Induction of the alkyltransferase (MGMT) gene by DNA damaging agents and the glucocorticoid dexamethasone and comparison with the response of base excision repair genes

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Repair of alkylated bases in DNA is performed by \( O^6 \)-methylguaninedNA methyltransferase (MGMT) and a set of enzymes of the base excision repair pathway involving N-methylpurine-DNA glycosylase (MPG), apurinic endonuclease (APE), DNA polymerase \( \beta \) (Pol \( \beta \)) and DNA ligase. The level of expression of these enzymes may exert a profound effect on resistance of cells towards alkylating drugs. We have comparatively analyzed the expression of MGMT and the different base excision repair genes in rat hepatoma cells (line H4IIE) after exposure to alkylating agents, X-rays and the glucocorticoid hormone dexamethasone. Furthermore, the effect of these agents on the activity of the cloned human MGMT promoter was assayed. Exposure of cells to \( N \)-methyl-N'-nitro-N-nitrosoguanidine (MNNG) or ionizing radiation increased MGMT mRNA levels up to 4.5-fold. Under the same conditions of treatment, exerting only a weak toxic effect, MPG and DNA ligase I mRNA levels were not enhanced, whereas the amounts of APE and Pol \( \beta \) mRNA transiently increased by -2-fold after X-ray and MNNG treatment, respectively. Dexamethasone induced both MGMT, APE and Pol \( \beta \) mRNA and the induction paralleled the increase in mRNA of the glucocorticoid-dependent gene tyrosine aminotransferase. The observed increase in MGMT mRNA was due to promoter activation, which was shown in transient transfection assays with MGMT promoter-CAT reporter constructs in H4IIE cells. In these assays, the human MGMT promoter was found to be induced by methylating agents (MNNG and methyl methanesulfonate), ionizing radiation and dexamethasone. Weak induction of the promoter was observed after UV irradiation. Treatment with the tumor promoter 12-O-tetradecanoyl-phorbol-13-acetate was ineffective in promoter activation. The transfected MGMT promoter was not inducible by mutagens in HeLa S3 cells, which do not respond with induction of the endogenous MGMT gene. This is the first report showing hormone induction of a DNA repair gene (MGMT). The induction of MGMT and other genes encoding enzymes involved in DNA alkylation damage repair may be relevant in cancer therapy by causing resistance of tumor cells to alkylating drugs.

Introduction

Alkylated bases induced in DNA spontaneously, upon exposure of cells to carcinogens and after treatment with various anticancer drugs are repaired by two different pathways. The highly premutagenic and precarcinogenic lesions \( O^6 \)-methylguanine and, to lesser extent, \( O^4 \)-methylthymine are repaired by \( O^6 \)-methylguanine-DNA methyltransferase (MGMT*) in a fast and single step reaction (for reviews see 1,2). In contrast, \( N \)-methylpurines, the major adducts induced by alkylating agents, are removed from DNA by an excision repair process involving the action of at least five enzymes: \( N \)-methylpurine-DNA glycosylase (MPG), apurinic endonuclease (APE), exonuclease (e.g. dRPase), DNA polymerase \( \beta \) (Pol \( \beta \)) and DNA ligase (for overviews see 3,4). The specificity of this repair process is directed by the base recognizing enzyme MPG, which removes not only 7-methylguanine, 3-methyladenine and 3-methylthymine, but also 8-hydroxyguanine, 1,6-ethanoadenine and hypoxanthine from the DNA backbone (5-10). The resulting apurinic sites as well as those sites generated by spontaneous loss or enzymatic removal of other bases are subsequently repaired in several steps, starting with cleavage of the DNA strand at the abasic site by APE, sugar removal and resealing of the resulting gap by the enzymes listed above.

In Escherichia coli both MGMT (Ada protein) and MPG (Alk A protein) are induced upon exposure of cells to alkylating agents, which is the underlying cause of the adaptive response (11). For mammalian cells induction of repair genes is less clear. Most convincing are data obtained for MGMT showing that this repair protein is inducible at the mRNA and activity level, giving rise to increased protection of cells against the mutagenic effects of alkylating agents (12-16). Whether induction of MGMT in mammalian cells is accompanied by co-induction of alklylation damage excision repair genes is a matter of debate. Conflicting results have been obtained, especially for MPG (2,17-19).

