Enzymatic MPG DNA repair assays for two different oxidative DNA lesions reveal associations with increased lung cancer risk

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DNA repair is a major mechanism for minimizing mutations and reducing cancer risk. Here, we present the development of reproducible and specific enzymatic assays for methylpurine DNA glycosylase (MPG) repairing the oxidative lesions 1,N6-ethenoadenine (εA) and hypoxanthine (Hx) in peripheral blood mononuclear cells protein extracts. Association of these DNA repair activities with lung cancer was determined using conditional logistic regression with specimens from a population-based case–control study with 96 lung cancer cases and 96 matched control subjects. The mean MPG-εA in case patients was 15.8 units/μg protein (95% CI 15.3–16.3), significantly higher than in control subjects—15.1 (14.6–15.5), *p = 0.011. The adjusted odds ratio for lung cancer associated with a one SD increase in MPG-εA activity (2.48 units) was significantly bigger than 1 (OR = 1.6, 95% CI = 1.1–2.4; *p = 0.013). When activity of OGG1, a different DNA repair enzyme for oxidative damage, was included in the model, the estimated odds ratio/SD for a combined MPG-εA-OGG1 score was 2.6 (95% CI 1.6–4.2) *p = 0.0001, higher than the odds ratio for each single assay. The MPG enzyme activity assays described provide robust functional risk biomarkers, with increased MPG-εA activity being associated with increased lung cancer risk, similar to the behavior of MPG-Hx. This underscores the notion that imbalances in DNA repair, including high DNA repair, usually perceived as beneficial, can cause cancer risk. Such DNA repair risk biomarkers may be useful for risk assessment of lung cancer and perhaps other cancer types, and for early detection techniques such as low-dose CT.

Introduction

Cancer prevention requires the identification of risk factors, such that measures can be taken to reduce the risk. Although there was a major progress in prevention of heart diseases, fueled by the discovery that cholesterol is a risk biomarker and the development of drugs to treat it (1), progress in cancer prevention is rather slow (2–4). One of the reasons for this is the scarcity of robust risk biomarkers that can be used to assess one’s risk to develop cancer.

DNA repair is one of the key mechanisms for removing DNA damage and preventing mutations, and is therefore a critical mechanism in safeguarding humans from cancer (5). This is clearly manifested by a series of cancer predisposition syndromes, which are caused by germ-line mutations in DNA repair genes (5–8). Similarly, inter-individual variations in DNA repair capacity were found to be associated with the risk of cancer (9–17), however, additional robust host risk factors (risk biomarkers) are required to promote progress in the field.

DNA damage comprises a broad spectrum of types of chemical alterations in DNA, which are dealt with by an array DNA repair pathways, each exhibiting a certain degree of DNA damage specificity (5,18). Oxidative DNA damage is one of the main and important classes of DNA damage, caused by reactive oxygen species originating in metabolism, inflammation and external agents such as tobacco smoke and heavy metals (19–21). A key mechanism that repairs oxidative DNA damage is base excision repair, in which a DNA glycosylase identifies the damaged base and cuts it out from DNA, leaving behind, as an intermediate, an abasic (apurinic/apyrimidinic) site, which is then incised and processed to restore the original sequence based on the intact complementary strand (Figure 1A). The specificity of this repair pathway is determined by the DNA glycosylase, an enzyme class with 11 distinct members in humans. Each DNA glycosylase acts on a relatively limited number of types of DNA lesions, which often share common chemical features (22–24).

