Supplementary Materials and Methods

N-Methypurine DNA Glycosylase and OGG1 DNA Repair Activities: Opposite Associations With Lung Cancer

Yael Leitner-Dagan¹, Ziv Sevilya¹, Mila Pinchev², Ran Kramer³, Dalia Elinger¹, Laila C. Roisman¹, Hedy S. Rennert², Edna Schechtman⁴, Laurence Freedman⁵, Gad Rennert², Zvi Livneh*¹, and Tamar Paz-Elizur*¹

¹Dept of Biological Chemistry, Weizmann Institute of Science, Rehovot 76100, Israel; ²Dept of Community Medicine and Epidemiology, Carmel Medical Center, Bruce Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, and Clalit Health Services National Cancer Control Center, Haifa, Israel. ³Dept of General Thoracic Surgery, Rambam Health Care Campus, Haifa, Israel; ⁴Dept. of Industrial Engineering and Management, Ben Gurion University of the Negev, Beer Sheva 84105, Israel; ⁵Biostatistics Unit, Gertner Institute for Epidemiology and Public Health Policy Sheba Medical Center Tel Hashomer 52621, Israel.

DNA substrates

The DNA substrates were prepared by annealing two complementary synthetic oligonucleotides as previously described (1, 2), except that different oligonucleotides were used. The oligonucleotide containing the site-specific Hx was 5' ³²P-labeled using γ-³²P ATP and T4 polynucleotide kinase, and annealed to the complementary oligonucleotide. The radiolabeled duplex was purified by polyacrylamide gel electrophoresis (PAGE) on a native 10% gel. The oligonucleotide containing the site-specific 8-oxoguanine was 3’ labeled with a Yakima Yellow fluorophore (Glen Research, Catalog# 205921), annealed to the complementary oligonucleotide,
and the fluorescent duplex was purified by PAGE on a native 10% gel. The concentration of the substrates was determined by NanoDrop® ND-1000 Spectrophotometer.

**Oligonucleotides.** Oligonucleotides were synthesized using an Expedite 8909 DNA Synthesizer (Applied Biosystems, Foster City, CA) with modified bases purchased from Glen Research. Oligonucleotides were also purchased from several commercial sources including Sigma, IDT, Proligo and Metabion. For the MPG assay the substrate was the 34-mer oligonucleotide 5’- GT CCG GTG CAT GAC ACT GTX ACC TAT CCT CAG CG -3’ (X = hypoxanthine) annealed to 5’- CG CTG AGG ATA GGT TAC AGT GTC ATG CAC CGG AC -3’. For the fluorescence-based OGG assay the substrate was the 32-mer oligonucleotide 5’-CCG GTG CAT GAC ACT GTZ ACC TAT CCT CAG CG Y -3’ (Z = 8-oxoG; Y = Yakima yellow tag) annealed to 5’- CGC TGA GGA TAG GT CACA GTG TCA TGC ACC GG -3’.

**Isolation of peripheral blood mononuclear cells**

PBMC were isolated essentially as previously described (1). Each study participant provided a sample (10mL) of peripheral blood collected in a 50mL tube containing 1.4mL of an anticoagulant citrate-phosphate-dextrose-adenine solution (CPDA-1; Teva Medical, Ashdod, Israel). Blood samples were processed 18-24 hours after collection1. The whole blood was centrifuged at 400xg for 10 minutes at 20°C, and plasma rich with contaminating platelets was removed. PBS (Dulbecco’s phosphate buffered saline, Sigma) supplemented with 2mM EDTA was added to the remaining whole blood portion to a final volume of 30ml, and PBMC were isolated by density gradient centrifugation of the diluted whole blood on a polysucrose-sodium metrizoate medium in UNI-SEP tube (NOVAmed, Jerusalem, Israel) at 1000xg for 30 minutes at

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1 This was done for logistics reasons, since blood specimens arrived in the evening to the lab in which analysis was performed. We have established that keeping blood specimens for up to 24 hours after collection does not alter their DNA repair activity.
20°C. Following centrifugation, the PBMC fraction was rinsed with PBS buffer + 2mM EDTA, and red blood cells were eliminated by lysis in 5 ml of 155mM NH₄Cl, 10mM KHCO₃, 0.1mM EDTA for 4 minutes at room-temperature. The PBMC were washed with PBS + 2mM EDTA, and suspended in 1ml PBS. The number of white blood cells in this suspension was determined using a Cobas Micros (Roche Diagnostic System) blood cell counter. The PBMC were pelleted by centrifugation at 5000 rpm, for 4 minutes at room temperature. The cells pellet was then re-suspended to a concentration of 50,000 cells/µl in 50mM Tris·HCl (pH 7.1), 1mM EDTA, 0.5mM spermidine, 0.1mM spermine, and a protease inhibitor cocktail (Sigma). The cells were incubated on ice for 30 minutes, after which they were frozen in liquid nitrogen. The frozen lymphocytes were stored at -80°C.

**Preparation of a protein extract**

The frozen lymphocytes were thawed at 30°C, after which their protein content was extracted with 220mM KCl for 30 minutes on ice. Cell debris were removed by centrifugation at 13,200 rpm for 15 minutes at 4°C. Glycerol was added to the protein extract to a final concentration of 10%, and the extract was frozen in liquid nitrogen. Determination of protein concentration was adapted to a robotic platform using the BCA assay kit (Pierce) and bovine γ-globulin as a standard. Liquid handling was performed by Freedom EVO 200 robot (Tecan) and the absorbance at 562nm was measured in an Infinite M200 plate reader (Tecan).

