Reduced O6-Methylguanine Repair in Fibroblast Cultures from Patients with Lung Cancer

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ABSTRACT

The activity of O6-methylguanine-DNA methyltransferase was determined in fibroblast cultures from 45 patients with lung cancer, 39 patients with cutaneous malignant melanoma, and 29 healthy controls. This enzyme is a critical parameter for the capacity to repair O6-methylguanine (O6-mGua) adducts in DNA, and a decreased activity might therefore be responsible for an enhanced susceptibility to cancer. The assay was performed with 8 × 10^6 fibroblasts which were homogenized and incubated with a known amount of O6-mGua containing DNA. The remaining substrate was determined fluorometrically after high performance liquid chromatographic separation. O6-mGua repair was significantly reduced in lung cancer patients [6.64 ± 4.32 (SD) pmol O6-methylguanine repaired/8 × 10^6 cells] as compared to healthy controls [10.35 ± 5.42, P < 0.0022] or patients with cutaneous malignant melanoma [10.83 ± 6.66]. The lowest mean values were detected in a subgroup of 16 lung cancer patients with a tumor manifestation between 46 years of age (5.06 ± 3.89). Fibroblasts from 4 patients with lung cancer had no detectable repair. We conclude that a reduced capacity to remove O6-mGua adducts may represent a further mechanism of individually enhanced lung cancer risk.

INTRODUCTION

There is convincing evidence that lung cancer is induced by exogenous carcinogenic influences like smoking (1), ionizing radiation (2), and various occupational hazards (3). Beside this, several large family studies have demonstrated that genetic factors may influence individual lung cancer risk (4-6). These are thought to increase a person’s sensitivity to carcinogens (7, 8) via mechanisms such as chronic obstructive pulmonary disease (9), altered metabolic activation of procarcinogens (10), or enhanced formation of DNA adducts after exposure to procarcinogenic aromatic hydrocarbons (8, 11). Here we report a decreased capacity of cultured fibroblasts from lung cancer patients to repair DNA methylation at the O6 position of guanine. If not removed by a specific O6-mGua-DNA methyltransferase, these adducts are likely to cause stable mutations because they frequently lead to a guanine-thymine mispairing during replication (12). Since O6-mGua-DNA methyltransferase binds the cleaved alkyl group and is thereby inactivated, it reacts stoichiometrically in a 1:1 ratio with modified nucleotides. Therefore, the number of O6-mGua-DNA methyltransferase molecules per cell is critical for the cellular capacity to repair these adducts (13, 14).

MATERIALS AND METHODS

Probands. Twenty-four female and 21 male patients with lung cancer between 33 and 85 years of age (mean, 56 years) were studied. They were considered cancer prone because they either had an early age of cancer manifestation (n = 16) or had at least one first degree relative with lung cancer (n = 15) or had never smoked (n = 10). Five patients belonged to more than one group, and 8 patients did not meet these criteria. Age below 46 years was considered an early tumor manifestation because 10% of all lung cancer patients at the Department of Internal Medicine, University of Hamburg, between 1974 and 1984 were this age. Patients were referred to us by three departments of pulmonary diseases in the area of Hamburg prior to the onset of therapy. Diagnoses were established by histological or cytological techniques.

Results were compared to two control groups: 39 patients with histologically established cutaneous malignant melanoma (25 females, 14 males, age between 20 and 84 years (mean, 35 years)) who were derived from the Department of Dermatology, University of Hamburg, and 29 healthy probands (12 females, 17 males, age between 23 and 84 years (mean, 47 years), with a negative family history of cancer.

Skin biopsies of all probands were taken from the inner side of the upper arm, and fibroblast cultures were initiated by standard techniques (15). Informed consent was obtained in all cases.

Cell cultures. We used Dulbecco’s modified Eagle’s medium (Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum (Gibco), 0.1 g/liter streptomycin, 0.1 g/liter neomycin, and 7.78 × 10^4 units/liter penicillin and buffered with 1 g/liter NaHCO3 plus 20 mM (2-hydroxyethyl)-1-piperazineethanesulfonic acid. Prior to sterile filtration, pH was adjusted to 7.5. The cells were grown in 175-cm² plastic flasks (Falcon, Oxnard, CA) and were stored deep frozen in liquid nitrogen after 4-8 subcultures until they were thawed and grown again for the assay procedure.

