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Altered expression of the DNA repair protein, *N*-methylpurine-DNA glycosylase (MPG), in breast cancer

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Abstract We examined expression of *N*-methylpurine-DNA glycosylase (MPG), a DNA repair enzyme that removes *N*-alkylpurine damage, in normal, malignant, and immortalized breast epithelial cells, and breast cancer cell lines (MDA-MB-231, MCF7, T47D). Northern analysis showed increased expression in cancer versus normal breast epithelial cells (2–24-fold). Southern blots revealed no gene amplification or polymorphisms. Immunofluorescence, immunohistochemistry, and Western blot analysis demonstrated increased MPG protein expression in the tumor cells that correlated with elevated glycosylase activity. Since MPG overexpression has been shown to be paradoxically associated with increased susceptibility to DNA damage, up-regulation of this gene may suggest a functional role in breast carcinogenesis.

Key words: *N*-methylpurine-DNA glycosylase; DNA repair; Alkylpurine; Breast cancer

1. Introduction

The human genome is continually exposed to exogenous and endogenous alkylating and oxidative agents that result in damage to DNA. If left unrepaired, DNA damage may lead to carcinogenesis, cell death, and aging [1,2]. The nitrogen moieties of the purine rings are the main target for alkylation damage from environmental methylating agents [3]. These *N*-alkylpurines: 7-methylguanine, 3-methyladenine, and 3-methylguanine, are among the most prevalent DNA adducts produced by simple alkylating agents and are removed by ubiquitous base excision repair processes involving the action of various enzymes [3]. The first step of the *N*-alkylpurine repair process is the glycolytic removal of the modified base by *N*-methylpurine-DNA glycosylase [2]. Removal of *N*-alkylpurines leads to the formation of apurinic sites, that are subsequently removed by excision base repair [2,3]. Although *N*-alkylpurines have not been found to be directly mutagenic, apurinic sites left by this repair process can block replication and lead to mutation [4]. In addition to the repair of *N*-alkylpurines and other related adducts, *N*-methylpurine-DNA glycosylase also participates in repair of 8-hydroxyguanine [5] and hypoxanthine [6].

Human *N*-methylpurine-DNA glycosylase has been cloned and characterized by several groups [7–10]. Although increased expression of this enzyme can be transcriptionally induced in rat hepatoma cells by DNA-damaging agents [11], it appears that during the multi-step repair of *N*-alkylpurines, MPG activity may not be rate limiting, and cells may contain this protein in excess. There are conflicting data regarding the biological role of *N*-methylpurine-DNA glycosylase in determining resistance to alkylating agents. Increased repair due to overexpression of MPG has not been shown to give rise to increased resistance to methylating agents in various cell lines [12–15]. Instead, overexpression of this enzyme was found to contribute to the formation of sister chromatid exchanges, chromosomal aberrations, and gene mutations, possibly due to incomplete excision repair [13]. While the expression of this enzyme has not been found to be a determinant of resistance of cells to cytotoxic alkylating drugs in either human breast cancer [15] or rat hepatoma [14] cell lines, repair deficient MPG homozygous mutant mouse cells exhibited increased sensitivity to alkylation-induced chromosome damage and cell killing [16]. In sum, these studies suggest that the balance between glycosylase activity, leading to apurinic sites and subsequent formation of strand breaks, and subsequent excision repair processes may play an important role in determining cellular cytotoxicity and resistance to alkylating agents.

In considering the proposed role of DNA base lesions in mutagenesis and carcinogenesis, we investigated expression of this enzyme in human breast cancer. Levels of *N*-methylpurine-DNA glycosylase messenger RNA were found to be overexpressed up to 24-fold in breast cancer as compared to normal primary breast epithelium. MPG protein expression and glycosylase activity was evaluated in the breast cancer cell lines and was found to be increased as well.

2. Materials and methods

2.1. Materials

Breast tissues were obtained from the National Cancer Institute Cooperative Human Tissue Network (Columbus, OH) and included normal breast from reduction mammoplasty patients and infiltrating ductal carcinoma. Specimens were collected fresh from surgery and either processed for tissue culture as described previously [17] or frozen down in liquid nitrogen within 1 h of surgery. Tissue culture reagents were obtained from GibcoBRL (Gaithersburg, MD) and supplements were either from Sigma (St. Louis, MO) or Collaborative Research Inc. (Bedford, MA). Blotting membranes and reagents used for labeling of probes for Northern and Southern analysis were from Amersham (Arlington Heights, IL). Immunoblotting reagents were from Bio-Rad Labs (Hercules, CA). The human cloned cDNA encoding MPG [8], kindly provided by Dr. S. Mitra (University of Texas Medical Branch), was used as a probe for Northern and Southern analysis. Two rabbit polyclonal antisera (HRP Inc., Denver, PA), raised to peptides near the N-(SRGGRQTPRNTGM)- or C-