**Assays of DNA glycosylase repair activity**

The assays were adapted to a robotic platform, in which liquid handling of the nicking reactions were performed automatically by a Freedom EVO 200 robot (Tecan) and Freedom EVOware
software (Tecan). Denatured radioactive DNA products were analyzed by electrophoresis on a 15 % polyacrylamide gel containing 8M urea, in 89mM Tris-borate, 2.5mM EDTA pH 8.0, at 1500 V for 2 hours at 45-50°C. The distribution of radiolabeled DNA products was visualized and quantified using a Fuji BAS 2500 phosphorimager. Fluorescent denatured DNA products were analyzed by capillary gel electrophoresis, using the ABI3130XL genetic analyzer (Applied Biosystems), and the GeneMapper (Applied Biosystems) and PeakAnalyzer (Robiotec, Rehovot, Israel) softwares. Reaction conditions are described below.

**MPG assay.** The standard reaction mixture (20µl) contained 50mM PIPES (pH 6.7), 10mM Tris (pH 7.1), 2mM EDTA, 0.5mM MgCl₂, 30mM KCl, 1µg/µl bovine γ-globulin, 0.1% polyvinyl alcohol (PVA), 7.5nM substrate and 15µg/ml protein extract. The reaction was carried out at 37°C for 15 minutes, after which it was stopped by heat inactivation at 95°C for 2 minutes. The proteins were degraded by incubation with proteinase K (20µg) for 30 minutes at 37°C, after which they were treated with 100mM NaOH for 30 minutes at 37°C, fractionated by PAGE and quantified by phosphorimaging. One unit of MPG activity is defined to cleave 1fmol of DNA substrate in 1 hour at 37°C, under these conditions.

**OGG-F assay.** The standard reaction mixture (10µl) contained 50mM Tris (pH 7.1), 1mM EDTA, 115mM KCl, 1µg/µl bovine γ-globulin, 100nM PolydA-polydT, 12.5nM substrate and 0.2-0.5mg/ml protein extract. The reaction was carried out at 37°C for 30 minutes, after which it was stopped by heat inactivation at 95°C for 2 minutes. The reactions were treated with 100mM NaOH for 30 minutes at 37°C, followed by fractionation and quantification using capillary gel
electrophoresis (the ABI 3130XL machine). One unit of OGG-F activity is defined to cleave 1 fmol of DNA substrate in 1 hour at 37°C, under these reaction conditions.

**Statistical analysis.** Mean levels of MPG were compared between case patients and control subjects using analysis of covariance controlling for membership of a matched pair and smoking status. The significance test was based on a F-test that yields a two-sided comparison. The same approach was used to compare mean test levels between histological types (within case patients), controlling for smoking status, gender, and age, and also between smoking, gender or age subgroups, controlling for disease status and the other covariates. Interactions between disease status and the other covariates were also tested using the F-test from the analysis of covariance.

The odds ratio of lung cancer was estimated for MPG and OGG tests using conditional logistic regression models. This method takes account of the matched design of the study and therefore automatically adjusts also for the matching variables, sex, age, area of residence and ethnicity. Odds ratios were estimated for each test as a continuous variable (assuming a linear relation with the log odds), expressing variables in standard deviation (SD) units (the log odds ratios being standardized coefficients) to allow meaningful comparison (e.g., (3)). Two of the control subjects and 11 of the case patients had previous other types of cancer. Re-calculation of odds ratios with adjustment to previous cancer yielded results very similar to those obtained without this adjustment.

The odds ratio of lung cancer was estimated for the combinations of continuous OGG with a continuous MPG test using conditional logistic regression models with smoking status as an adjusting variable. A combined OGG-MPG score was calculated from the regression coefficients in this model and was used to estimate the odds ratio per 1 SD. P-values were based
on the two-sided Wald test. All P-values presented in this paper are two-sided. All the statistical analyses were performed using S-Plus 2000, Professional Release 1 (1988-99 Mathsoft, Inc.) and/or SAS software (version 9.2; SAS Institute Inc.).

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3. Charakida M, Besler C, Batuca JR, Sangle S, Marques S, Sousa M, et al. Vascular abnormalities, paraoxonase activity, and dysfunctional HDL in primary antiphospholipid syndrome. JAMA. 2009;302(11):1210-7.
Supplementary Figure 1
N-Methylpurine DNA Glycosylase and OGG1 DNA Repair Activities: Opposite Associations With Lung Cancer

Supplementary Figure 1. Distribution of MPG and OGG enzyme activities in lung cancer cases and control subjects. Relative frequency plots for MPG and OGG activities were determined in 100 case patients (continuous line) and 100 matched control subjects (dashed line). The relative frequencies as percent were plotted using GraphPad Prism version 5.00, with bin width automatically chosen by the software. The relative frequency plots were smoothed by two neighbors on each size, zero order of polynomial smoothing. A. Relative frequency plots for MPG activity. Bin width was 20 units. Case patients exhibit a shift to higher values of MPG. B. Relative frequency plots for OGG activity. Bin width was 0.5 unit. Case patients exhibit a shift to lower values of OGG.