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Overexpressed human metallothionein IIA gene protects Chinese hamster ovary cells from killing by alkylating agents

(γ irradiation/bleomycin/methyltransferase/methylnitrosourea/UV response)

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ABSTRACT **Experiments were designed to detect survival advantages that cells gain by overexpressing metallothionein (MT). Chinese hamster ovary K1-2 cells and an x-ray-sensitive derivative were transfected with a bovine papillomavirus (BPV)-linked construct carrying the human metallothionein IIA (hMT-II_A) gene. Transfectants survived 40-fold higher levels of cadmium chloride, harbored at least 30 copies of hMT-II_A, and contained 25- to 166-fold more MT than the parent cells. Even under conditions of reduced glutathione synthesis, the transfectants were not more resistant to the lethal effects of ionizing radiation and bleomycin than the parent cells. Thus free radicals generated by these agents cannot be scavenged efficiently by MT *in vivo*. The hMT-II_A transfectants, however, but not control transfectants harboring a BPV-MT promoter-neo construct, tolerated significantly higher doses of the alkylating agents *N*-methyl-*N*-nitrosourea and *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine. Resistance and MT overexpression occurred irrespective of selection and cultivation in cadmium and zinc. There was no increase in resistance to methyl methanesulfonate and *N*-hydroxyethyl-*N*-chloroethylnitrosourea. MT did not affect the degree of overall DNA methylation after *N*-methyl-*N*-nitrosourea treatment nor the level of O^6-methylguanine-DNA methyltransferase. The results suggest that MT participates as a cofactor or regulatory element in repair or tolerance of toxic alkylation lesions.**

Metallothioneins (MTs) are expressed in all cells of a large variety of species. Their physiologic function is not understood (1). Expression of the MT genes is inducible. The synthesis of the human MT-II_A gene *in vivo* or in cell culture is enhanced by cadmium (ref. 2; see also earlier references in the review articles, refs. 1 and 3), interleukin 1 (4, 5), glucocorticoid hormones (6, 7), phorbol ester (8), ultraviolet (UV) irradiation (8), mitomycin C (MMC) (8), and the UV-induced extracellular protein synthesis-inducing factor EPIF (8, 9). The type of regulation suggests a protective function and a role in cell growth and proliferation. Only one example of protection has yet been verified: the protection against the toxic effects of certain heavy metals, including Cd (1–3, 10). Purified MT protein was reported to act as a scavenger of free hydroxyl radicals (11), a major radiolysis product of water. Cd treatment of human cells in culture (12) or of mice (13, 14) caused slight increases of radiation tolerance. Furthermore, tumor cells resistant to cisplatin often had high MT levels (15–18), which has been interpreted to indicate a role of direct MT–drug interaction in the development of resistance.

The experiments presented here were designed to test the question of radiation protection by MTs. To this end we introduced an extrachromosomally encoded hMT-II_A gene into two types of rodent cells: the "wild-type" strain Chinese hamster ovary (CHO) K-1 and a derivative with increased sensitivity to ionizing radiation. These transfectants and their parent cells allowed us to examine variations in the content of MTs by more than two orders of magnitude for an influence on radiation protection. Neither in hypoxic nor oxygenated conditions nor with reduced levels of glutathione could we detect any protective effect of MT against ionizing radiation. With the idea that MT could serve as an intracellular zinc donor for a number of enzymes and thus assist indirectly in the repair of lethal damage, we tested a series of other toxic agents. In these experiments we detected a type of protection that may have implications for the development of drug resistance in cancer treatment. Several MT-overexpressing cell lines were dramatically more resistant to the monofunctional alkylating agents *N*-methyl-*N*-nitrosourea (MNU) and *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG) (19, 20). In a similar approach, increased resistance to cisplatin, merphalan, and chlorambucil has recently been reported (18).

MATERIALS AND METHODS

Cell Culture. CHO wild-type cells K1 (subclone 2; K1-2) and the x-ray-sensitive derivative, xrs-2, were obtained from P. Jeggo (21). Bcl1 is a subclone of xrs-2. Cells were kept in alpha-MEM supplemented with 10% inactivated fetal calf serum and 30 μg of gentamycin per ml in a humidified atmosphere containing 5% CO_2. HeLa S3 cells were from the American Type Culture Collection. HeLa MR was kindly provided by R. Goth-Goldstein (Berkeley). HeLa cells were cultivated in F10 medium/Dulbecco's modified Eagle medium (F10/DMEM) (1:1) with 10% inactivated fetal calf serum.

