

Decreased Repair Activities of 1,*N*⁶-Ethenoadenine and 3,*N*⁴-Ethenocytosine in Lung Adenocarcinoma Patients¹

Elżbieta Speina, Maja Zielińska, Alain Barbin, Daniel Gackowski, Janusz Kowalewski, Maria A. Grażewicz, Janusz A. Siedlecki, Ryszard Oliński, and Barbara Tudek²

Department of Molecular Biology, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Pawińskiego 5a, 02-106 Warsaw, Poland [E. S., M. Z., M. A. G., B. T.]; International Agency for Research on Cancer, 150 cours Albert Thomas, 69372 Lyon Cedex 08, France [A. B.]; Department of Clinical Biochemistry [D. G., R. O.] and Department and Clinic of Thoracic Surgery and Tumours [J. K.], The Ludwik Rydygier Medical University in Bydgoszcz, Karłowicza 24, 85-092 Bydgoszcz, Poland; and Department of Molecular Biology, Cancer Center-Institute, Roentgena 5, 02-781 Warsaw, Poland [J. A. S.]

ABSTRACT

To assess the role of oxidative stress and lipid peroxidation (LPO) in the pathogenesis of lung cancer, we measured the levels of 1,*N*⁶-ethenoadenine (εA) and 3,*N*⁴-ethenocytosine (εC) in the DNA by immunoaffinity/³²P postlabeling (33 cases). We also measured the capacity for εA and εC repair (by the nicking assay) in normal and tumor lung tissues, as well as in blood leukocytes of lung cancer patients (56 cases). Repair activities for εA and εC were also assayed in leukocytes of healthy volunteers, matched with cancer patients for age, sex, and smoking habit (25 individuals).

Up to 10-fold variations among individuals were observed both in adducts level and repair activities. No differences in εA and εC levels between tumor and nonaffected lung tissues were recorded. However, leukocytes accumulated a significantly higher number of DNA adducts than the lung tissues. Repair activities for both εA and εC were significantly higher in tumor than in normal lung tissue. No significant differences in εA and εC repair activities were associated with age, sex, or smoking habit. However, a significant difference in repair capacity was observed between two histological types of lung cancer, squamous cell carcinoma (SQ) and adenocarcinoma (AD). In individuals suffering from lung AD, εA- and εC-repair activities in normal lung and blood leukocytes were significantly lower than in SQ patients. Moreover, in nonaffected lung tissue of AD patients, the ratio εA/εC adducts was lower than in SQ patients. Differences have also been found between εA and εC repair activities of cancer patients and healthy volunteers. Repair capacity for εA was significantly lower in blood leukocytes of lung cancer patients than in leukocytes of healthy volunteers ($P = 0.012$). This difference was even larger between healthy volunteers and patients developing inflammation-related AD ($P = 0.00033$). Repair activities for εC were the same in leukocytes of healthy controls, all lung cancer patients, and SQ patients. However, individuals with ADs revealed significantly lower εC-repair activity ($P = 0.013$).

These results suggest that oxidative stress-mediated lipid peroxidation might contribute to induction and/or progression of lung cancer. Decreased activity of base excision repair pathway for εA and εC is associated particularly with inflammation-related lung AD.

INTRODUCTION

Formation of ROS³ and RNS, which accompanies inflammatory processes and LPO, is involved in many pathological conditions, including certain types of human cancers, *e.g.*, lung, breast, and colon (1–3). Lung cancer is the most frequent cancer all over the world and in Poland (4, 5). More than 80% of lung cancer patients are tobacco smokers or ex-smokers (6). Tobacco smoke contains over 4,000 compounds, and some of them generate the formation of ROS. Moreover, cigarette smoking causes chronic lung inflammation (7). ROS and RNS can cause LPO, with products such as malondialdehyde, crotonaldehyde, and *trans*-4-hydroxy-2-nonenal; these species can also cause oxidation of DNA and protein thiols and protein nitrosylation (8, 9). Individual susceptibility to the compounds contained in tobacco smoke is an important determinant of risk, because only 10–15% of smokers develop lung cancer (10). A balance between metabolic activation and detoxification of B[a]P, one of the major toxic components of tobacco smoke, is an important determinant of lung cancer risk related to tobacco smoking. Polymorphisms of cytochrome P-450 and glutathione *S*-transferase have been discovered and well characterized (11–13). However, the role of oxidative DNA damage and its repair in pathogenesis of lung cancer is not well understood, and such damage might be highly important in certain histological types of lung cancer. Two major histological subtypes of NSCLC have been described: SQ and AD. SQ is located mainly in major bronchi and is strongly associated with cigarette smoking (14, 15). It often has mutations in the *p53* gene (16) and overexpresses genes involved in cellular detoxification or anti-oxidation (17). Most ADs arise in the periphery of the lung and in comparison to SQs are characterized by an up-regulation of some oncogenes and a down-regulation of several drug resistance proteins (18). ADs are more frequently mutated in *Ki-ras* oncogene (19, 20) and have a high level expression of small airway-associated or immunologically related proteins (17). Etiology of this lung tumor is linked to prolonged inflammations and healing of scars (21, 22). However, the majority of patients suffering from both types of lung cancer are tobacco smokers.

Oxidative stress and LPO generate DNA lesions such as εA and εC (Fig. 1). Ethenoadenine and ethenocytosine reveal high miscoding potential in mammalian cells (23, 24). They also cause chromosomal aberrations and recombination (25). In humans, εA is eliminated from DNA by ANPG (26) and εC by mismatch specific TDG (27). Both enzymes are monofunctional DNA-glycosylases and require AP-endonuclease to incise DNA at the site of the removed base. Moreover, the activity of TDG is stimulated several fold by HAP-1, which increases the turnover of the enzyme on damaged DNA (28). Thus,

³ The abbreviations used are: ROS, reactive oxygen species; RNS, reactive nitrogen species; LPO, lipid peroxidation; B[a]P, benzo[a]pyrene; SQ, squamous cell carcinoma; AD, adenocarcinoma; NSCLC, non-small cell lung carcinoma; εA, 1,*N*⁶-ethenoadenine; εC, 3,*N*⁴-ethenocytosine; ANPG, alkylpurine-DNA-*N*-glycosylase; TDG, thymine-DNA-glycosylase; HAP-1, human AP-endonuclease; AP, apurinic/aprimidinic; BER, base excision repair; TLC, thin layer chromatography; εAdA, 1,*N*⁶-ethenodeoxyadenosine; εdC, 3,*N*⁴-ethenodeoxycytidine; HPLC, high-performance liquid chromatography; MUG, mismatch specific uracil-DNA-glycosylase; OGG1, 8-oxoguanine-DNA-glycosylase.

Received 2/19/03; accepted 5/19/03.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¹ Supported by grants from the State Committee for Scientific Research (Poland) 6 PO4A 065 18 and the European Commission grant ENV4-CT97-0505 through a collaborative research agreement between the International Agency for Research on Cancer, Lyon, France and the Institute of Biochemistry and Biophysics, Polish Academy of Sciences (PAS; GI/43/4). This work has been supported by a Union International Centre Cancer Technology Transfer Fellowship (No 358/2000) and was conducted within the framework of the activity of the Center of Excellence in Molecular Biotechnology, Institute of Biochemistry and Biophysics PAS (WP10).

² To whom requests for reprints should be addressed, at Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Pawińskiego 5a, 02-106 Warsaw, Poland. Phone: 48-22-658-47-24; Fax: 48-22-658-46-36; E-mail: tudek@ibb.waw.pl.