Preparation of O6-Methylguanine-DNA Oligonucleotides. Methylated DNA substrate was prepared by incubation of 40 mg calf thymus DNA (Boehringer, Mannheim, Federal Republic of Germany) together with an excess of 0.86 g N-methyl-N-nitrosourea (Sigma, Munich, Federal Republic of Germany) for 1 h. The DNA was heated to 95°C for 1 h to hydrolyze temperature sensitive N-methyl purines and allowed to cool down to room temperature. Subsequently it was dialyzed against water and brought to a final concentration of 2.97 mg/ml on Amicon YM 10 ultrafiltration membranes as measured spectrophotometrically at a wavelength of 260 nm. The DNA contained between 9 and 16 pmol O6-mGua/µg in various preparations.

Determination of O6-Methylguanine-DNA Methyltransferase Activity. Cell cultures were grown to confluence, harvested, and counted. They consisted of fibroblasts only as controlled microscopically. Cell pellets were suspended in fresh ice cold assay buffer (100 mM NaH2PO4·0.1 ml EDTA·1 mmol dithiothreitol, pH 7.4) at a concentration of 40 × 10^6 cells/ml and sonicated for 1 min at 4°C in an oxygen free atmosphere.

Aliquots of the cell homogenate corresponding to 8 × 10^6 cells were incubated with methylated DNA substrate containing 80 pmol of O6-mGua for 1 h at 37°C. Blanks contained buffer instead of homogenate. Protein was denatured by heating at 95°C for 1 h, and samples were cooled on ice and centrifuged at 10,000 × g for 10 min.

The supernatant was removed and stored overnight at −20°C. Before the assay, pH was adjusted to 1.0 with HCl and the samples were hydrolyzed at 80°C for 30 min. After centrifugation (10,000 × g, 10 min) the supernatant was used for high performance liquid chromatography. Chromatographic separation was performed using a cation exchange column (Nucleosil SA 10; Knauer, Berlin, Federal Republic of Germany) and eluting with 50 mM ammonium formate buffer, pH 2.7,
RESULTS

A linear reduction of \( O^6\)-mGua fluorescence was obtained up to \( 3.2 \times 10^7 \) cells/individual assay (Fig. 1). A 5-fold determination with the same cell line revealed a coefficient of variation of 9.8%; four independent determinations with the same cell strain in weekly intervals revealed a coefficient of variation of 15.1%. The activity of \( O^6\)-mGua methyltransferase expressed as pmol \( O^6\)-mGua removed per \( 8 \times 10^6 \) cells, of all cell lines tested, is shown in Fig. 2. There is a broad interindividual variability of \( O^6\)-mGua methyltransferase which is larger than 1 order of magnitude in all three groups of probands.

The mean repair capacity did not differ between normal healthy controls [10.35 ± 5.42 (SD) pmol \( O^6\)-mGua removed] and patients with cutaneous malignant melanoma [10.83 ± 6.66]. In contrast, the mean repair capacity of cells from lung cancer patients was clearly reduced (6.64 ± 4.32), the difference being statistically significant with \( P < 0.0022 \) as compared to healthy controls and \( P < 0.0005 \) compared to the combined data of melanoma patients and healthy controls (two-tailed Mann-Whitney test). It is particularly noteworthy that four of the lung cancer patients but none of the controls had no detectable \( O^6\)-mGua repair (<1.0 pmol \( O^6\)-mGua repair/\( 8 \times 10^6 \) cells). These results were each confirmed by testing two independently processed cell cultures.

When subgroups of lung cancer patients were evaluated separately (Fig. 3), the mean repair capacity was lowest in patients with an early age of manifestation (5.06 ± 3.89 pmol \( O^6\)-mGua removed, \( P < 0.0009 \)), as compared to patients with a family history of lung cancer (7.58 ± 4.71, \( P < 0.07 \)) and to nonsmoking patients (7.44 ± 3.88, \( P < 0.13 \)). We did not obtain a correlation between \( O^6\)-mGua methyltransferase activity and smoking history, age, or sex of the probands.