Chemicals and Enzymes. Bleomycin was a gift of Mack Corp. (Heidelberg). MNU, MNNG, and methyl methanesulfonate (MMS) were obtained from Sigma. MNU was recrystallized before use. *N*-Hydroxyethyl-*N*-chloroethylnitrosourea (HeCNU) was kindly provided by G. Eisenbrand (Heidelberg). [^{14}C]MNU (specific activity, 56 mCi/mmol; 1 Ci = 37 GBq), the nick-translation kit, and [^{32}P]dCTP were supplied by Amersham. Restriction enzymes were from Boehringer Mannheim. Buthionine sulfoximine (BSO) was from Chemical Dynamics (South Plainfield, NJ). Protein concentrations were estimated using the assay of Bio-Rad.

DNA-Mediated Gene Transfer. One day prior to transfection, 7×10^5 cells were plated in a 10-cm Petri dish. Two micrograms of the plasmid pMTII-BPV(+) (where BPV indicates bovine papillomavirus) (10) and 20 μg of CHO carrier DNA per plate were transfected as described (22, 23).

Abbreviations: MT, metallothionein; MMC, mitomycin C; MNNG, *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine; MNU, *N*-methyl-*N*-nitrosourea; ENU, *N*-ethyl-*N*-nitrosourea; MMS, methyl methanesulfonate; HeCNU, *N*-hydroxyethyl-*N*-chloroethylnitrosourea; BSO, buthionine sulfoximine; CHO, Chinese hamster ovary; BPV, bovine papillomavirus.

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Two days after transfection $CdCl_2$ (20 μM) and $ZnCl_2$ (70 μM) were added to the medium to select for MT-overexpressing cells. Alternatively, MT overexpressors were obtained by cotransfection of 15 μg of pMTII-BPV and 5 μg of pSV2neo (24) and selection with G418 at 1.5 mg/ml. Transfectants were grown routinely in alpha-MEM containing $CdCl_2$ and $ZnCl_2$ or G418, respectively. $CdCl_2$ and $ZnCl_2$ were omitted, where indicated. In control experiments cells were transfected with 5 μg of pdBPV-MMTneo (25) and 15 μg of carrier DNA per dish. Selection was performed 2 days later with G418 (1.5 mg/ml).

Preparation of Nucleic Acids and Hybridization. Extrachromosomal DNA was extracted as described (26). Genomic DNA was prepared after isolation of nuclei, lysis, proteinase K treatment, and phenol/chloroform extraction (27). Total RNA was extracted after lysis of cells in 7 M urea (27). Hybridizations were performed on nitrocellulose-immobilized DNA or RNA (28). The amounts of MT RNA were quantitated as described (29).

Determination of Cell Survival. *γ Irradiation.* Cells (1.5×10^5) were plated in 50-ml culture flasks 24 hr prior to γ irradiation at a dose rate of 0.75 Gy/min (^{60}Co-Gammacell 200; Atomic Energy, Ottawa). Immediately thereafter the cells were trypsinized, counted, and plated for surviving colony determination.

Irradiation under hypoxic conditions. One hour prior to irradiation, the cells were equilibrated with oxygen-free nitrogen, kept oxygen-free during irradiation, and, after irradiation, treated like oxygenated samples.

Glutathione depletion and γ-glutamylsynthetase inhibition. Cells were incubated with BSO at 5 μg/ml for 24 hr (30).

Bleomycin. Cells were exposed for 1 hr, washed with phosphate-buffered saline (PBS), trypsinized, and reseeded for colony formation.

Alkylating agents. MNNG and MNU were solubilized in a small volume of dimethyl sulfoxide followed by the addition of distilled water. MMS and HeCNU were dissolved in distilled water directly. Batches of mutagen stocks were stored at −80°C and thawed only once just before use (31). Three hundred cells were seeded per 5-cm dish and 4 hr later treated with the mutagens at 37°C for 60 min. Thereafter cells were rinsed with PBS and fed with fresh medium. Colonies were fixed 8 days after seeding.

