repair of O^6-methylguanine indicate that by far the best substrate is double-stranded B-DNA. Substrate DNA containing O^6-methylguanine in the Z-conformation is a much poorer substrate (51) and RNA has only marginal activity (52). [A report that tRNA containing O^6-methylguanine was an active substrate (53) could not be confirmed.] Oligodeoxynucleotides containing O^6-methylguanine are acted upon by the alkyltransferase and are demethylated rapidly, provided they are double-stranded under the assay conditions. Even tetramers were substrates, but these single-stranded substrates reacted considerably more slowly (54). Double-stranded dodecadeoxynucleotides containing O^6-methylguanine are excellent substrates for the alkyltransferase (41, 54–58) and the rate of repair follows second-order kinetics (41, 56). Comparisons of the rate of repair of such substrates can, therefore, be made by determination of the second-order rate constants. The rate of repair was not affected significantly by the base opposite the O^6-methylguanine (54), but there was some sequence specificity in repair rates (56, 59).

Such oligodeoxynucleotides can be used as substrates for convenient, highly sensitive assays for alkyltransferase activity. The oligodeoxynucleotide can be labeled at high specific activity by reaction with polynucleotide kinase and [γ-^{32}P]ATP and the methylated substrate and nonmethylated product can be separated by high pressure liquid chromatography (41, 54, 56), immunoprecipitation of the methylated oligodeoxynucleotide (57, 58), or a change in the sensitivity to restriction enzymes (55). These assays may be useful for the determination of alkyltransferase activity in very small tissue samples including tumor biopsies. Such sensitive assays can also be used to determine the content of O^6-alkylguanine adducts in DNA samples, if a known amount of alkyltransferase is allowed to react with the DNA sample and the residual activity is determined (39, 60). The mammalian alkyltransferase is preferable for such purposes, since the *E. coli* proteins react not only with O^6-alkylguanine but also with O^4-methylthymine.

Sequence Specificity of Alkylation and Repair

The activation of the Ha-*ras* or K-*ras* oncogenes in rodent tumors induced by *N*-nitroso- carcinogens is known to occur by means of a $G \cdot C \rightarrow A \cdot T$ transition at the guanine (indicated by *) in the second base of codon 12 in the sequence 5′-CTGG*A-3′ (61–63). This is consistent with the formation and lack of repair of O^6-methylguanine at this site. There is also a high frequency of $G \cdot C \rightarrow A \cdot T$ transition mutations induced by *N*-nitroso- compounds at sequences having the sequence 5′-RG-3′ (64–67). Several factors may contribute to the sensitivity of these sequences, including the increased formation of O^6-methylguanine at such sites (59, 68, 69) and an effect of neighboring bases on miscoding by DNA polymerase (70). A reduced rate of repair by the alkyltransferase may also play a role. Some indirect evidence for sequence specificity in repair by *E. coli* alkyltransferase was obtained by Topal *et al.* (71, 72), who found that a sequence similar to that surrounding the 12th codon in the *ras* oncogene was poorly repaired. Studies with oligodeoxynucleotide substrates of different sequences indicated that there is some sequence specificity in the rate of repair by the mammalian alkyltransferase (56, 59). Although incomplete, these results suggest that repair is slowest when the O^6-methylguanine is located on the 3′ side of another guanine residue. No sequences were found that were not repaired, and differences in the rate of repair are only likely to have much effect under conditions in which the total number of alkylation lesions exceeds the number of available alkyltransferase molecules. The differential rate of repair could lead to a majority of the alkyltransferase being used up on the most favored sites and the residual adducts would then be present at a high frequency in the sites which are repaired more slowly. No evidence was found for a difference in repair of O^6-methylguanine between the two positions in the 12th codon in the *ras* gene inserted into Rat4 fibroblasts (73), but the ratio of inserted O^6-methylguanine to the alkyltransferase repair capacity of the cells is unknown.

Cellular Distribution of Alkyltransferase

Alkyltransferase activity has been detected in a wide variety of vertebrate species (8, 74). There is considerable variation in

[4] M. E. Dolan, A. E. Pegg, and R. C. Moschel, unpublished observations.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/50/19/6119/2440997/cr0500196119.pdf by guest on 24 August 2022

the content of alkyltransferase between different species and cell types (1, 6, 8, 74–78). The liver and spleen contain relatively high levels and the brain and mammary gland activities are very low. Although alkyltransferase activities have frequently been expressed as a function of the protein present, it is probably more relevant to express these values in terms of DNA content, since mammalian cells differ significantly in size (75). More importantly, the total activity obtained from a tissue homogenate may not be an accurate reflection of the alkyltransferase present within individual cells in the tissue. The alkyltransferase activity in rat hepatocytes is much higher than in the nonparenchymal cells in the liver (76), and there are striking differences in the levels present in different cell types isolated from the lung, with the Clara cells having particularly low activity, especially after exposure to 4-(*N*-methyl-*N*-nitrosamino)-1-(3-pyridyl)-1-butanone (78). These differences may be of great importance in determination of the sensitivity towards tumor initiation by alkylating agents (46, 76–78). It should be noted that the capacity to repair O^6-alkylguanine formed in DNA is related to both the content of the alkyltransferase at the time of carcinogen exposure and the rate at which the alkyltransferase can be synthesized to replace that used up in the repair reaction.

The number of individual cell types derived from mammalian tissues which have been assayed for alkyltransferase activity is limited because, until very recently, the only means available for this assay was to isolate the cells, prepare an extract, and measure the activity. Only a few cell types can be separated and purified in sufficient numbers for alkyltransferase assay without loss of enzymatic activity. Recently, both monoclonal antibodies and rabbit antiserum towards alkyltransferase peptides have become available, and these should prove useful in studies of the distribution and turnover of the alkyltransferase and will enable immunohistochemical determination of the distribution of alkyltransferase activity. Brent and colleagues have isolated a number of monoclonal antibodies towards the human alkyltransferase (26, 79). Antibodies which react well with the alkyltransferase have been prepared in rabbits to peptides which correspond to the carboxyl and amino termini of the human protein.[5]

Although the relative distribution of alkyltransferase activity between different tissues appears to be similar, human cells have much higher levels of alkyltransferase than their rodent cell counterparts (1, 6, 80–82). There are significant intraindividual variations in human alkyltransferase activities (1, 2, 81–85). Patients developing a therapy-related acute nonlymphocytic leukemia were found to have low alkyltransferase activity in peripheral blood lymphocytes (84). This may indicate that the tendency to develop a secondary malignancy after treatment with alkylating agents is associated with a low ability for repair of O^6-alkylguanine. Fibroblasts from patients with lung cancer were reported to have a lower alkyltransferase activity than healthy controls or melanoma patients, and it was suggested that a reduced capacity to repair O^6-alkylguanine may, therefore, be a risk factor for lung cancer (85). Further studies of this possibility and of the variation of alkyltransferase activity within the population are needed.

Induction of Alkyltransferase Activity

The alkyltransferase activity in rat liver is increased after treatment with a variety of alkylating carcinogens (reviewed in Refs. 1, 5, and 8). This increase occurs only in the hepatocytes (76) and amounts to maximally about a 3-fold increase. It is, therefore, in no way comparable to the much larger elevation in the *ada* gene product as part of the adaptive response (9–11). Furthermore, a rise in the alkyltransferase activity in rat liver occurs as a response to either partial hepatectomy or a variety of hepatotoxins and carcinogens that are not simple alkylating agents (1, 4). Changes in alkyltransferase levels in rat liver and kidney also occur in response to endocrine stimuli (86) and interferon (87). It is possible that the apparent induction of alkyltransferase in rats treated with carcinogens is a secondary response to the cellular damage caused by them. Attempts to demonstrate similar changes in other mammals, including mice, hamsters, and gerbils, have been unsuccessful (1, 4). The regulation of alkyltransferase activity in the rat may, therefore, not be a good model for generalization. However, it is worth pointing out that the ability of the rat to increase its hepatic alkyltransferase in response to exposure to alkylating carcinogens may contribute to the relative resistance of this species to carcinogenesis by these agents, compared to other species such as hamsters that lack this ability.

The underlying mechanism by which the alkyltransferase activity is increased in the rat is not known, but the availability of cDNA and antibody probes should permit this phenomenon to be studied in more detail. Increased alkyltransferase activity occurs in cultured rat hepatoma H4 cells in response to MNNG and other alkylating agents (88). It is not known whether the alkyltransferase activity in human tissues responds to exposure to alkylating agents, and the induction in cultured cells from species other than rats is controversial. Some reports have indicated significant increases in such cells in response to MNNG, but other laboratories have not been able to repeat these observations (reviewed in Refs. 6, 89, and 90). Multiple factors may influence the alkyltransferase activity in such experiments. Changes in alkyltransferase activity during the cell cycle were reported to occur in mouse embryo cells [C3H/10T1/2] (91) and in normal human fibroblasts but not in cells from a Bloom's syndrome patient (92). The inhibition of poly(ADP-ribose) synthesis in H4 cells increased the enhancement of alkyltransferase in response to MNNG (93).

Exposure to ionizing radiation increases alkyltransferase in a variety of mammalian species and cell types (94–97) and bleomycin induces alkyltransferase in rat liver (98). It is possible that the formation of strand breaks in DNA in response to radiation leads to the increase in alkyltransferase. Anaerobic conditions, which may reduce the occurrence of single-strand breaks, also prevented the radiation-mediated rise in alkyltransferase in C3H/10T1/2 cells (96).