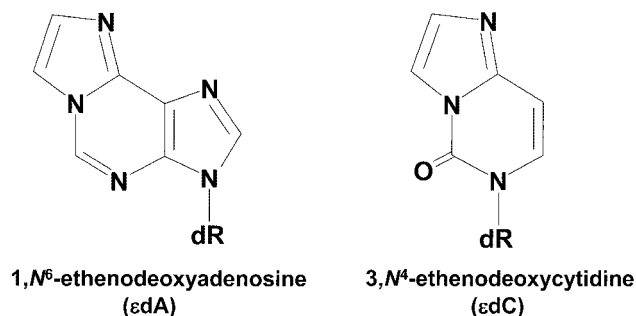


Fig. 1. Chemical structures of ϵ DNA adducts.

tissue repair capacity for ϵ C and ϵ A probably depends on the availability of DNA-glycosylases and AP-endonuclease.

In the present study, we show that, in comparison with healthy volunteers, BER for ethenoadenine and for ethenocytosine is decreased in lung cancer patients and in patients developing lung AD, respectively. On basis of this finding, we suggest that deficiency of repair enzymes eliminating oxidation-induced exocyclic DNA adducts from DNA may be a risk factor for developing lung cancer, particularly the inflammation-related lung AD.

MATERIALS AND METHODS

Materials. RNases A and T1, micrococcal nuclease, deoxyribonucleoside 3'-monophosphates, 3'-dUMP, Histopaque 1119 solution, and Bradford reagent were purchased from Sigma (St. Louis, MO); phenol and chloroform were from Roche Molecular Biochemicals. Phenylmethyl sulfonyl fluoride was obtained from SERVA (Heidelberg, Germany). Calf-spleen phosphodiesterase was from Worthington Biochemical Corp., and anti- β -actin antibody was from Santa Cruz Biotech., Inc. T4 polynucleotide kinase, [γ -³²P]ATP, Hybond-C membrane, horseradish peroxidase-conjugated goat antirabbit IgG, the ECL Plus Western blotting detection reagents, and Rainbow Molecular Weight Markers were obtained from Amersham-Pharmacia Biotech. *Escherichia coli* Exonuclease III was from Promega (Madison, WI). Polyethyleneimine-cellulose TLC sheets were from Machery-Nalgen (Germany). Micro Bio-Spin P-30 columns were obtained from Bio-Rad Laboratories. Monoclonal antibodies EM-A-1 (ϵ dA) and EM-C-1 (ϵ dC) were provided by Dr. M. Rajewsky (Institute of Cell Biology, University of Essen, Essen, Germany). Oligodeoxynucleotides (40-mers) containing a single ϵ A or ϵ C at position 20 in the sequence 5'-d(GCT ACC TAC CTA GCG ACC T \underline{X} C GAC TGT CCC ACT GCT CGA A)-3', where \underline{X} indicates that ϵ A or ϵ C was obtained from Eurogentec Herstal (Herstal, Belgium) or Genset Oligos (Paris, France), respectively. Complementary oligodeoxynucleotides containing T opposite ϵ A or G opposite ϵ C were synthesized according to standard procedures using an Applied Biosystems synthesizer (Oligonucleotide Synthesis Laboratory, Institute of Biochemistry and Biophysics, Polish Academy of Sciences).

Study Group and Tissue Sampling. The study was performed in two groups. The control group consisted of 25 healthy volunteers, all cigarette smokers with comparable smoking status, 19 males and 6 females of the mean age of 60 years (range, 42–79 years). The second group comprised 56 patients, with previously untreated primary non-small cell lung cancer, undergoing pulmonary surgery at the Medical University Hospital in Bydgoszcz, Poland. Each person received an individual code number. Information concerning age, sex, lifestyle habits, type of tumor, and treatment of patients became available only before statistical analysis of parameters, which were measured blind. A group of 51 lung cancer patients did not receive any treatment before surgery. For five patients, all of them developing SQs, the information concerning chemotherapy was not clear; however, no treatment was performed at least 3 weeks before surgery. The lung cancer patients group comprised 41 males and 15 females; 32 smokers and 24 ex-smokers (those who refrained from smoking for at least 2 years). The mean age was 60 years (range, 41–82). All individuals answered a questionnaire concerning smoking, diet, lifestyle habits, and illnesses. There were no significant differences in diet, body weight, or antioxidant use between the patients and control group. The histological type of

cancer was determined according to the WHO classification (29). In the patient group, 36 patients were diagnosed with SQ and 14 with AD. For 6 patients, information about cancer type was not obtained.

The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the medical ethics committee of L. Rydygier Medical University, Bydgoszcz, Poland (in accordance with Good Clinical Practice, Warsaw 1998). All participants of the study signed informed consent.

Samples of lung tumor and marginal lung tissue, which did not show neoplastic changes in histological examination (and, for simplicity, will further be called "nonaffected" or "normal lung"), were obtained after therapeutic surgery, immediately frozen under liquid nitrogen and stored at -80°C until analyzed. A day before surgery, 20 ml of venous blood were drawn into heparinized tubes. The blood was carefully applied on top of Histopaque 1119 solution, and leukocytes were isolated by centrifugation according to the manufacturer's procedure.

Preparation of Tissue Extracts. Lung tissues and leukocyte pellets were homogenized with 4 volumes of buffer containing 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 10 mM DTT, and 1 mM phenylmethyl sulfonyl fluoride. Cells were disrupted by sonication (three times with 15-s pulses with 30-s intervals). The cell debris was removed by centrifugation, and the supernatant served as a source of enzymes. Protein concentration was determined by the method of Bradford (30).

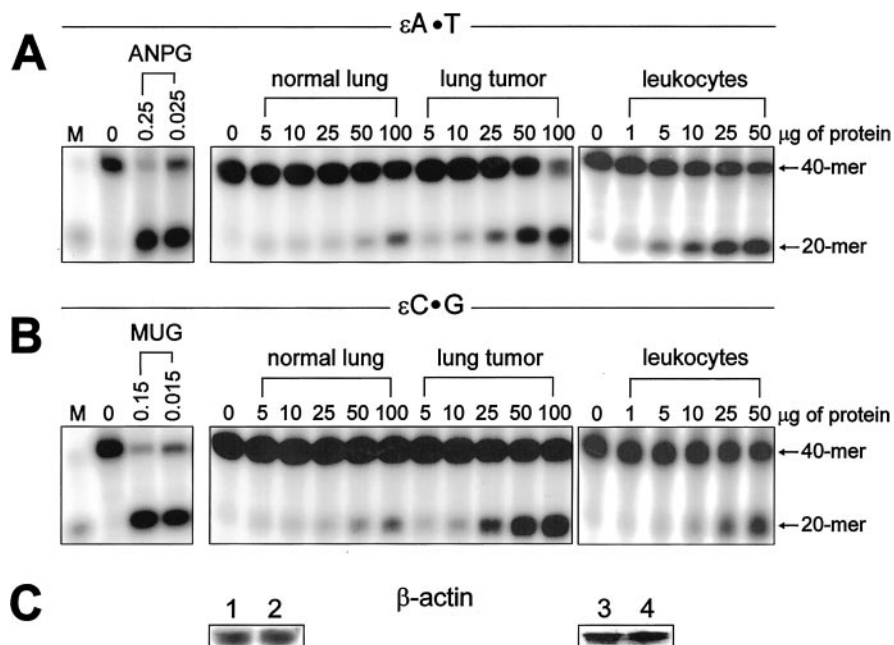
Analysis of ϵ A and ϵ C Level in DNA. DNA was extracted from lung tissues and leukocytes according to standard phenol/chloroform extraction procedure (31), with a modification involving repetitive digestion with RNases A/T1 mixture. Quality and quantity of the DNA was determined spectrophotometrically with $A_{260}:A_{280}$ ratio ranging from 1.8 to 2.0.