Determination of DNA Alkylation. Cells (2×10^6) were seeded into a 10-cm dish. After 18 hr of cultivation cells were rinsed and treated with medium (2.5 ml per plate) containing 1% serum and [^{14}C]MNU for 60 min (diluted with unlabeled MNU to a specific activity of 31 μCi/mg; 37°C; 6% CO_2). After alkylation cells were rinsed with PBS and lysed with 1% SDS/10 mM Tris/1 mM EDTA, pH 8.0. DNA was purified by treatment with proteinase K and RNase and extracted with phenol/chloroform (27).

Quantitation of O^6-Methylguanine-DNA Methyltransferase and MT. Cells of subconfluent cultures were rinsed twice with cold PBS, scraped off, and pelleted by centrifugation. Crude cell extracts were prepared by sonication in 20 mM Tris·HCl, pH 8.5/1 mM EDTA/1 mM 2-mercaptoethanol/5% glycerol, frozen in liquid nitrogen, and stored at −80°C. Methyltransferase activity was assayed as ^{3}H transfer to protein (32) from salmon sperm DNA that had been alkylated by [^{3}H]MNU and partially depurinated (33). The DNA (1.3×10^3 cpm per assay) contained 70% of ^{3}H activity as O^6-methylguanine. Values were corrected using bovine serum albumin as a control. The amount of MT in cell extracts was determined by the Cd-saturation assay as described (34).

RESULTS

Generation of MT-Overexpressing Transfectants. CHO K1-2 and the x-ray-sensitive mutant Bc11 were transfected with the plasmids pMTII-BPV (10), pdBPV-MMTVneo (25), pSV2neo (24), or a combination of pMTII-BPV and pSV2neo. Stable Cd-resistant pMTII-BPV transfectants were isolated either by Cd or by G418 selection. Four pMTII-BPV transfectants (Cd/Zn-selected K1-2MT, Bc11MT, and K1-2MT-C3 and the G418-selected clone K1-2 pMTII-BPV/pSV2neo) and several control transfectants were chosen for further experiments. The pMTII-BPV transfectants contained ≈30 copies of extrachromosomal pMTII-BPV DNA per cell (examples in Fig. 1*A*). In K1-2MT, a small fraction of the plasmid had concatemerized. Neither integration of hMT-II_A nor amplification of the endogenous MT genes was detectable by Southern analysis of restricted chromosomal DNA (not shown). The population doubling time of the MT transfectants was slightly prolonged (e.g., 15 hr for K1-2MT and 17.6 hr for Bc11MT as compared to 11.4 hr and 13.2 hr for the parent cells).

In agreement with previous results with other cells (10), the transfectants contained transcripts of the hMT-II_A gene even in the absence of heavy metal induction (Fig. 1*B*). This is due to the strong enhancer present in the BPV fragment of the construct (10) and to the large copy number. The size of the transcripts was identical to that of human MT mRNA (not shown), and the probe did not cross-hybridize with CHO sequences. MT transcripts were induced by Cd and Zn about 5- and 2-fold in the strains K1-2MT and Bc11MT, respectively. The weak MT induction by Cd in the transfectants is consistent with previous findings (10).

An important parameter for the intended study of MT function is the overall level of MT protein. Based on Cd binding activity of partially purified extracts, parental K1-2 and Bc11 contained <0.2 μg of MT per mg of total protein, whereas the Cd-selected transfectants K1-2MT, K1-2MT-C3, and Bc11MT contained 30.8, 15.2, and 33.2 μg of MT per mg, respectively. The G418-selected clone K1-2 pMTII-BPV/pSV2neo contained 22.6 μg of MT per mg. Independently, MT was quantitated by labeling with [^{35}S]cysteine. Less than 4% of the radioactivity incorporated into protein was in MT in K1-2, whereas 98% was in MT in the noninduced K1-2MT. Thus, the overexpressors contained between 25 and 166 times the control cell level of MT.

MT Overexpression Does Not Protect from Toxic Effects of γ Irradiation and Bleomycin. To cover direct DNA damage, which requires the presence of oxygen, and indirect damage