Absence of Alkyltransferase in Some Cultured Cell Lines

A particularly interesting feature of the cellular physiology of alkyltransferase expression is that a considerable number of human cultured cell lines appear to lack this activity. This phenomenon was discovered independently in two laboratories and has been extended in many other studies. Day and colleagues (99) found that some cell lines were unable to support the growth of adenovirus 5 which had been damaged by reaction with MNNG. This phenotype was described as Mer$^-$ and subsequent work established that the underlying basis for this phenotype was the absence of alkyltransferase activity (6, 99). Strauss *et al.* (100, 101) noted that certain virally transformed lymphoblastoid lines were lacking alkyltransferase and described these lines as Mex$^-$. Although based on these two

[5] A. E. Pegg, L. Wiest, C. Mummert, and M. E. Dolan, unpublished observations.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/50/19/6119/2440997/cr0500196119.pdf by guest on 24 August 2022

different operational definitions, there is no difference between the Mer⁻ and the Mex⁻ phenotypes and Mer⁻ is used in the remainder of this article.

The origin of the phenotype is not clear. Fibroblasts from patients whose tumor cells gave rise to Mer⁻ tumor cell lines were Mer⁺ (6). Virtually all human tumors so far examined have some alkyltransferase activity (1, 80–84). Although it is possible that growth in culture selects for a small subpopulation of tumor cells which are Mer⁻, it seems more likely that loss of alkyltransferase activity occurs as a result of culture conditions. The reason for this is presently unknown. Immortalization of cell lines via viral transformation appears to increase the frequency of the appearance of the Mer⁻ phenotype. About 60% of the virus-transformed lines were Mer⁻, whereas only 20% of the other human tumor cell lines were similarly lacking in alkyltransferase activity (99, 101, 102). Viral transformation of some murine cells also led to the appearance of cells lacking alkyltransferase (103). However, not all such virally transformed cells were deficient (103). It should also be stressed that at least two nontransformed fibroblast lines were completely lacking in alkyltransferase (6, 44). Studies of the development of the Mer⁻ phenotype during the establishment of immortalized human fibroblast cell lines by SV40 or by Epstein-Barr virus indicate that it was not associated with transformation or any particular stage of the immortalization process (104, 105). Genetic analysis of the Mer⁻ condition has been inconclusive. Although some experiments with crosses of Mer⁻ and Mer⁺ cell lines suggested that the Mer⁻ characteristic was recessive, other experiments showed a more complex pattern (6, 101). Karran *et al.* (106) have found recently that the loss of alkyltransferase activity which occurred during culture of two human lymphoblastoid cell lines is accompanied by a loss of thymidine kinase and galactokinase activity. They also observed a link between the expression of these three activities in Raji cell lines and they suggest that alkyltransferase expression may be coordinately regulated with that of these genes, which are present on chromosome 17.

Early attempts to explain the Mer⁻ phenotype on the basis of the rate of resynthesis of the protein following exhaustion by alkylating agents (107) have not been supported by the weight of experimental evidence, which in general consensus shows that the activity is actually lacking from the Mer⁻ cells (1, 6, 8). No protein corresponding to the alkyltransferase was observed in Western blots from six Mer⁻ cell lines probed with a monoclonal antibody which readily detected the protein in blots from four Mer⁺ lines (79). Similar results indicating a lack of alkyltransferase protein in Mer⁻ cells were obtained from Western blots developed with antibodies raised to peptide sequences located at the amino and carboxyl termini of the human alkyltransferase.[5] Also, the content of mRNA corresponding to the alkyltransferase was either not detectable or greatly reduced on Northern blots of RNA isolated from Mer⁻ cell lines developed using oligonucleotide probes based on the human cDNA sequence (31, 108, 109). These results suggest that the absence of alkyltransferase activity is due to a lack of synthesis of the protein and tnat this, in turn, may be due to the lack of transcription of the alkyltransferase gene.

Several experiments suggest that at least some Mer⁻ cells do contain an intact alkyltransferase gene. When hybridized to alkyltransferase cDNA, Southern blots of *Eco*RI-digested DNA from L33 lymphoblastoid cells, which are Mer⁻, showed the same bands as did the Mer⁺ HeLa S3, although HeLa M (another Mer⁻ line) did not (31, 109). DNA from another four Mer⁻ lines tested in similar Southern blots also showed no differences from Mer⁺ cells (109). Exposure of Ha821 cells (murine sarcoma virus-transformed NIH3T3 cells) to 5-azacytidine led to a restoration of alkyltransferase activity to the level seen in the parental NIH3T3 cells (110). Significant alkyltransferase activity was detected in extracts from cells produced by exposing Mer⁻ V79 cells to increasing concentrations of mitozolomide and selecting for cells able to survive this alkylating agent (111). Furthermore, transfection of human DNA from either Mer⁻ or Mer⁺ cells into CHO cells, which have no alkyltransferase, led to the production of cells possessing the activity (112), although it should be pointed out that there is no independent evidence that it is the human rather than the hamster alkyltransferase which was expressed.

Expression of Bacterial Alkyltransferase in Mammalian Cells

Several groups have reported the results of experiments in which the *ada* gene product was inserted into an appropriate vector driven by a mammalian promoter and expressed in mammalian cells. Such expression leads to a reduction in the cytotoxicity, mutagenicity, and SCE-inducing ability of both monofunctional and bifunctional alkylating agents, providing additional evidence for the importance of O^6-alkylguanine and/or O^4-alkylthymine in these processes (113–116). Similar protection from MNNG was obtained when the *ada* gene product was expressed in yeast (24). These experiments were carried out using constructs which express the entire *ada* gene product. This also repairs the *S*-stereoisomer of alkylated phosphodiester residues. However, expression in mammalian cells of a truncated *ada* product, containing only the carboxyl domain, that lacks the site for repair of alkylphosphotriesters yielded similar results, indicating that the base adducts are responsible for the toxic effects (117–119). Converse experiments in which only the amino terminal domain of the *ada* protein was expressed have provided mammalian cells synthesizing an alkylphosphotriester repair function, but no significant effects of this expression in cellular responses to alkylating agents have yet been established (115, 119, 120).

Studies in which the *ada* gene product was placed in a mammalian expression vector under the control of a glucocorticoid-inducible MMTV promoter and transfected into HeLa MR cells have been used to illustrate the importance of the expression of this activity in permitting survival from the toxic effects of MNNG (121). These studies also indicate the importance of both *de novo* synthesis and the existing pool of the alkyltransferase in removal of O^6-methylguanine from the cellular DNA after exposure to MNNG.

Retroviral vectors have also been used for expression of the *E. coli* alkyltransferase in mammalian cells (122). These vectors offer significant advantages with regard to the level of expression and the ease of transfection of various cell types, but all of the studies in which the *ada* protein is expressed in mammalian cells suffer from the disadvantage that the *E. coli* alkyltransferase differs significantly from the mammalian protein in a number of properties and that the cellular localization of the expressed bacterial protein may also not be the same. Indeed, immunohistochemical studies on NIH-3T3 cells expressing *ada* indicated that the protein was predominantly cytoplasmic (122). These cells were only slightly more resistant to BCNU than the control cells, despite a 15-fold rise in total alkyltransferase activity (as measured in cell extracts), suggesting that the additional protein may not be fully functional in the cell (122).

Several attempts have been made to produce transgenic mice

Downloaded from http://aacrjournals.org/cancerres/article-pdf/50/19/6119/2440997/cr0500196119.pdf by guest on 24 August 2022

expressing the *ada* protein. Studies using a construct in which the gene is combined with a metallothionein promoter have yielded some animals in which the alkyltransferase activity in liver is about 3 times that of controls and in which the *ada* protein can be detected by immunoblotting (123). However, the activity was not greatly stimulated by exposure to heavy metals to activate the promoter (123) and this construct may not be ideal, since such metals are also good inhibitors of the alkyltransferase (35, 36). Transgenic mice expressing *ada* controlled from the promoter-regulatory region of the phosphoenolpyruvate carboxykinase gene have also been derived (124). These mice showed an increase in alkyltransferase activity in the liver and kidney in response to a high protein diet and a reduction with a high carbohydrate diet, which is in accord with the known effects on phosphoenolpyruvate carboxykinase expression. However, even the maximal level was only 3 times that of the control mice and such dietary manipulations may complicate the results of studies aimed at evaluating the importance of the alkyltransferase in carcinogenesis.

Transfection of Mammalian Alkyltransferase Activity

Conversion of Mer^- cells into Mer^+ has been accomplished by transfection with mammalian DNA followed by selection of clones resistant to chloroethylating agents (112, 125–127). These clones possess alkyltransferase activity which presumably arises from the expression of a gene present in the transfected DNA. There was integration of human DNA into CHO cells in these experiments, but attempts to retrieve the transfected human alkyltransferase gene from these cells have not yet been successful (112, 126, 127) and it remains possible that the hamster gene has been reactivated. The expression of the human alkyltransferase activity in CHO cells does lead to an increased resistance to the toxicity, mutagenicity, and SCE induction by monofunctional alkylating agents (112, 125–129) which again emphasizes the importance of the rapid repair of O^6-methylguanine in such resistance. Direct studies of the mutagenicity of O^6-alkylguanine, in which a plasmid containing this lesion at a defined site was introduced into control CHO cells or CHO cells expressing alkyltransferase after transfection with human DNA, confirm a major role for this protein in the prevention of mutations (130). However, it should be noted that some colonies of transfected cells selected for resistance to monofunctional alkylating agents do not express alkyltransferase (112, 127, 131, 132) and other mechanisms for this resistance must be operating in these cells.