There are data indicating that MGMT expression is not only inducible by DNA damaging agents. It may also be modulated through physiological changes and is affected by the hormonal status of the organism (20-22). The human MGMT promoter has been cloned (23), which allows a more precise analysis of regulation and, notably, induction of this repair gene. Whether the MGMT promoter can be activated by genotoxic stress and hormones has not yet been proven. Here we report that MGMT mRNA is induced in rat liver cells in vitro not only by DNA damaging treatments but also by the glucocorticoid hormone dexamethasone. Under conditions of treatment with DNA damaging agents or dexamethasone inducing MGMT mRNA, the human MGMT promoter (cloned in front of a reporter gene) was found to be activated, which strongly indicates that

*Abbreviations: MGMT, \( O^6 \)-methylguaninedNA methyltransferase; MPG, \( N \)-methylpurine-DNA glycosylase; APE, apurinic endonuclease; Pol \( \beta \), DNA polymerase \( \beta \); MNNG, \( N \)-methyl-N'-nitro-N-nitrosoguanidine; MMS, methyl methanesulfonate; TPA, 12-O-tetradecanoylphorbol-13-acetate; DMSO, dimethylsulfoxide; TAT, tyrosine aminotransferase; GAPDH, glyceraldehyde phosphate dehydrogenase; CAT, chloramphenicol acetyltransferase.
Fig. 1. Dose dependence of MGMT and MPG mRNA induction. H4IIE cells were treated with different doses of X-rays and MNNG. Twenty four hours later cells were harvested for RNA preparation. Filters were hybridized with radioactively labeled probes of MGMT, MPG and GAPDH. Quantitation was performed by laser densitometric scanning of the autoradiograms. Rehybridization with GAPDH cDNA served as an internal standard to correct for differences in amount of RNA on the filter. Induction factors were calculated in relation to the MGMT and MPG mRNA level of untreated cells.

the increases in MGMT mRNA observed upon mutagen and glucocorticoid hormone treatment are due to transcriptional activation of the MGMT gene.

To address the question of whether induction of the MGMT gene is accompanied by co-induction of other alkylation repair genes, we compared the expression of MGMT, MPG, APE, Pol β and DNA ligase I at the mRNA level. This is the first study in which the various DNA repair genes have been comparatively assayed for their inducibility. The data are taken to indicate that MGMT and the various genes involved in base excision repair are not coordinately up-regulated upon induction of DNA damage. Some of these genes seem, however, to be expressed simultaneously at an elevated level after exposure of cells to glucocorticoids.

Material and methods

Cell culture and transient transfection
Rat hepatoma cell line H4IIE, obtained from K.-L.Lee (Oak Ridge National Laboratories), was cultivated in alpha medium containing 5% fetal calf serum and 50 µg/ml gentamycin. HeLa S3 cells were grown in Dulbecco's MEM with 10% fetal calf serum. Cells were transiently transfected by calcium phosphate co-precipitation as described previously (24).

Drugs and cell treatment
N-Methyl-N'-nitro-N-nitrosoguanidine (MNNG), methyl methanesulfonate (MMS), dexamethasone and 12-O-tetradecanoylphorbol-13-acetate (TPA) were obtained from Sigma. MNNG [dissolved in dimethylsulfoxide (DMSO)] and MMS were diluted in sterile distilled water to a concentration of 10 and 100 mM respectively. Batches were stored at −80°C. Dexamethasone was dissolved in ethanol at a concentration of 10⁻⁴ M and stored at −20°C. TPA was dissolved in DMSO at 200 mg/ml and also stored at −20°C. These agents were added directly from the stocks to exponentially growing cells. The maximal final concentration of DMSO and ethanol in the medium did not exceed 0.1%. Exponentially growing cells were treated with ionizing radiation onto the culture dishes without removal of the medium using a ⁶⁰Co source (Atomic Energy of Canada Ltd) or a MOD 301/4 X-ray source (Phillips). For UV irradiation, cells were washed with phosphate-buffered saline and fresh medium was added immediately after irradiation.