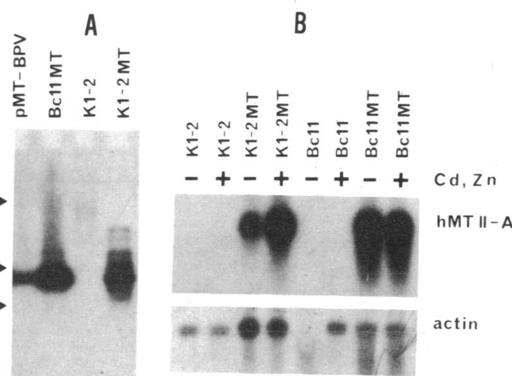

FIG. 1. (*A*) Blot hybridization of *Eco*RI-digested DNA of Hirt extracts from K1-2 and from the transfectants Bc11MT and K1-2MT with [^{32}P]dCTP-labeled pMTII-BPV DNA. Linearized pMTII-BPV DNA served as control. The size markers indicate 23.7, 10.0, and 6.7 kilobase pairs. (*B*) Blot hybridization of RNA extracted from K1-2 and Bc11 and the corresponding transfectants, K1-2MT and Bc11MT. Cells were incubated in the presence (+) or absence (−) of Cd and Zn for at least 1 week before RNA extraction. Ten micrograms of total RNA was applied per slot. A [^{32}P]dCTP-labeled *Hin*dIII fragment of the hMT-IIA gene isolated from pMTII-BPV was used as hybridization probe. To demonstrate the presence of RNA on the filter, rehybridization was performed with an actin probe.

by hydroxyl radicals, survival after γ irradiation under oxygenated and anoxic conditions was determined. Under oxygenated conditions (Fig. 2 *A* and *D*), there was no increase in the survival of the MT-overexpressing cells. The wild-type strain K1-2 was even slightly more resistant than its derivative K1-2MT. Also the x-ray-sensitive strain Bc11 did not benefit from overexpressing hMT-II_A. Accordingly, the induction of the transfected MT genes by low concentrations of Cd (2 μM) and Zn (10 μM) did not alter the sensitivity to killing by γ rays (data not shown, and Fig. 2 *A* and *D*). Under hypoxic conditions, the toxic effects of γ rays were reduced (Fig. 2 *B* and *E*). Again, no increase of resistance by MT overproduction could be detected: the pairs K1-2/K1-2MT and Bc11/Bc11MT have identical survival curves. To rule out the possibility that a contribution of MT-II_A to radiation protection was concealed by high levels of the radical scavenger glutathione, Bc11 and Bc11MT cells were depleted of glutathione by cultivation in medium containing BSO. BSO pretreatment sensitized the cells, but overexpression of the hMT-II_A gene did not improve survival (Fig. 2*E*).

Results similar to those obtained after γ irradiation were obtained with the radiomimetic drug bleomycin (consistent with data shown in ref. 18). Bc11 was more sensitive to bleomycin than K1-2 (Fig. 2 *C* and *F*). Overexpression of hMT-II_A did not improve survival, irrespective of pretreatment with Cd and Zn. The results suggest that MT does not protect against the toxic effects of radicals generated by either radiation or bleomycin.

MT Transfectants Are Resistant to MNNG/MNU-Induced Killing. The sensitivity of the transfectants to alkylating agents was measured after cultivation either in the presence or absence of Cd and Zn. The MT overexpressors were significantly more resistant to MNU (examined for only two transfectants; Fig. 3 *A* and *E*) and MNNG (Fig. 3 *B* and *F* and Fig. 4) than the parental strains K1-2 and Bc11 or the control transfectants. The degree of resistance was the same whether or not the cells had been cultivated in medium with Cd and Zn several days prior to alkylation. This is consistent with the high basal MT expression in the transfectants. Overexpression of MT did not improve survival after treatment with two

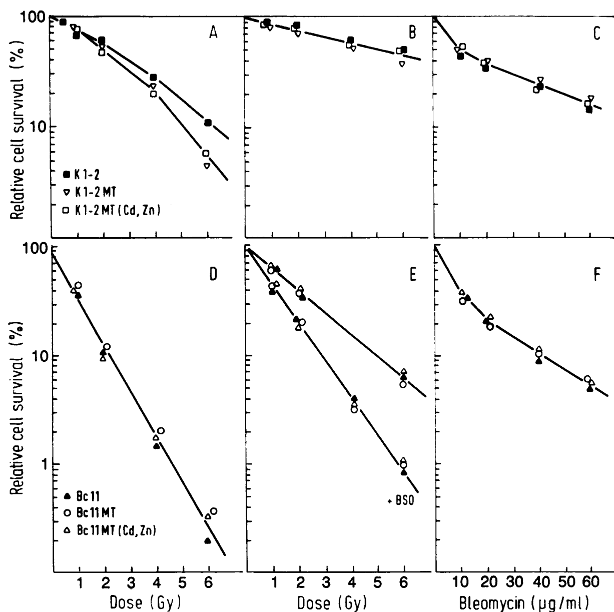

FIG. 2. Survival of parental cells and transfectants of strain K1-2 (*A–C*) and Bc11 (*D–F*) as a function of dose of γ radiation and bleomycin. Irradiation was performed under oxygenated (*A* and *D*) or anoxic (*B* and *E*) conditions. Transfectants were grown, before plating, in medium with or without the addition of Cd or Zn.