Effect of Alkyltransferase on Sensitivity to Chloroethylating Agents

The alkyltransferase activity is able to protect cells from the lethal effects of exposure to chloroethylating agents such as BCNU, 1-(4-amino-2-methyl-5-pyrimidinyl)methyl-3-(2-chloroethyl)-3-nitrosourea [ACNU], and 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea [CCNU] (133–136). The mechanism underlying this protection is not fully understood, but it is clear that the alkyltransferase can prevent the formation of DNA interstrand cross-links (7, 137–140). These cross-links arise from O^6-chloroethylguanine, which first undergoes an intramolecular rearrangement to form O^6,N^1-ethanoguanine. This then reacts with the complementary cytosine on the opposite strand to form the N^1-guanine-N^3-cytosine ethano cross-link (7). It was thought initially that the alkyltransferase interfered with this process by removing the chloroethyl group from the O^6-position, but more recently Brent and colleagues (141, 142) have found that it can also react with O^6, N^1-ethanoguanine in DNA. This reaction leads to the alkyltransferase becoming covalently linked to the DNA, presumably by an ethano group attached at the other end to the N^1 position of guanine, but this adduct has not yet been characterized. An elegant scheme for the purification of the alkyltransferase has been devised to take advantage of this covalent linkage (142). A substrate for the protein was made by reacting BCNU with a labeled synthetic oligodeoxynucleotide with a polyadenylated 3′ terminus. The complex between the alkyltransferase and this substrate was then purified using oligothymidylate-cellulose. Although the yield of protein was not high, a very high degree of purification was achieved in this way (142).

It is also well established that the alkyltransferase activity prevents the formation of SCEs in response to these chloroethylating agents (143–145). The presence of interstrand cross-links may, therefore, give rise to these SCEs.

Although it is likely that the prevention of cross-link formation as a result of alkyltransferase activity may be a critical feature of the reduced sensitivity of Mer^+ cells to killing by chloroethylating agents (138–140, 146), other factors may also be involved. Treatment with α-difluoromethylornithine, an inhibitor of polyamine biosynthesis, enhances the sensitivity of Mer^+ but not Mer^- cells to chloroethylating agents, and this enhancement apparently occurs without an increase in the detectable cross-links (147, 148).

Depletion of Alkyltransferase Activity

The reduction of alkyltransferase activity by administration of appropriate drugs is of interest for two reasons. Firstly, such treatments, if specific and effective, could be used to evaluate the importance of the alkyltransferase reaction in the pathological responses to alkylating agents. Secondly, reduction of alkyltransferase activity in tumor cells may be used to improve the clinical effectiveness of the chemotherapeutic chloroethylating agents since, as described above, this activity protects against the cytotoxic effects of these agents.

The latter has also been approached by pretreatment with a monofunctional methylating agent (149–152). In cultured cells and animal tumor models, this produces sufficient O^6-methylguanine in the DNA that the cellular alkyltransferase activity is exhausted and the formation of cytotoxic cross-links in response to BCNU is increased. It has also been documented that the alkyltransferase activity can be depleted in cells of patients treated with methylating agents (83, 84, 151). On this basis, the combination of streptozotocin and BCNU has been placed into clinical trials (151, 152). However, a significant drawback is the potential long term damage due to the alkylating agent, including the risk of initiation of additional tumors, since both streptozotocin and *N*-methyl-*N*-nitrosourea are potent carcinogens.

Therefore, there is a clear need for a less dangerous way in which to deplete the alkyltransferase. Several years ago it was reported that exposure of cultured cells to the free base, O^6-methylguanine, produced a substantial drop in alkyltransferase activity (53, 153). Although this drop was not complete, it was sufficient to demonstrate that reduction of the alkyltransferase increased mutagenesis in response to MNNG and increased the toxicity of BCNU, Clomesone [2-chloroethyl(methylsulfonyl)-methanesulfonate], and related compounds (153–159). The fall in alkyltransferase in cells treated with O^6-methylguanine is not related to the base incorporating into DNA or RNA and then acting as a substrate (53). No such incorporation could be

Downloaded from http://aacrjournals.org/cancerres/article-pdf/50/19/6119/2440997/cr0500196119.pdf by guest on 24 August 2022

demonstrated in growing cells and the same extent of alkyltransferase reduction occurs even when nucleic acid synthesis is blocked (153). Studies *in vitro* with the isolated alkyltransferase showed that activity was lost on incubation with O^6-methylguanine. The loss of activity was dependent on both time and the concentration of the base and it was irreversible (153, 157). This suggests that O^6-methylguanine is a weak substrate for the protein. This was confirmed by the demonstration that [^{3}H]guanine is indeed formed from O^6-methyl[^{3}H]guanine in stoichiometric amounts with the number of molecules of the alkyltransferase which were inactivated (153). The rate of inactivation is sufficiently slow that accurate calculations cannot be made, but it appears that the first-order rate constant for the free base is 10^7 to 10^8 times less than for O^6-methylguanine in double-stranded DNA (56, 59, 157). The maximal reduction in alkyltransferase which can be achieved in cultured cells exposed to O^6-methylguanine is about 80%. This value is probably due to the new steady state set up between the synthesis of the protein and its inactivation by O^6-methylguanine.

A number of other O^6-alkylguanine derivatives were examined for their abilities to deplete alkyltransferase in cultured cells. Significant depletion was obtained with the ethyl, *n*-propyl, and *n*-butyl derivatives, whereas the isopropyl, isobutyl, and 2-hydroxyethyl compounds had lesser activity (153). The potencies of these compounds were in approximate parallel with their abilities to act as substrates for the protein when present as adducts in DNA (38–40), with the exception that O^6-*n*-butylguanine was slightly more active than expected, but this is probably explained by the improved uptake of the *n*-butyl derivative (153).

The limited solubility and uptake of O^6-methylguanine, combined with its low potency, has hampered attempts to use this compound to reduce alkyltransferase in intact animals. Although two recent studies have demonstrated that depletion is achieved in tissues of mice given large doses of O^6-methylguanine (160, 161) and substantial depletion was produced in human tumor xenografts of treated nude mice (160), the doses needed, 440 mg/kg by i.p. injection (160) and 160 mg/kg i.v. or a 15-h infusion of 79 μg/kg/min (161), are unlikely to be practicable.

Some reduction of the hepatic alkyltransferase activity has been reported to occur following i.p. injection of DNA methylated by *N*-methyl-*N*-nitrosourea (162), and exposure of cultured cells to oligodeoxynucleotides containing O^6-methylguanine leads to a fall in alkyltransferase (59). However, the uptake of such alternative substrates is very poor unless facilitated by electroporation or complexing with lipids, and it is unlikely that such approaches will be of any practical therapeutic value.

Very recently, it has been found that O^6-benzylguanine is a vastly better inactivator of the alkyltransferase than O^6-methylguanine (43). Exposure of the alkyltransferase *in vitro* to 2.5 μM O^6-benzylguanine led to more than 90% of the activity being lost within 10 min, whereas 200 μM O^6-methylguanine for 60 min was required to get the same reduction. When added to cultured cells, complete loss of alkyltransferase activity was produced within 15 min of addition of O^6-benzylguanine to the culture medium and a maximal effect was obtained with 5 μM concentrations. The cytotoxicity of 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea or Clomesone towards tumor cells was enormously enhanced by pretreatment with 10 μM O^6-benzylguanine for 2 h (43). Virtually complete depletion of the alkyltransferase in mouse liver and kidney was achieved by i.p. injection of 10 mg/kg doses. Thus, O^6-benzylguanine and its *p*-chloro and *p*-methyl derivatives, which are equally effective, have promise as agents with which to enhance the chemotherapeutic effectiveness of chloroethylating agents or to evaluate the role of the DNA repair alkyltransferase in protection against the effects of alkylating agents.

The inability of the alkyltransferase to repair S^6-thiomethylguanine adducts may provide an alternative approach to enhance the effectiveness of the chloroethylnitrosoureas. Pretreatment of growing cells with 6-thioguanine prior to exposure to these agents increased their cytotoxicity (50, 163). This may be due to two factors: an enhanced extent of reaction of the alkylating agent with the mercapto group of the incorporated 6-thioguanine and the absence of repair of these adducts.

Consequences of Lack of Alkyltransferase Activity

Although the situation is complex and other factors clearly can play a significant role, the balance of the experimental evidence described above and studies correlating the alkyltransferase activity with the sensitivity to alkylating agents (1, 6, 44, 99, 156, 164) suggest that this activity can protect mammalian cells from mutations, cytotoxicity, and induction of SCEs after exposure to monofunctional alkylating agents. However, this correlation is not universal. Clearly, other factors can contribute to the lethality of methylating agents (6, 131, 165, 166). A number of mammalian cell mutants or variants having altered sensitivities to the toxic and mutagenic effects of monofunctional alkylating agents that do not show parallel changes in the alkyltransferase activity have been isolated (165–171). A more exact correlation is observed between the expression of alkyltransferase activity and the resistance to toxicity and SCE induction by bifunctional chloroethylating reagents *in vitro* (7, 103, 105, 133, 134, 136, 144, 172, 173) and *in vivo* (174). This indicates that interaction with the O^6-position of guanine is of major importance in these properties.

Acknowlegments

I thank T. Long for secretarial assistance and Dr. M. E. Dolan for her help with the manuscript and in carrying out many of the experiments described here.

Note Added in Proof

Since the time of submission of this review, two other groups have reported the cloning of cDNAs for the human alkyltransferase and the gene has been localized to chromosome 10 (H. Hayakawa, G. Koike and M. Sekiguchi. Expression and cloning of complementary DNA for a human enzyme that repairs O^6-methylguanine in DNA. J. Mol. Biol., *213:* 739–747, 1990; B. Rydberg, J. Hall, and P. Karran. Active site amino acid sequence of the bovine O^6-methylguanine-DNA methyltransferase. Nucleic Acids Res., *18:* 17–21, 1990).