The level of ϵ A and ϵ C in DNA was measured by immunoaffinity purification and ³²P-postlabeling. DNA aliquots of 50 μg were digested to 3'-dNMPs using micrococcal nuclease and calf spleen phosphodiesterase. Ethenonucleotides were separated from normal 3'-monophosphates by immunoaffinity chromatography on columns containing monoclonal antibodies against ϵ dA or ϵ dC. Purified etheno adducts were ³²P-postlabeled with [γ -³²P]ATP (10 μCi , 5000 Ci/mmol) and T4 polynucleotide kinase (10 units) under conditions yielding 5'-[³²P]monophosphates (32), with 3'-UMP added as an internal standard. Radiolabeled 5'-monophosphates were resolved by two-dimensional TLC on polyethyleneimine-cellulose using 1 M ammonium acetate (pH 3.5) for the first direction and saturated ammonium sulfate (pH 3.5) for the second direction. Radioactive spots were visualized in PhosphorImager (Molecular Dynamics), and adducts were quantified against standards using ImageQuant Software. The normal nucleotides were quantified by separating aliquots of DNA digests and 3'-dNMP standards by reversed-phase HPLC using isocratic elution with 100 mM NH_4HCO_2 (pH 7.5) and UV absorbance at 260 nm. The numbers of adducts per parent nucleotide were obtained from the ratio of the quantity of ϵ dA and ϵ dC, measured by TLC to the quantity of parent nucleotide in the corresponding sample obtained by HPLC analysis (32). The results are expressed as adducts/ 10^8 nucleotides, based on the medians of three independent assays.

Repair Activity Assay for Etheno Adducts. Forty-mer oligodeoxynucleotides containing ϵ A or ϵ C at position 20 were ³²P-labeled at the 5'-end by polynucleotide kinase and the excess of [γ -³²P]ATP (3000 Ci/mmol). Radiolabeled oligomers were purified from unincorporated radioactivity using Micro Bio-Spin P-30 columns as described by the manufacturer. These oligomers were annealed, at double molar excess, to complementary oligonucleotides containing T opposite ϵ A or G opposite ϵ C, by incubating at 95°C for 3 min and subsequent cooling at $<28^{\circ}\text{C}$ during at least 2 h. Formation of duplexes was verified by nondenaturing PAGE.

The repair activities of lung tissue or leukocytes excising ϵ A and ϵ C were measured by the nicking of oligodeoxynucleotides at the site of the lesion by the tissue extract, which supplied both DNA-glycosylases and AP-endonucleases. The reaction mixture in a total volume of 20 μl contained 25 mM Tris-HCl (pH 7.8), 50 mM NaCl, 5 mM β -mercaptoethanol, 1 mM EDTA, 1 pmol of ³²P-labeled duplex and increasing amounts of tissue extract (1–100 μg protein/sample). Samples were incubated at 37°C for 1 h and reactions were stopped by digestion with proteinase K (1 $\mu\text{g}/\mu\text{l}$ of reaction mixture, 1 h, 37°C). The cleavage products were separated by denaturing 20% PAGE (see Fig. 2, A and B), and quantified in PhosphorImager using ImageQuant Software. From each data set, a Michaelis-Menten curve was plotted and the enzymes activities were calculated from the initial velocity. All estimations

Fig. 2. A and B, typical autoradiograms of denaturing PAGE showing nicking of ϵ A- and ϵ C-containing oligodeoxynucleotides by human tissue extracts. The 5'-labeled ϵ A- and ϵ C-oligonucleotides annealed to complementary strands were incubated with protein extracts from blood leukocytes and normal and tumor lung tissues from one of lung cancer patients. Annealing and the identification of damaged bases in ϵ A:T and ϵ C:G duplexes were verified by digestion with pure DNA-repair glycosylases, ANPG and MUG, respectively. 20-mer denotes oligomer cleaved at the position of the modified base. The amount of cleaved oligomer was measured by phosphorimaging. Spontaneous breaks were measured in the control Lane and subtracted from tissue cleavage products. Numbers above each Lane indicate the amount of tissue extract or pure protein added per assay (in micrograms of protein). M, 20-mer marker. C, an example of Western blot analysis of β -actin level in extracts from normal lung (Lane 1), lung tumor (Lane 2), and leukocytes (Lane 3) from the lung cancer patient mentioned above and from leukocytes from a healthy donor (Lane 4). Aliquots of proteins (50 or 10 μ g/lane for lung and leukocytes, respectively) were separated by SDS-PAGE, electrotransferred to Hybond-C membranes (Amersham) and probed with anti- β -actin antisera (Santa Cruz Biotech., Inc.) as described in "Materials and Methods." β -actin is shown as the control for protein integrity and enrichment.



were performed at least in triplicate and are presented as picomoles of cleaved 32 P-oligo (20-mer) per hour per milligrams of protein. In each experiment, two control samples were used: a negative one, with nontreated oligonucleotides subjected to denaturing PAGE, and a positive one, with oligonucleotides digested with an excess of damage-specific repair glycosylase/AP-endonuclease-ANPG-40 for ϵ A and MUG (bacterial homologue of the TDG) for ϵ C, followed by *E. coli* Exonuclease III. ANPG and MUG glycosylases were a kind gift from Drs. Jacques Laval and Murat Saparbaev (LA 147 CNRS, Institute Gustave Roussy, Villejuif, France).

Additionally, protein extraction and integrity were controlled by Western blot analysis of β -actin levels in the corresponding samples of normal and tumor lung tissues, as well as in leukocytes of lung cancer patients and healthy controls. Equal amounts of tissue-protein extracts (50 or 10 μ g/lane for lung and leukocytes, respectively) were resolved on SDS-polyacrylamide gels and electrotransferred to Hybond-C membranes. The membranes were sequentially blocked with 5% nonfat dry milk, probed with anti- β -actin antibody at 1 μ g/ml, and visualized with horseradish peroxidase-conjugated secondary antibody and an Amersham-enhanced chemiluminescence system according to the manufacturer's recommendation.

Statistical Analysis. All data are presented as means \pm SD. The STATISTICA (version 5.1) computer software (Stat Soft, Inc., Tulsa, OK) was used for the statistical analysis. All parameters were tested for normal distribution with the tests of the Shapiro-Wilk and the Kolmogorov-Smirnov with Lilliefors correction. The homogeneity of variances was evaluated by the *F* and the Leven tests. Differences among groups with normal value distribution were evaluated using ANOVA and analyzed within groups using Student's *t* test. The Mann-Whitney *U* test was carried out to compare the means of variables without normal distributions. For those variables, which were not homogenous or consisted of different number of cases, tests with separate variance estimation were used. The relationship between different variables was evaluated by Spearman's rank correlation test and the Pearson linear regression analysis. Statistical significance was considered at $P < 0.05$.

RESULTS

Ethno Adduct Level and Repair Efficiency in Normal Lung, Lung Tumor, and Leukocytes of Lung Cancer Patients. DNA adducts levels were measured by immunoaffinity/ 32 P-postlabeling in DNA samples obtained from 33 lung cancer patients. The level of ϵ A was similar in normal lung (1.18 ± 0.64 , range, 0.34 to 3.33 ϵ A/ 10^8 of unmodified A) to that in lung tumor tissue (1.28 ± 0.51 ; range, 0.43 to 2.47 ϵ A/ 10^8 A). Similarly, the levels of ϵ C did not differ

between normal lung (2.63 ± 1.42 ; range, 0.67–5.71 ϵ C/ 10^8 C) and in lung tumor (2.74 ± 1.65 ; range 0.78–6.18 ϵ C/ 10^8 C). Leukocytes accumulated more ϵ A (1.83 ± 1.16 ; range, 0.64–6.15 ϵ A/ 10^8 A) than normal lung (two-tailed $P = 0.012$) or lung tumor (two-tailed $P = 0.048$). For ϵ C adduct level (3.64 ± 2.04 ; range, 0.72–9.19) the same tendency, albeit nonsignificant, was observed among lung tissues and leukocytes (two-tailed $P = 0.054$ and 0.076 for normal lung and tumor, respectively; Fig. 3A).

