

Research Article

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Low Integrated DNA Repair Score and Lung Cancer Risk

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Abstract

DNA repair is a prime mechanism for preventing DNA damage, mutation, and cancers. Adopting a functional approach, we examined the association with lung cancer risk of an integrated DNA repair score, measured by a panel of three enzymatic DNA repair activities in peripheral blood mononuclear cells. The panel included assays for AP endonuclease 1 (APE1), 8-oxoguanine DNA glycosylase (OGG1), and methylpurine DNA glycosylase (MPG), all of which repair oxidative DNA damage as part of the base excision repair pathways. A blinded population-based case-control study was conducted with 96 patients with lung cancer and 96 control subjects matched by gender, age (± 1 year), place of residence, and ethnic group (Jews/non-Jews). The three DNA repair activities were measured, and an integrated DNA repair OMA (OGG1, MPG, and APE1) score was calculated for each individual. Conditional logistic regression analysis revealed that individuals in the lowest tertile of the integrated DNA repair OMA score had an increased risk of lung cancer compared with the highest tertile, with OR = 9.7; 95% confidence interval (CI), 3.1–29.8; $P < 0.001$, or OR = 5.6; 95% CI, 2.1–15.1; $P < 0.001$ after cross-validation. These results suggest that pending validation, this DNA repair panel of risk factors may be useful for lung cancer risk assessment, assisting prevention and referral to early detection by technologies such as low-dose computed tomography scanning. *Cancer Prev Res*; 7(4); 398–406. ©2013 AACR.

Introduction

Prevention is arguably the most effective way to overcome disease and improve human health. This has been demonstrated by the dramatic decrease in heart disease mortality over the past 20 years, a significant part of which was because of the identification of mechanism-based risk biomarkers, and taking effective measures to treat them (1). Despite the advance in the molecular mechanisms that underlie cancer development, progress in cancer prevention has been slow (2). A key reason is the lack of effective mechanism-based host-risk factors (e.g., risk biomarkers) that estimate one's risk to develop cancer (3). DNA repair plays a critical role in preventing cancer via its DNA safeguarding activities, which prevent mutations and genomic instability (4) that are critical for cancer (5–8). This suggests

that DNA repair is a promising target for mechanism-based cancer prevention approaches (9–12).

Aiming at harnessing DNA repair for cancer prevention, it was hypothesized that variations exist among individuals in their ability to repair DNA, and that those variations translate into differences in cancer predisposition, such that low effectiveness in DNA repair capacity will cause higher cancer risk. To measure such interindividual variations in DNA repair, we (13–18) and others (19–21) took the approach of measuring the enzymatic activity of specific DNA repair enzymes, using peripheral blood mononuclear cell (PBMC) as the reporter tissue. Our primary focus was on DNA repair enzymes that target oxidative DNA damage, which has been implicated as a major factor in carcinogenesis (22). This approach assumes that (i) DNA repair activity in PBMC is a legitimate surrogate for DNA repair in other tissues, and (ii) each individual has a personal baseline capacity to repair DNA. These assumptions are supported by previous results showing that the activity of the DNA repair enzyme OGG1 in PBMC correlates to the activity in lung tissue (13), and that individuals exhibit a personal OGG1 activity that is stable over several years (13) and does not significantly change years after recovery from cancer (15). Using this approach we found that low OGG1 enzymatic activity is associated with the risk of lung cancer (13) and head and neck cancer (15). In addition, we found that increased enzymatic activity of MPG, a DNA repair enzyme of the DNA glycosylases family, which removes from DNA multiple alkylation and oxidative lesions, is associated with increased lung cancer risk (18). This surprising finding illustrates the notion that imbalances in DNA repair

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(23–25), and not necessarily low activity of DNA repair, can be involved in cancer risk.