Northern blot analysis
Total RNA from H4IIE cells was isolated by guanidinium isothiocyanate/phenol/chloroform extraction (25). Twenty micrograms of RNA from each sample were denatured with formaldehyde at 56°C and electrophoretically separated on a 1% agarose gel with 40 mM MOPS, 10 mM sodium acetate, 1 mM EDTA, pH 7.0, as running buffer. After transfer to a nylon membrane (Hybond N⁺; Amersham, Braunschweig, Germany) the RNA was fixed onto the membrane by UV cross-linking and sequentially hybridized with different cDNA probes: rat MGMT and MPG cDNAs were from Dr F.Laval (Paris), tyrosine amino transferase (TAT) from Dr G.Schütz (Heidelberg), Pol β from Dr S.Wilson (Galveston) and DNA ligase I from Dr T.Lindahl (London). The APE cDNA was cloned in our laboratory (B.K.) by PCR. cDNAs were labeled to a specific activity of 5×10⁶–10⁸ c.p.m./µg following the random priming procedure with Klenow DNA polymerase I (Prim It Kit; Stratagene, Heidelberg, Germany). Hybridization was performed in 1 M Na₂HPO₄, pH 7.2, 1 mM EDTA, pH 8, 7% SDS at 60°C. The filters were washed at 60°C with decreasing concentrations of SSC (2X, 1X, 0.5X and 0.1X SSC) in 0.5%
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Fig. 2. Time dependence of expression of DNA repair genes after X-ray treatment. Cells were exposed to X-rays (2 Gy) and cells were harvested for RNA extraction at the time points indicated. Northern hybridizations were performed with cDNAs of MGMT, MPG, APE, DNA ligase I, Pol β and GAPDH as probes. The signals were quantified by laser densitometric scanning of the autoradiograms and normalized in relation to GAPDH. Induction factors indicate the change in the amount of mRNA in relation to the corresponding non-treated control.

SDS until the signal-to-noise ratio did not decrease any further. The wet filters were wrapped in sealable plastic bags and exposed to X-ray films. For rehybridization, filters were stripped in 1% SDS at 80°C for 0.5 h, washed in 2X SSC and resealed in the wet state. mRNA induction factors were calculated from quantitation of signals in relation to glyceraldehyde phosphate dehydrogenase (GAPDH). Factors reproducibly above 1.8 were taken to indicate significant induction.

Reported gene analysis
H4IIE cells were co-transfected with 5–10 μg pCAT basic vector-derived plasmid pMGMT-CAT containing the 2.6 kb SstI–SstI human MGMT promoter fragment (23) and 1 μg plasmid pRSV-β-gal by calcium phosphate co-precipitation as described above. Eight to 15 h later cells were treated as indicated and harvested 48 h later to assay for the enzyme activities in the cell extracts. Variation in the transfection efficiency was corrected for by co-transfection with pRSV-β-gal, containing the bacterial β-galactosidase gene under control of the RSV promoter, and measuring the β-galactosidase activity in the same cell extract that was analyzed for chloramphenicol acetyltransferase (CAT) activity. The RSV promoter was not inducible under the treatment conditions applied.

β-Galactosidase activity measurement
In a standard reaction 50–250 μg protein were incubated in a 200 μl reaction solution (60 mM Na₂HPO₄, 40 mM NaH₂PO₄, 50 mM 2-mercaptoethanol, 1 mM MgCl₂) with O-nitrophenyl-β-D-galactoside overnight at 37°C. The addition of 250 μl 1 M Na₂CO₃ stopped the reaction. Relative β-galactosidase activity was determined by measuring absorption at 405 nm. As a control, β-galactosidase activity in cell extracts from non-transfected cells was measured.

CAT activity assay
CAT activity in cell extracts was quantified by measuring the transfer of acetyl groups from acetyl-CoA to [¹⁴C]chloramphenicol (26). Cell extract protein (50–250 μg) was incubated in a final volume of 200 μl in 0.25 M Tris–HCl, pH 8.0, at 37°C after addition of 20 μl 4 mM acetyl-CoA and 0.5 μl [¹⁴C]chloramphenicol (0.1 μCi final concentration; Amersham). The reaction was stopped and extracted with 1 ml ethyl acetate. The solvent was evaporated and the dried chloramphenicol was redissovled in 20 μl ethyl acetate. Separation of acetylated from non-acetylated [¹⁴C]chloramphenicol was performed by thin layer chromatography on a silica gel using chloroform/methanol (9:1 v/v). The relative amount of acetylated [¹⁴C]chloramphenicol was determined by autoradiography using an automated phosphoimager (Fuji Corp.) CAT activity was calculated as percentage conversion of chloramphenicol to the acetylated forms. CAT activities of control extracts from cells that were transfected with the promoterless pCAT basic vector were negligible, but were nevertheless subtracted from the CAT activities obtained with the expression vectors.