When lung cancer patients are grouped by the histological type of their tumors according to Kreyberg's classification (16) as group I containing squamous cell and small cell cancers (\( n = 23 \)) and group II containing all others, predominantly adenocarcinomas and large cell cancers (\( n = 23 \)), the repair capacities were 6.02 ± 4.81 and 7.49 ± 3.79 pmol \( O^6\)-mGua removed per \( 8 \times 10^6 \) cells, respectively (Fig. 4). One patient with two independent lung tumors, one squamous cell carcinoma and one adenocarcinoma, was evaluated in either group. The difference between histological types was statistically not significant.

DISCUSSION

Humans are exposed to methylating agents like nitrosamines from various sources, and there is evidence that nitrosamines are also present in tobacco and tobacco smoke (17). Since the persistence of \( O^6\)-mGua is known to produce mutations (18) and cellular transformation (19), the decreased repair capacity in fibroblasts from lung cancer patients could well be interpreted as an endogenously predisposing factor in some individuals increasing their sensitivity against carcinogenic DNA methylating agents. This conclusion is supported by the observation of particularly decreased repair in young patients because endogenously susceptible individuals could be expected to develop cancer at an early age (20).

Studies on the correlation between tumor histology and carcinogenic exposure have revealed that smoking as the main source of lung cancer causing agents produces predominantly Kreyberg type I cancers (16). Therefore, the lower repair capacities in patients with Kreyberg type I as compared to Kreyberg type II cancers support the concept of \( O^6\)-mGua repair as a factor determining lung cancer susceptibility.

A reduced repair capacity will increase individual risk only in the presence of methylating agents. It may be indifferent with respect to numerous other carcinogenic influences that may be responsible for lung cancer in nonsmokers, who in our study had only a slightly decreased repair (7.44 ± 3.88 \( O^6\)-mGua removed, \( P < 0.13 \)). Unexpectedly, the repair capacity in lung cancer patients with at least one first degree relative having lung cancer was only slightly reduced (7.58 ± 4.71 \( O^6\)-mGua removed, \( P < 0.07 \)). This might be due to an insufficient definition of this group, which was not standardized according to family size and to carcinogenic exposure in relatives, so that an affected relative in the majority of cases does not necessarily mean enhanced genetic predisposition.

The four patients who had no detectable \( O^6\)-mGua repair in their cultured fibroblasts are particularly interesting because we did not yet observe such individuals among normal healthy

![Fig. 1. Linear reduction of \( O^6\)-mGua fluorescence after 1 h incubation with fibroblast lysate of 0.3-3.2 \( \times 10^7 \) cells (○). O controls with boiled extract; bars, SD of the mean of a triple determination.](image-url)


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Fig. 2. Activity of O'-mGua methyltransferase expressed as pmol O6-mGua removed per 8 x 10^6 fibroblasts from 29 normal healthy controls, 39 patients with cutaneous malignant melanoma, and 45 patients with lung cancer. Bars, means.

Fig. 3. Repair capacity expressed as pmol O6-mGua removed per 8 x 10^6 fibroblasts in subgroups of lung cancer patients: manifestation <46 years of age, n = 16; at least one first degree relative with lung cancer, n = 15; lifelong non-smokers, n = 10. Bars, means.

Fig. 4. Repair capacity expressed as pmol O6-mGua removed per 8 x 10^6 fibroblasts in lung cancer patients with squamous cell and small cell cancer (type I according to Kreyberg, n = 23) and others (type II, n = 23). One patient with two independent tumors, one type I and the other type II, was evaluated in either group. Bars, means.

controls. It is possible, however, that cells other than fibroblasts of these four probands may exhibit a sufficient repair activity, since repair levels were reported to differ between cells (20). In addition, this is an inducible enzyme (21) and we do not yet have data about inducible repair activities in low repair probands.

A study of cultured fibroblasts has the advantage that in vivo conditions such as the presence of growth or inhibitory factors are unlikely to persist. Fibroblast cultures have proved useful for the study of various genetic markers and defects, although these cells often do not represent relevant cellular targets in vivo. A definite precursor cell for lung cancer cannot be defined in any case.

In conclusion, our findings suggest a reduced repair of O'-mGua DNA adducts in fibroblasts from a group of patients with lung cancer which might reflect a further mechanism of constitutionally increased lung cancer risk. Further studies are needed to evaluate the predictive power of this parameter.

REFERENCES


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