Here we present evidence for a third enzymatic DNA repair risk factor for lung cancer, namely low enzyme activity of AP endonuclease 1 (APE1). APE1 is of special interest because of its central role in base excision repair, a pathway to which OGG1 and MPG also belong (refs. 26–29; Fig. 1A). Moreover, we show that a personalized integrated DNA repair score, composed of a combination of the new APE1 risk factor and the OGG1 and MPG risk factors, is strongly associated with lung cancer risk, and pending validation may provide a tool for improved selection criteria of extra-high-risk individuals for early detection approaches such as low-dose computed tomography (LD-CT) scanning (30, 31).

Materials and Methods

Study participants

The study participants have been previously described (18). They included 100 patients recently diagnosed with lung cancer (case patients) recruited from the Rambam Medical Center in Haifa, located in northern Israel, and 100 subjects with no diagnosis of lung cancer (control subjects) who were randomly selected enrollees of Clalit Health Services (CHS) identified from the same geographical area. The controls were individually matched to the cases by gender, year of birth, place of residence (defined by primary clinic location), and ethnic group (Jews vs. non-Jews). Controls were excluded only if they had a former diagnosis of lung cancer. Participants provided written informed consent at the 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 were made independently by the diagnosing hospitals and included information on histologic type, TNM staging, and tumor grade. The institutional review board at Carmel Medical Center, Haifa, approved all procedures. Blood samples were drawn from cases before the operative procedure or any treatment intervention. The case patients and control subjects had mean ages of 67 years, included 60% males and 40% females, and were 77% Jews and 23% non-Jews. The controls comprised 22%, 28%, and 50% current, former, and never smokers, respectively, and the cases 37%, 36%, and 24%, respectively (18). There was no matching for smoking status, but adjustment was performed for smoking in the statistical analyses.

Isolation of peripheral blood mononuclear cells, and preparation of protein extracts

Each study participant provided a sample (17 mL) of peripheral blood collected in 2 ACD blood collection tubes (BD vacutainers, Catalog No. 364606). Blood samples were kept at room temperature, and processed 18 to 24 hours after collection to isolate PBMC, essentially as previously described (18). Protein extracts were prepared from PBMC as previously described (18).