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Abbreviations: MPG, *N*-methylpurine-DNA glycosylase

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(DQRDLAQDEAVWLERG)-terminal ends of the human cloned MPG [8], were used for immunoassays. Purified human MPG was kindly donated by Dr. T. O'Connor (City of Hope, Beckman Research Institute, CA). 1,N^6-Ethenoadenine and 1,N^6-Etheno-2′-deoxyadenosine standards were purchased from Sigma (St. Louis, MO). Etheno-dA-CE phosphoramidite, used in the synthesis of the (εA)-containing 25-base oligonucleotide utilized for DNA glycosylase activity assays, was from Glen Research (Sterling, VA). The (εA)-containing 25-base oligonucleotide sequence 5′-CCG-CT(εA)-GCG-GGT-ACC-GAG-CTC-GAA-T-3′, and its complementary strand, 3′-GGC-GAT-CGC-CCA-TGG-CTC-CAG-CTT-A-5′, were taken from Rydberg et al. [27]. Oligonucleotides were synthesized by the Biotechnology Laboratories at Northwestern University. All other reagents used in this study were from Sigma unless otherwise specified.

2.2. Cell culture

Normal human mammary epithelial cells used in this study were primary cultures derived from individual surgical specimens. Briefly, cells were culled and grown in MCDB-170 medium plus serum-free supplements as described previously [17]. Human breast cancer and immortalized epithelial cells were established cell lines obtained from the American Tissue Culture Collection (Rockville, MD). MCF10A, an immortalized, non-transformed breast epithelial cell line derived from a normal reduction mammoplasty specimen, was maintained in a 1:1 mix of Ham's F12 and DMEM supplemented with 5% heat inactivated equine serum, 500 ng/ml hydrocortisone, 20 ng/ml epidermal growth factor (EGF), 100 ng/ml cholera toxin, and 0.01 mg/ml insulin. Breast cancer cell lines were cultured in RPMI-1640 medium plus 10% fetal bovine serum (FBS) supplemented with either 0.01 mg/ml insulin, for T47D cells, or 0.292 mg/ml glutamine, for MCF7 and MDA-MB-231 cells. Cells were routinely passaged at near-confluence with Trypsin-EDTA.

2.3. Northern blot analysis

Total RNA was prepared from breast cancer tissues and from exponentially growing cells using trizol reagent (GibcoBRL), according to manufacturer's directions. Samples of 30 μg of total RNA were electrophoresed in denaturing 1.2% agarose/formaldehyde gels and transferred by capillary action to Hybond-N (Amersham) in 20×SSC, following the manufacturer's recommendations. Blots were fixed and pre-hybridized at 42°C for 3 h in hybridization buffer (5×SSPE, 5×Denhardt's, 0.5% SDS, 50% formamide, 100 μg/ml Herring sperm DNA). Hybridization followed for 24 h at the same temperature with a ^{32}P-MPG cDNA probe, prepared by the random primer Redivue method (Amersham). Membranes were successively washed following hybridization in 2×SSC/0.1% SDS (twice quickly at room temperature and once for 20 min at 60°C), 1×SSC/0.1% SDS (once 20 min at 60°C), and 0.1×SSC/0.1% SDS (once 20 min at 60°C). Autoradiographs were exposed for periods of time to ensure that signal saturation did not occur. Blots were stripped and reprobed with ^{32}P-human glyceraldehyde phosphate dehydrogenase (GAPDH), as internal reference.

2.4. Southern blot analysis

Genomic DNA was prepared from cultured cells according to Maniatis et al. [18]. DNA samples (15 μg) were digested with *Eco*RI, *Bam*HI, or *Hin*dIII (GibcoBRL), electrophoresed in 0.7% agarose, and capillary blotted on to Hybond-N+ (Amersham, IL) in 0.4 M NaOH. The hybridization and washing protocols for blots were the same as those described for Northern blotting with the exception of a 16 h hybridization period at 42°C of ^{32}P-MPG cDNA probe. Following washes, blots were autoradiographed.

