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FEBS Letters 341 (1994) 59–64

FEBS 13752

FEBS LETTERS

Oxidative DNA base damage and antioxidant enzyme activities in human lung cancer

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Received 27 December 1993; revised version received 2 February 1994

Abstract

We have investigated levels of antioxidant enzymes and free radical-induced DNA base modifications in human cancerous lung tissues and in their cancer-free surrounding tissues. Various DNA base lesions in chromatin of lung tissues were measured by gas chromatography-mass spectrometry. Activities of superoxide dismutase, catalase and glutathione peroxidase were also measured in lung tissues. Higher levels of DNA lesions were observed in cancerous tissues than in cancer-free surrounding tissues. Antioxidant enzyme levels were lower in cancerous tissues. The results indicate an association between decreased activities of antioxidant enzymes and increased levels of DNA lesions in cancerous tissues. Higher levels of DNA lesions suggest that free radical reactions may be increased in malignant tumor cells.

Key words: Oxidative DNA damage; Hydroxyl radical; Mutation; Modified base; Antioxidant enzyme

1. Introduction

DNA damage is thought to be involved in all stages of carcinogenesis (reviewed in [1,2]). Thus any agent capable of chemically modifying DNA in cells could be carcinogenic. Oxygen-derived free radicals formed in cells by endogenous and exogenous sources may modify cellular components including DNA by a variety of mechanisms (reviewed in [3]). Free radicals, most notably hydroxyl radical (•OH), can cause extensive chemical modifications in DNA and nucleoprotein, including modified bases, modified sugars, strand breaks and DNA-protein cross-links (reviewed in [3–6]). Thus free radicals may be mutagenic and carcinogenic.

Antioxidant enzymes exist in cells to protect against the toxic effects of oxygen-derived species produced during normal cellular metabolism or during oxidative stress (reviewed in [7]). These antioxidant enzymes include superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). Tumor cells have been shown to have abnormal levels of antioxidant enzymes and activities when compared with normal cells [7]. However, antioxidant enzyme activities also differ among individual tumors.

Typical free radical-induced DNA base modifications in cancerous human tissues have been investigated recently [8,9]. However, no insight has been provided thus far, regarding a possible relationship between these modifications and antioxidant enzyme activities in cancerous tissues and in surrounding cancer-free tissues. In the present work, we have investigated DNA base modifications and antioxidant enzyme activities in normal and cancerous human lung tissues to check whether an association exists between these two possibly important factors in cancer.

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Abbrevations: $O_2^{\bullet-}$, superoxide radical; •OH, hydroxyl radical; 5-OH-Hyd, 5-hydroxyhydantoin; 5-OH-5-MeHyd, 5-hydroxy-5-methylhydantoin; Thy glycol, thymine glycol; 5-OHMe-Ura, 5-(hydroxymethyl)uracil; 5-OH-Ura, 5-hydroxyuracil; 5-OH-Cyt, 5-hydroxycytosine; 5,6-diOH-Ura, 5,6-dihydroxyuracil; 2-OH-Ade, 2-hydroxyadenine; 8-OH-Ade, 8-hydroxyadenine; FapyAde, 4,6-diamino-5-formamidopyrimidine; 8-OH-Gua, 8-hydroxyguanine; FapyGua, 2,6-diamino-4-hydroxy-5-formamidopyrimidine; GC/MS-SIM, gas chromatography-mass spectroscopy with selected-ion monitoring; GPx, glutathione peroxidase; SOD, superoxide dismutase; CAT, catalase.

2. Materials and methods

2.1. Materials, human tissues, measurement of enzyme activities and isolation of chromatin

Certain commercial equipment or materials are identified in this paper in order to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

Materials were as described previously [10]. The human tissues were obtained from Department of Surgery, Medical School, Gdansk, Poland, during surgery on five lung cancer patients. Approximately 0.7–1.2 g of tissues were collected for chromatin isolation and measurement

SSDI 0014-5793(94)00137-K

of enzyme activities. After surgical removal, all tissues were quickly frozen and stored in liquid nitrogen. Histopathological evaluation of surgically removed tissues revealed that the surgical margins (removed from the most distant part of the lung which had no connection to the cancer) were disease-free while the affected tissues exhibited lung squamous cell carcinoma. Every tissue sample was divided into three separate parts, transferred to a Potter homogenizer and disrupted with several strokes of first loose and then a tighter pestle in 10 ml of 20 mM phosphate buffer (pH 7.4), containing 0.1 mM phenylmethanesulfonyl fluoride. An aliquot (6 ml) of the resulting homogenate was used for chromatin isolation [9,11]. Another aliquot (4 ml) was used to assay antioxidant enzyme activity. Catalase was assayed by a colorimetric method using dichromate/acetic acid [12]. The total activity of SOD (CuZnSOD and MnSOD) was measured using xanthine–xanthine oxidase system with Nitroblue tetrazolium [13]. The activity of Se-GPx was assayed using *t*-butylhydroperoxide as a substrate [14]. Protein content in tissue extracts was determined according to Smith et al. [15].