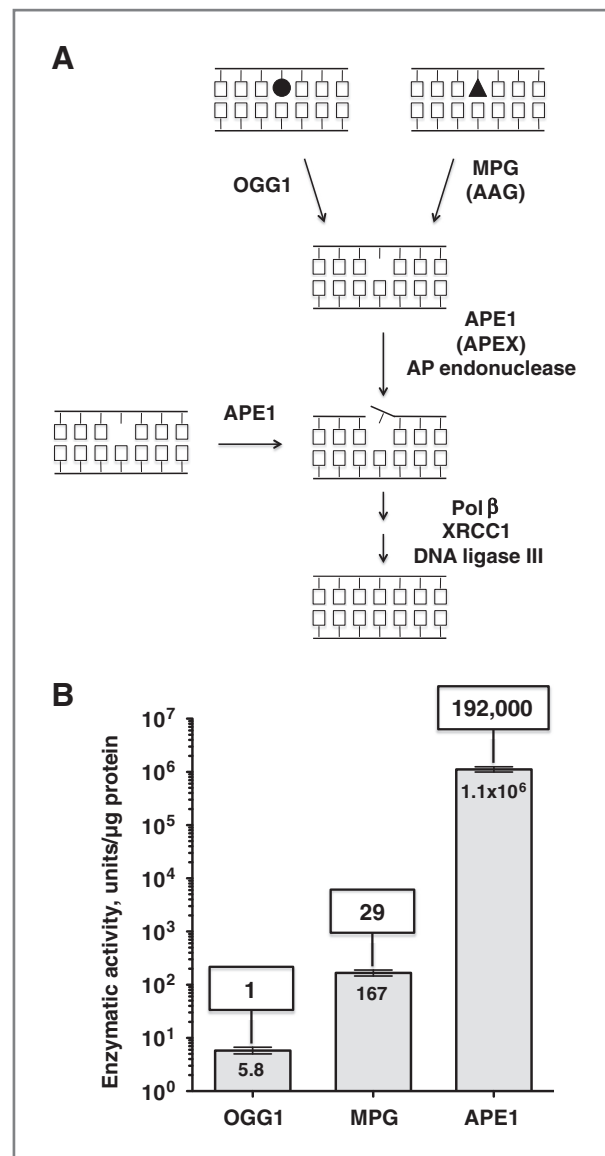


Figure 1. Base excision repair and enzymes studied in this work. A, each of the DNA glycosylases OGG1 and MPG initiates a subpathway of BER by removing from DNA its damaged base target (the black circle and triangle). The abasic site thus formed is incised by the AP endonuclease APE1, followed by further processing, repair synthesis, and ligation to complete the repair. APE1 also initiates the repair of primary abasic sites formed in DNA by spontaneous or oxidation-induced base loss. B, mean DNA repair enzyme activity in PBMC specimens obtained from 10 control subjects, which were assayed for OGG1 acting on 8-oxoguanine, MPG acting on hypoxanthine, and APE1 acting on a furanyl abasic site. The numbers inside the columns are the values of the mean enzymatic activity in units/μg protein, whereas the numbers above the columns represent the activities relative to OGG1 activity. Assay conditions are described under Materials and Methods.

Assays of enzymatic DNA repair activity

The fluorescence-based DNA repair assays were done with DNA substrates tagged with a 3'-Yakima yellow fluorescent tag, each containing the appropriate site-specific lesion: a synthetic abasic site (also called apurinic/

aprimidinic site; AP site) for APE1, an hypoxanthine for MPG, and 8-oxoguanine (8-oxoG) for OGG1. The assays monitor the specific incision at the lesion, followed by capillary gel electrophoresis analysis using an AB3130XL genetic analyzer. The assays were done using 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).

APE1 assay. The assay used for measuring APE1 enzymatic activity is in principle similar to previously published assays (32, 33). Its development will be described elsewhere, but the final reaction conditions were as follows: the DNA substrate used was 5'-G GTG CAT GAC ACT GTF ACC TAT CCT CAG CG Y-3' (F = furanyl abasic site; Y = Yakima yellow tag) annealed to 5'-CG CTG AGG ATA GGT CAC AGT GTC ATG CAC C-3'. The reaction mixture (20 μ L) contained 75 mmol/L Tris (pH 7.8), 0.1 mmol/L EDTA, 9 mmol/L MgCl₂, 42.4 mmol/L KCl, 0.25 μ g/ μ L bovine γ -globulin, 0.25% polyvinyl alcohol (PVA), 0.25 mmol/L Spermidine; 0.05 mmol/L Spermine; 35 nmol/L substrate, and 0.015 ng/ μ L 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. One unit of APE1 activity is defined to cleave 1 fmol of DNA substrate in 1 hour at 37°C, under these conditions.

MPG assay. The DNA substrate used for the MPG assay was 5'-GT CCG GTG CAT GAC ACT GTX ACC TAT CCT CAG CG Y-3' (X = hypoxanthine; Y = Yakima yellow tag) annealed to 5'-CG CTG AGG ATA GGT TAC AGT GTC ATG CAC CGG AC-3'. The standard reaction mixture (20 μ L) contained 50 mmol/L MOPS (pH 6.8), 30 mmol/L Tris (pH 7.1), 2 mmol/L EDTA, 0.5 mmol/L MgCl₂, 36 mmol/L KCl, 1 μ g/ μ L bovine γ -globulin, 0.3% PVA, 15 nmol/L substrate, and 45 ng/ μ L 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 reactions were treated with 100 mmol/L NaOH for 30 minutes at 37°C. One unit of MPG activity is defined to cleave 1 fmol of DNA substrate in 1 hour at 37°C, under these conditions.

OGG1 assay. The DNA substrate used for the OGG1 assay was 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 GTC ACA GTG TCA TGC ACC GG-3'. The standard reaction mixture (10 μ L) contained 50 mmol/L Tris (pH 7.1), 1 mmol/L EDTA, 115 mmol/L KCl, 1 μ g/ μ L bovine γ -globulin, 100 nmol/L PolydA.polydT, 12.5 nmol/L substrate, and 0.2 to 0.5 mg/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 100 mmol/L NaOH for 30 minutes at 37°C. One unit of OGG1 activity is defined to cleave 1 fmol of DNA substrate in 1 hour at 37°C, under these reaction conditions.