2.5. Immunoblotting assays

Total protein was obtained from whole cell lysates from exponentially growing cells using RIPA buffer (50 mM Tris/HCl pH 8.0, 150 mM NaCl, 1% NP-40, 0.5% Na deoxycholate, 0.1% SDS) and protease inhibitors. Briefly, cells were seeded in 100×20 mm tissue culture dishes (VWR, McGaw Park, IL) and cultured to 50% confluence, rinsed with phosphate buffered saline (PBS), and lysed in 0.5 ml of RIPA buffer for 45 min on ice. 60 μg protein/lane were subjected to SDS-PAGE on 12% polyacrylamide gels, transferred to nitrocellulose membranes, and processed for immunoblotting as previously described [19], using polyclonal rabbit antiserum directed against the N-terminal end of human MPG. Red ponceau staining of blots was routinely performed to verify equal loading of lanes. Blots were developed by chemiluminescence using the ECL detection Western blotting system (Amersham) and autoradiographed.

2.6. Immunofluorescence

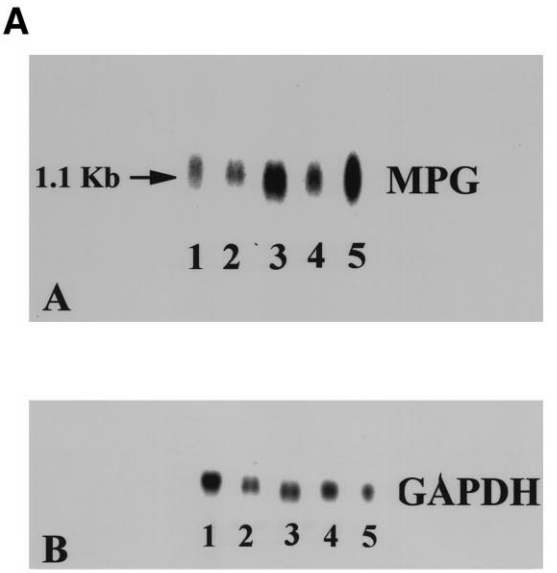
Normal or malignant breast cells were seeded on culture slides at (VWR) 4×10^3 cells/well and grown for 24–48 h to 30% confluence. Slides were washed three times in PBS, pH 7.4, fixed in 100% methanol for 5 min, permeabilized in acetone for 2 min at −20°C, and allowed to air dry. Fixed cells were incubated with rabbit polyclonal antiserum raised to the C-terminal end of the human MPG (diluted 1:2000 in PBS 10% FBS) for 1 h at 37°C in a humidified atmosphere. Following incubation, cells were rinsed 3× with PBS, incubated 30 min with 5% normal goat serum (Jackson ImmunoResearch, West Grove, PA) to block non-specific staining, washed 3× with PBS, and incubated with goat anti-rabbit rhodamine conjugated secondary antibody (Southern Biotech. Associates, Birmingham, AL) diluted in PBS 1:40 for 45 min at 37°C. Following staining, cells were rinsed 3× with PBS, once with Tris-saline (pH 8.2) at 4°C, and blot dried. Coverslips were then mounted on slides with gelvatol (vinol 203; Air Products and Chemicals, Allentown, PA), containing 25 mg/ml of 1,4-Diazabicyclo[2.2.2]octane (Sigma) to reduce fluorescence quenching, and allowed to dry. Control slides with non-immune serum were also prepared. Fluorescence samples were visualized using a Zeiss Photomicroscope III fitted with epifluorescence optics (Zeiss Inc., Thornwood, NY). Photographs were taken using the same exposure, images were stored on Sony optical discs and printed on a Tektronix printer (Tektronix, Wilsonville, OR).

2.7. Immunohistochemistry

For immunohistochemical analysis of MPG, a titration assay was used to estimate the approximate amount of MPG [20]. Immunohistochemical assays were performed on both thin prep cytology samples fixed for 10 min as well as formalin-fixed paraffin-embedded cell pellets processed and cut at 4 microns. Briefly, formalin-fixed paraffin-embedded cell lines were deparaffinized in xylene and appropriately fixed as previously described [20]. Non-specific staining was blocked by pretreating with normal goat serum (Vector Laboratories Inc, Burlingame, CA) in PBS 1% bovine serum albumin (BSA) for 15 min in a humidity chamber at room temperature. Slides were incubated overnight at 4°C with primary antibody, raised to a peptide near the C-terminal end of human MPG, in PBS 1% BSA or preimmune rabbit serum. Following rinses, 1× in 1% Tween 20 for 10 min and 3× in PBS 5 min each, immunoreactivity was made visible by incubating with biotinylated goat anti-rabbit secondary antibody (Vector) in PBS 1% BSA for 30 min at room temperature. After incubation, cells were rinsed 3× in PBS for 10 min each and incubated with streptavidin-horseradish peroxidase (HRP) (Zymed Laboratories, Inc., South San Francisco, CA) in PBS 1% BSA for 30 min. After three rinses in PBS for 10 min, working diaminobenzidine (DAB, Sigma) was added as substrate for 5 min at room temperature, consisting of 0.6% DAB and 0.01% hydrogen peroxide in PBS 1% BSA. Cells were then rinsed 3× in PBS for 10 min total, 2× in distilled water, and counterstained with 1% methyl green for 30 s. Slides were washed at least four times in distilled water until stain no longer came off and dehydrated by dipping ten times each through two changes of 70%, 95%, and absolute ethanol. Slides were cleared in four changes of xylene for 1 min each and coverslips were mounted with permount or other xylene miscible mounting medium.