Results

Induction of MGMT mRNA
MGMT mRNA is clearly inducible in H4IIE rat hepatoma cells upon treatment with ionizing radiation or MNNG. As shown in Figure 1, the amount of MGMT mRNA in H4IIE cells increased in a dose-dependent fashion, reaching saturation at ≥60 μM for MNNG. For ionizing radiation linearity of mRNA induction was observed with doses up to 8 Gy. A threshold in the response was not obvious. For comparison with MGMT, the dose dependence of expression of MPG mRNA was determined. There was a slight increase in MPG mRNA level with increasing dose of ionizing radiation (maximal 2-fold with high doses). No significant induction of
Fig. 3. Time dependence of expression of DNA repair genes after MNNG treatment. H4IIE cells were exposed to 50 μM MNNG and harvested for RNA preparation at the time points indicated. RNA was processed for Northern blot analysis as described in the legend to Figure 2.

MPG mRNA was observed 24 h after MNNG treatment with doses up to 60 μM.

**Kinetics of expression of MGMT and other base excision repair genes**

To elucidate whether induction of MGMT mRNA is accompanied by increased expression of other products of genes involved in repair of DNA alkylation damage, we studied at the mRNA level the simultaneous expression of MGMT, MPG, APE, Pol β and DNA ligase I. Various time points after mutagen treatment were chosen in order to monitor short- and long-term effects. The time dependence of mRNA expression for these genes after treatment with ionizing radiation and MNNG is shown in Figures 2 and 3, respectively. MGMT mRNA was induced by X-rays and MNNG with different kinetics. For ionizing radiation, induction occurred 6 h after exposure and reached a maximum 24 h later. For MNNG, induction is initiated at a later time (16 h) and the mRNA level gradually increased until 72 h after treatment. A slight increase in APE mRNA (~2-fold) was observed 0.5-24 h after irradiation, but not after MNNG exposure. Weak induction of Pol β mRNA was observed 72 h after MNNG treatment. Neither MPG nor DNA ligase I mRNA appeared to be significantly induced by these treatments.

**Effect of dexamethasone on expression of alkylation damage repair genes**

As shown in Figure 4, dexamethasone induced MGMT mRNA. The induction was significant (~5-fold) and transient, reaching a maximum at 16 h after addition of the hormone. It paralleled nearly exactly the time course of induction of TAT, which is a well-characterized glucocorticoid-responsive gene in liver cells and was therefore included as a control here. Induction of APE and Pol β mRNA (up to 2.6- and 1.8-fold, respectively) was also observed, which followed the same kinetics as MGMT and TAT.

**Induction of the human MGMT promoter**

The strong induction of MGMT mRNA after mutagen and dexamethasone treatment raised the question of whether these treatments lead to activation of the MGMT promoter. On transfecting H4IIE cells with a MGMT promoter–CAT construct which harbors the complete human MGMT promoter (see Figure 5A) and subsequently treating the cells with MNNG or ionizing radiation, a significant induction of CAT activity was observed (Figure 5A). Induction of MMS (4.5-fold induction; in some individual experiments an induction of up to 8-fold was observed). Treatment with dexamethasone also activated the MGMT–CAT reporter construct significantly (on average 4-fold). Irradiation with UV light (10 J/m²) exerted low promoter inducing activity (2.2-fold), whereas TPA was ineffective in inducing the MGMT promoter in these cells (Figure 5B). It should be noted that UV was effective in inducing MGMT mRNA, whereas TPA was not, in H4IIE cells (13,16; unpublished data). There was virtually no induction of the MGMT promoter–CAT construct transfected into HeLa S3 (Mex+ cells treated with MNNG,
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Fig. 4. Expression of DNA repair genes after dexamethasone treatment. Cells were treated with dexamethasone (10^{-7} M) and harvested for RNA preparation at the time points indicated. Northern hybridization was performed with cDNAs of MGMT, MPG, APE, Pol β, TAT and GAPDH as probes. The signals were quantified as described in the legend to Figure 1.