All enzyme activities measured in the case-control specimens were assayed also in 2 standard extracts that were used in each round of the assays, and served as reference normalizing extracts. These standard extracts were prepared from blood bags obtained from the blood bank.

Measurement of baseline APE1, OGG1, and MPG enzyme activities. Baseline enzymatic activities of APE1, OGG1, and MPG were measured in the same PBMC specimens, obtained from 10 blood bank bags, using the assays described above. The results obtained for the average activities of OGG1 and MPG were 5.8 (range: 5.0–6.7) and 167 (range: 146–189) units/ μ g protein, respectively, whereas surprisingly, APE1 activity was 1,121,000 units/ μ g protein (range: 994,000–1,247,000), 5 orders of magnitude higher than OGG1 activity (Fig. 1B).

Statistical analysis

The OR of lung cancer was estimated for APE1 tests using conditional logistic regression models adjusting for smoking status with or without OGG1 and MPG activities. 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. Two models were used to estimate the OR. In the first model, the explanatory variable was treated as a continuous variable (assuming a linear relation with the log odds); and in the second model, the variable was categorized into 3 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 to 3 for the 3 tertile groups.

The OR of lung cancer was estimated for the OMA score using conditional logistic regression models adjusting for smoking status. Odds ratios were estimated for the continuous variable (assuming a linear relation with the log odds), and categorized into 3 groups, as with APE1. Because the weights for each component used in the OMA score were chosen to optimize strength of association of the score with lung cancer for the observed data, a cross-validation procedure was applied to remove the resulting bias in the ORs. A "cross-validated" OMA score was obtained for each individual by omitting the individual and his/her matched pair and running the conditional logistic regression model on the remaining pairs so as to obtain new weights for the OMA score. The cross-validated OMA score for the omitted pair was then calculated using these new weights. We did this for each matched pair, and then ran the conditional logistic regression on the set of cross-validated OMA scores instead of the original scores. All the statistical analyses were performed using S-Plus (TIBCO Software Inc.) and/or SAS software (version 9.2; SAS Institute Inc.).

Results

APE1 activity characteristics in lung cancer case patients and matched control subjects

We first concentrated on examining whether interindividual variations in APE1 activity, like OGG1 and MPG, are also associated with lung cancer risk, thereby increasing the number of risk biomarkers to 3, and likely provide a better risk estimate. The results of measuring APE1 enzyme activity of the patients with lung cancer and matched control subjects are presented in Table 1 and Supplementary Fig. S1A. The interindividual variation among the controls was 4.9-fold, ranging from 435 to 2137 units/ng protein,

Table 1. Distribution of selected characteristics and APE1 activity value in patients with lung cancer and control subjects^a

Variable	Control subjects (n = 100)		Case patients (n = 100)		P
	No.	APE1 mean (95% CI)	No.	APE1 mean (95% CI)	
All ^b	99	896 (849–943)	99	787 (752–823)	0.0007 ^c
SQCC			30	747 (686–807)	
Adenocarcinoma			45	805 (757–854)	
				P = 0.089 ^d	
P interaction ^e					0.75
Age, y					
≤65	40	933 (867–1,000)	40	828 (772–884)	
>65	59	871 (807–936)	59	760 (715–805)	
P comparing subgroups ^f					0.018
P interaction ^e					0.78
Sex					
Male	59	859 (811–907)	59	783 (738–828)	
Female	40	951 (860–1,042)	40	794 (736–852)	
P comparing subgroups ^f					0.14
P interaction ^e					0.13
Smoking status					
Never smoked	50	927 (851–1,002)	24	784 (721–847)	
Former smoker	27	867 (792–942)	36	787 (722–852)	
Current smoker	22	863 (780–945)	36	785 (733–837)	
P comparing subgroups ^f					0.40
P interaction ^e					0.55

^aAPE1 activity was measured as described under "Materials and Methods." One participant did not have a known APE1 value. This participant and the matched control were excluded from the analysis. Three case participants did not have a known smoking status.