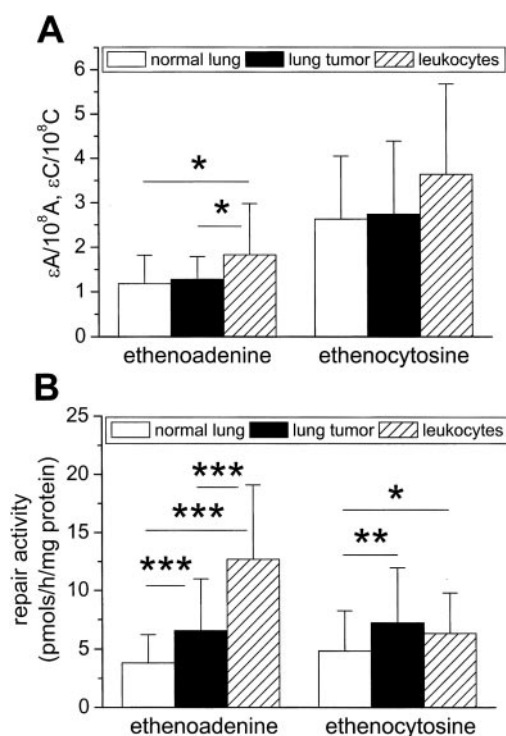


Fig. 3. A, levels of ethenoadenine and ethenocytosine, determined by immunoaffinity/ 32 P-postlabeling, in DNA from normal lung tissue, lung tumor, and leukocytes of lung cancer patients. B, repair activities of ϵ A and ϵ C, as measured by nicking assay, in extracts from normal lung, lung tumor, and leukocytes of lung cancer patients. *, $P < 0.05$; **, $P < 0.005$; ***, $P < 0.0001$.

The repair activities for ϵ A and ϵ C were measured by nicking assay in tissues of 56 lung cancer patients; the group included 33 cases, for which the adducts level was estimated. The means \pm SD of ϵ A repair activities were 3.82 ± 2.4 pmols/h/mg protein (range, 0.92–9.56) in normal lung tissue, 6.56 ± 4.45 (range, 1.1–24.05) in tumor tissue, and 12.69 ± 6.4 (range, 2.43–37.23) in leukocytes. Repair activity for ϵ C was 4.83 ± 3.42 (range, 0.55–17.35) in nonaffected lung, 7.23 ± 4.73 (range, 1.35–28.11) in lung tumor, and 6.32 ± 3.47 (range, 1.67–17.53) in leukocytes (Fig. 3B). Repair of both etheno adducts was significantly higher in tumor than in normal lung (one-tailed $P = 0.000053$ for ϵ A and 0.0018 for ϵ C). Repair activity in leukocytes from cancer patients was significantly higher than in normal lung tissue for both adducts studied (one-tailed $P = 0.0000$ and 0.0098 for ϵ A and ϵ C, respectively).

The possibility that the differences in ϵ A and ϵ C incision activities among normal and tumor lung tissues, as well as among leukocytes of cancer patients and controls, could be attributable to differential enzyme protein enrichment was excluded by Western blot analysis, which showed equal amount of β -actin in the same amount of protein extract (Fig. 2C).

The level of adducts probably reflected oxidative stress, to which individuals were exposed, and individual differences in repair capacity. A strong positive linear relationship between ϵ A and ϵ C adduct levels in DNA from normal and tumor lung tissues and leukocytes of lung cancer patients ($r = 0.621$, $P = 0.00019$; $r = 0.563$, $P = 0.00078$; $r = 0.693$, $P = 0.0005$, respectively) was observed. There was also a positive linear association in etheno adduct repair activities between normal and tumor lung tissue (for ϵ A, $r = 0.36$ and $P = 0.0047$; for ϵ C, $r = 0.49$ and $P = 0.00014$).

Effect of Tobacco Smoking. No effect of smoking was found for ϵ A and ϵ C levels, as well as their repair activities in normal lung and leukocytes of lung cancer patients. Surprisingly, in tumor tissues from smoking patients ϵ C-repair activity was lower than in ex-smokers (two-tailed $P = 0.032$, not shown).

Gender and Age. No significant differences in ϵ A and ϵ C levels and their repair activities between males and females were observed. Similarly, no effect of age was found for above parameters.

Histological Type of Tumor. Within the studied group of patients, 36 and 14 individuals developed smoking-related SQ and AD, respectively, postulated to be inflammation-related. In patients with AD, repair capacity for ϵ A and ϵ C was significantly lower than in SQ patients, both in nonaffected lung tissue and leukocytes (Figs. 4 and 5; in nonaffected lung tissue two-tailed $P = 0.000004$ and 0.002 for ϵ A and ϵ C, respectively; in leukocytes for ϵ A, two-tailed $P = 0.0083$ and for ϵ C, two-tailed $P = 0.0046$). Normal lung tissue of AD patients revealed higher deficiency in ϵ A-glycosylase activity (2.33-fold decrease) than in ϵ C-glycosylase activity (1.72-fold) in comparison with normal lung of SQ patients.

No significant difference in the levels of ϵ A and ϵ C was found between AD and SQ patients, both in DNA from normal lung tissue and from leukocytes. However, there was a significant difference in the ratio ϵ A/ ϵ C in normal lung tissue, which was lower for AD patients compared with SQ patients (two-tailed $P = 0.0035$; Fig. 6). Such a ratio can reflect decreased level of ϵ A or increased level of ϵ C. In fact, we found lower ϵ A and higher ϵ C level in normal lung tissue of AD patients, although this finding was below statistical significance.

Lung Cancer Patients versus Healthy Volunteers. Etheno adduct repair activities in leukocytes were compared between lung cancer patients and 25 healthy volunteers matched for age, sex, and smoking habit. Repair activity of ϵ A varied from 1.88 to 45 pmols/h/mg protein (mean \pm SD: 19.41 ± 11.76). The mean \pm SD of ϵ C-repair activity was 6.68 ± 3.77 (range, 0.25–17.62). Repair activ-

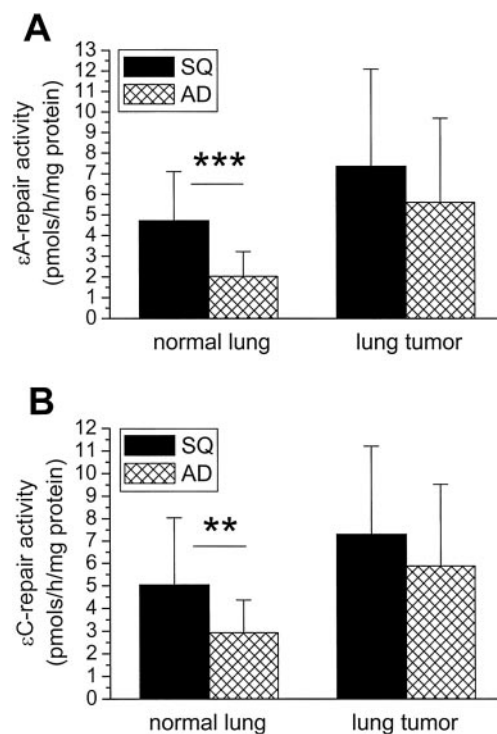


Fig. 4. Ethenoadenine (A) and ethenocytosine (B) repair activities in lung tissues of lung cancer patients diagnosed with different histological types of cancer: SQ or AD. ** $P < 0.005$; *** $P < 0.0001$.

ity of ϵ A was significantly lower in leukocytes of lung cancer patients than in leukocytes of healthy volunteers (mean 12.69 versus 19.41 , two-tailed $P = 0.012$; Fig. 5A). There was no significant difference between the groups in repair activity of ϵ C adduct (Fig. 5B).