Adopting a functional approach we have previously developed an epidemiology-applicable enzymatic assay for the OGG1 DNA glycosylase, which removes the common and mutagenic DNA lesion 8-oxoguanine (25). Using this assay in a case-control study with protein extracts isolated from peripheral blood mononuclear cells (PBMC) we have shown that low OGG1 activity is associated with increased lung cancer risk (14), as well as head and neck cancer risk (15). The chemical diversity of oxidative DNA damage prompted us to explore the enzyme methylpurine DNA glycosylase (MPG), also termed 3-alkyladenine DNA glycosylase or alkyl-N-purine-DNA glycosylase, as an additional potential risk biomarker. MPG has a broader DNA damage specificity than OGG1, and removes from DNA a variety of oxidative lesions such as 1,N6-ethenoadenine (εA) and hypoxanthine (Hx), as well as alkylolation DNA damage including 3-methyladenine and 7-methylguanine (26,27). The methylated adducts can be事故发生ly formed in DNA by intracellular agents such as S-adenosyl methionine (18), or by alkylating agents that are used for cancer therapy such as temozolomide (28). The exocyclic DNA adducts such as εA are secondary oxidative lesions, generated by endogenous processes through the interaction of lipid peroxidation-derived aldehydes and hydroxyalkenals with DNA, or by reactions with oxidized metabolites of vinyl chloride, 2-chloroacetaldehyde and 2-chloroethylen oxide (29,30). Here we describe the development and characterization of robust and reproducible assays for the enzymatic activity of MPG in PBMC extracts, acting on Hx (MPG-Hx) or on εA (MPG-εA). In developing the two assays, we sought to examine whether there is a different manifestation of the two, which differ in activity by an order of magnitude (26), in estimating lung cancer risk. Here, we present results showing that high activity of MPG-εA is associated with increased lung cancer risk, consistent with our previous finding with MPG-Hx (16). These results underscore the notion that cancer risk is not necessarily caused by low DNA repair; rather, imbalances in DNA repair, including high DNA repair, usually perceived as beneficial, may cause cancer risk. Our results suggest that MPG enzymatic activity is a biomarker for lung cancer risk, and can be used in combination with other risk biomarkers as a useful tool for lung cancer prevention and early detection, and perhaps for other cancer types as well.
from which multiple matched candidates were available and one was randomly assigned as a control subject. Case patients and control subjects were excluded only if they had a former diagnosis of lung cancer. Participants provided written informed consent at time of recruitment, and were interviewed in-person to obtain information about their personal and family history of cancer, and smoking history. Diagnoses of lung cancer, all of primary origin, were made independently by the diagnosing hospitals and included information on histological type, TNM staging and tumor grade. The institutional review board at Carmel Medical Center, Haifa, approved all procedures.

**Blood samples**

Each study participant provided a sample (10 ml) of peripheral blood collected with CPDA1 as anticoagulant. Blood samples were kept at room temperature, and processed 18–24 h after collection to isolate PBMC, essentially as previously described (16). Isolation of PBMC and preparation of protein extracts were as previously described (16). Importantly, both the isolated PBMC and protein extracts can be stored frozen for at least 1.5 years at −80°C with essentially no loss of the DNA repair activity.

**DNA substrates**

The preparation of 32P-labeled duplex oligonucleotides was previously described (14,16,25). The oligonucleotides used in this study were as follows: MPG-Hx oligonucleotides. The 34-mer Liv34-Hx oligonucleotide was 5′-GT CCG GTG CAT GAC GCT GTX ACC CCT ACC TAG CGC CG-3′ (X = hypoxanthine), and its complementary oligonucleotide was 5′-CG CTG AGG ATA GGT TAC AGT GTC ATG CAC CGG AC-3′. The control 34-mer oligonucleotide was 5′-GT CCG GTG CAT GAC GCT GTA ACC TAT CCT CAG CG-3′, and its complementary oligonucleotide was 5′-CG CTG AGG ATA GGT TAC AGT GTC ATG CAC CGG AC-3′. The 32-mer Tad32-Hx oligonucleotide (11) was 5′-CCT ACC TAG CGA CCT XCG ACT GTC GTC CCA CCA CTG CT-3′ (X = hypoxanthine), and its complementary oligonucleotide was 5′-AGC AXT GGG GCA TCA GTG CTC TAG GTA GG-3′. MPG-ea oligonucleotides. The 32-mer Tad32-ea sequence was 5′-CCT ACC TAG CGA CCT ZCG ACT GTC CCA CTG CT-3′ (Z = ea), and its complement ary oligonucleotide was 5′-AGC AXT GGG GCA TCA GTG CTC TAG GTA GG-3′. The control 32-mer oligonucleotide was 5′-CCT ACC TAG CGA CCT AGC ACT GTC CCA CTG CT-3′ and its complementary oligonucleotide was 5′-AGC AGT GGG GCA TCA GTG CTC TAG GTA GG-3′. The 34-mer Liv34-ea oligonucleotide was 5′-GT CCG GTG CAT GAC ACT GTZ ACC TAT CCT CAG CG-3′ (Z = ea), and its complementary oligonucleotide was 5′-CG CTG AGG ATA GGT TAC AGT GTC ATG CAC CGG AC-3′.