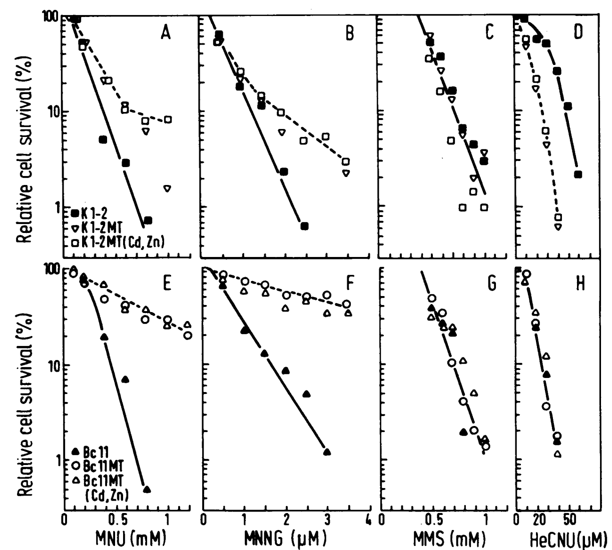

FIG. 3. Survival of parental cells and MT-overexpressing transfectants of strain K1-2 (*A–D*) and Bc11 (*E–H*) as a function of dose of MNU, MNNG, MMS, and HeCNU. Transfectants were grown before plating either continuously in Cd/Zn medium or, for at least 2 weeks, in medium not supplemented with Cd/Zn.

other alkylating agents: MMS and the chloroethylation-inducing agent HeCNU (Fig. 3 *C* and *G* and Fig. 3 *D* and *H*). For *N*-ethyl-*N*-nitrosourea (ENU) there was only a very slight increase of survival [D_o values (dose at which survival = $1/e$): K1-2, 1.1 mM; K1-2MT, 1.2 mM; Bc11, 0.8 mM; Bc11MT, 1.2 mM]. The alkylation resistance observed is a function of the MT coding region and not of the BPV vector. All control transfectants with pdBPV-MMTneo (25), a construct similar to pMTII-BPV (10) except for replacement of promoter and coding region, were MNNG sensitive (Fig. 4).

To distinguish whether MT overexpression acted prior to or after DNA alkylation, the extent of DNA methylation was analyzed. Parental cells and the transfectants K1-2MT and Bc11MT were treated with [^{14}C]MNU using the same dose range as in the survival experiments. Alkylation was a linear function of dose and there was no difference of the degree of overall DNA methylation between the parental cells and the transfectants (Fig. 5). Resistance to MNU of the MT-overproducing strains is therefore not due to a general reduction in the initial amounts of methylation derivatives in the DNA.

O^6-Methylguanine-DNA methyltransferase has been shown to be a determinant of cytotoxicity of MNNG and MNU in mammalian cells (35, 36). Therefore an influence of hMT-II_A on methyltransferase was considered. The activities in cell extracts were compared with those in a methyltransferase-proficient strain (HeLa S3) and in its methyltransferase-deficient derivative (HeLa MR). Extracts of the CHO

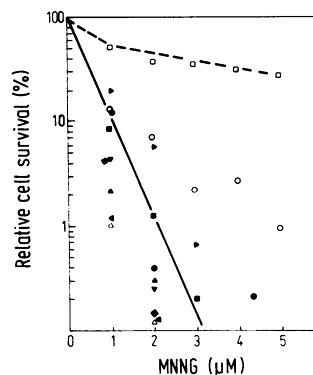

FIG. 4. Survival as a function of MNNG concentration of K1-2 cells (■), a pMTII-BPV transfectant clone (K1-2MT-C3) selected with Cd/Zn (□), a pMTII-BPV + pSVneo transfectant selected with G418 (○), a K1-2 pSV2neo transfectant (△), and several pdBPV-MMTneo-transfected clones selected with G418 (▲, ●, ▲, ▼, ◆, ▲).

