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Lung cancer in smoking patients inversely alters the activity of hOGG1 and hNTH1

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Abstract

N-Glycosylases excise the damaged adducts from DNA. 7,8-Dihydro-8-oxoguanine in human cells is repaired by OGG1 and hNTH1. The activities of hOGG1 and hNTH1 were measured, using modified and ^{32}P labelled oligonucleotides, in bronchial biopsy samples of smoking patients with non-small cell lung carcinoma. The activity of hOOG1 was significantly higher in biopsies from tumour tissues compared with intra-individual control samples. On the contrary, the activity of endonuclease III homologue, hNTH1, was lower in tumours compared to controls. These opposing alterations in DNA repair enzymes may affect cancer growth due to the increased formation of AP sites.

Keywords: Lung cancer; Base excision repair; DNA repair; Smoking; Formamidopyrimidine-DNA; OGG1; hNTH1; Mutation

1. Introduction

Mammalian DNA is exposed to continuous attack by reactive oxygen species (ROS) and significant oxidative damage occurs. Endogenous and exogenous agents such as radiation, genotoxic chemicals and smoking are known to be linked to increased oxidative DNA damage [1]. The role of endogenous DNA damage in certain diseases, such as cancer, might have significant influence on the fate and/or progression of the disease [2]. DNA damage, if it is unrepaired, might lead to mutation and it has been proposed by Loeb et al. [3–5], that cancer cells exhibit a mutator phenotype.

Protective systems have been developed to maintain the vital integrity of DNA. Among these protective systems, base excision repair (BER) is specialized to remove the oxidatively damaged bases [6,7]. The first step of BER is executed by DNA glycosylases that recognize and remove the damaged base leaving an abasic site (AP site); the AP site is recognized and cleaved by AP endonuclease, which

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introduces a DNA strand break 5′ to the baseless sugar: DNA polymerase β (Pol β) fills the one nucleotide gap, and the process is completed by DNA ligase [8]. BER enzymes have preferred substrates. The 8-oxoguanine (8-oxoG) is repaired primarily by 8-oxoG-DNA glycosylase (OGG1) in mammalian cells and by formamidopyrimidine-DNA glycosylase (Fpg) in bacteria [9]. Formamidopyrimidines, derived from purines by ROS, are also mutagenic, like 8-oxoG, and in bacteria are repaired by endonuclease III (Endo III). The mammalian homolog of the Endo III is hNTH1, which catalyses the excision of a variety of types of DNA damage, including *cis–trans*-thymine glycol, 5,6-dihydrothymine, 5, 6-dihydrouracil, 5-hydroxyuracil, and AP sites [10–12].

BER is started by DNA glycosylases by removal of the damaged base and thus leaves an AP site. This site is then cleaved by AP endonuclease. Thus OGG1 and NTH1 appear to have significant importance in DNA integrity [13]. It has been suggested that NTH1 exerts its function by preventing OGG1 reassociation to the AP site [14]. Therefore, an alteration of the activities of these enzymes could affect the mutation rate of DNA, and hence effect the viability of cells. It has been observed that OGG1 knockout mice have developed an adenoma/carcinoma [15]. In addition, Paz-Elizur et al. [16] noted that low OGG activity is associated with an increased risk of lung cancer.

Lung cancer has been shown to inversely effect the expression of superoxide dismutase and catalase [17] possibly leading to increased hydrogen peroxide concentration, which could cause a manyfold increase in mutation [18]. This mutation could be due to the significant increase in unrepaired oxidative damage to DNA may accelerate the progress of lung cancer. Therefore, the present study was driven by the hypothesis that lung cancer in patients who smoke regularly, is associated with inadequate activity of hOGG1 and hNTH1.

2. Materials and methods

2.1. Subjects

Patients undergoing bronchoscopy for diagnostic purposes were examined: eight subjects (age 64 ± 3 years; six male, two female) with a smoking history of more than 15 pack-years with no symptoms suggestive of chronic obstructive pulmonary disease and with normal lung function (FEV1 91 ± 4% predicted, FEV1/FVC 79.3 ± 3%). Pulmonary function tests were performed within the week of bronchoscopy. Bronchoscopies were performed at the National Koranyi Institute for Pulmonology. The study conformed to the Declaration of Helsinki, was approved by the local Ethics Committee, and written informed consent was obtained from each subject.