References

1. Pegg, A. E. Alkylation and subsequent repair of DNA after exposure to dimethylnitrosamine and related carcinogens. Rev. Biochem. Toxicol., *5:* 83–133, 1983.
2. Magee, P. N. The experimental basis for the role of nitroso compounds in human cancer. Cancer Surv., *8:* 208–239, 1989.
3. Pegg, A. E., and Singer, B. Is O^6-alkylguanine necessary for initiation of carcinogenesis by alkylating agents? Cancer Invest., *2:* 221–238, 1984.
4. Saffhill, R., Margison, G. P., and O'Connor, P. J. Mechanism of carcinogenesis induced by alkylating agents. Biochim. Biophys. Acta, *823:* 111–145, 1985.
5. Singer, B. *O*-Alkyl pyrimidines in mutagenesis and carcinogenesis: occurrence and significance. Cancer Res., *46:* 4879–4885, 1986.
6. Yarosh, D. B. The role of O^6-methylguanine-DNA methyltransferase in cell survival, mutagenesis and carcinogenesis. Mutat. Res., *145:* 1–16, 1985.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/50/19/6119/2440997/cr0500196119.pdf by guest on 24 August 2022

7. Brent, T. P. Isolation and purification of O^6-alkylguanine-DNA alkyltransferase from human leukemic cells: prevention of chloroethylnitrosourea-induced cross-links by purified enzyme. Pharmacol. Ther., *31:* 121–140, 1985.
8. Pegg, A. E., and Dolan, M. E. Properties and assay of mammalian O^6-alkylguanine-DNA alkyltransferase. Pharmacol. Ther., *34:* 167–179, 1987.
9. Demple, B., and Karran, P. Death of an enzyme: suicide repair of DNA. Trends Biol. Sci., *8:* 137–139, 1983.
10. Demple, B. Self-methylation by suicide DNA repair enzymes. *In:* W. M. Paik and S. Kim (eds.), Protein Methylation, pp. 285–304. Boca Raton, FL: CRC Press, Inc., 1990.
11. Lindahl, T., Sedgwick, B., Sekiguchi, M., and Nakabeppu, Y. Regulation and expression of the adaptive response to alkylating agents. Annu. Rev. Biochem., *57:* 133–157, 1988.
12. Potter, P. M., Wilkinson, M. C., Fitton, J., Carr, F. J., Brennand, J., Cooper, D. P., and Margison, G. P. Characterization and nucleotide sequence of *ogt*, the O^6-alkylguanine-DNA-alkyltransferase gene of *E. coli.* Nucleic Acids Res., *15:* 9177–9193, 1987.
13. Wilkinson, M. C., Potter, P. M., Cawkwell, L., Georgiadis, P., Patel, D., Swann, P. F., and Margison, G. P. Purification of the *E. coli ogt* gene product to homogeneity and its rate of action on O^6-methylguanine, O^6-ethylguanine and O^4-methylthymine in dodecadeoxyribonucleotides. Nucleic Acids Res., *17:* 8475–8484, 1989.
14. Morohoshi, F., Hayashi, K., and Munakata, N. *Bacillus subtilis* gene coding for constitutive O^6-methylguanine-DNA alkyltransferase. Nucleic Acids Res., *17:* 6531–6543, 1989.
15. Kodama, K., Nakabeppu, Y., and Sekiguchi, M. Cloning and expression of the *Bacillus subtilis* methyltransferase gene in *Escherichia coli ada*$^-$-cells. Mutat. Res., *218:* 153–163, 1989.
16. Bhattacharyya, D., Tano, K., Bunick, G. J., Uberbacher, E. C., Bhenke, W. D., and Mitra, S. Rapid, large-scale purification and characterization of 'ada protein' (O^6 methylguanine-DNA methyltransferase) of *E. coli.* Nucleic Acids Res., *16:* 6397–6410, 1988.
17. Sedgwick, B., Robins, P., Totty, N., and Lindahl, T. Functional domains and methyl acceptor sites of the *Escherichia coli* ada protein. J. Biol. Chem., *263:* 4430–4433, 1988.
18. Takano, K., Nakabeppu, Y., and Sekiguchi, M. Functional sites of the Ada regulatory protein of *Escherichia coli.* J. Mol. Biol., *201:* 261–271, 1988.
19. Tano, K., Bhattacharyya, D., Foote, R. S., Mural, R. J., and Mitra, S. Site-directed mutation of the *Escherichia coli ada* gene: effects of substitution of methyl acceptor cysteine-321 by histidine in ada protein. J. Bacteriol., *171:* 1535–1543, 1989.
20. Takahashi, K., Kawazoe, Y., Sakumi, K., Nakabeppu, Y., and Sekiguchi, M. Activation of Ada protein as a transcriptional regulator by direct alkylation with methylating agents. J. Biol. Chem., *263:* 13490–13492, 1988.
21. Tsimis, J., and Yarosh, D. B. Adaptive response induction by bacterial catalysis of nitrosation. Environ. Mol. Mutagen., *15:* 69–70, 1990.
22. Rebeck, G. W., Smith, C. M., Goad, D. L., and Samson, L. Characterization of the major DNA repair methyltransferase activity in unadapted *Escherichia coli* and identification of a similar activity in *Salmonella typhimurium.* J. Bacteriol., *171:* 4563–4568, 1989.
23. Ceccoli, J., Rosales, N., Goldstein, M., and Yarosh, D. B. Polyclonal antibodies against O^6-methylguanine-DNA methyltransferase in adapted bacteria. Mutat. Res., *194:* 219–226, 1988.
24. Brozmanova, J., Kleibl, K., Vlckova, V., Skorvaga, M., Cernakova, L., and Margison, G. P. Expression of the *E. coli ada* gene in yeast protects against the toxic and mutagenic effects of *N*-methyl-*N'*nitro-*N*-nitrosoguanidine. Nucleic Acids Res., *18:* 331–335, 1990.
25. Sassanfar, M., and Samson, L. Identification and preliminary characterization of an O^6-methylguanine DNA repair methyltransferase in the yeast *Saccharomyces cerevisiae.* J. Biol. Chem., *265:* 20–25, 1990.
26. Brent, T. P., von Wronski, M., Pegram, C. M., and Bigner, D. D. Immunoaffinity purification of human O^6-alkylguanine-DNA alkyltransferase using newly developed monoclonal antibodies. Cancer Res., *50:* 58–61, 1990.
27. von Wronski, M., Bigner, D. D., and Brent, T. P. Amino acid sequence data from immunoaffinity-purified human O^6-methylguanine-DNA methyltransferase. Proc. Am. Assoc. Cancer Res., *31:* 446, 1990.
28. Major, G. N., Gardner, E. F., Carne, A. F., and Lawley, P. D. Purification to homogeneity and partial amino acid sequence of a fragment which includes the methyl acceptor site of the human DNA repair protein for O^6-methylguanine. Nucleic Acids. Res., *18:* 1351–1359, 1990.
29. Wilkinson, M. C., Cooper, D. P., Southan, C., Potter, P. M., and Margison, G. P. Purification to apparent homogeneity and partial amino acid sequence of rat liver O^6-alkylguanine-DNA-alkyltransferase. Nucleic Acids Res., *18:* 13–16, 1990.
30. Rydberg, B., Hall, J., and Karran, P. Active site amino acid sequence of the bovine O^6-methylguanine-DNA methyltransferase. Nucleic Acids Res., *18:* 17–21, 1990.