2.8. Construction of recombinant GST-MPG fusion proteins

Construction, expression, and purification of recombinant GST-MPG fusion proteins was carried out according to the GST gene fusion system (Pharmacia Biotech, New Jersey). Construction of the pGEX-3XMPG fusion vector was performed using standard cloning techniques and procedures [18]. *E. coli* strain BL21 containing pGEX-3XMPG fusion vector was grown in 2X-YTA medium at 30°C to $A_{600} = 0.9$. Isopropyl-β-D-thiogalactopyranoside (IPTG) was added to a final concentration of 0.4 mM and cultures were harvested 2 h later. GST-MPG fusion proteins were prepared from bacterial sonicates and purified using glutathione sepharose 4B according to the instructions for the Bulk GST Purification Module (Pharmacia). Fusion proteins were eluted with 50 mM Tris-HCl (pH 8), 5 mM reduced glutathione, 20% glycerol, 1 mM PMSF, and 2 μg aprotinin. The



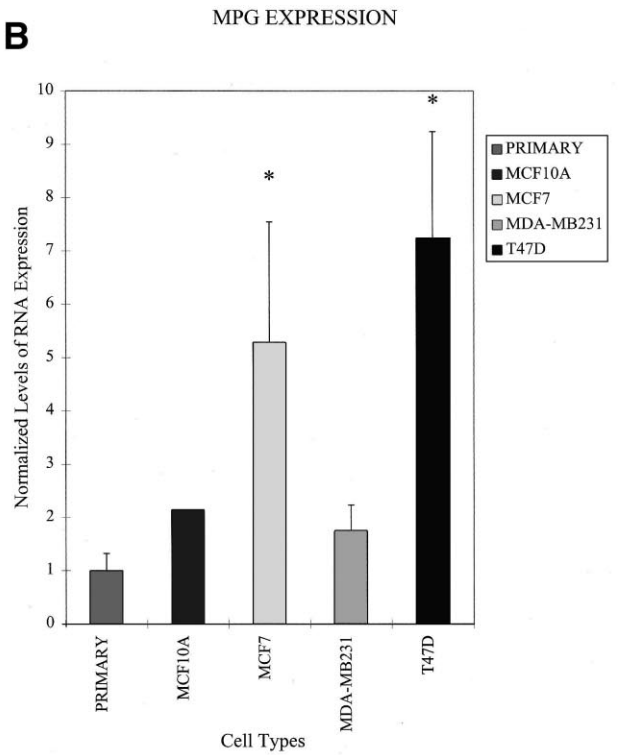


Fig. 1. Expression of MPG mRNA in cultured normal and tumor cell lines. Panel A: Northern blot analysis of MPG in human normal and tumor breast cells. 30 μg of total RNA from cultured tumor cell lines and from normal breast epithelial cells were electrophoresed in 1.2% agarose and transferred to nylon membrane. Blots were hybridized with an MPG cDNA probe, washed, and reprobed with human glyceraldehyde phosphate dehydrogenase (GAPDH) to control for differences in loading. Upper panel, MPG transcripts from normal breast epithelial cells (lane 1) and cultured normal and tumor cell lines, MCF10A, MCF7, MDA-MB-231, and T47D (lanes 2–5, respectively). Lower panel, filter reprobed for GAPDH as internal reference. Panel B: Data were collected from densitometric measurement of the amount of MPG and GAPDH mRNAs and values expressed in arbitrary units as normalized levels of MPG expression calculated from the quotient of MPG/GAPDH within each experiment. Error bars represent the standard deviation of the mean of five different experiments. Significance of findings was evaluated by Student's *t*-test comparing groups to the normal primary epithelial cell control group, $P < 0.05$ is indicated by an asterisk (*).

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supernatant was removed, aliquoted, and frozen in liquid nitrogen, and stored at −70°C. The supernatant contained GST activity, measured using the CDNB assay from Pharmacia (data not shown). GST-MPG fusion proteins were analyzed by SDS-PAGE and detected by Western blot with both the anti-GST antibody contained in the GST Detection Module (Pharmacia) and rabbit polyclonal antisera raised against human MPG (data not shown).