**DNA repair enzyme activity assays**

The optimization of the MPG activity reaction conditions is described in Supplementary Materials and Methods, available at Carcinogenesis Online. The optimized final enzyme reaction conditions are presented below.

**MPG-ea activity assay.** The reaction mixture (20 μl) contained 50 mM PIPES (pH 6.7), 10 mM Tris (pH 7.1), 2 mM ethylenediaminetetraacetic acid, 0.5 mM MgCl2, 30 mM KCl, 1 μg/ml bovine -globulin, 0.1% polyvinyl alcohol (PVA), 0.5 nM DNA substrate and 15 ng/μl protein extract. The reaction was carried out at 37°C for 15 min, after which it was stopped by heat inactivation at 95°C for 2 min. The proteins were degraded by incubation with proteinase K (20 μg) for 30 min at 37°C, after which they were treated with 100 mM NaOH for 30 min at 37°C, fractionated by urea-polyacrylamide gel electrophoresis and quantified by phosphorimaging. One unit of MPG-ea activity cleaves 1 fmol of DNA substrate in 1 h at 37°C, under these conditions.

**MPG-Hx assay.** The reaction mixture (20 μl) was as described for MPG-ea, except that the DNA substrate concentration was 7.5 nM substrate. One unit of MPG-Hx activity is defined to cleave 1 fmol of DNA substrate in 1 h at 37°C, under these conditions.

**OGG1 assay.** The fluorescence-based OGG1 assay conditions were previously described (16).

**Statistical analysis**

Statistical analysis was performed essentially as previously described (16). The odds ratio of lung cancer was estimated for MPG-Hx and MPG-ea tests using conditional logistic regression models adjusting for smoking status with or without OGG1 activity. This method takes account of the matched design of the study and therefore automatically adjusts also for the matching variables, sex, age, place of residence and ethnicity. Odds ratios were estimated for each test as a continuous variable (assuming a linear relationship with the log odds), a binary variable using the median of the controls as the threshold, and categorized into three groups according to the tertiles of the controls. In the latter case, a test for a linear trend across tertiles in the log odds was conducted using scores of 1, 2 and 3 for the three tertile groups.

**Materials and methods**

**Study participants**

The study participants were previously described (16,17). Case patients (100) were recruited from the Rambam Medical Center (Haifa, Israel). Control subjects were enrollees of Clalit Health Services identified from the same geographical area. Clalit Health Service is the largest health care provider in Israel and covered, during the study years, approximately four million persons in Israel. Health care coverage in Israel is mandatory and is provided by four groups akin to not-for-profit health maintenance organizations. Thus, all study participants (case patients and control subjects) had similar basic health insurance plans and access to health services. Specimens were collected between April 2008 and December 2009. About 89% of the blood samples were collected from the case patients within 2 months of the diagnosis. For all the case patients, blood samples were drawn prior to the operative procedure or any treatment intervention. One hundred control subjects were individually matched to the case patients by gender, year of birth (±1 year), place of residence and ethnic group (Jews vs. non-Jews). Control subjects were selected from the enrollee list of Clalit,
The odds ratio of lung cancer was estimated for pairwise combinations of MPG tests or OGG1 and MPG tests using conditional logistic regression models with smoking status as an adjusting variable.

Significance tests for odds ratios and for comparing the combined assay with each individual assay were performed using the likelihood ratio test. All the statistical analyses were performed using S-Plus (TIBCO Software Inc.) and/or SAS software (version 9.2; SAS Institute Inc.).