31. Tano, K., Shiota, S., Collier, J., Foote, R. S., and 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, *87:* 686–690, 1990.
32. Moody, P. C. E., and Demple, B. Crystallization of O^6-methylguanine-DNA methyltransferase from *Escherichia coli.* J. Mol. Biol., *200:* 751–752, 1988.
33. Pegg, A. E., Wiest, L., Foote, R. S., Mitra, S., and Perry, W. Purification and properties of O^6-methylguanine-DNA transmethylase from rat liver. J. Biol. Chem., *258:* 2327–2333, 1983.
34. Boulden, A. M., Foote, R. S., Fleming, G. S., and Mitra, S. Purification and some properties of human DNA-O^6-methylguanine methyltransferase. J. Biosci. (Bangalore), *11:* 215–224, 1987.
35. Scicchitano, D. A., and Pegg, A. E. Inhibition of O^6-alkylguanine-DNA-alkyltransferase by metals. Mutat. Res., *192:* 207–210, 1987.
36. Bhattacharyya, D., Boulden, A. M., Foote, R. S., and Mitra, S. Effect of polyvalent metal ions on the reactivity of human O^6-methylguanine-DNA methyltransferase. Carcinogenesis (Lond.), *9:* 683–685, 1988.
37. Krokan, H., Grafstrom, R. C., Sundqvist, K., Esterbauer, H., and Harris, C. C. Cytotoxicity, thiol depletion and inhibition of O^6-methylguanine-DNA methyltransferase by various aldehydes in cultured human bronchial fibroblasts. Carcinogenesis (Lond.), *6:* 1755–1759, 1985.
38. Pegg, A. E., Scicchitano, D., and Dolan, M. E. Comparison of the rates of rates of repair of O^6-alkylguanines in DNA by rat liver and bacterial O^6-alkylguanine-DNA alkyltransferase. Cancer Res., *44:* 3806–3811, 1984.
39. Pegg, A. E., Dolan, M. E., Scicchitano, D., and Morimoto, K. Studies of the repair of O^6-alkylguanine and O^4-alkylthymine in DNA by alkyltransferases from mammalian cells and bacteria. Environ. Health Perspect., *62:* 109–114, 1985.
40. Morimoto, K., Dolan, M. E., Scicchitano, D., and Pegg, A. E. Repair of O^6-propylguanine and O^6-butylguanine in DNA by O^6-alkylguanine-DNA alkyltransferases from rat liver and *E. coli.* Carcinogenesis (Lond.), *6:* 1027–1031, 1985.
41. Graves, R. J., Li, B. F. L., and Swann, P. F. Repair of O^{6-} methylguanine, O^6-ethylguanine, O^6-isopropylguanine and O^4-methylthymine in synthetic oligodeoxynucleotides by *Escherichia coli* ada gene O^6-alkylguanine-DNA-alkyltransferase. Carcinogenesis (Lond.), *10:* 661–666, 1989.
42. Samson, L., Thomale, J., and Rajewsky, M. F. Alternative pathways for the *in vivo* repair of O^6-alkylguanine and O^4-alkylthymine in *Escherichia coli*: the adaptive response and nucleotide excision repair. EMBO J., *7:* 2261–2267, 1988.
43. Dolan, M. E., Moschel, R. C., and Pegg, A. E. Depletion of mammalian O^6-alkylguanine-DNA alkyltransferase activity by O^6-benzylguanine provides a means to evaluate the role of this protein in protection against carcinogenic and therapeutic alkylating agent. Proc. Natl. Acad. Sci. USA, 5368–5372, 1990.
44. Domoradzki, J., Pegg, A. E., Dolan, M. E., Maher, V. M., and McCormick, J. J. Correlation between O^6-methylguanine-DNA-methyltransferase activity and resistance of human cells to the cytotoxic and mutagenic effect of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. Carcinogenesis (Lond.), *5:* 1641–1647, 1984.
45. Dolan, M. E., Oplinger, M., and Pegg, A. E. Use of a dodecadeoxynucleotide to study repair of the O^4-methylthymine lesion. Mutat. Res., *193:* 131–137, 1988.
46. Brent, T. P., Dolan, M. E., Fraenkel-Conrat, H., Hall, J., Karran, P., Laval, F., Margison, G. P., Montesano, R., Pegg, A. E., Potter, P. M., Singer, B., Swenberg, J. A., and Yarosh, D. B. Repair of *O*-alkylpyrimidines in mammalian cells: a present consensus. Proc. Natl. Acad. Sci. USA, *85:* 1759–1762, 1988.
47. Boucheron, J. A., Richardson, F. C., Morgan, P. H., and Swenberg, J. A. Molecular dosimetry of O^4-ethyldeoxythymidine in rats continuously exposed to diethylnitrosamine. Cancer Res., *47:* 1577–1581, 1987.
48. Swenberg, J. A., Richardson, F. C., Boucheron, J. A., Deal, F. H., Belinsky, S. A., Charbonneau, M., and Short, B. G. High- to low-dose extrapolation: critical determinants involved in the dose response of carcinogenic substances. Environ. Health Perspect., *76:* 57–63, 1987.
49. Ludlum, D. B., Kent, S., and Mehta, J. R. Formation of O^6-ethylthioethylguanine in DNA by reaction with the sulfur mustard, chloroethyl sulfide, and its apparent lack of repair by O^6-alkylguanine-DNA alkyltransferase. Carcinogenesis (Lond.), *7:* 1203–1206, 1986.
50. Yarosh, D. B. Regulation and inhibition of O^6-methylguanine-DNA methyltransferase in human cells. *In:* B. Myrnes and H. Krokan (eds.), Repair of DNA Lesions Introduced by *N*-nitroso Compounds, pp. 135–153. Oslo: Norwegian University Press, 1986.
51. Boiteaux, S., and Laval, J. Repair of O^6-methylguanine, by mammalian cell extracts, in alkylated DNA and poly(dG-m^{5}dC) in B and Z forms. Carcinogenesis (Lond.), *6:* 805–807, 1985.
52. Pegg, A. E., Morimoto, K., and Dolan, M. E. Investigation of the specificity of O^6-alkylguanine-DNA alkyltransferase. Chem. Biol. Interact., *65:* 275–281, 1988.
53. Karran, P. Possible depletion of a DNA repair enzyme in human lymphoma cells by subversive repair. Proc. Natl. Acad. Sci. USA, *82:* 5285–5289, 1985.
54. Scicchitano, D., Jones, R. A., Kuzmich, S., Gaffney, B., Lasko, D. D., Essigmann, J. M., and Pegg, A. E. Repair of oligodeoxynucleotides containing O^6-methylguanine by O^6-alkylguanine-DNA-alkyltransferase. Carcinogenesis (Lond.), *7:* 1383–1386, 1986.
55. Wu, R. S., Hurst-Calderone, S., and Kohn, K. W. Measurement of O^6-alkylguanine-DNA alkyltransferase activity in human cells and tumor tissues by restriction endonuclease inhibition. Cancer Res., *47:* 6229–6235, 1987.
56. Dolan, M. E., Scicchitano, D., and Pegg, A. E. Use of oligodeoxynucleotides containing O^6-alkylguanine for the assay of O^6-alkylguanine-DNA-alkyltransferase activity. Cancer Res., *48:* 1184–1188, 1988.
57. Souliotis, V. L., Giannopoulos, A., Koufakis, I., Kaila, S., Dimopoulos, C., and Kyrtopoulos, S. A. Development and validation of a new assay for O^6-