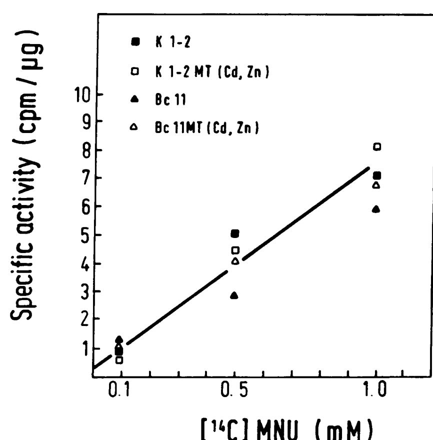

FIG. 5. Alkylation of DNA after treatment with [^{14}C]MNU in the parental strains and the transfectants (grown in the presence of Cd/Zn).

parental cells and the transfectants contained nonsignificant levels of methyltransferase activity (K1-2 was indistinguishable from HeLa MR; Bc11 and the transfectants were slightly above HeLa MR; Fig. 6). This suggests that the level of methyltransferase is, within the limit of detection, not affected by MT.

DISCUSSION

The hypothesis that expression of MT could protect cells from the lethal effect of ionizing radiation rests on the observation that purified MT *in vitro* is an efficient scavenger of free hydroxyl radicals (11). Theoretically, MT could be involved in radiation protection in two ways. (*i*) After radiation about 10% of the DNA damage is due to direct energy absorption and subsequent cleavage of C—H bonds from short-lived C radicals that react with oxygen to form peroxo structures. Electrophilic sulfhydryl groups as in MT could restore the original DNA structure by hydrogen donation. (*ii*) The predominant origin of radiation-induced radicals is water. The hydroxyl radicals contribute to about 90% of the radiation-induced damage to DNA (37). Consequently, scavenging of radiation-induced hydroxyl radicals by MT could be

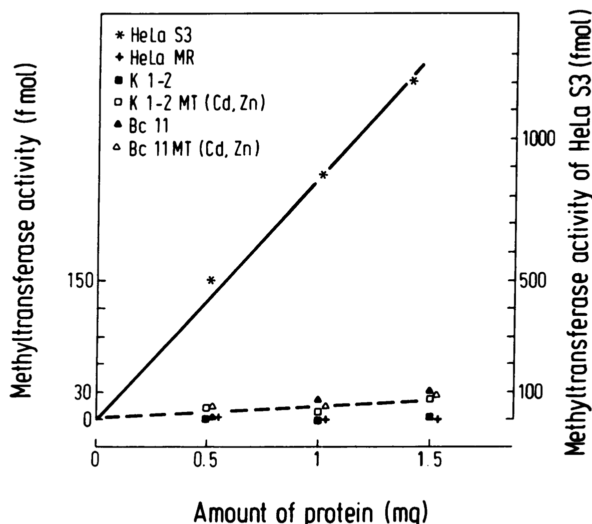

FIG. 6. O^6-Methylguanine-DNA methyltransferase activity in K1-2, K1-2MT, Bc11, and Bc11MT. Transfectants were cultivated in Cd/Zn medium. For comparison, the methyltransferase activities of HeLa S3 and HeLa MR are shown. Data are the average of two to four determinations.

an efficient mechanism of protection. The hypothesis would require, however, that DNA damage is the cytotoxic event and that MT is in close proximity to the DNA to neutralize free hydroxyl radicals in the nucleus. Previous experiments that seemed to support the hypothesis include the observation that mice that were fed a heavy metal-containing diet showed increased tolerance to γ irradiation (13, 14). In human and murine cell lines heavy metal resistance was reported to go along with slightly better γ survival (12). Furthermore, simian virus 40-transformed human cells showed elevated MT expression and concomitantly were more resistant to ionizing radiation than the parental cells (38). Whether, indeed, MT was the cause for these increases in radiation tolerance could not be derived from these indirect and uncontrolled experiments because chronic exposure to heavy metals may have led to a large variety of alterations.