Results

Overview of the MPG activity assays

Because of the broad spectrum of DNA lesions removed by MPG, we developed in parallel two independent assays for MPG activity: one for the removal of hypoxanthine (MPG-Hx; Figure 1A), the deamination product of adenine, and the other for 1,N6-ethenoadenine (MPG-eA; Figure 1A), a secondary oxidation adduct formed via lipid peroxidation, which is repaired by MPG by an order of magnitude less effectively than Hx (26). The substrates were short synthetic DNAs, each carrying the corresponding site-specific lesion, as well as a common radiolabel on its 5′-end to enable detection of the reaction products (Figure 1B and Supplementary Figure 2, available at Carcinogenesis Online). MPG activity in the protein extract removes the lesions, leaving in the DNA an abasic site, which was subsequently incised by the AP endonuclease (APE1) activity present in the protein extract, leading to the conversion of the intact oligonucleotide into the nicked product (Figure 1B and Supplementary Figure 2, available at Carcinogenesis Online). The reaction products can be monitored by electrophoresis in denaturing urea-polyacrylamide gels followed by visualization and quantification by phosphorimaging (Supplementary Figure 2, available at Carcinogenesis Online). To specifically measure MPG activity, regardless of the level of APE1 activity, the reaction products were treated with NaOH, which effectively breaks all abasic sites.

Optimization of protein extraction

Using for the MPG assays, the protein extracts optimized for the OGG1 DNA repair assay is clearly advantageous, because it enables the measurement of all activities in the same extract. To explore this possibility we prepared PBMC protein extracts using several methods, and assayed them for MPG-Hx and MPG-eA activities. The methods tested were freeze-thaw cycles in a hypotonic buffer, which was originally used to prepare PBMC extracts for the OGG1 assay, and in addition shearing by passage through a syringe needle, sonication and detergent treatment (NP40 and Triton X-100). In addition, we tested the salt concentration and incubation times needed for efficient extraction of the DNA repair enzymes from the nucleus. We found that the total MPG activities in extracts prepared with a detergent were 30–40% higher than extracts prepared by the freeze/thaw method, however, the specific activities were similar (Supplementary Figure 1A and B, available at Carcinogenesis Online). Because the detergents strongly inhibited OGG1 activity (25), we chose the freeze/thaw method for assaying both OGG1 and MPG activities. Analysis of MPG activities in extracts prepared from six blood samples from the same individual yielded a mean MPG-Hx activity of 98.9 ± 9.7
(CV = 10%) and a mean MPG-εA activity of 6.9 ± 0.6 (CV = 8%) (Supplementary Figure 1C, available at Carcinogenesis Online). These low CV values indicate a high reproducible extraction procedure. In addition we found that MPG activities were stable in thawed extracts that were stored for 24h on ice (Supplementary Figure 1D, available at Carcinogenesis Online). Thus, the freeze and thaw extraction procedure is indeed suitable for both MPG and OGG1 activities.

Optimization of assay conditions

MPG assays conditions were rigorously optimized to be quantitative, reproducible and robust, suitable for epidemiological studies. Optimization included substrates concentration, substrate length and sequence, salts concentration, metal ions, stabilizing agents like bovine γ-globulin and nuclease inhibitors like ethylenediaminetetraacetic acid. Here we present typical examples for optimization of the MPG assays. As can be seen in Figure 2A and B, both buffer type and the pH-affected MPG activities, being highest at slightly acidic pH, consistent with the activity of purified MPG enzyme, reported to be at pH 6–6.5 (31). We chose as our standard pH 6.7, which was optimal for MPG-Hx activity, and slightly suboptimal for MPG-εA nicking activity, in order not to deviate too much from neutral pH. PIPES was chosen as the standard buffer because pH 6.7 is well within its buffering capacity range.

We compared MPG activities for Hx and εA in two sequence context: Liv34, a randomly selected DNA sequence context used for the OGG1 test, and Tud32 sequence based on a sequence previously used to study MPG activity on εA (11). Interestingly, MPG-Hx activity was 50–80% higher with the Liv34 sequence (Average 68%, Figure 2C), whereas MPG-εA activity was 40–80% higher with the Tud32 sequence (average 60%, Figure 2D). Therefore, the Liv34 substrate was selected for the MPH-Hx assay, and the Tud32 substrate was selected for the MPG-εA assay.