Downloaded from http://aacrjournals.org/cancerres/article-pdf/50/19/6119/2440997/cr0500196119.pdf by guest on 24 August 2022

alkylguanine-DNA-alkyltransferase based on the use of an oligonucleotide substrate, and its application to the measurement of DNA repair activity in extracts of biopsy samples of human urinary bladder mucosa. Carcinogenesis (Lond.), *10:* 1203–1208, 1989.
58. Mironov, N. M., Wild, C. P., Martel-Planche, G., Swann, P. F., and Montesano, R. Measurement of the removal of O^6-methylguanine and O^4-methylthymine from oligodeoxynucleotides using an immunoprecipitation technique. Anal. Biochem., *183:* 74–79, 1989.
59. Pegg, A. E., and Dolan, M. E. Investigation of sequence specificity in DNA alkylation and repair using oligodeoxynucleotide substrates. *In:* M. W. Lambert and J. Laval (eds.), DNA Repair Mechanisms and their Biological Implications in Mammalian Cells, pp. 45–59. New York: Plenum Publishing Corp., 1989.
60. Souliotis, V. L., and Kyrtopoulos, S. A. A novel, sensitive assay for O^6-ethylguanine in DNA, based on repair by the enzyme O^6-alkylguanine-DNA-alkyltransferase in competition with an oligonucleotide containing O^6-methylguanine. Cancer Res., *49:* 6997–7001, 1989.
61. Zarbl, H., Sukumar, S., Arthur, A. V., Martin-Zanca, D., and Barbacid, M. Direct mutagenesis in Ha-*ras*-1 oncogenes by *N*-nitroso-*N*-methylurea during initiation of mammary carcinogenesis in rats. Nature (Lond.), *315:* 382–385, 1985.
62. Belinsky, S. A., Devereux, T. R., Maronpot, R. R., Stoner, G. D., and Anderson, M. W. Relationship between the formation of promutagenic adducts and the activation of the K-*ras* protooncogene in lung tumors from A/J mice treated with nitrosamines. Cancer Res., *49:* 5305–5311, 1989.
63. Wang, Y., You, M., Reynolds, S. H., Stoner, G., and Anderson, M. W. Mutational activation of the cellular Harvey *ras* oncogene in rat esophageal papillomas induced by methylbenzylnitrosamine. Cancer Res., *50:* 1591–1595, 1990.
64. Richardson, K. K., Crosby, R. M., Richardson, F. C., and Slopek, T. R. DNA base changes induced following *in vivo* exposure of unadapted, adapted or Ada$^-$ *Escherichia coli* to *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. Mol. Gen. Genet., *209:* 526–532, 1987.
65. Glickman, B. W., Horsfall, M. J., Gordon, A. J. E., and Burns, P. A. Nearest neighbor affects G:C to A:T transitions induced by alkylating agents. Environ. Health Perspect., *76:* 29–32, 1987.
66. Horsfall, M. J., Gordon, A. J. E., Burns, P. A., Zielenska, M., van der Vliet, G. M. E., and Glickman, B. W. Mutational specificity of alkylating agents and the influence of DNA repair. Environ. Mol. Mutagen., *15:* 107–122, 1990.
67. Sikpi, M. O., Waters, L. C., Kraemer, K. H., Preston, R. J., and Mitra, S. *N*-Methyl-*N*-nitrosourea-induced mutations in a shuttle plasmid replicated in human cells. Mol. Carcinogen., *3:* 30–36, 1990.
68. Dolan, M. E., Oplinger, M., and Pegg, A. E. Sequence specificity of guanine alkylation and repair. Carcinogenesis (Lond.), *9:* 2139–2143, 1988.
69. Richardson, F. C., Boucheron, J. A., Skopek, T. R., and Swenberg, J. A. Formation of O^6-methyldeoxyguanosine at specific sites in a synthetic oligonucleotide designed to resemble a known mutagenic hotspot. J. Biol. Chem., *264:* 838–841, 1989.
70. Singer, B., Chavez, F., Goodman, M. F., Essigmann, J. M., and Dosanjh, M. K. Effect of 3' flanking neighbors on kinetics of pairing of dCTP or dTTP opposite O^6-methylguanine in a defined primed oligonucleotide when *Escherichia coli* DNA polymerase I is used. Proc. Natl. Acad. Sci. USA, *86:* 8271–8274, 1989.
71. Topal, M. D., Eadie, J. S., and Conrad, M. O^6-Methylguanine mutation and repair is nonuniform. Selection for DNA most interactive with O^6-methylguanine. J. Biol. Chem., *261:* 9879–9885, 1986.
72. Topal, M. D. DNA repair, oncogenes and carcinogenesis. Carcinogenesis (Lond.), *9:* 691–696, 1988.
73. Mitra, G., Pauly, G. T., Kumar, R., Pei, G. K., Hughes, S. H., Moschel, R. C., and Barbacid, M. Molecular analysis of O^6-substituted guanine-induced mutagenesis of *ras* oncogenes. Proc. Natl. Acad. Sci. USA, *86:* 8650–8654, 1989.
74. Woodhead, A. D., Grist, E., Carlson, C., White, T. E., and Waldstein, E. Presence of O^6-methylguanine acceptor protein in the tissues of different classes of vertebrates and invertebrates. Comp. Biochem. Physiol. B, *85:* 125–130, 1986.
75. Gerson, S. L., Trey, J. E., Miller, K., and Berger, N. A. Comparison of O^6-alkylguanine-DNA alkyltransferase activity based on cellular DNA content in human, rat and mouse tissues. Carcinogenesis (Lond.), *7:* 745–749, 1986.
76. Swenberg, J. A., Bedell, M. A., Billings, K. C., Umbenhauer, D. R., and Pegg, A. E. Cell specific differences in O^6-alkylguanine DNA repair activity during continuous carcinogen exposure. Proc. Natl. Acad. Sci. USA, *79:* 5499–5502, 1982.
77. Fong, L. Y. Y., Jensen, D. E., and Magee, P. N. DNA methyl-adduct dosimetry and O^6-alkylguanine-DNA alkyltransferase activity determinations in rat mammary carcinogenesis by procarbazine and *N*-methyl-*N*-nitrosourea. Carcinogenesis (Lond.), *11:* 411–417, 1990.
78. Belinsky, S. A., Dolan, M. E., White, C. W., Maronpot, R. R., Pegg, A. E., and Anderson, M. E. Cell specific differences in O^6-methylguanine-DNA methyltransferase activity and removal of O^6-methylguanine in rat pulmonary cells. Carcinogenesis (Lond.), *9:* 2053–2058, 1988.
79. von Wronski, M. A., Bigner, D. D., and Brent, T. P. Expression of O^6-alkylguanine-DNA alkyltransferase in mer$^+$ and mer$^-$ human cell extracts probed with specific monoclonal antibodies. Cancer Commun., *1:* 323–327, 1989.
80. Wiestler, O., Kleihues, P., and Pegg, A. E. O^6-Alkylguanine-DNA alkyltransferase activity in human brain and brain tumors. Carcinogenesis (Lond.), *5:* 121–124, 1984.
81. Myrnes, B., Giercksky, K., and Krokan, H. Interindividual variation in the activity of O^6-methylguanine-DNA methyltransferase and uracil-DNA methyltransferase and its application to human neoplastic tissues. Carcinogenesis (Lond.), *4:* 1565–1568, 1983.
82. Graftstrom, R. C., Pegg, A. E., Trump, B. F., and Harris, C. C. O^6-Alkylguanine-DNA-alkyltransferase activity in normal human tissues and cells. Cancer Res., *44:* 2855–2857, 1984.
83. Sagher, D., Karrison, T., Schwartz, J. L., Larson, R., Meier, P., and Strauss, B. Low O^6-alkylguanine DNA alkyltransferase activity in the peripheral blood lymphocytes of patients with therapy-related acute nonlymphocytic leukemia. Cancer Res., *48:* 3084–3089, 1988.
84. Sagher, D., Karrison, T., Schwartz, J. L., Larson, R. A., and Strauss, B. Heterogeneity of O^6-alkylguanine-DNA alkyltransferase activity in peripheral blood lymphocytes: relationship between this activity in lymphocytes and in lymphoblastoid lines from normal controls and from patients with Hodgkin's disease or non-Hodgkin's lymphoma. Cancer Res., *49:* 5339–5344, 1989.
85. Rüdiger, H. W., Schwartz, U., Serrand, E., Stief, M., Krause, T., Nowak, D., Doerjer, G., and Lehnert, G. Reduced O^6-methylguanine repair in fibroblast cultures from patients with lung cancer. Cancer Res., *49:* 5623–5626, 1989.
86. Pegg, A. E., and Wiest, L. Regulation of O^6-methylguanine-DNA methyltransferase levels in rat liver and kidney. Cancer Res., *43:* 972–975, 1983.
87. Bertini, R., Coccia, P., Pagani, P., Marinello, C., Salmona, M., and D'Incalci, M. Interferon inducers increase O^6-alkylguanine-DNA alkyltransferase in the rat liver. Carcinogenesis (Lond.), *11:* 181–183, 1990.
88. Frosina, G., and Laval, F. The O^6-methylguanine-DNA methyltransferase activity of rat hepatoma cells is increased after a single exposure to alkylating agents. Carcinogenesis (Lond.), *8:* 91–95, 1987.
89. Frosina, G., and Abbondandolo, A. The current evidence for an adaptive response to alkylating agents in mammalian cells, with special reference to experiments with *in vitro* cell cultures. Mutat. Res., *154:* 85–100, 1985.
90. Wani, G., Wani, A. A., Gibson-D'Ambrosio, R., Samuel, M., Lowder, E., and D'Ambrosio, S. M. Absence of DNA damage-mediated induction of human methyltransferase specific for precarcinogenic O^6-methylguanine. Teratogen. Carcinogen. Mutagen., *9:* 259–272, 1989.
91. Dunn, W. C., Foote, R. S., Hand, J. R. E., and Mitra, S. Cell-cycle-dependent modulation of O^6-methylguanine-DNA methyltransferase in C3H/10T1/2 cells. Carcinogenesis (Lond.), *7:* 807–812, 1986.
92. Kim, S., Vollberg, T. M., Ro, J. Y., Kim, M., and Sirover, M. A. O^6-Methylguanine methyltransferase increases before S phase in normal human cells but does not increase in hypermutable Bloom's syndrome cells. Mutat. Res., *173:* 141–145, 1986.
93. Lefebvre, P., and Laval, F. Potentiation of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine-induced O^6-methylguanine-DNA-methyltransferase activity in a rat hepatoma cell line by poly(ADP-ribose) synthesis inhibitors. Biochem. Biophys. Res. Commun., *163:* 599–604, 1989.
94. Margison, G. P., Butler, J., and Hoey, B. O^6-Methylguanine methyltransferase activity is increased in rat tissues by ionizing radiation. Carcinogenesis (Lond.), *6:* 1699–1702, 1985.
95. Schmerold, I., and Wiestler, O. D. Induction of rat liver O^6-alkylguanine-DNA alkyltransferase following whole body X-irradiation. Cancer Res., *46:* 245–249, 1986.
96. von Hofe, E., and Kennedy, A. R. *In vitro* induction of O^6-methylguanine-DNA methyltransferase in C3H/10T1/2 cells by X-rays is inhibited by nitrogen. Carcinogenesis (Lond.), *9:* 679–681, 1988.
97. Stammberger, I., Schmahl, W., and Nice, L. The effects of X-irradiation, *N*-ethyl-*N*-nitrosourea or combined treatment on O^6-alkylguanine-DNA alkyltransferase activity in fetal rat brain and liver and the induction of CNS tumours. Carcinogenesis (Lond.), *11:* 219–222, 1990.
98. Schmerold, I., and Spath, A. Induction of rat liver O^6-alkylguanine-DNA alkyltransferase by bleomycin. Chem. Biol. Interact., *60:* 297–304, 1986.
99. Day, R. S., III, Ziolkowski, C. H. J., Scudiero, D. A., Meyer, S. A., Lubiniecki, A. S., Girardi, A. J., Galloway, S. M., and Bynum, G. D. Defective repair of alkylated DNA by human tumor and SV-40-transformed human cell strains. Nature (Lond.), *288:* 724–727, 1980.
100. Sklar, R., Brady, K., and Strauss, B. Limited capacity for the removal of O^6-methylguanine and its regeneration in a human lymphoma line. Carcinogenesis (Lond.), *2:* 1293–1298, 1981.
101. Strauss, B. S. Cellular aspects of DNA repair. Adv. Cancer Res., *45:* 45–105, 1985.
102. Babich, M. A., and Day, R. S., III. Synergistic killing of virus-transformed human cells with interferon and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. Carcinogenesis (Lond.), *10:* 265–268, 1989.
103. Yagi, T., and Day, R. S., III. Differential sensitivities of transformed and untransformed murine cell lines to DNA cross-linking agents relative to repair of O^6-methylguanine. Mutat. Res., *184:* 223–227, 1987.
104. Arita, I., Tachibana, A., Takebe, H., and Tatsumi, K. Predominance of Mex$^+$ cells in newly established human lymphoblastoid cell lines. Carcinogenesis (Lond.), *10:* 2067–2073, 1989.
105. Green, M. H. L., Lowe, J. E., Petit-Frere, C., Karran, P., Hall, J., and Kataoka, H. Properties of *N*-methyl-*N*-nitrosourea-resistant, Mex$^-$ deriva-

Downloaded from http://aacrjournals.org/cancerres/article-pdf/50/19/6119/2440997/cr0500196119.pdf by guest on 24 August 2022