^bOf the 100 lung cancer cases, 30 had squamous cell carcinoma (SQCC), 46 had adenocarcinoma, 14 BAC, 4 adenosquamous carcinoma, 4 adenoBAC, 1 small cell carcinoma, and 1 unknown histology.

^cAnalysis of covariance comparing cases with controls, with matched pair and smoking status as a covariate.

^dAnalysis of covariance comparing histological type within cases, with smoking status, age (continuous), or gender as covariates.

^eTest for interaction between case-control status and the variable of interest.

^fAnalysis 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.

and among case patients 3.3-fold, ranging from 432 to 1410 units/ng protein. The mean APE1-specific activity in case patients was 787 (95% CI, 752–823) units/ng protein, significantly lower than the 896 (95% CI, 849–943) units/ng protein mean activity in control subjects ($P = 0.0007$; Table 1). APE1 activity in the controls seemed lower in males (859 units/ng protein) than in females (951 units/ng protein), but not among cases (783 units/ng protein in males vs. 794 in females; test for gender-disease interaction: $P = 0.13$; Table 1). In addition, APE1 activity was lower in those ages >65 years than in those ages ≤65 years ($P = 0.018$; Table 1). There was no difference in APE1 activity between adenocarcinoma and squamous cell carcinoma (Table 1). To determine whether smoking status is associated with APE1 activity level, the mean APE1 activity level was calculated for each smoking status in case patients and control subjects. As can be seen in Table 1, the mean levels of APE1 activity among current, past and never smokers were

similar, suggesting that smoking did not significantly affect the level of APE1 enzyme activity in PBMC. In addition, no significant interaction between smoking status and APE1 activity was found when a test for interaction was performed (Table 1).

Decreased APE1 enzymatic activity is associated with increased risk of lung cancer

The association between the level of activity of APE1 and the probability of having lung cancer, adjusted for smoking status, was further explored using conditional logistic regression. When APE1 activity was used as a continuous variable, the adjusted OR for lung cancer associated with 1 SD (239 units)¹ decrease in APE1

¹Expressing the variable in SD units (standardized coefficients) allows meaningful comparisons to other DNA repair assays.

Table 2. Conditional logistic regression analysis of APE1 activity value in patients with lung cancer and control subjects

Variable	No. of control subjects (%)	No. of case patients (%)	Adjusted ^a OR (95% CI)
APE1 (per 239 U decrease) ^{b,c}	96 (100.0)	96 (100.0)	2.0 (1.3–3.1), <i>P</i> = 0.002
APE1 (by tertiles) ^d			
>961 U	32 (33.3)	14 (14.6)	1.0 (referent)
795–961 U	32 (33.3)	29 (30.2)	1.7 (0.7–3.9), <i>P</i> = 0.22
<795 U	32 (33.3)	53 (55.2)	3.7 (1.5–8.8), <i>P</i> = 0.003
			Trend test ^d <i>P</i> = 0.003

^aConditional logistic regression for matched sets adjusted for smoking status (smoker, former-smoker, never smoked). Three case patients did not have known smoking status and 1 control subject did not have known APE1 value. These participants along with their matched subjects were excluded from the analysis.

^bAPE1 activity was measured as described in the "Materials and Methods" section and was first fitted in the conditional logistic regression model as a continuous variable and with adjustment for smoking status. The OR for smoking that was obtained with this model, was: former smoker vs. never smoked: 3.2 (95% CI, 1.3–7.6); current smoker vs. never smoker: 3.0 (95% CI, 1.3–6.8).

^c239 U represents 1 SD in the control group.

^dTertiles of the control subjects' values. The upper tertile was chosen as the referent.

activity was 2.0 (95% CI, 1.3–3.1; *P* = 0.002; Table 2). When APE1 activity values were divided into tertiles using the controls' values, the adjusted OR for the lowest tertile versus the highest was 3.7 (95% CI, 1.5–8.8; *P* = 0.003). A test for trend over the 3 tertiles was also significant (*P* = 0.003; Table 2). The significant associations obtained with the 2 different models strengthen the conclusion that low APE1 enzymatic activity is associated with lung cancer risk.