When analyzing repair activity in leukocytes of healthy volunteers and patients developing different histological types of tumor, we observed that ϵ A-repair activity in leukocytes of AD patients was much lower than for all cancer patients (two-tailed $P = 0.00033$), for healthy controls, and SQ patients (two-tailed $P = 0.027$, Fig. 5A). Repair activities for ϵ C were similar in leukocytes of healthy controls, all lung cancer patients, and SQ patients. However, individuals with ADs revealed significantly decreased ϵ C-repair activity (two-tailed $P = 0.013$; Fig. 5B).

DISCUSSION

DNA repair plays an important role in protecting individuals from cancer. Lung cancer in Poland is attributed mainly to tobacco smoking. Two different pathways of tobacco smoke genotoxic activity can be distinguished: (a) the direct pathway, related to DNA adducts formation of tobacco smoke carcinogens (e.g., B[a]P metabolites or nitrosoamines); and (b) indirect pathway, related to tobacco smoke induced inflammation and oxidation of DNA, lipids, and proteins. The first pathway has been studied extensively, showing repair deficiencies in cancer patients attributable to loss of heterozygosity or polymorphism in DNA repair genes (33, 34). For example, in relation to healthy controls, lung cancer patients had 5-fold lower repair activity against B[a]P diol epoxide (35). However, the second pathway, particularly LPO in pathogenesis of lung cancer, is still poorly understood. This prompted us to study the level of etheno DNA adducts, ϵ A and ϵ C, as well as overall repair activities for these adducts in a population of NSCLC patients and a control group of healthy volunteers matched for age, sex, and smoking habit. We observed among individuals from cancer patients and control group up to 10-fold

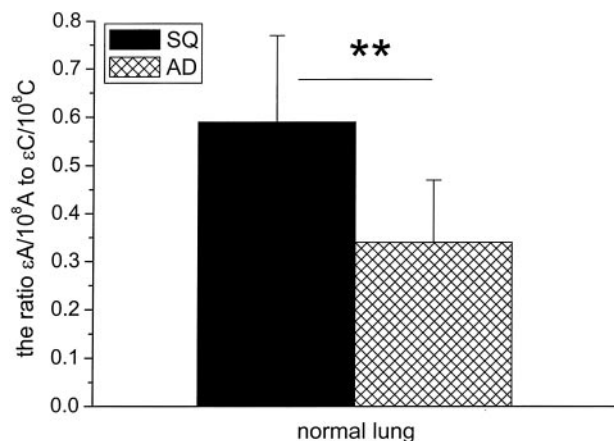


Fig. 5. Repair activities of ethenoadenine (A) and ethenocytosine (B) in leukocytes of lung cancer patients *versus* healthy volunteers. Comparison includes all cancer patients and patients of groups developing different histological types of cancer: SQ or AD. *, $P < 0.05$; **, $P < 0.005$.

differences in repair activities and adducts levels in all tissues studied. Several previous studies have shown similar variation in the level of other adducts related to tobacco smoking (36) and DNA repair capacity (37, 38). For example, the activities of O^6 -alkylguanine-DNA-alkyltransferase and uracil-DNA-glycosylase differ by as much as 180-fold and 300-fold, respectively, in a human population (39). Individual variations in repair enzymes activities may depend on their expression, splicing, and activation or inhibition. ANPG protein excising ϵ A from DNA can exist as several alternatively spliced forms (40). The truncated form, ANPG-40, was found to be less active against 1, N^2 -ethenoguanine in comparison with the full-length and splice variant proteins, ANPG-60 and ANPG-70 (41). Individual variations can also be attributable to the extent of the expression of other proteins comprising BER, *e.g.*, HAP-1. It was shown that the activity of TDG is stimulated by the major human AP endonuclease HAP-1 (28, 42) and is also regulated by posttranslational modification-sumoylation by ubiquitin-like proteins SUMO-1 and SUMO-2/3 (43). The levels of etheno DNA adducts measured in this study were also shown to be highly variable but similar to those reported previously (44, 45).

We have found, for the first time, that BER activity for ϵ A is lower in leukocytes of all lung cancer patients in comparison to healthy individuals and still much lower in the group developing inflammation-related AD. Repair activity of ϵ C was lower only in leukocytes of patients developing ADs. Also lung tissues, cancer free from a histological point of view, showed lower ϵ A and ϵ C repair activities in patients with ADs than with SQ tissues. The same tendency was observed in tumor tissues. Although, the nicking assay used in this study did not allow for the discovery of whether DNA-glycosylases or AP-endonuclease deficiencies were responsible for the observed decrease in overall repair activities in AD patients, more probable candidates were DNA-glycosylases for the following reasons: (a) different degrees of deficiency were observed for ϵ A and ϵ C repair capacities in the same tissues and (b) oxidative stress was expected to increase, not decrease HAP-1 activity, because of stimulation of *HAP-1* gene transcription by ROS (46). At least two different mechanisms can lead to lowering of ϵ A and ϵ C repair activities in AD patients: (a) genetic polymorphism and existence of proteins of lower activity and (b) inhibition of repair glycosylases and/or AP-endonuclease by ROS or RNS formed during inflammation. Human *hOGG1* genes were found to be frequently mutated in lung AD (47). Decreased repair of 8-oxoguanine was also observed in lung AD because of alternative splicing of *hOGG1* mRNA and producing a protein of

lower activity (48). Genetic studies also revealed that polymorphism in *hOGG1* exon 1 predisposed to AD of lung (49). Exogenous nitric oxide and peroxy nitrite have been shown to inhibit DNA repair enzymes such as ligase (50), formamidopyrimidine-DNA-glycosylase (51), *hOGG1* (52), and O^6 -alkylguanine-DNA-alkyltransferase (53), by direct nitrosylation. Other oxidative stress intermediates secreted by human neutrophils, hypochlorous acid, and *N*-chloramines inhibit ADP-ribosylation, unscheduled DNA synthesis, and DNA strand-break repair (54). Increased production of nitric oxide in human lung AD is, however, not well documented. Fujimoto *et al.* (55) reported higher inducible nitric oxide synthase activities in AD than in other types of lung cancer and normal lung, whereas Ambts *et al.* (56) did not find an up-regulation of nitric oxide synthase isoforms during NSCLC progression. Although it is difficult to judge which mechanism is responsible for the observed differences, we have shown, for the first time, an association between the development of an inflammation-related type of lung cancer (AD) and decreased repair of LPO-induced ϵ A and ϵ C DNA damage by DNA-glycosylases/AP-endonuclease, not only in target tissue, but also in peripheral leukocytes. Enhanced LPO was observed in human lung cancer tissues in comparison with matched lung parenchymas, suggesting that LPO is important in pathology of lung cancer, at least for cancer progression (57, 58).