Fig. 3. Kinetics and protein titration of the optimized MPG DNA repair assays. (A and B) Time course and protein extract titration, respectively, of MPG-Hx DNA repair activity under optimized reaction conditions in protein extracts prepared from peripheral blood mononuclear cells. (C and D) Time course and protein extract titration, respectively, of MPG-εA DNA repair activity under optimized reaction conditions in protein extracts prepared from peripheral blood mononuclear cells. Quantification was done by phosphorimaging of gels such as presented in Supplementary Figure 2, available at Carcinogenesis Online. Closed symbols, substrates with Hx or εA, as indicated; Open symbols, control DNAs without any lesion. (E) Mean DNA repair enzyme activity measured using the DNA repair assays was 161 units/μg protein (95% CI 154–168) for MPG-Hx (average of 97 control subjects) and 15.1 units/μg protein (95% CI 14.6–15.5) for MPG-εA (average of 99 control subjects). Assay conditions are described in Materials and Methods. (F) Relative frequency plots for MPG-εA activities were determined in 99 case patients (continuous line) and 99 matched controls subjects (dashed line). The relative frequencies as percent were plotted using GraphPad Prism version 5.00, with bin width of 1 unit that was automatically chosen by the software. The relative frequency plots were smoothed by two neighbors on each size, zero order of polynomial smoothing. Case patients exhibit a shift to higher values of MPG-εA.
The coefficient of variation of the MPG-εA assay was determined in two different ways (1): The assay was repeated 34 times, each performed on a different day with two different extracts, yielding CV = 8.5 (2). The assay was conducted twice with 18 different extracts on two different days, yielding CV = 8.1. The average of the two CVs, 8.3, was taken as the CV of the MPG-εA assay. The CV of the MPG-Hx assay (11.1%), similarly determined, was previously reported (16).

Figure 3A–D show the performance of the MPG assays after the optimization. MPG activities were linear for up to 2 h of reaction time (MPG-Hx, Figure 3A; \( R^2 = 0.994 \); MPG-εA, Figure 3C; \( R^2 = 0.993 \)). Cleavage was observed with the substrate carrying the lesion (Hx or εA), but not with the control oligonucleotides, which carried an A instead of the lesion (Figure 3A–D; Supplementary Figure 2, available at Carcinogenesis Online). The assay was also linear with increasing protein concentration for both MPG-Hx (Figure 3B; \( R^2 = 0.994 \); Supplementary Figure 2C, available at Carcinogenesis Online), and MPG-εA (Figure 3D; \( R^2 = 0.998 \); Supplementary Figure 2F, available at Carcinogenesis Online).

Specificity of the MPG assays
The MPG assays monitor the repair of Hx and εA carried out by proteins present in the extracts, and could therefore be affected by other DNA repair pathways. Specifically, nucleotide incision repair can act as a backup for base excision repair (32), and the human AlkB homologue 2 (ABH2) was shown to repair εA by direct reversal (33,34). We therefore used an anti-MPG antibody to deplete PBMC extracts of endogenous MPG. As can be seen in Figure 4A, the antibody successfully removed MPG from the extract, whereas non-specific IgG had no effect. In parallel, while there was normal MPG enzyme activity in extracts treated with IgG, there was a strong reduction in the MPG-depleted extracts: More than 90% reduction of MPG-Hx activity (Figure 4B) and 70% of MPG-εA activity (Figure 4C). Addition of purified human MPG enzyme to the depleted extract restored the MPG activities in the depleted extracts (Figure 4B and C). These results indicate that MPG is the major repair enzyme of Hx and εA in PBMC extracts. In addition, we did not detect any ABH2 activity in the extracts under MPG assay conditions (Supplementary Figure 3, available at Carcinogenesis Online).