The integrated DNA repair OMA score is strongly associated with lung cancer risk

When measuring the 3 DNA repair activities in an individual, it is useful to form an integrated DNA repair score of the 3 with appropriate weights, because this takes into account situations in which only 1 or 2 of the DNA repair activities are in the range that is associated with higher cancer risk. The weights of the score were obtained directly from the estimated log ORs in the fit of the conditional logistic regression model, including OGG1, MPG, and APE1 (with smoking status included in the model; Supplementary Table S1), and the score was defined as $0.00425 \times \text{APE1} + 0.5419 \times \text{OGG1} - 0.02541 \times \text{MPG}$. We term this the OMA score (for OGG1, MPG, and APE1) and calculated its value for each study participant. The distributions of the OMA score among study participants showed a clear shift of case patients to lower OMA score values compared with control subjects (Supplementary Fig. S1B). The OMA scores ranges were 1.40 to 10.42 and 0.53 to 7.01 units in controls subjects and case patients, respectively. The mean OMA score in patients was 2.8 (95% CI, 2.6–3.0), significantly lower than the value of 3.6 (95% CI, 3.4–3.8) in control subjects (*P* < 0.0001; Table 3). The integrated DNA repair OMA score in adenocarcinoma cases was 2.9 (95% CI,

2.7–3.2), higher than in SCC cases, where it was 2.5 (95% CI, 2.2–2.7; *P* = 0.04). There was no appreciable difference in the OMA score between males and females, and between subjects >65 and ≤65 years old. In addition, there was no appreciable difference between never, former, and current smokers, and no interaction between the OMA score and smoking status.

The association between the OMA score and the probability of having the disease, adjusted for smoking status, was calculated using conditional logistic regression with the OMA score as a continuous variable. As can be seen in Table 4, the adjusted OR for lung cancer associated with a 1.18 unit decrease (1 SD) in OMA score was significantly greater than 1 (OR = 3.2; 95% CI, 1.7–5.7; *P* < 0.001; Table 4). We used cross-validation to adjust the estimated ORs so that they will reflect the likely performance of the score in a new dataset and obtained OR of 2.7 (95% CI, 1.6–4.5; *P* < 0.001), which is smaller, but still very strong (Table 2). The strength of the association of reduced OMA score with lung cancer risk is further illustrated in a logistic regression model using tertiles of OMA score among control subjects. After adjusting for smoking status, the OR for lung cancer of individuals in the lowest tertile (vs. the highest tertile) of the DNA repair OMA score was 9.7 (95% CI, 3.1–29.8; *P* < 0.001), and after cross-validation OR 5.6 (95% CI, 2.1–15.1; *P* < 0.001), compared with individuals in the upper tertile (Table 4).

Discussion

Because of the important role of oxidative stress in carcinogenesis, imbalances in DNA repair enzymes that repair oxidative DNA damage are attractive candidate risk factors for lung cancer. The diversity of oxidative DNA damage suggests that a single DNA repair risk factor will

Table 3. Distribution of selected characteristics and combined OMA-integrated DNA repair score in patients with lung cancer and control subjects^a

Variable	Control subjects (n = 100)		Case patients (n = 100)		P
	No.	OMA score mean (95% CI)	No.	OMA score mean (95% CI)	
All ^b	99	3.6 (3.4–3.8)	99	2.8 (2.6–3.0)	<0.0001 ^c
SQCC			30	2.5 (2.2–2.7)	
Adenocarcinoma			45	2.9 (2.7–3.2)	
P comparing subgroups ^d					0.04
P interaction ^e					0.35
Age, y					
≤65	40	3.6 (3.3–3.9)	40	2.8 (2.5–3.1)	
>65	59	3.6 (3.2–3.9)	59	2.8 (2.5–3.1)	
P comparing subgroups ^f					0.67
P interaction ^e					0.91
Sex					
Male	59	3.5 (3.3–3.8)	59	2.7 (2.5–3.0)	
Female	40	3.7 (3.3–4.2)	40	2.9 (2.5–3.2)	
P comparing subgroups ^f					0.33
P interaction ^e					0.62
Smoking status					
Never smoked	50	3.7 (3.4–4.1)	24	2.8 (2.4–3.2)	
Former smoker	27	3.6 (3.2–4.1)	36	2.7 (2.4–3.1)	
Current smoker	22	3.3 (2.9–3.7)	36	2.8 (2.5–3.1)	
P comparing subgroups ^f					0.39
P interaction ^e					0.52