All the subjects were free of acute upper respiratory tract infections and none had received glucocorticosteroids or antibiotics within the month preceding examination. All subjects had negative skin tests for common allergen extracts and no past history of asthma or allergic rhinitis.

Bronchial biopsy samples were taken from the site of the tumour (experimental) and also from a nearby visually unaffected (control) area. Both tumour and control tissues were examined histologically. All tumours had squamous cell carcinoma. Samples for excision assays were immediately frozen in liquid nitrogen and stored in a low-temperature freezer at -70 °C until further use.

2.2. Excision assays

The biopsy samples were homogenized with buffer containing 20 mM of Tris (pH 8.0), 1 mM of EDTA, 1 mM of dithiothereitol, 0.5 mM of spermidine, 0.5 mM spermine, 50% glycerol and protease inhibitors. Homogenates were rocked for 30 min after the addition of a 1/10 v/v 2.5 M of KCl and centrifuged at 14,000 rpm for 30 min. The supernatant was aliquoted and stored at -80 °C. Protein levels were measured by the BCA method. The assay was carried out according to the protocol described by Cardozo-Pealez et al. [19]. In brief, twenty picomoles of synthetic probe containing either 8-oxodG or AP site (Trevigen, Gaithersburg, MD, USA) were labeled with P^{32} at the 5‴ end using polynucleotide T4 kinase (Boehringer Mannheim, Germany). To separate the unincorporated free [γ-P^{32}]ATP, the reaction mixtures were spun through a Sephadex G-25 column.

For the nicking reaction, 4 μg protein extract were mixed with reaction mixture containing 10 mM HEPES–KOH (pH 7.4), 100 mM KCl and 10 mM EDTA (for the OGG1 activity 0.1 mg/ml BSA was

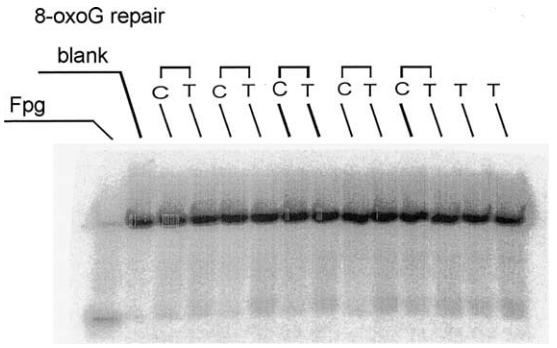

Fig. 1. The oligonucleotide sequence for 8-oxoG repair was 5′-GAACTAGTG**OA**TCCCCCGGGCTGC-3′. Fpg enzyme was loaded in the first lane followed by blank, control (C) and tumour (T) samples. Five patient-paired samples were followed by two patient tumour samples (control sample sizes were insufficient for the assay). The activity of hOGG1 increased in tumour samples. Fpg and hOGG1 were used synonymously as Fpg is the readily available bacterial antibody used for this analysis.

added) and labelled probe (approximately 2000 cpm). The reaction was carried out at 30 °C for 15 min and stopped by placing the solution in ice.

The amount of protein used and the incubation time were in the liner activity phase of the enzymes. Next, 30 μl chloroform were added and samples were centrifuged and 15 μl taken and mixed with loading buffer containing 90% formamide, 10 mM NaOH, and blue-orange dye. After 3 min heating at 95 °C, samples were chilled and loaded into polyacrylamide gel (20%) with 7 M urea and 1×TBE and run at 400 mV for 2 h. Commercially available Fpg (Trevigen Gaithersburg, MD, USA) was loaded as a standard. Gels were quantified using BAS 2000 Bioimaging Analyzer (Fuji Film Co., Japan). Radioactive signal densities were determined using the software designed for this system.

A paired Student's '*t*' test was used to compare the data. Significance was set at $P<0.05$.

3. Results and discussion

The activity of 8-oxoG repair enzyme increased in that portion of the lung containing the tumour, in all patients (Fig. 1). This suggests that lung cancer is associated with an enhanced formation of 8-oxoG. On the other hand, the activity of hNTH1 decreased in the cancer hosting sites of the patients when compared to their healthy counterparts (Fig. 2). The excision assay data were quantified by densitometer and the data revealed significant inverse changes in the activity of hOOG1 and hNTH1 (Fig. 3). This alteration indicates an accumulation of AP sites in the lung tumours.