High MPG-εA activity is associated with increased lung cancer risk
An interesting question is whether the two MPG assays, which are conducted under different reaction conditions and reflect repair activities with different efficiencies will have similar manifestation as far as lung cancer risk is concerned. To that end the specimens from the blinded population-based case–control study that were assayed for MPG-Hx and OGG1 activities were assayed for MPG-εA activity. These included 100 lung cancer patients and 100 control subjects matched for gender, age (±1 year), place of residence and ethnicity. The mean MPG activity on εA in 99 specimens of healthy individuals was 15.1 units/µg protein (95% CI 14.6–15.5) (Table I) compared to an MPG-Hx average activity of 161 units/µg protein (95% CI 154–168) (Figure 3E (16)). Thus, MPG-Hx activity in human PBMC is 10.7-fold higher than MPG-εA activity, similar to the behavior of the purified MPG enzyme (26). Figure 3F shows the distribution of MPG-εA activity, spanning 8.8–20.4 and 8.1–21.9 units/µg protein for control subjects and case patients, respectively. A shift to higher MPG-εA values was observed in case patients compared to control subjects (Figure 3F).

The mean value of MPG-εA in cases was 15.8 (95% CI 15.3–16.3), significantly higher than in control subjects, with \( P = 0.011 \) (Table I). Comparing mean MPG-εA activity in current, past and never smokers showed no significant difference (Table I). There was also no significant difference in mean MPG-εA activity between males and females, between subjects of ages ≤65 and >65 years, and between adenocarcinoma and SQCC patients (Table I).

![Image](https://academic.oup.com/carcin/article-abstract/35/12/2763/335931) by guest on 28 March 2019

**Fig. 4.** Specificity of the MPG assays. (A) Depletion of MPG from PBMC extracts by an anti-MPG antibody. Western blot analysis of the treated samples is presented. GAPDH served as a loading control. Ext, PBMC extract; Sup, supernatant left after immune-precipitation; Beads, immunoprecipitated fraction. UT, untreated; Ab-MPG, immunoprecipitated with anti-MPG antibody; IgG, control treated with IgG. A cropped image is presented. The full gel image is presented in the Supplementary Material, available at Carcinogenesis Online. (B) MPG activity in two different immune-depleted samples. Treatment with the anti-MPG antibody strongly decreased DNA repair activity on Hx-containing (B) and εA-containing (C) DNA substrates (columns marked ‘Deplt.’), compared to IgG-treated or untreated (UT) controls. Complementing the depleted extracts with purified MPG enzyme (0.001 units) restored the two activities (B and C; columns marked ‘Comp.’).
We used conditional logistic regression to examine whether there is an association between MPG-eA enzyme activity and the disease adjusted for smoking status. When MPG-eA activity was used as a continuous variable, the adjusted odds ratio for lung cancer associated with a one SD decrease in MPG-eA activity (2.48 units) was statistically significantly greater than 1 (OR = 1.6, 95% CI = 1.1–2.4; $P = 0.013$) (Table II). This is comparable to the result obtained with the MPG-Hx assay, where OR = 1.8, 95% CI = 1.2–2.6; $P = 0.006$ (16). Thus, increased MPG activity is associated with an increased risk of lung cancer.

The combination of MPG-eA and OGG1 DNA repair activities is more strongly associated with lung cancer than each DNA repair activity alone

Table II shows that when both continuous OGG1 and MPG-eA activity values were included in the logistic regression model, both were statistically significant (for OGG1, OR = 2.0 (95% CI 1.3–3.0) $P = 0.0008$; for MPG-eA, OR = 2.3 (95% CI 1.4–3.8) $P = 0.0009$). Thus, combining the two tests strengthened both assay odds ratios and the level of statistical significance. Forming a combined MPG-eA-OGG1 score from this model, the estimated odds ratio per one