Interestingly, we did not observe any effect of tobacco smoking on repair activity in leukocytes and unaffected lung tissue. It is known that tobacco smoke up-regulates the activity of several resistance-related proteins that may protect lung cells from carcinogens (59–61). For example, tobacco smoke induces O^6 -alkylguanine-DNA-alkyltransferase in human lung tissue, and this induction is maintained for 1 year after quitting smoking (60), probably because of particulate deposits in the lung, resulting in continued metabolic activation of tobacco carcinogens after smoking cessation (62). Similar to repair activities, we did not observe any significant differences in etheno

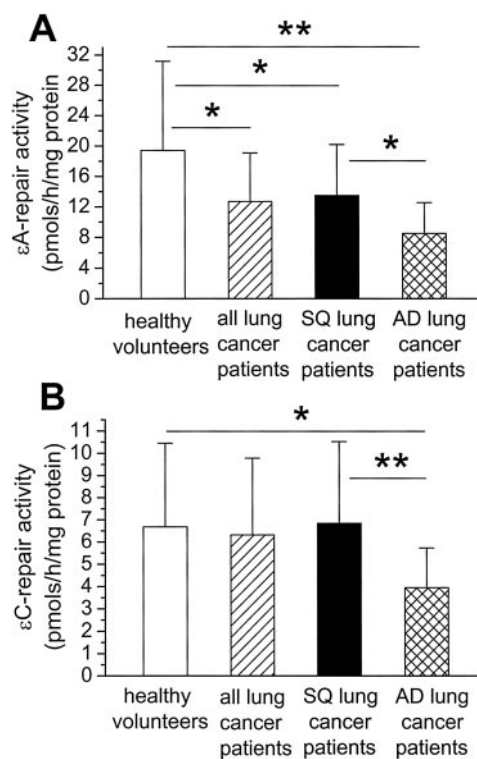


Fig. 6. The ratio of ethenoadenine to ethenocytosine in normal lung tissues from patients with different histological types of cancer: SQ or AD. **, $P < 0.005$.

adduct levels in lung tissues and leukocytes between smokers and ex-smokers. This is consistent with results of Godschalk *et al.* (45), who did not find any effect of smoking on ϵ A and ϵ C levels in lung tissue.

We also investigated whether exocyclic adducts can contribute to tumor progression. On average, we found the same levels of ϵ A and ϵ C in tumor as in nonaffected lung tissue. However, we found 1.7- and 1.5-fold greater repair activities for ϵ A and ϵ C, respectively, in tumor lung tissue in comparison with corresponding normal lung. The higher DNA repair activity in tumor was probably able to maintain etheno adduct lesions at an unchanged level (Fig. 3). Similarly, a higher rate of DNA repair has been described for rapidly dividing foetal tissue of neonatal rodents in comparison with normal adult liver or brain tissue, both by higher levels of transcript and the incision activity (63, 64). This higher repair activity was also able to maintain the oxidative lesion, 8-oxoguanine, in foetal tissue at the same basal level as in adults (63). Tumors have a higher content of cells in the S phase. Expression of some DNA-glycosylases and AP-endonuclease genes was shown to be cell-cycle dependent (65). The mRNA levels of ANPG, human thymine glycol-DNA-glycosylase, uracil-DNA-glycosylase, and HAP-1 increase 2.5–3.5-fold during the G₁ phase of the cell cycle, remain constant during the S phase, and decrease to the basal level after mitosis. However, expression levels of genes for TDG and OGG1 are not regulated during the cell cycle (65, 66). The nicking assay used in this study does not allow to distinguish between glycosylase and AP-endonuclease activity. Hence, the possibility that the total increase of at least ϵ C-repair activity in tumor reflects only an increase in AP-endonuclease expression during the S phase of the cell cycle cannot be excluded. HAP-1 is the limiting enzyme for ROS-induced DNA damage repair in extracts of human cells (67). The enzyme transcript levels have been found to be elevated in a number of cancers (68, 69). Accelerated repair of ϵ A and ϵ C in tumor accompanied by accelerated cell proliferation might prevent apoptotic or necrotic tumor cell death and contribute to tumor metastasis.

In conclusion, this study shows impaired repair of ϵ A and ϵ C in patients developing the AD type of lung tumor, the etiology of which is linked to inflammatory processes. For this reason, we postulate that lipid peroxidation is more important in the pathogenesis of lung adenocarcinoma than in the other type of NSCLC, squamous cell carcinoma. Deficiency of repair enzymes for ϵ A and ϵ C may be a risk factor for developing AD type of lung cancer.