MMS or X-rays. The MGMT–CAT construct was also induced, however, in these cells by dexamethasone (Figure 5C).

Discussion

It has been shown in various studies that expression of MGMT is cell- and tissue-type specific, related to the status of differentiation and may undergo alteration during immortalization and malignant transformation (2). The highly variable expression of MGMT indicates that multiple factors are involved in regulation of the basal level of expression of this gene. An important peculiarity of MGMT pertains to its inducibility by various stress factors. Induction of MGMT mRNA and repair activity has been shown in rat, mouse and human cell lines in vitro (12–16,27) and in rat and mouse tissue in vivo (28–30) upon exposure to ionizing radiation and chemical mutagens. Interestingly, all DNA damaging treatments tested so far, including electroporation of restriction enzymes, were able to induce MGMT mRNA and protein in H4IIE cells (16), which suggests that MGMT induction is part of a general cellular response to DNA damage. Enhancement of MGMT mRNA and repair activity was shown to be inhibited by actinomycin D and cycloheximide (13,16,18), which indicates that de novo RNA and protein synthesis are required for induction. To our knowledge, run-on data, which would give final proof of transcriptional activation of the gene, are not available for MGMT, probably due to low transcriptional activity of the gene (13).

This study was aimed at elucidating whether: (i) mutagenic treatments that give rise to induction of MGMT mRNA are able to activate the MGMT promoter, as measured by transfection of a reporter construct in competent cells; (ii) MGMT mRNA and its promoter can be induced by dexamethasone, which is frequently utilized in the therapy of tumors and other diseases; (iii) induction of MGMT is accompanied by an increase in mRNA level of genes involved in excision repair of DNA alkylation lesions. Here we provide evidence that the human MGMT promoter can be activated by ionizing radiation and alkylating agents in rat hepatoma H4IIE cells, which respond to these treatments by induction of MGMT mRNA. The level of activation of the MGMT promoter was comparable with the induction level of MGMT mRNA (~5-fold). These data strongly suggest that the observed increases in MGMT mRNA and the corresponding repair activity were due to transcriptional activation of the MGMT gene. Interestingly, there was no MGMT promoter–CAT induction by MNNG, MMS and ionizing radiation in HeLa S3 cells, which are, like H4IIE cells, MGMT proficient (Mex^{+}), but unable to respond to these treatments with increase in MGMT mRNA level (13). Hence we infer that in HeLa S3 cells one or various factors that stimulate MGMT promoter activity are not present.
reproducibly induced and at a high level, as compared with these studies because in this cell line MGMT mRNA is most compared it with MGMT. We have chosen H4IIE cells for and DNA ligase I up to 73 h after mutagen treatment and studies, however, has the expression of different genes involved hypoxic conditions in colon cancer cells (34). In none of these human fibroblasts (33) and APE expression increased under also, DNA ligase I was induced by UV light in primary cells by alkylating agents and hydrogen peroxide (31,32).

Thus, Pol (3 was shown to be inducible in Chinese hamster on induction of DNA repair genes by DNA damaging agents.

This difference in time course supports a model according to which DNA strand breaks are involved in triggering MGMT induction (16). These may be induced either immediately by ionizing radiation or indirectly and delayed by MNNG as a consequence of repair of damaged bases and replication blocks.

A substantial new finding provided here pertains to the induction of the MGMT gene by dexamethasone, which was shown both in MGMT promoter–CAT transfection experiments and by analysis of MGMT mRNA level. Interestingly, the time course of induction of MGMT mRNA exactly paralleled TAT mRNA expression, indicating co-regulation of both glucocorticoid-dependent genes. It should be noted that the MGMT promoter contains two glucocorticoid-responsive elements (23) and is therefore potentially inducible by glucocorticoids. It was also of interest to see that in HeLa S3 cells the MGMT promoter can be stimulated by dexamethasone, but not by DNA damaging agents, which is in line with the view that different signal transduction pathways are involved in induction of MGMT by glucocorticoid hormone and DNA damaging agents.