^aCombined OMA score was defined as: $0.00425 \times \text{APE1} + 0.5419 \times \text{OGG1} - 0.02541 \times \text{MPG}$.

One participant did not have known test values. This participant and the matched control were excluded from the analysis.

^bOf the 100 lung cancer cases, 30 had squamous cell carcinoma (SQCC), 46 had adenocarcinoma, 14 BAC, 4 adenosquamous carcinoma, 4 adenoBAC, 1 small cell carcinoma, and 1 unknown histology.

^cAnalysis of covariance comparing cases with controls, with matched pair and smoking status as a covariate. For smoking status subgroups, the covariates were age (continuous) and gender.

^dAnalysis of covariance comparing histological type within cases, with smoking status, age (continuous), and gender as covariates.

^eTest for interaction between case-control status and the variable of interest.

^fAnalysis 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.

not provide a sufficiently strong risk prediction, and rather a panel of risk factors will be needed. We have thus chosen APE1 enzymatic activity, in addition to the OGG1 and MPG activities that we have previously studied, because of its key function in BER (4, 27–29, 34), and its involvement in single-strand break repair (35, 36) and nucleotide incision repair (NIR; 37). In addition, APE1 is a redox factor via its REF domain (38).

Somewhat surprisingly, APE1 enzyme activity in PBMC was 4 to 5 orders of magnitude higher than the activities of OGG1 and MPG (Fig. 1B), likely because its involvement in multiple DNA repair pathways as described above. These high APE1 activities are not saturating, and the 4.9-fold range of APE1 activity that we have observed amongst control subjects seems to have biologic significance, as indicated by the significant association of low APE1 activity and lung cancer risk.

This association provides support for the importance of oxidative DNA damage in the development of lung cancer, and is intuitively understandable because low repair causes higher mutation rates because of unrepaired oxidative DNA damage, thereby facilitating carcinogenesis. A role of low APE1 in facilitating carcinogenesis is at first glance inconsistent with the observations that APE1 is overexpressed in several types of cancers, also associated with resistance to chemotherapy (38–41). To explain this, we hypothesize a dual role for DNA repair in carcinogenesis. In normal cells, low APE1 activity facilitates carcinogenesis by increasing mutations and genomic instability, however, as cells progress along the carcinogenesis path, which involves robust proliferation, cells with upregulated DNA repair may be selected because of the advantage that it provides during replication.

Table 4. Conditional logistic regression analysis of combined OMA score based on OGG1, MPG, and APE1 values in patients with lung cancer and control subjects^a

Variable	No. of control subjects (%)	No. of case patients (%)	Adjusted ^b OR (95% CI)
Score (per 1 SD decrease, 1.18U) ^c	96 (100.0)	96 (100.0)	3.2 (1.7–5.7), <i>P</i> < 0.001
Cross-validation			
Score (per 1 SD decrease, 1.16 U)	96 (100.0)	96 (100.0)	2.7 (1.6–4.5), <i>P</i> < 0.001
Score (by tertiles) ^d			
>3.98	31 (32.3)	8 (8.3)	1.0 (referent)
3.15–3.98	32 (33.3)	23 (24.0)	3.3 (1.1–9.5), <i>P</i> = 0.03
≤3.14	33 (34.4)	65 (67.7)	9.7 (3.1–29.8), <i>P</i> < 0.001
			Trend test <i>P</i> < 0.001
Cross-validation			
Highest tertile	30 (31.2)	12 (12.5)	1.0 (referent)
Middle tertile	33 (34.4)	19 (19.8)	1.5 (0.6–3.7), <i>P</i> = 0.42
Lowest tertile	33 (34.4)	65 (67.7)	5.6 (2.1–15.1), <i>P</i> < 0.001
			Trend test <i>P</i> < 0.001