tives of an SV40-immortalized human fibroblast cell line. Carcinogenesis (Lond.), *10:* 893–898, 1989.
106. Karran, P., Stephenson, C., Macpherson, P., Cairns-Smith, S., and Priestley, A. Coregulation of the human O^6-methylguanine-DNA methyltransferase with two unrelated genes that are closely linked. Cancer Res., *50:* 1532–1537, 1990.
107. Waldstein, E. A., Cao, E. H., and Setlow, R. B. Adaptive resynthesis of the O^6-methylguanine acceptor protein can explain the differences between mammalian cells proficient and deficient in methyl excision repair. Proc. Natl. Acad. Sci. USA, *79:* 5117–5121, 1982.
108. Fornace, A. J., Papathanasiou, M., Hollander, M. C., and Yarosh, D. B. Expression of the O^6-alkylguanine-DNA alkyltransferase gene in mer$^+$ and mer$^-$ human tumor cells. Proc. Am. Assoc. Cancer Res., *31:* 346, 1990.
109. Pieper, R. O., Futscher, B. W., Dong, Q., Ellis, T. M., and Erickson, L. C. Comparison of O^6-methylguanine DNA methyltransferase (MGMT) mRNA levels in Mer$^+$ and Mer$^-$ human tumor cell lines containing the *MGMT* gene by the polymerase chain reaction technique. Cancer Commun., *2:* 13–20, 1990.
110. Mitani, H., Yagi, T., Leiler, C. Y., and Takebe, H. 5-Azacytidine-induced recovery of O^6-alkylguanine-DNA alkyltransferase activity in mouse Ha821 cells. Carcinogenesis (Lond.), *10:* 1879–1882, 1989.
111. Morten, J. E. N., and Margison, G. P. Increased O^6-alkylguanine-DNA alkyltransferase activity in Chinese hamster V79 cells following selection with chloroethylating agents. Carcinogenesis (Lond.), *9:* 45–49, 1988.
112. Dolan, M. E., Norbeck, L., Clyde, C., Hora, N. K., Erickson, L. C., and Pegg, A. E. Expression of mammalian O^6-alkylguanine-DNA alkyltransferase in a cell line sensitive to alkylating agents. Carcinogenesis (Lond.), *10:* 1613–1619, 1989.
113. Brennand, J., and Margison, G. P. Reduction of the toxicity and mutagenicity of alkylating agents in mammalian cells harboring the *Escherichia coli* alkyltransferase gene. Proc. Natl. Acad. Sci. USA, *83:* 6292–6296, 1986.
114. Samson, L., Derfler, B., and Waldstein, E. A. Suppression of human DNA alkylation-repair defects by *Escherichia coli* DNA-repair genes. Proc. Natl. Acad. Sci. USA, *83:* 5607–5610, 1986.
115. Kataoka, H., Hall, J., and Karran, P. Complementation of sensitivity to alkylating agents in *Escherichia coli* and Chinese hamster ovary cells by expression of a cloned bacterial repair gene. EMBO J., *5:* 3195–3200, 1986.
116. Ishizaki, K., Tsujimura, T., Yawata, H., Fujio, C., Nakabeppu, Y., Sekiguchi, M., and Ikenaga, M. Transfer of the *E. coli* O^6-methylguanine methyltransferase gene into repair-deficient human cells and restoration of cellular resistance to *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. Mutat. Res., *166:* 135–141, 1986.
117. Brennand, J., and Margison, G. P. Expression in mammalian cells of a truncated *Escherichia coli* gene coding for O^6-alkylguanine alkyltransferase reduces the toxic effects of alkylating agents. Carcinogenesis (Lond.), *7:* 2081–2084, 1986.
118. Ishizaki, K., Tsujimura, T., Fujio, C., Yangpei, Z., Yawata, H., Nakabeppu, Y., Sekiguchi, M., and Ikenaga, M. Expression of the truncated *E. coli* O^6-methyltransferase gene in repair-deficient human cells and restoration of cellular resistance to alkylating agents. Mutat. Res., *184:* 121–128, 1987.
119. Hall, J., Kataoka, H., Stephenson, C., and Karran, P. The contribution of O^6-methylguanine and methylphosphate triesters to the cytotoxicity of alkylating agents in mammalian cells. Carcinogenesis (Lond.), *9:* 1587–1593, 1988.
120. Brennand, J., and Margison, G. P. Expression of the *E. coli* O^6-methylguanine-methylphosphotriester methyltransferase gene in mammalian cells. Carcinogenesis (Lond.), *7:* 185–188, 1986.
121. Waldstein, E. A. Effect of O^6-methylguanine-DNA methyltransferase gene activity on repair in human cells transformed by a regulatable *ada* gene. Carcinogenesis (Lond.), *11:* 21–26, 1990.
122. Dumenco, L. L., Warman, B., Hatzoglou, M., Lim, I. K., Abboud, S. L., and Gerson, S. L. Increase in nitrosourea resistance in mammalian cells by retrovirally mediated gene transfer of bacterial O^6-alkylguanine-DNA alkyltransferase. Cancer Res., *49:* 6044–6051, 1989.
123. Matsukuma, S., Nakatsuru, Y., Nakagawa, K., Utakoji, T., Sugano, H., Kataoka, H., Sekiguchi, M., and Ishikawa, T. Enhanced O^6-methylguanine-DNA methyltransferase activity in transgenic mice containing an integrated *E. coli ada* repair gene. Mutat. Res., *218:* 197–206, 1989.
124. Lim, I. K., Dumenco, L. L., Yun, J., Donovan, C., Warman, B., Gorodetzkaya, N., Wagner, T. E., Clapp, W., Hanson, R. W., and Gerson, S. L. High level, regulated expression of the chimeric P-enolpyruvate carboxykinase (GTP)-bacterial O^6-alkylguanine-DNA alkyltransferase (*ada*) gene in transgenic mice. Cancer Res., *50:* 1701–1708, 1990.
125. Ding, R., Ghosh, K., Eastman, A., and Bresnick, E. DNA-mediated transfer and expression of a human DNA repair gene that demethylates O^6-methylguanine. Mol. Cell. Biol., *5:* 3293–3296, 1985.
126. Yarosh, D. B., Barnes, D., and Erickson, L. C. Transfection of DNA from a chloroethylnitrosourea-resistant tumor cell line (Mer$^+$) to a sensitive tumor cell line (Mer$^-$) results in a tumor cell line resistant to MNNG and CNU that has increased O^6-methylguanine-DNA methyltransferase levels and reduced levels of DNA interstrand crosslinking. Carcinogenesis (Lond.), *7:* 1603–1606, 1986.
127. Barrows, L. R., Borchers, A. H., and Paxton, M. B. Transfectant cells expressing O^6-alkylguanine-DNA-alkyltransferase display increased resistance to DNA damage other than O^6-guanine alkylation. Carcinogenesis (Lond.), *8:* 1853–1859, 1987.
128. Bignami, M., Terlizzese, M., Zijno, A., Calcagnile, A., Frosina, G., Abbondandolo, A., and Dogliotti, E. Cytotoxicity, mutations and SCEs induced by methylating agents are reduced in CHO cells expressing an active mammalian O^6-methylguanine-DNA methyltransferase gene. Carcinogenesis (Lond.), *8:* 1417–1421, 1987.
129. Bignami, M., Dogliotti, E., Aquilina, G., Zijno, A., Wild, C. P., and Montesano, R. O^6-Methyltransferase-deficient and -proficient CHO cells differ in their responses to ethyl- and methyl-nitrosourea-induced DNA alkylation. Carcinogenesis (Lond.), *10:* 1329–1332, 1989.
130. Ellison, K. S., Dogliotti, E., Connors, T. D., Basu, A. K., and Essigmann, J. M. Site-specific mutagenesis by O^6-alkylguanines located in the chromosomes of mammalian cells: influence of the mammalian O^6-alkylguanine-DNA alkyltransferase. Proc. Natl. Acad. Sci. USA, *86:* 8620–8624, 1989.
131. Scudiero, D. A., Meyer, S. A., Clatterbuck, B. E., Mattern, M. R., Ziolkowski, C. H. J., and Day, R. S., III. Relationship of DNA repair phenotypes of human fibroblasts and tumor strains to killing by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. Cancer Res., *44:* 961–969, 1984.
132. Kaina, B., Van Zeeland, A. A., Backendorf, C., Thielmann, H. W., and Van de Putte, P. Transfer of human genes conferring resistance to methylating mutagens, but not to UV irradiation and cross-linking agents into Chinese hamster ovary cells. Mol. Cell. Biol., *7:* 2024–2030, 1987.
133. Erickson, L. C., Bradley, M. O., Ducore, J. M., Ewig, R. A., and Kohn, K. W. DNA cross-linking and cytotoxicity in normal and transformed human cells treated with antitumor nitrosoureas. Proc. Natl. Acad. Sci. USA, *77:* 467–471, 1980.
134. Tsujimura, T., Zhang, Y. P., Fujio, C., Chang, H., Watatani, M., Ishizaki, K., Kitamura, H., and Ikenaga, M. O^6-Methylguanine methyltransferase activity and sensitivity of Japanese tumor cell strains to 1-(4-amino-2-methyl-5-pyrimidinyl)methyl-3-(2-chloroethyl)-3-nitrosourea hydrochloride. Jpn. J. Cancer Res., *78:* 1207–1215, 1987.
135. Satoh, M. S., Huh, N. H., Horie, Y., Thomale, J., Rajewsky, M. F., and Kuroki, T. Acquisition of resistance to 1-(4-amino-2-methyl-5-pyrimidinyl)methyl-3-(2-chloroethyl)-3-nitrosourea hydrochloride in V79 cells through increased removal of O^6-alkylguanine. Jpn. J. Cancer Res., *78:* 1094–1099, 1987.
136. Smith, D. G., and Brent, T. P. Response of cultured human cell lines from rhabdomyosarcoma xenografts to treatment with chloroethylnitrosoureas. Cancer Res., *49:* 883–886, 1989.
137. Robins, P., Harris, A. L., Goldsmith, I., and Lindahl, T. Cross-linking of DNA induced by chloroethylnitrosourea is prevented by O^6-methylguanine-DNA methyltransferase. Nucleic Acids Res., *11:* 7743–7758, 1983.
138. Bodell, W. J., Tokuda, K., and Ludlum, D. B. Differences in DNA alkylation products formed in sensitive and resistant human glioma cells treated with *N*-(2-chloroethyl)-*N*-nitrosourea. Cancer Res., *48:* 4489–4492, 1988.
139. Brent, T. P., Lestrud, S. O., Smith, D. G., and Remack, J. S. 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.
140. Dolan, M. E., Pegg, A. E., Hora, N. K., and Erickson, L. C. Effect of O^6-methylguanine on DNA interstrand cross-link formation by chloroethylnitrosoureas and 2-chloroethyl(methylsulfonyl)methanesulfonate. Cancer Res., *48:* 3603–3606, 1988.
141. Brent, T. P., and Remack, J. S. Formation of covalent complexes between human O^6-alkylguanine-DNA alkyltransferase and BCNU-treated defined length synthetic oligodeoxynucleotides. Nucleic Acids Res., *16:* 6779–6788, 1988.
142. Gonzaga, P. E., and Brent, T. P. Affinity purification and characterization of human O^6-alkylguanine-DNA alkyltransferase complexed with BCNU-treated, synthetic oligonucleotide. Nucleic Acids Res., *17:* 6581–6590, 1989.
143. Tokuda, K., and Bodell, W. J. Cytotoxicity and induction of sister chromatid exchanges in human and rodent brain tumor cells treated with alkylating agents. Cancer Res., *48:* 3100–3105, 1988.
144. Schwartz, J. L., Turkula, T., Sagher, D., and Strauss, B. The relationship between O^6-alkylguanine alkyltransferase activity and sensitivity to alkylation-induced sister chromatid exchanges in human lymphoblastoid cell lines. Carcinogenesis (Lond.), *10:* 681–685, 1989.
145. Trey, J. E., and Gerson, S. L. The role of O^6-alkylguanine DNA alkyltransferase in limiting nitrosourea-induced sister chromatid exchanges in proliferating human lymphocytes. Cancer Res., *48:* 1899–1903, 1989.
146. Gibson, N., Zlotogorski, C., and Erickson, L. C. Specific DNA repair mechanisms may protect some human tumor cells from DNA interstrand crosslinking by chloroethylnitrosoureas but not from crosslinking by other anti-tumor alkylating agents. Carcinogenesis (Lond.), *6:* 445–450, 1985.
147. Ducore, J. α-Difluoromethylornithine effects on nitrosourea-induced cytotoxicity and crosslinking in a methylation excision repair positive (mer$^+$) human cell line. Biochem. Pharmacol., *36:* 2169–2174, 1987.
148. Seidenfeld, J., and Sprague, W. S. Comparisons between sensitive and resistant human tumor cell lines regarding effects of polyamine depletion on chloroethylnitrosourea efficacy. Cancer Res., *50:* 521–526, 1990.
149. Zlotogorski, C., and Erickson, L. C. Pretreatment of human colon tumor cells with DNA methylating agents inhibits their ability to repair chloroethyl monoadducts. Carcinogenesis (Lond.), *5:* 83–87, 1984.
150. Zeller, W. J., Berger, M. R., Henne, T., and Weber, E. More than additive toxicity of the combination of 1-methyl-1-nitrosourea plus 1,3-bis(2-chloroethyl)-1-nitrosourea in the rat. Cancer Res., *46:* 1714–1716, 1986.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/50/19/6119/2440997/cr0500196119.pdf by guest on 24 August 2022