A comparison of the effect of dexamethasone on the mRNA level of MGMT with that of excision repair genes revealed that, in addition to MGMT, APE and Pol β mRNA were increased by the glucocorticoid hormone, although to a much lesser extent than MGMT. In a previous study we have concluded from the effect of overexpression of MPG that this repair enzyme is probably not rate limiting in excision repair (35). However, if APE and Pol β were limiting in amount, their induction under conditions of genomic stress might increase the overall excision repair capacity of the cell. In fact, changes in APE and Pol β activity in transfected and knockout cells increased their sensitivity to methylating agents (36,37). Whether the increases in APE and Pol β mRNA observed here actually lead to an increase in overall excision repair capacity of the induced cells remains to be determined.

Dexamethasone is frequently utilized as an anti-inflammatory and immunosuppressive drug. It is applied during tumor therapy in combination with antineoplastic agents, including nitrosoureas (e.g. carmustine) and hydrazine derivatives (e.g. procarbazine and dacarbazine), against which MGMT

or cannot be activated upon mutagen exposure. Taken together, the data clearly allow us to conclude that MGMT is a DNA damage-inducible repair gene in mammalian cells whose response appears to depend on particular, not yet defined, cell type-specific factors.

Apart from the studies on MGMT, there are only few reports on induction of DNA repair genes by DNA damaging agents. Thus, Pol β was shown to be inducible in Chinese hamster cells by alkylating agents and hydrogen peroxide (31,32). Also, DNA ligase I was induced by UV light in primary human fibroblasts (33) and APE expression increased under hypoxic conditions in colon cancer cells (34). In none of these studies, however, has the expression of different genes involved in repair of a specific DNA damage been studied comparatively. We have monitored the mRNA levels of MPG, APE, Pol β and DNA ligase I up to 73 h after mutagen treatment and compared it with MGMT. We have chosen H4IIE cells for these studies because in this cell line MGMT mRNA is most reproducibly induced and at a high level, as compared with other cell lines (13,19). Under the treatment conditions which increased MGMT mRNA significantly, only slight increases in mRNA level have been found for APE (~2-fold) after ionizing irradiation and for Pol β after MNNG exposure. MPG and DNA ligase I mRNA levels were not significantly enhanced after MnNG or X-ray treatment with doses that were sufficiently high to induce MGMT. Obviously, induction of the MGMT gene by X-rays and MnNG is not accompanied by a general and significant co-induction of other genes involved in excision repair of DNA alkylolation lesions.

It should be noted that the induction experiments with Pol β reported previously (31,32) were performed with CHO cells that are Mex- (MGMT-deficient) and therefore more sensitive to the genotoxic effect of MnNG than the cell line we have used here. The doses utilized in our time course experiments exerted only slight toxic effects. Since most cells were able to survive, the observed induced responses may be biologically relevant. We should also stress that induction of MGMT mRNA after treatment with MnNG and ionizing radiation followed different kinetics. Thus, after irradiation the increase in mRNA level was observed 6 h after irradiation and achieved a maximum 24 h thereafter, whereas after MnNG treatment induction occurred at a significantly later stage, with peak level 72 h after addition of the mutagen. This difference in time course supports a model according to which DNA strand breaks are involved in triggering MGMT induction (16). These may be induced either immediately by ionizing radiation or indirectly and delayed by MnNG as a consequence of repair of damaged bases and replication blocks.

A substantial new finding provided here pertains to the induction of the MGMT gene by dexamethasone, which was shown both in MGMT promoter–CAT transfection experiments and by analysis of MGMT mRNA level. Interestingly, the time course of induction of MGMT mRNA exactly paralleled TAT mRNA expression, indicating co-regulation of both glucocorticoid-dependent genes. It should be noted that the MGMT promoter contains two glucocorticoid-responsive elements (23) and is therefore potentially inducible by glucocorticoids. It was also of interest to see that in HeLa S3 cells the MGMT promoter can be stimulated by dexamethasone, but not by DNA damaging agents, which is in line with the view that different signal transduction pathways are involved in induction of MGMT by glucocorticoid hormone and DNA damaging agents.
was shown to exert protection (38–40). Data are available to indicate that of cells enzymes involved in the base excision repair pathway may also affect on the level of protection against alkylating cytostatic drugs (41,42). Thus the possibility arises that induction by demethasone of MGMT as well as genes participating in excision repair in tumors may reduce the curative effect of alkylating tumor therapeutic drugs when applied in combination with glucocorticoids. It would thus be of importance to elucidate in further studies whether the expression of MGMT and other DNA repair genes increases in human normal and tumor tissue upon treatment of individuals with glucocorticoid hormones.

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