REFERENCES

- Migliore, L., and Coppede, F. Genetic and environmental factors in cancer and neurodegenerative diseases. *Mutat. Res.*, *512*: 135–153, 2002.
- Oliński, R., Gackowski, D., Foksiński, M., Różalski, R., Roszkowski, K., and Jaruga, P. Oxidative DNA damage: assessment of the role in carcinogenesis, atherosclerosis, and acquired immunodeficiency syndrome. *Free Radic. Biol. Med.*, *33*: 192–200, 2002.
- Marnett, L. J. Oxyradicals and DNA damage. *Carcinogenesis (Lond.)*, *21*: 361–370, 2000.
- Greenlee, R. T., Murray, T., Bolden, S., and Wingo, P. A. Cancer statistics, 2000. *CA Cancer J. Clin.*, *50*: 7–33, 2000.
- Didkowska, J., Wojciechowska, W., Tarkowski, W., and Zatoński, W. A. Cancer in Poland. Warsaw, Poland: Centre of Oncology Scientific Publication, 2002.
- Hecht, S. S. Tobacco smoke carcinogens and lung cancer. *J. Natl. Cancer Inst.*, *91*: 1194–1210, 1999.
- Saetta, M. Airway inflammation in chronic obstructive pulmonary disease. *Am. J. Respir. Crit. Care Med.*, *160*: 17–20, 1999.
- Singh, R. J., Hogg, N., Joseph, J., and Kalyanaraman, B. Mechanism of nitric oxide release from S-nitrosothiols. *J. Biol. Chem.*, *271*: 18596–18603, 1996.
- Bartsch, H. Keynote address: exocyclic adducts as new risk markers for DNA damage in man. In: B. Singer and H. Bartsch (eds.), *Exocyclic DNA Adducts in Mutagenesis and Carcinogenesis*, pp. 1–16. Lyon, France: International Agency for Research on Cancer Scientific Publication No. 150, 1999.
- Mattson, M. E., Pollack, E. S., and Cullen, J. W. What are the odds that smoking will kill you? *Am. J. Public Health*, *77*: 425–431, 1987.
- Stücker, I., Hirvonen, A., de Waziers, I., Cabelguenne, A., Mitrunen, K., Cénéé, S., Koum-Besson, E., Hémon, D., Beaune, P., and Lorient, M. A. Genetic polymorphisms of glutathione S-transferases as modulators of lung cancer susceptibility. *Carcinogenesis (Lond.)*, *23*: 1475–1481, 2002.
- Bartsch, H., Nair, U., Risch, A., Rojas, M., Wikman, H., and Alexandrov, K. Genetic polymorphism of CYP genes, alone or in combination, as a risk modifier of tobacco-related cancers. *Cancer Epidemiol. Biomarkers Prev.*, *9*: 3–28, 2000.
- Rojas, M., Cascorbi, I., Cascorbi, I., Alexandrov, K., Kriek, E., Auburtin, G., Mayer, L., Kopp-Schneider, A., Roots, I., and Bartsch, H. Modulation of benzo[a]pyrene diol-epoxide-DNA adduct levels in human white blood cells by CYP1A1, GSTM1 and GSTT1 polymorphism. *Carcinogenesis*, *21*: 35–41, 2000.
- Shimosato, Y. Pathology of lung cancer: development, progression and function. In: D. N. Carney (ed.), *Lung cancer*, pp. 28–42. Arnold, London, 1995.
- Hammer, S. P. Common neoplasms. In: D. H. Dail (ed.), *Pulmonary Pathology*, pp. 1123–1278. Berlin: Springer-Verlag, 1993.
- Niklińska, W., Chyczewski, L., Ludański, J., Sawicki, B., and Nikliński, J. Detection of p53 abnormalities in non-small cell lung cancer by yeast functional assay. *Folia Histochem. Cytobiol.*, *39*: 147–148, 2001.
- Nacht, M., Dracheva, T., Gao, Y., Fujii, T., Chen, Y., Player, A., Akmaev, V., Cook, B., Dufault, M., Zhang, M., Zhang, W., Guo, M., Curran, J., Han, S., Sidransky, D., Buetow, K., Madden, S. L., and Jen, J. Molecular characteristics of non-small cell lung cancer. *Proc. Acad. Sci. USA*, *98*: 15203–15208, 2001.
- Volm, M., Koomagi, R., Efferth, T., and Mattern, J. Protein expression profiles of non-small cell lung carcinomas: correlation with histological subtype. *Anticancer Res.*, *22*: 2321–2324, 2002.
- Graziano, S. L., Gamble, G. P., Newman, N. B., Abbott, L. Z., Rooney, M., Mookherjee, S., Lamb, M. L., Kohman, L. J., and Poiesz, B. J. Prognostic significance of K-ras codon 12 mutations in patients with resected stage I and II non-small cell lung cancer. *J. Clin. Oncol.*, *17*: 668–675, 1999.
- Gazdar, A. F. The molecular and cellular basis of human lung cancer. *Anticancer Res.*, *14*: 261–267, 1994.
- Fujimoto, H., Sasaki, J., Matsumoto, M., Suga, M., Ando, Y., Iggo, R., Tada, M., Saya, H., and Ando, M. Significant correlation of nitric oxide synthase activity and p53 gene mutation in stage I lung adenocarcinoma. *Jpn. J. Cancer Res.*, *89*: 696–702, 1998.
- Madri, J. A., and Carter, D. Scar cancers of the lung: origin and significance. *Hum. Pathol.*, *15*: 625–631, 1984.
- Levine, R. L., Yang, I. Y., Hossain, M., Pandya, G. A., Grollman, A. P., and Moriya, M. Mutagenesis induced by a single 1,N⁶-ethenodeoxyadenosine adduct in human cells. *Cancer Res.*, *60*: 4098–4104, 2000.
- Moriya, M., Pandya, G. A., Johnson, F., and Grollman, A. P. Cellular response to exocyclic DNA adducts. In: B. Singer and H. Bartsch (eds.), *Exocyclic DNA Adducts in Mutagenesis and Carcinogenesis*, pp. 263–70. Lyon, France: International Agency for Research on Cancer Scientific Publication No. 150, 1999.
- Bartsch, H., Barbin, A., Marion, M. J., Nair, J., and Guichard, Y. Formation, detection, and role in carcinogenesis of ethenobases in DNA. *Drug Metab. Rev.*, *26*: 349–371, 1994.
- Saparbav, M., Kleibl, K., and Laval, J. *Escherichia coli*, *Sacharomyces cerevisiae*, rat and human 3-methyladenine DNA glycosylases repair 1,N⁶-ethenoadenine when present in DNA. *Nucleic Acids Res.*, *23*: 3750–3755, 1995.
- Saparbav, M., and Laval, J. 3,N⁴-ethenodeoxycytidine, a highly mutagenic adduct, is a primary substrate for *Escherichia coli* double-stranded uracil-DNA-glycosylase. *Proc. Acad. Sci. USA*, *95*: 8508–8513, 1998.
- Privezentzev, C. V., Saparbav, M., and Laval, J. The HAP1 protein stimulates the turnover of human mismatch-specific thymine-DNA-glycosylase to process 3,N⁴-ethenocytosine residues. *Mutat. Res.*, *480–481*: 277–284, 2001.
- World Health Organization. Histological typing of lung tumors. *Am. J. Clin. Pathol.*, *77*: 123–136, 1982.
- Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilising the principle of protein-dye binding. *Anal. Biochem.*, *72*: 248–255, 1976.
- Sambrook, J., Fritsch, E. F., and Maniatis, T. *Molecular Cloning*, Ed. 2. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 1989.
- Nair, J., Barbin, A., Guichard, Y., and Bartsch, H. 1,N⁶-ethenodeoxyadenosine and 3,N⁴-ethenodeoxycytidine in liver DNA from humans and untreated rodents detected by immunoaffinity/³²P-postlabeling. *Carcinogenesis*, *3*: 613–617, 1995.
- de Boer, J. G. Polymorphisms in DNA repair and environmental interactions. *Mutat. Res.*, *509*: 201–210, 2002.
- Chen, L., Spitz, M. R., Hong, W. K., and Wie, Q. Reduced expression levels of nucleotide excision repair genes in lung cancer: a case-control analysis. *Carcinogenesis (Lond.)*, *218*: 1527–1530, 2000.
- Wei, Q., Cheng, L., Hong, W. K., and Spitz, M. R. Reduced DNA repair capacity in lung cancer patients. *Cancer Res.*, *56*: 4103–4107, 1996.
- Szyfter, K., Banaszewski, J., Jałoszyński, P., Pabiszczak, M., Szyfter, W., and Szmaja, Z. Carcinogen: DNA adducts in tobacco smoke-associated cancer of the upper respiratory tract. *Acta Biochim. Pol.*, *46*: 275–287, 1999.
- Wei, Q., Cheng, L., Amos, C. I., Wang, L. E., Guo, Z., Hong, W. K., and Spitz, M. R. Repair of tobacco carcinogen-induced DNA adducts and lung cancer risk: a molecular epidemiologic study. *J. Natl. Cancer Inst.*, *92*: 1764–1772, 2000.
- Mitra, S., and Kaina, B. Regulation of repair of alkylation damage in mammalian genomes. *Prog. Nucleic Acid Res. Mol. Biol.*, *44*: 109–142, 1993.
- Vahakangas, K., Trivers, G. E., Plummer, S., Haye, R. B., Krokan, H., Rowe, M., Swartz, R. P., Yeager, H., Jr., and Harris, C. C. O⁶-methylguanine-DNA methyltransferase and uracil DNA glycosylase in human broncho-alveolar lavage cells and peripheral blood mononuclear cells from tobacco smokers and non-smokers. *Carcinogenesis*, *12*: 1389–1394, 1991.
- Pendlebury, A., Frayling, I. M., Santibanez Koref, M. F., Margison, G. P., and Rafferty, J. A. Evidence for the simultaneous expression of alternatively spliced