2.9. DNA glycosylase activity assay

The (εA)-containing 25-base oligonucleotide sequence and conditions used for the determination of DNA glycosylase activity were according to Rydberg et al. [27]. The duplex oligodeoxynucleotide was annealed by combining equal moles of the 25-base (εA)-oligo and its complementary strand in 150 mM Tris pH 8, 30 mM $MgCl_2$, heating for 2 min at 95°C, and allowing the mixture to cool down slowly to room temperature. Crude cell extracts were prepared from exponentially growing cells. Briefly, cells were washed three times with cold PBS, scraped from dishes, and collected by centrifugation at 4°C ($500 \times g$, 5 min). Cells were then resuspended in cold buffer containing 50 mM Tris-HCl (pH 8.5), 1 mM EDTA, 1 mM DTT, and 10% glycerol, supplemented with protease inhibitors as indicated above, and disrupted by sonication on ice. After centrifugation ($14\,000 \times g$, 20 min) at 4°C to pellet debris, supernatants were aliquoted and stored at −80°C. Equal amounts of crude cell extracts (100 μg of protein), purified human MPG protein (1 μg), or purified recombinant MPG-GST (3 μg), were incubated with 20 pmol of (εA)-containing double-stranded oligonucleotide in 35 mM HEPES-KOH, 0.5 mM EDTA, 0.5 mM DTT, and 40 mM KCl (pH 7.2), for 4 h at 37°C. Low molecular weight products were then separated from bulk protein and oligonucleotide by ultrafiltration using Ultrafree-MC NMWL 30,000 (Millipore, Bedford, MA). Analysis of εA released from the 25-mer oligonucleotide was performed by reverse-phase HPLC on a Prodigy 5 μm ODS 2 column (Phenomenex Inc., Torrance, CA), using a gradient of 0–20% methanol for 15 min, followed by constant 20% methanol for an additional 15 min at a flow rate of 0.9 ml/min. Fluorescence detection and quantitation of εA was accomplished using a Shimadzu RF-551 spectrofluorometric detector at

Table 1
Immunohistochemical reactivity: intensity of nuclear staining

Cell line	Pre 1:500	Pre 1:1000	1:500	1:1000	1:2000
T47D	neg[a] bg	neg	3+[b]	2+	1+
MCF7	neg bg	neg	2+	1+	±
MDA-MB-231	neg bg	neg	±	neg	neg

Immunohistochemical data: formalin-fixed paraffin-embedded cell lines. Expression of MPG protein using polyclonal rabbit antiserum and preimmune bleed ('Pre').

[a]neg, no staining; bg, cytoplasmic or perimembranous background staining, faint.

[b]± to 3+, intensity score; positive, nuclear brown stain.

These findings were supported by end-point titration assays as well, which demonstrated loss of staining by MCF7 prior to T47D with antibody dilution.

an excitation wavelength of 290 nm and an emission wavelength of 410 nm, coupled with a Hitachi HSM model D-7000 data analysis program.

3. Results

3.1. Expression of N-methylpurine-DNA glycosylase mRNA in human breast cell lines

To investigate the role of *N*-methylpurine-DNA glycosylase in breast cancer, we examined its expression in normal breast epithelial cells from primary culture ($n = 6$), an immortalized breast epithelial cell line (MCF10A), and three breast cancer cell lines (MDA-MB-231, MCF7, and T47D). Analysis of steady-state RNA indicated the presence of a single 1.1 kb transcript (Fig. 1A). A 1.1 kb MPG transcript was also observed by Chakravarti et al. [8] in a variety of rat and human cell lines using the same MPG cDNA probe and by others in different human transformed cell lines [10]. The abundance of the mRNA in all cells was determined by densitometric measurement of the amount of MPG transcript standardized to GAPDH. *N*-methylpurine-DNA glycosylase mRNA in immortalized (non-transformed) breast epithelial MCF10A cells and breast cancer cell lines MDA-MB-231, MCF7, and T47D, was increased by approximately 2-, 2-, 5-, and 7-fold, respectively, above normal breast epithelial cells (Fig. 1B). Southern analysis of genomic DNAs from these lines generated identical patterns, suggesting a lack of genetic polymorphism among the lines, and indicated no MPG amplification (data not shown).

Fig. 2. Western blot analysis of MPG protein in whole-cell extracts of human normal and tumor breast cells. 60 μg of total protein were resolved by SDS-PAGE on a 12% polyacrylamide gel, transferred to nitrocellulose membranes, and immunoblotted with rabbit polyclonal anti-human MPG antiserum. 300 ng of purified human MPG, kindly provided by Dr. T. O'Connor (City of Hope, Beckman Research Institute, CA), served as a marker in lane 1. Sizes of molecular weight standards (kDa) are indicated on the right side. The arrow denotes the MPG protein band (32 kDa). Western blot analysis of MPG detected a 32 kDa protein that was increased 3- and 6-fold in T47D and MCF7 cells respectively, as compared to normal breast epithelial cells.