151. Gerson, S. L. Modulation of human lymphocyte O^6-alkylguanine-DNA alkyltransferase by streptozotocin *in vivo*. Cancer Res., *49:* 3134–3138, 1989.
152. Futscher, B. W., Micetich, K. C., Barnes, D. M., Fisher, R. I., and Erickson, L. C. Inhibition of a specific DNA repair system and nitrosourea cytotoxicity in resistant human cancer cells. Cancer Commun., *1:* 65–73, 1989.
153. Dolan, M. E., Morimoto, K., and Pegg, A. E. Reduction of O^6-alkylguanine-DNA-alkyltransferase activity in HeLa cells treated with O^6-alkylguanines. Cancer Res., *45:* 6413–6417, 1985.
154. Dolan, M. E., Corsico, C. D., and Pegg, A. E. Exposure of HeLa cells to O^6-alkylguanines increases sensitivity to the cytotoxic effects of alkylating agents. Biochem. Biophys. Res. Commun., *132:* 178–185, 1985.
155. Dolan, M. E., Young, G. S., and Pegg, A. E. Effect of O^6-alkylguanine pretreatment on the sensitivity of human colon tumor cells to the cytotoxic effects of chloroethylating agents. Cancer Res., *46:* 4500–4504, 1986.
156. Domoradzki, J., Pegg, A. E., Dolan, M. E., Maher, V. M., and McCormick, J. J. Depletion of O^6-methylguanine-DNA-methyltransferase in human fibroblasts increases the mutagenic response to *N*-methyl-*N'*-nitro-*N*-nitroguanidine. Carcinogenesis (Lond.), *6:* 1823–1826, 1985.
157. Yarosh, D. B., Hurst-Calderone, S., Babich, M. A., and Day, R. S., III. Inactivation of O^6-methylguanine-DNA methyltransferase and sensitization of human tumor cells to killing by chloroethylnitrosourea by O^6-methylguanine as a free base. Cancer Res., *46:* 1663–1668, 1986.
158. Dempke, W., Nehls, P., Wandl, U., Soll, D., Schmidt, C. G., and Osieka, R. Increased cytotoxicity of 1-(2-chloroethyl)-1-nitroso-3-(4-methyl)-cyclohexylurea by pretreatment with O^6-methylguanine in resistant but not in sensitive human melanoma cells. J. Cancer Res. Clin. Oncol., *113:* 387–391, 1987.
159. Thielmann, H. W., Edler, L., Müller, N., and Eisenbrand, G. 6-Methylguanine and 6-methylguanosine inhibit colony forming ability in a malignant xeroderma pigmentosum cell line but not in other xeroderma pigmentosum and normal human fibroblast strains after treatment with 1-(2-chloroethyl)-1-nitroso-3-(2-hydroxyethyl)-urea. J. Cancer Res. Clin. Oncol., *113:* 67–72, 1987.
160. Dolan, M. E., Larkin, G. L., English, H. F., and Pegg, A. E. Depletion of O^6-alkylguanine-DNA alkyltransferase activity in mammalian tissues and human tumor xenografts in nude mice by treatment with O^6-methylguanine. Cancer Chemother. Pharmacol., *25:* 103–108, 1989.
161. Dexter, E. U., Yamashita, T. S., Donovan, C., and Gerson, S. L. Modulation of O^6-alkylguanine-DNA alkyltransferase in rats following intravenous administration of O^6-methylguanine. Cancer Res., *49:* 3520–3524, 1989.
162. Likhachev, A., Anisimov, V., Zhukovskaya, N., and Petrov, A. Inactivation of O^6-alkylguanine-DNA alkyltransferase in the rat liver due to an intraperitoneal administration of methylated DNA. Carcinogenesis (Lond.), *9:* 1139–1142, 1988.
163. Bodell, W. J., Morgan, W. F., Rasmussen, J., Williams, M. E., and Deen, D. F. Potentiation of 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU)-induced cytotoxicity in 9L cells by pretreatment with 6-thioguanine. Biochem. Pharmacol., *34:* 515–520, 1985.
164. Kalamegham, R., Warmels-Rodenhiser, S., MacDonald, H., and Ebisuzaki, K. O^6-Methylguanine-DNA methyltransferase-defective human cell mutant: O^6-methylguanine, DNA strand breaks and cytotoxicity. Carcinogenesis (Lond.), *9:* 1749–1753, 1988.
165. Samson, L., and Linn, S. DNA alkylation repair and the induction of cell death and sister chromatid exchange in human cells. Carcinogenesis (Lond.), *8:* 227–230, 1987.
166. Goth-Goldstein, R., and Hughes, M. Characterization of a CHO variant in respect to alkylating agent-induced biological effects and DNA repair. Mutat. Res., *184:* 139–146, 1987.
167. Goldmacher, V. S., Cuzick, R. A., and Thilly, W. G. Isolation and partial characterization of human cell mutants differing in sensitivity to killing and mutation by methylnitrosourea and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. J. Biol. Chem., *261:* 12462–12471, 1986.
168. Ishida, R., and Takahashi, T. *N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine-resistant HeLa S3 cells still have little O^6-methylguanine-DNA methyltransferase activity and are hypermutable by alkylating agents. Carcinogenesis (Lond.), *8:* 1109–1113, 1987.
169. Aquilina, G., Frosina, G., Zijno, A., Di Muccio, A., Dogliotti, E., Abbondandolo, A., and Bignami, M. Isolation of clones displaying enhanced resistance to methylating agents in O^6-methylguanine-DNA methyltransferase-proficient CHO cells. Carcinogenesis (Lond.), *9:* 1217–1222, 1988.
170. Green, M. H. L., Karran, P., Lowe, J. E., Priestly, A., Arlett, C. F., and Mayne, L. Variation in the loss of O^6-methylguanine-DNA methyltransferase during immortalization of human fibroblasts. Carcinogenesis (Lond.), *11:* 185–187, 1990.
171. Kaina, B., van Zeeland, A. A., de Groot, A., and Natarajan, A. T. DNA repair and chromosomal stability in the alkylating agent-hypersensitive Chinese hamster cell line 27-1. Mutat. Res., *243:* 219–224, 1990.
172. Bodell, W. J., Aida, T., Berger, M. S., and Rosenblum, M. L. Increased repair of O^6-alkylguanine DNA adducts in glioma-derived human cells resistant to the cytotoxic and cytogenetic effects of 1,3-bis(2-chloroethyl)-1-nitrosourea. Carcinogenesis (Lond.), *7:* 879–883, 1986.
173. Ikenaga, M., Tsujimura, T., Chang, H. R., Fujio, C., Zhang, Y. P., Ishizaki, K., Kataoka, H., and Shima, A. Comparative analysis of O^6-methylguanine methyltransferase activity and cellular sensitivity to alkylating agents in cell strains derived from a variety of animal species. Mutat. Res., *184:* 161–168, 1987.
174. Fujio, C., Chang, H. R., Tsujimura, T., Ishizaki, K., Kitamura, H., and Ikenaga, M. Hypersensitivity of human tumor xenografts lacking O^6-alkylguanine-DNA alkyltransferase to the anti-tumor agent (1-(4-amino-2-methyl-5-pyrimidinyl)methyl-3-(2-chloroethyl)-3-nitrosourea. Carcinogenesis (Lond.), *10:* 351–356, 1989.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/50/19/6119/2440997/cr0500196119.pdf by guest on 24 August 2022