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**Table I. Distribution of selected characteristics and MPG-eA activity value in lung cancer patients and control subjects**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control subjects (n = 100)</th>
<th>Case patients (n = 100)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>MPG-eA mean (95% CI)</td>
<td>No.</td>
</tr>
<tr>
<td>All</td>
<td>99</td>
<td>15.1 (14.6–15.5)</td>
<td>99</td>
</tr>
<tr>
<td>SQCC</td>
<td>30</td>
<td>15.9 (15.2–16.6)</td>
<td>46</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>30</td>
<td>15.9 (15.2–16.6)</td>
<td>46</td>
</tr>
<tr>
<td>Age, years</td>
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<td>15.1 (14.5–15.7)</td>
<td>65</td>
</tr>
<tr>
<td>&gt;65</td>
<td>40</td>
<td>15.0 (14.3–15.7)</td>
<td>59</td>
</tr>
<tr>
<td>Sex</td>
<td>59</td>
<td>14.8 (14.2–15.4)</td>
<td>59</td>
</tr>
<tr>
<td>Male</td>
<td>40</td>
<td>15.4 (14.6–16.3)</td>
<td>40</td>
</tr>
<tr>
<td>Female</td>
<td>16</td>
<td>15.3 (14.5–16.0)</td>
<td>24</td>
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<tr>
<td>Smoking status</td>
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<td>14.4 (13.6–15.2)</td>
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<tr>
<td>Never smoked</td>
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<td>15.4 (14.4–16.4)</td>
<td>37</td>
</tr>
<tr>
<td>Past smoker</td>
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<td>14.4 (13.6–15.2)</td>
<td>35</td>
</tr>
<tr>
<td>Current smoker</td>
<td>22</td>
<td>15.4 (14.4–16.4)</td>
<td>37</td>
</tr>
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**Table II. Conditional logistic regression analyses of MPG and OGG1 activities in lung cancer patients and control subjects**

<table>
<thead>
<tr>
<th>Variable</th>
<th>SD of variable</th>
<th>Adjusted for</th>
<th>No. of case patients</th>
<th>No. of control subjects</th>
<th>Adjusted odds ratio (95% confidence interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(units/μg protein)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MPG-eA (per 1 SD increase)</td>
<td>2.48</td>
<td>Smoking</td>
<td>96</td>
<td>96</td>
<td>1.6 (1.1–2.4) $P = 0.013$</td>
</tr>
<tr>
<td>MPG-Hx (per 1 SD increase)</td>
<td>34.0</td>
<td>Smoking</td>
<td>94</td>
<td>94</td>
<td>1.8 (1.2–2.6) $P = 0.006$</td>
</tr>
<tr>
<td>Combined model: MPG-eA and OGG1</td>
<td>2.48</td>
<td>Smoking and OGG</td>
<td>96</td>
<td>96</td>
<td>2.3 (1.4–3.8) $P = 0.0009$</td>
</tr>
<tr>
<td>MPG-eA (per 1 SD increase)</td>
<td>1.08</td>
<td>Smoking</td>
<td>96</td>
<td>96</td>
<td>2.0 (1.3–3.0) $P = 0.0008$</td>
</tr>
<tr>
<td>OGG (per 1 SD decrease)</td>
<td>0.94</td>
<td>Smoking</td>
<td>96</td>
<td>96</td>
<td>2.6 (1.6–4.2) $P = 0.0001$</td>
</tr>
</tbody>
</table>

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1. MPG-eA activity was measured as described in Materials and methods. One control participant, for which the MPG-eA value was unknown, and his matched case were excluded from the analysis. Three case patients did not have a known smoking status.
2. Of the 100 lung cancer cases, 30 had squamous cell carcinoma (SQCC), 46 had adenocarcinoma, 14 BAC, 4 adenosquamous carcinoma, 4 adenBAC; 1 small cell carcinoma and 1 unknown histology.
3. Analysis of covariance comparing cases with controls, with matched pair and smoking status as covariate.
4. Analysis of covariance comparing histological type within cases, with smoking status, age (continuous) and gender as covariates.
5. Test for interaction between case-control status and the variable of interest. This estimates whether the difference in mean MPG between cases and controls differs between the subgroups of the variable in question (e.g. between males and females). None of the differences were statistically significant, indicating no interaction.
6. Analysis of covariance comparing subsets defined by the variable of interest and stratified by cases and controls, with smoking status, age (continuous) or gender as covariates, as appropriate. This estimates whether there are real differences in MPG between the subgroups of the variable in question (e.g. between ages ≤65 and >65 years) after adjustment for disease status, and other appropriate covariates from among age, sex and smoking status (see reference (48)).
standard deviation was 2.6 (95% CI 1.6–4.2) \( P = 0.0001 \) (Table II), higher than the odds ratio for each single assay. This is comparable to the odds ratio of 2.3 (95% CI 1.4–3.6) \( P < 0.001 \) obtained with MPG-Hx assay (16).