3.2. MPG protein expression in human breast cell lines

Protein expression in human breast cells was investigated in whole cell protein extracts by Western blot, using rabbit poly-

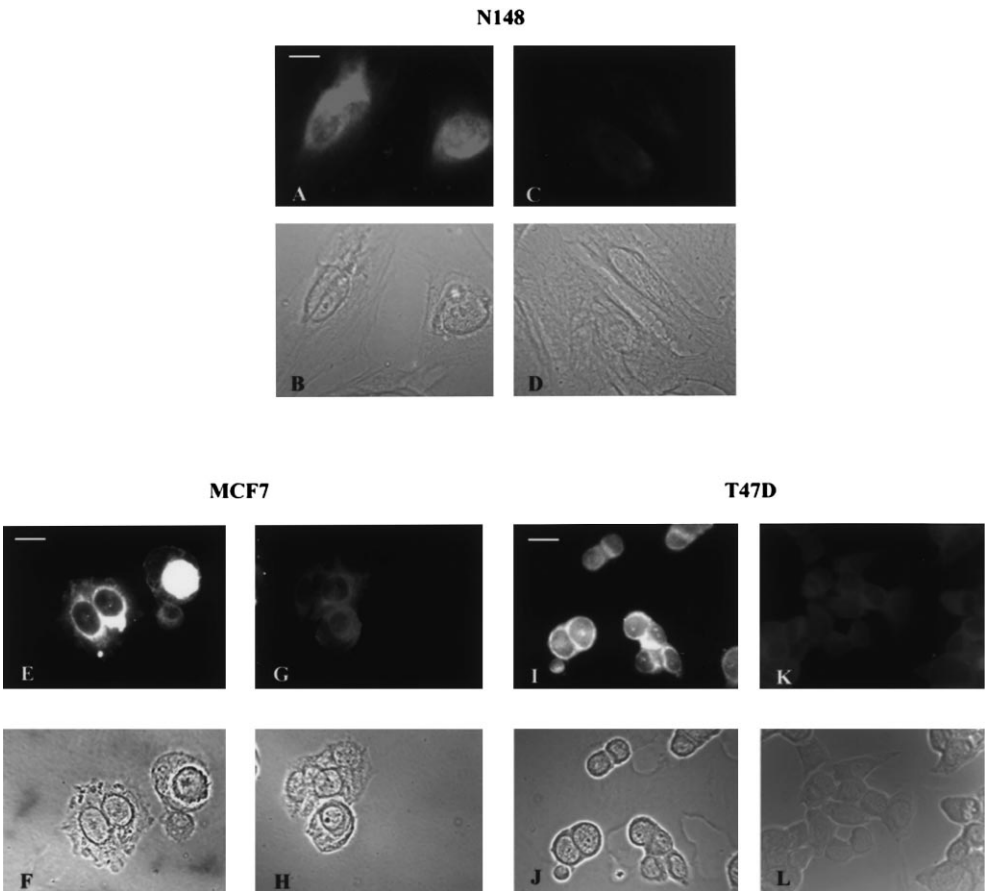

Fig. 3. Immunofluorescent staining for MPG in human breast cells. Human normal (N148) and malignant (MCF7, T47D) breast cells, were cultured on glass slides, fixed, and processed for indirect immunofluorescence microscopy using rabbit polyclonal MPG antiserum (A, E, I) or preimmune serum (C, G, K). Fluorescence samples were visualized using a Zeiss Photomicroscope III fitted with epifluorescence optics (Zeiss Inc., Thornwood, NY). Photographs were taken using the same exposure, images were stored on Sony optical discs and printed on a Tektronix printer (Tektronix, Wilsonville, OR). B, D, F, H, J, L: Phase contrast images of the cells. Bar, 25 μm.

clonal anti-human MPG antiserum (Fig. 2). MPG protein resolved as a 32 kDa protein band, as previously shown using purified full length protein [21]. MPG protein was found to be increased approximately 3- and 6-fold in T47D and MCF7 cells, as compared with normal breast epithelial cells. Although the differences in protein expression between cells were smaller than with mRNA, possibly due to post-translational modifications in these lines, MPG protein expression was found to be higher in breast cancer cells as compared with normal breast epithelial cells.