Our experiments rule out a radiation-protective effect of MT *in vivo*. We have compared isogenic pairs of wild-type and x-ray-sensitive CHO cells that differed only in MT content and had been grown in the absence of heavy metal. In spite of a large increase in the cellular content of MT (and correspondingly elevated Cd resistance), the hMT-II$_A$ transfectants did not exhibit increased resistance to ionizing radiation. Our experiments with BSO and bleomycin confirm this result. Treatment with BSO, which leads to depletion of cellular glutathione (30, 39), increased the sensitivity of hypoxic Bc11MT cells to ionizing radiation. This effect should not be expected if MT could substitute for glutathione as a radical scavenger. The radiomimetic drug bleomycin produces strand breaks in the cellular DNA through the generation of hydroxyl radicals (40). As with γ irradiation, MT overproduction did not prevent killing by bleomycin. Since hydroxyl radicals have a very short half-life (10^{-13} sec) and are neutralized by reaction with molecules in their immediate vicinity (37), MT concentrations in the nucleus, even in these MT overexpressors, were apparently insufficient for protection.

The increased resistance of the MT-overexpressing transfectants to MNNG and MNU suggests an involvement of MT protein in a new type of protection from alkylation toxicity. Although the protection depended on the presence of the MT coding region and not on the presence of or selection by Cd, nor on the vector sequences, we have yet no hint on how direct is this action of MT. It should be noted that the level of MT did not seem to parallel the degree of protection: (*i*) The 2- to 3-fold increase of MT induced by Cd/Zn is not accompanied by increased resistance. (*ii*) There is no clear-cut correlation between the amount of MT in the overexpressing cells and their degree of resistance. This may indicate that in the transfectants the MT levels are saturating and/or that additional yet unknown factors are involved. These seem to be MT related since MT transfectants selected with G418 yielded equal numbers of surviving colonies after MNNG and Cd treatment (not shown). Assuming a direct involvement, MT protein could scavenge electrophilic chemical groups through its abundant nucleophilic sulfhydryl groups. Carbenium ions generated by MNU/MNNG decomposition could be prevented from reacting with DNA. However, the total degree of DNA methylation after treatment with toxic doses of MNU was the same in parent cells and MT overexpressors. The overall determination of methylation cannot distinguish whether a minor critical alkylation product was reduced in the MT-overexpressing cells. The spectrum of resistance to several alkylating agents indicates, however, that MT prevents toxicity by *O*-alkylation products through a post-alkylation event. The MT-overproducing transfectants were resistant to MNNG and MNU but not to MMS. Although alkylating DNA at the same positions, MNNG and MNU induce considerably more methylations at the O^6 position of guanine in DNA than MMS [7.5% O^6-methyl-

guanine for MNU and MNNG as compared to 0.3% for MMS (41)]. Therefore one could argue that the resistance of the transfectants to MNNG and MNU is due to increased removal of O^6-methylguanine from DNA, which has been suggested to be a toxic lesion (35, 36, 42). The protection must be generated at a step other than methyltransferase. All of our cell clones had nonsignificant methyltransferase activity (detection limit, ≈3000 molecules per cell). That MT does not increase the amount of methyltransferase above the control levels is in agreement with the observed lack of resistance to HeCNU. This agent induces chloroethylations in the O^6 position of guanine that are removed by preexisting methyltransferase before forming toxic crosslinks (45). We conclude that hMT-II_A protects from MNNG/MNU toxicity by some other yet unknown mechanism.

The MT transfectants were characterized by a slightly enhanced population doubling time as compared to the parental cells. This could be of importance because the slower the growth rate, the greater chance the cells have to repair critical DNA damage before fixation during replication. It is unlikely, however, that an altered growth rate is responsible for the observed resistance since it was observed only for MNNG and MNU but not for MMS, γ rays, and bleomycin. One may expect that cells should profit from all types of repair during slow growth, although the nature and half-lives of critical toxic lesions are unknown.

The fate of toxic alkylation damage in mammalian cells is apparently subjected to several pathways. This is illustrated by the different patterns of alkylation resistance of cell strains transfected with cloned genes. (*i*) hMT-II_A protects from MNNG and MNU (our data) and from the alkylating agents melphalan and chlorambucil (16) but not from MMS and HeCNU (there was weak resistance to cisplatin and MMC in the strain Bc11MT; data not shown). (*ii*) The bacterial O^6-methylguanine-DNA methyltransferase protects from MNNG- (36, 42, 43), MNU- (35), and chloroethylnitrosourea- (36) induced killing. Data for MMS are controversial (35, 42). (*iii*) A gene (not coding for methyltransferase or MT) transferred from human diploid fibroblasts to CHO cells by genomic transfection causes resistance to MNNG, MNU, and MMS but not to HeCNU and MMC (44). A resolution of the mechanisms of alkylation repair and the role of MT in damage removal or tolerance will only be possible through the characterization of the genes involved.

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