**Discussion**

The interest in MPG stems from its broad DNA damage specificity, which includes both oxidative and alkylation types of DNA damage, making it relevant for both risk assessment, as oxidative DNA damage is important in carcinogenesis, and cancer therapy, where alkylating agents are used (26–28). MPG enzyme activity on different lesions varies considerably, which prompted us to develop assays for two different lesions: the secondary oxidative lesion εA and the spontaneous and oxidation-induced lesion Hx. Under individually optimized conditions, MPG activity on Hx was about 10-fold higher than on εA. The identity of the enzymatic activity measured in our assay is determined by the lesion present in the DNA substrate. This means that any enzyme that can repair Hx or εA will contribute to the observed repair. However, as the immune-depletion experiments show, MPG was the main enzyme responsible for the repair of over 90% of the Hx lesion. Somewhat surprisingly, activity of the extract on the εA lesion was decreased by only 70% upon immune-depletion of MPG. A possible explanation is based on the much lower \( K_m \) of MPG towards the εA substrate compared to Hx (31,35). The residual MPG left in the depleted extract may have been sufficient to bind the εA substrate and partially remove it, but too low for binding to the Hx substrate. Moreover, there was no interference from the activity of ABH2, an enzyme that can perform oxidative reversal repair of the εA adduct directly to Adenine (33,34), because there was no significant ABH2 enzyme activity under our assay conditions.

Despite the differences discussed above between the two MPG enzymatic activities, they were highly correlated (Pearson correlation coefficient = 0.76), in line with the notion that the same enzyme was responsible for the removal of both. This is also manifested in a logistic regression analysis in which the MPG-εA assay was included in addition to the MPG-Hx assay, which showed that the added MPG-εA assay was not statistically significant (\( P = 0.44 \)). Thus, the two assays provide essentially the same information as far as lung cancer risk is concerned. Moreover, the logistic regression analysis showed that high activity of MPG-εA is associated with increased lung cancer risk, similarly to the results that we have reported for MPG-Hx activity (16). It should be pointed out that a prospective study is needed to validate that high MPG activity is predictive of increased risk of lung cancer.

Previous studies performed with the εA substrate reported conflicting results, with MPG being lower in lung cancer cases than in healthy subjects in an earlier study (11), and higher in a more recent study (36). The fact that our results were obtained with two different assays strengthens our conclusion that increased MPG activity is associated with increased lung cancer risk. It should be pointed out that most, if not all, biological processes operate within a defined optimal range; DNA repair is no different, and imbalances in DNA repair were previously reported to have adverse effects (37–39). Due to its broad specificity for damaged DNA, MPG may be sequestered to lesions to which it can bind, but not repair, thereby both interfering with their repair, and failing to repair its own substrate lesions (40,41). In addition increased amounts of MPG were reported to cause frameshift mutations and microsatellite instability (42), as well as increased sensitivity to alkylating agents (43,44). Yet, it is expected that very low activities of MPG will also be associated with increased lung cancer risk, as do high activities. To examine whether MPG indeed exhibits such a bimodal risk behavior will require bigger epidemiological studies.

In conclusion, enzyme activity of MPG is expected to be a robust functional biomarker for lung cancer risk, and warrants further investigations as a potential risk factor in other types of cancer. We have recently found that low enzyme activity of APE1 is a risk factor for lung cancer (17). Importantly, a panel composed of the three DNA repair risk biomarkers, namely OGG1, MPG and APE1, was used to calculate an integrated DNA repair score. Low values of this integrated DNA score were strongly associated with lung cancer risk (17,45). Such risk assessment tests based on DNA repair activity are expected to be useful for risk assessment of lung cancer and perhaps other cancer types, and for early detection of lung cancer by techniques such as low-dose CT (46,47).

**Supplementary Material**

Supplementary Figures 1–3 can be found at http://carcin.oxfordjournals.org.

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**Conflict of interest Statement:** T.P.E. and Z.L. have a patent on the OGG1 risk factor for lung cancer, and a patent application pending for the OGG1, MPG and APE1 panel of DNA repair biomarkers.

**References**

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