^aOR, odds ratio.^bConditional logistic regression for matched sets adjusted for smoking status (smoker, ex-smoker, never smoker). Three case patients did not have known smoking status and one control subject did not have known test values. These participants along with their matched subjects were excluded from the analysis.^cScore was defined as $0.00425 \times \text{APE1} + 0.5419 \times \text{OGG1} - 0.02541 \times \text{MPG}$ and was fitted in the conditional logistic regression model as a continuous variable. The value 1.18U is 1 SD in the control group. The odds ratio for smoking, obtained with this model, was: ex-smoker vs. never smoker: 2.6 (95% CI, 1.0–6.3); current smoker vs. never smoker: 3.0 (1.1–8.2). The value 1.16U is 1 SD of the cross-validated score in the control group.^dTertiles of the control subjects' values. The upper tertile was chosen as the referent.

The OMA score presented in this study enables to measure one's ability to repair oxidative DNA damage, representing a personalized DNA repair capacity. The strong association of low OMA score with lung cancer risk is highlighted in Supplementary Fig. S1C that shows the OR calculated as a function of the percentiles of the distribution of OMA score among the control subjects. The OR for a person at the 10th (lowest) percentile versus a person at the 90th percentile of the distribution was 11.5 (95% CI, 3.6–36.3) or 7.6 (95% CI, 2.7–20.9) after adjustment by cross-validation. The strong association of the integrated DNA repair score suggests that it can potentially be used for lung cancer risk assessment, thereby aiding lung cancer prevention. Importantly, the fact that low integrated DNA repair OMA score is a strong risk factor, which is independent of smoking status, suggests that it may serve as a powerful component, on top of smoking status, in evaluating one's risk to develop lung cancer. This can be used to estimate personalized risk of a smoker to develop lung cancer, and provide motivation for smoking cessation and other risk reduction activities.

Like in any epidemiologic study, validation of the results is needed in independent studies. In this context, it should be pointed out that the present OGG1 results validate a previous study that we have performed on different groups of patients with lung cancer and healthy control subjects

(13). A limitation of the present study is its relatively small sample size, which limits the ability to perform subgroup analysis. In addition, a prospective study is needed for definitively proving that low OMA DNA repair score has predictive power for lung cancer.

It was recently reported that LD-CT screening for early detection of lung cancer enabled identification and treatment of very early lung cancer, leading to a 20% decrease in lung cancer mortality. However, the low rate of 4% true positives in this detection method complicates its broad usage for screening purposes (ref. 30; NLST study; see also the I-ELCAP study, ref. 31). A useful paradigm would be to use the integrated DNA repair OMA score as a means to improve selection criteria for LD-CT screening for early detection of lung cancer, by identifying individuals at extra-high risk. Pending validation, such a strategy is expected to significantly increase the rate of true-positives, thereby avoiding unnecessary invasive procedures and improving cost-benefit (42–44). In addition, having the OMA personalized measure for DNA repair activity raises the possibility of searching for drugs that will improve the effectiveness of DNA repair, potentially providing protection against lung cancer.

In summary, our study identified a panel of DNA repair risk factors, which when combined produced an individual risk score that is strongly associated with lung cancer risk.

Pending validation it can be used to motivate or select individuals for intensive primary or secondary prevention intervention.

Disclosure of Potential Conflicts of Interest

T. Paz-Elizur and Z. Livneh have ownership interest (including patents) on low OGG1 as a lung cancer risk factor. Another patent on the 3 DNA repair activities was submitted. There was no income. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): Z. Sevilya, Y. Leitner-Dagan, M. Pinchev, D. Elinger, G. Rennert

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Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): D. Elinger, T. Paz-Elizur

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