- alkylpurine-DNA-*N*-glycosylase transcripts in human tissues and cells. *Carcinogenesis (Lond.)*, *15*: 2957–2960, 1994.
41. Saparbaev, M., Langouët, S., Privezentzev, C. V., Guengerich, F. P., Cai, H., Elder, R. H., and Laval, J. 1,*N*²-Ethenoguanine, a mutagenic DNA adduct, is a primary substrate of *Escherichia coli* mismatch-specific uracil-DNA glycosylase and human alkylpurine-DNA-*N*-glycosylase. *J. Biol. Chem.*, *277*: 26987–26993, 2002.
 42. Waters, T. R., Gallinari, P., Jiricny, J., and Swann, P. F. Human thymine DNA glycosylase binds to apurinic sites in DNA but is displaced by human apurinic endonuclease 1. *J. Biol. Chem.*, *274*: 67–74, 1999.
 43. Hardeland, U., Steinacher, R., Jiricny, J., and Schar, P. Modification of the human thymine-DNA glycosylase by ubiquitin-like proteins facilitates enzymatic turnover. *EMBO J.*, *21*: 1456–1464, 2002.
 44. Nair, J. Lipid peroxidation-induced etheno-DNA adducts in humans. In: B. Singer and H. Bartsch (eds.), *Exocyclic DNA Adducts in Mutagenesis and Carcinogenesis*, pp. 55–61. Lyon, France: International Agency for Research on Cancer Scientific Publication No. 150, 1999.
 45. Godschalk, R., Nair, J., van Schooten, F. J., Risch, A., Drings, P., Kayser, K., Dienemann, H., and Bartsch, H. Comparison of multiple DNA adduct types in tumor adjacent human lung tissue: effect of cigarette smoking. *Carcinogenesis (Lond.)*, *23*: 2081–2086, 2002.
 46. Ramana, C. V., Boldogh, I., Izumi, T., and Mitra, S. Activation of apurinic/aprimidinic endonuclease in human cells by reactive oxygen species and its correlation with their adaptive response to genotoxicity of free radicals. *Proc. Natl. Acad. Sci. USA*, *95*: 5061–5066, 1998.
 47. Chevillard, S., Radicella, J. P., Levalois, C., Lebeau, J., Poupon, M. F., Oudard, S., Dutrillaux, B., and Boiteux, S. Mutations in *OGG1*, a gene involved in the repair of oxidative DNA damage, are found in human lung and kidney tumours. *Oncogene*, *16*: 3083–3086, 1998.
 48. Kohno, T., Shinmura, K., Tosaka, M., Tani, M., Kim, S. R., Sugimura, H., Nohmi, T., Kasai, H., and Yokota, J. Genetic polymorphisms and alternative splicing of the *hOGG1* gene, that is involved in the repair of 8-hydroxyguanine in damaged DNA. *Oncogene*, *16*: 3219–3225, 1998.
 49. Ishida, T., Takashima, R., Fukayama, M., Hamada, C., Hippo, Y., Fujii, T., Moriyama, S., Matsuba, C., Nakahori, Y., Morita, H., Yazaki, Y., Kodama, T., Nishimura, S., and Aburatani, H. New DNA polymorphisms of human *MMH/OGG1* gene: prevalence of one polymorphism among lung-adenocarcinoma patients in Japanese. In: *J. Cancer*, *80*: 18–21, 1999.
 50. Grąziewicz, M., Wink, D. A., and Laval, F. Nitric oxide inhibits DNA ligase activity: potential mechanisms for NO-mediated DNA damage. *Carcinogenesis (Lond.)*, *17*: 2501–2505, 1996.
 51. Wink, D. A., and Laval, J. The Fpg protein, a DNA repair enzyme, is inhibited by the biomediator nitric oxide *in vitro* and *in vivo*. *Carcinogenesis (Lond.)*, *15*: 2125–2129, 1994.
 52. Jaiswal, M., LaRusso, N. F., Nishioka, N., Nakabeppu, Y., and Gores, G. J. Human OGG1, a protein involved in the repair of 8-oxoguanine, is inhibited by nitric oxide. *Cancer Res.*, *61*: 6388–6393, 2001.
 53. Laval, F., and Wink, D. A. Inhibition by nitric oxide of the repair protein. *O*⁶-methylguanine-DNA-methyltransferase. *Carcinogenesis*, *15*: 443–447, 1994.
 54. Pero, R. W., Sheng, Y., Olsson, A., Bryngelsson, C., and Lund-Pero, M. Hypochlorous acid/*N*-chloramines are naturally produced DNA repair inhibitors. *Carcinogenesis (Lond.)*, *17*: 13–18, 1996.
 55. Fujimoto, H., Ando, Y., Yamashita, T., Terazaki, H., Tanaka, Y., Sasaki, J., Matsumoto, M., Suga, M., and Ando, M. Nitric oxide synthase activity in human lung cancer. *Jpn. J. Cancer Res.*, *88*: 1190–1198, 1997.
 56. Amba, S., Bennett, W. P., Merriam, W. G., Ogunfusika, M. O., Oser, S. M., Kham, M. A., Jones, R. T., and Harris, C. C. Vascular endothelial growth factor and nitrite oxide synthase expression in human lung cancer and the relation to *p53*. *Br. J. Cancer (Phila.)*, *78*: 233–239, 1998.
 57. Zieba, M., Nowak, D., Suwalski, M., Piasecka, G., Grzelewska-Rzymowska, I., Tymiąska, K., Kroczyńska-Bednarek, J., and Kwiatkowska, S. Enhanced lipid peroxidation in cancer tissue homogenates in non-small cell lung cancer. *Monaldi Arch. Chest Dis.*, *56*: 110–114, 2001.
 58. Zieba, M., Suwalski, M., Kwiatkowska, S., Piasecka, G., Grzelewska-Rzymowska, I., Stolarek, R., and Nowak, D. Comparison of hydrogen peroxide generation and the content of lipid peroxidation products in lung cancer tissue and pulmonary parenchyma. *Respir. Med.*, *94*: 8000–8005, 2000.
 59. Volm, M., Mattern, J., and Koomagi, R. Expression of lung resistance-related protein (LRP) in non-small cell lung carcinomas of smokers and non-smokers and its predictive value for doxorubicin resistance. *Anticancer Drugs*, *8*: 931–936, 1997.
 60. Drin, I., Schoket, B., Kostic, S., and Vincez, I. Smoking-related increase in *O*⁶-alkylguanine-DNA alkyltransferase activity in human lung tissue. *Carcinogenesis (Lond.)*, *15*: 1535–1539, 1994.
 61. Wodrich, W., and Volm, M. Overexpression of oncoproteins in non-small cell lung carcinomas of smokers. *Carcinogenesis (Lond.)*, *14*: 1121–1124, 1993.
 62. Phillips, D. H. Smoking-related DNA and protein adducts in human tissues. *Carcinogenesis (Lond.)*, *23*: 1979–2004, 2002.
 63. Riis, B., Risom, L., Loft, S., and Poulsen, H. E. OGG1 mRNA expression and incision activity in rats are higher in foetal tissue than in adult liver tissue while 8-oxo-2'-deoxyguanosine levels are unchanged. *DNA Repair*, *1*: 709–717, 2002.
 64. Marietta, C., Palombo, F., Gallinari, P., Jiricny, J., and Brooks, P. J. Expression of long-patch and short-patch DNA mismatch repair proteins in the embryonic and adult mammalian brain. *Brain Res. Mol. Brain Res.*, *53*: 317–320, 1998.
 65. Bouziane, M., Miao, F., Bates, S. E., Somsouk, L., Sang, B. C., Denissenko, M., and O'Connor, T. R. Promoter structure and cell cycle dependent expression of the human methylpurine-DNA glycosylase gene. *Mutat. Res.*, *461*: 15–29, 2000.
 66. Dhenaut, A., Boiteux, S., and Radicella, J. P. Characterization of the hOGG1 promoter and its expression during the cell cycle. *Mutat. Res.*, *461*: 109–118, 2000.
 67. Izumi, T., Hazra, T. K., Boldogh, I., Tomkinson, A. E., Park, M. S., Ikeda, S., and Mitra, S. Requirement for human AP endonuclease 1 for repair of 3'-blocking damage at DNA single-strand breaks induced by reactive oxygen species. *Carcinogenesis (Lond.)*, *21*: 1329–1334, 2000.
 68. Evans, A. R., Limp-Foster, M., and Kelley, M. R. Going APE over ref-1. *Mutat. Res.*, *461*: 83–108, 2000.
 69. Xu, Y., Moore, D. H., Broshears, J., Liu, L., Wilson, T. M., Kelley, and M. R. The apurinic/aprimidinic endonuclease (APE/ref-1) DNA repair enzyme is elevated in premalignant and malignant cervical cancer. *Anticancer Res.*, *17*: 3713–3719, 1997.