Immunofluorescent staining for human MPG was subsequently investigated to study localization of MPG in human breast cells. While normal primary breast cells (N148) showed nuclear staining of MPG, the breast tumor cells exhibited both a perinuclear and nuclear staining pattern (Fig. 3). Intensity of nuclear staining was semi-quantitated by immunohistochemistry in the breast cancer cell lines and MPG nuclear staining was found to correlate with mRNA expression (Table 1).

3.3. DNA glycosylase activity by quantitation of εA release in human breast cell lines

Crude cell extracts from human breast cells were incubated with 20 pmol of a synthetic oligonucleotide containing εA and release of the free base was identified after reverse-phase HPLC using fluorescence detection as previously described [27]. Both standards 1,N^6-Ethenoadenine (εA) and 1,N^6-Etheno-2′-deoxyadenosine showed distinct peaks that were clearly separated by approximately 2 min and had retention times of approximately 16.8 and 19.45 min respectively. DNA glycosylase activity was expressed as pmoles of εA released per hour in 100 μg of protein from crude cell extracts made from normal (MCF10A) and cancer (MDA-MB-231, MCF7, and T47D) human breast cell lines (Table 2). Release of (εA) was also measured using purified human *N*-methylpurine-DNA glycosylase (1 μg) and recombinant GST-MPG fusion protein (3 μg) as controls. As shown in Table 2, DNA glycosylase activity was increased approximately 4-5-fold in T47D and MCF7 cells, as compared with MDA-MB-231 and MCF10A cells (Table 2).

3.4. Expression of N-methylpurine-DNA glycosylase mRNA in human breast tumor tissues

In vivo expression of *N*-methylpurine-DNA glycosylase mRNA was investigated in 13 human primary breast cancer tissues and breast epithelium from six normal individuals. Tissues included normal breast from reduction mammoplasty patients and infiltrating ductal carcinoma. Expression of MPG message in tumor tissues was found to be increased 2–4-fold in three of the samples tested and 5–24-fold above normal primary breast epithelium in the remaining samples tested, with no overlap between the normal and malignant groups (Fig. 4). In sum, these data suggest increased MPG expression in breast cancer tissues.

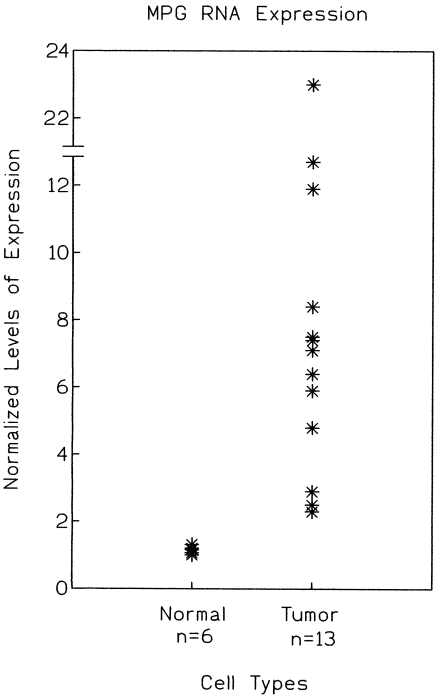

Fig. 4. Steady-state mRNA expression of MPG in primary tumors of the breast. 30 μg of total RNA collected from breast cancer tissues and from normal breast epithelial cells were electrophoresed in 1.2% agarose, blotted to nylon membrane, and hybridized with an MPG cDNA probe. Blots were then washed and reprobed with human glyceraldehyde phosphate dehydrogenase (GAPDH) to control for differences in loading. Data were collected from densitometric measurement of the amount of MPG and GAPDH mRNAs and values expressed in arbitrary units as normalized levels of MPG expression calculated from the quotient of MPG/GAPDH within each experiment. Data points are plotted in a scatter graph. No overlap between groups was observed. Expression of MPG message in tumors is increased 2–24-fold as compared with normal primary breast epithelial cells.

4. Discussion

The main target for alkylation damage from environmental methylating mutagens is purines in the DNA [3]. Cells possess specific DNA excision repair mechanisms that involve the action of various enzymes implicated in the recognition and

Table 2
DNA glycosylase activity releasing 1,N^6-Ethenoadenine from (εA)-containing 25-base oligonucleotide

	Total protein (μg)	Release of εA (pmol/h)
Purified MPG protein	1	2.892
Recombinant MPG-GST fusion protein	3	2.688
MCF10A	100	0.099
MDA-MB-231	100	0.154
T47D	100	0.469
MCF7	100	0.377

Data shown are averages of two to three independent experiments. Indicated amounts of protein extract were incubated for 4 h at 37°C in 120 μl of assay buffer containing 20 pmol of 25-mer synthetic oligonucleotide containing a single εA at the sixth position from the 5′ end [27]. Release of the free base was identified after reverse-phase HPLC using fluorescence detection at an excitation wavelength of 290 nm and an emission wavelength of 410 nm.