We are reporting for the first time, to our knowledge, that lung cancer in patients who smoke regularly is associated with an increased activity of 8-oxoG repair enzyme and a decreased activity of hNTH1. The induction of the enzyme responsible for the removal of 8-oxoG, which in mammalian tissue is cloned as 8-oxoguanine DNA glycosylase (OGG1) [20], suggests an adaptive response to the increased formation of 8-oxoG as a result of lung cancer. The up-regulation of 8-oxoG repair is an important process because of the mutagenic potential of 8-oxoG [21] which could induce G·C transversion to T·A [22].

The steady-state level of AP site damage, which is suggested to be 50,000–200,000 lesions per mammalian cell under normal physiological condition [23], reflects the balance between the formation and repair of AP sites. The repair of AP sites involves the orchestrated work of two-three pathways and it has been shown that the following order likely occurs: AP endonuclease, DNA synthesis, deoxyribonucleotide phosphate lyase activity, and the ligation of DNA [24].

In the present study the activity of hNTH1 was decreased significantly suggesting an accumulation of AP sites and possibly AP site derived mutations. The decrease in AP site repair in human lung cancer appears to be a causative factor in the progress of

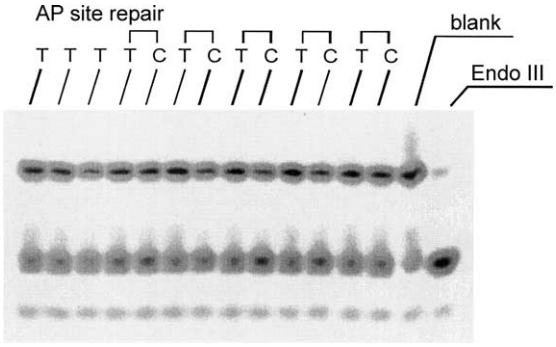

Fig. 2. For the AP site, the damage repair oligonucleotide with the following sequence CCTGCCCTG$_{\mathbf{AP}}$GCAGCTGTGGG, was labeled with ^{32}P. It appears that the activity of hNTH1 decreases in each individual tumour when compared with control samples.

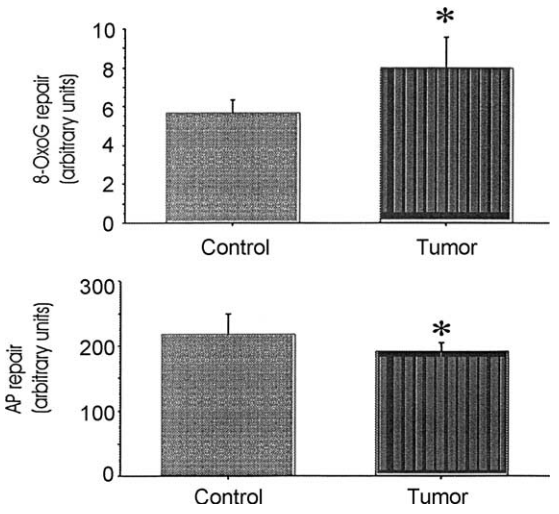

Fig. 3. The histogram shows the densitometric data obtained by the BAS 2000 system. The semiquantitive data reveal significant increases in 8-oxoG repair in tumour samples compared to control, healthy tissue. On the other hand, the activity of the AP site repair enzyme, hNTH1, was significantly lower in the cancer-affected cells. Values reported are means ± standard deviations ($n=5$). *$P<0.05$.

the disease. This finding is in accordance with the suggestion that cancer cells display a mutator phenotype [3,4,25]. Our data suggest that this mutation in lung cancer could be derived from unrepaired AP sites. The hypothesis, that targeted induction of the AP site repair process could have beneficial effects on the progress of the disease, needs to be investigated as a possible tool for the treatment of lung cancer.

The findings of the present investigation suggest, that lung cancer in patients who smoke regularly, is associated with an increased activity of 8-oxoG repair and a decreased activity of hNTH1. The down regulation of hNTH1 might cause increased mutation and progress of the disease. Therefore, the treatment-related induction of AP site repair might serve as an important tool to retard the progress of the disease.

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