removal of these *N*-methylation products [1,3]. In addition to repairing DNA alkylation damage, base excision repair mechanisms are involved in the repair of one of the most prevalent products of oxygen radical injury, the mutagenic DNA lesion 8-hydroxydeoxyguanosine [5,23]. When these defenses cannot keep pace with the degree of damage, such as under conditions of high oxidative or alkylating stress, or when repair is inefficient, the miscoding potential of these lesions can result in the accumulation of genetic defects that may contribute to carcinogenesis [22]. High concentrations of these hydroxyl radical-induced DNA base lesions have been found by others in human breast cancers [24]. In considering the proposed role of DNA base lesions in mutagenesis and carcinogenesis, we investigated expression of one of the enzymes responsible for repair of these DNA lesions, *N*-alkylpurine glycosylase, in breast cancer.

In this study we have shown that the steady-state level of MPG mRNA is consistently higher in human breast cancer samples: three (MCF7, MDA-MB-231, and T47D) cell lines and 13 primary tumors, as compared to normal human breast epithelium (Figs. 1 and 4). Northern blot analysis showed a 1.1-kb transcript in human normal and tumor breast cells, as previously seen for other human carcinoma cell lines [10] and rat hepatocytes [14]. MPG protein levels were also found to be higher in human breast cancer cell lines than in normal breast epithelial cells (Fig. 2). While expression of MPG gene has been shown to be constitutively expressed in different tissues tested [8,10,11,14,25], consistent with its function as housekeeping gene, high levels of MPG have also been reported in some tumors, such as HT-29 cells derived from a colon adenocarcinoma, when compared to other human transformed cell lines [10]. The authors suggested that the environmental exposure to alkylating agents in colon epithelium may be particularly high. In contrast, normal rat hepatocytes were found to exhibit increased levels of expression of this gene, as compared to rat malignant hepatoma cell lines [14]. Expression of MPG may be dictated by various types and levels of carcinogen exposure in different tissues.

The possibility that overexpression of MPG in the human breast tumor cell lines could be due to gene amplification was ruled out in Southern blot analysis, which also revealed no evidence of genetic polymorphisms. In contrast, the highly aneuploid human HeLa MR cell line has shown polymorphisms in this gene [8]. It has been reported that MPG mRNA can be weakly induced in some cells 24 and 48 h after mutagen exposure [11,25], while other studies on the inducibility of the rat MPG promoter have not shown a significant induction by DNA methylating agents and ionizing radiation [26]. However, activity of the rat MPG promoter can be induced by UV light and the tumor promoter 12-O-tetradecanoylphorbol 13-acetate [26]. While MPG deficient mouse cells are more sensitive to chromosome damage and cell killing induced by methylating agents [16], overexpression of this enzyme has not been demonstrated to increase protection against the cytotoxic and mutagenic effect of alkylating agents [12–15]. In fact, cells overexpressing MPG were found to exhibit increased chromosomal damage and gene mutations, possibly due to imbalance in the multi-step process of DNA excision repair [13]. If overall repair would then depend upon downstream repair pathways such as apurinic endonucleases, MPG overexpression, leading to increased apurinic sites not compensated by downstream repair pathways, might contribute to carcinogenesis. Our data suggest either that overexpression of this gene might contribute to breast cancer development through imbalanced repair or might be utilized as a protective mechanism by breast cancer cells in response to elevated levels of DNA adducts found in breast cancerous tissue [24]. Although all cell types exhibited nuclear MPG staining (Fig. 3), a high intensity of this staining was localized outside of the nucleus in the breast cancer cells, where DNA damage cannot be induced. This difference in nuclear localization/transport of MPG in breast tumor cells as compared to normal breast epithelial cells, possibly due to post-translation modifications, could reflect a deficiency in nuclear DNA repair mechanisms in the breast cancer cells investigated in this study. With this in mind, DNA glycosylase activity was determined in the breast cancer cell lines and was found to be increased 4-5-fold in T47D and MCF7 cells respectively, as compared with MCF10A and MDA-MB-231 cells, correlating with protein expression (Table 2 and Fig. 2). Although we cannot tell at this point whether increased expression of this enzyme is a helpful adaptation to increased adduct formation or decreased transport, or, conversely, a detrimental response, data suggest up-regulation of MPG in breast cancer. We are currently addressing this issue in our laboratory. Additionally, animal models using transgenic mice overexpressing this enzyme are being developed to determine the in vivo role of MPG in breast carcinogenesis.

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