2.2. *Hydrolysis, derivatization and gas chromatography/mass spectrometry*

Chromatin samples containing 100 μg DNA were hydrolyzed and trimethylsilylated, and subsequently analyzed by gas chromatography-mass spectrometry with selected-ion monitoring (GC/MS-SIM) as described previously [9,10,16]. The quantification of products was done by isotope-dilution mass spectrometry using their stable isotope-labeled analogues as internal standards [10,17].

3. Results

The aim of this study was to examine endogenous levels of typical free radical-induced DNA bases and antioxidant enzyme activities in human cancerous lung tissues and their cancer-free surrounding tissues. Chemical characterization and quantification of free radical-modified DNA bases in DNA or in chromatin can be achieved by GC/MS [16–19]. To this end, various DNA lesions derived from all four DNA bases are measured using the same DNA or chromatin sample.

Tissues samples were removed surgically from five lung cancer patients. All patients were heavy smokers. We were unable to find any nonsmoker patient among lung cancer patients examined in the clinic of Medical School in Gdansk, Poland. Twelve modified bases were identified and quantified in chromatin samples isolated from cancerous tissues and their cancer-free surrounding tissues. These were 5-OH-Hyd, 5-OH-5-MeHyd, Thy glycol, 5-OHMe-Ura, 5-OH-Ura, 5-OH-Cyt, 5,6-diOH-Ura, 2-OH-Ade, 8-OH-Ade, FapyAde, 8-OH-Gua and FapyGua. The quantities of these modified DNA bases in lung tissues are shown in Fig. 1. For each of the data points shown in Fig. 1, three independent measurements were carried out using chromatin samples isolated from three separate tissue samples taken from the same cancerous tissue or surrounding cancer-free tissue. Aliquots of the same tissues samples were used to measure the activities of SOD, CAT and GPx. The results are illustrated in Fig. 2.

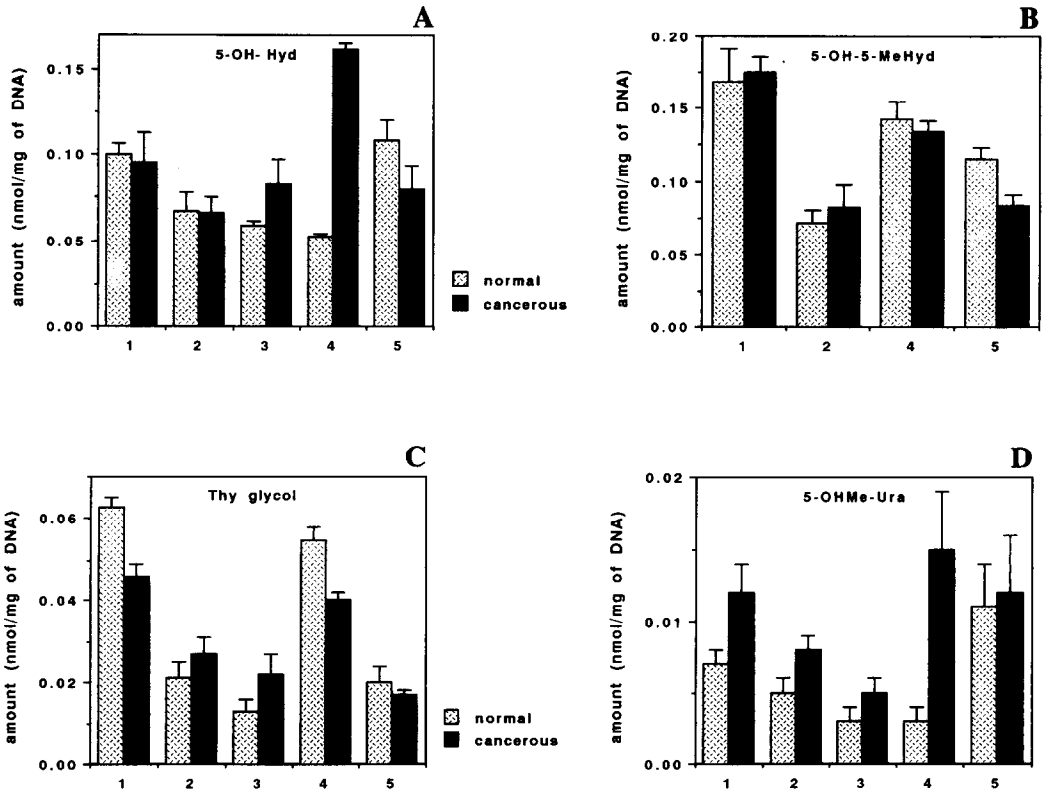

Fig. 1. Amounts of modified DNA bases in human cancerous lung tissues and their surrounding cancer-free tissues. Each data point represents the mean ± S.D. from measurement of chromatin samples isolated from three separate tissue samples (1 nmol of a modified base/mg of DNA ≈ 32 molecules of a modified base/10^5 DNA bases).

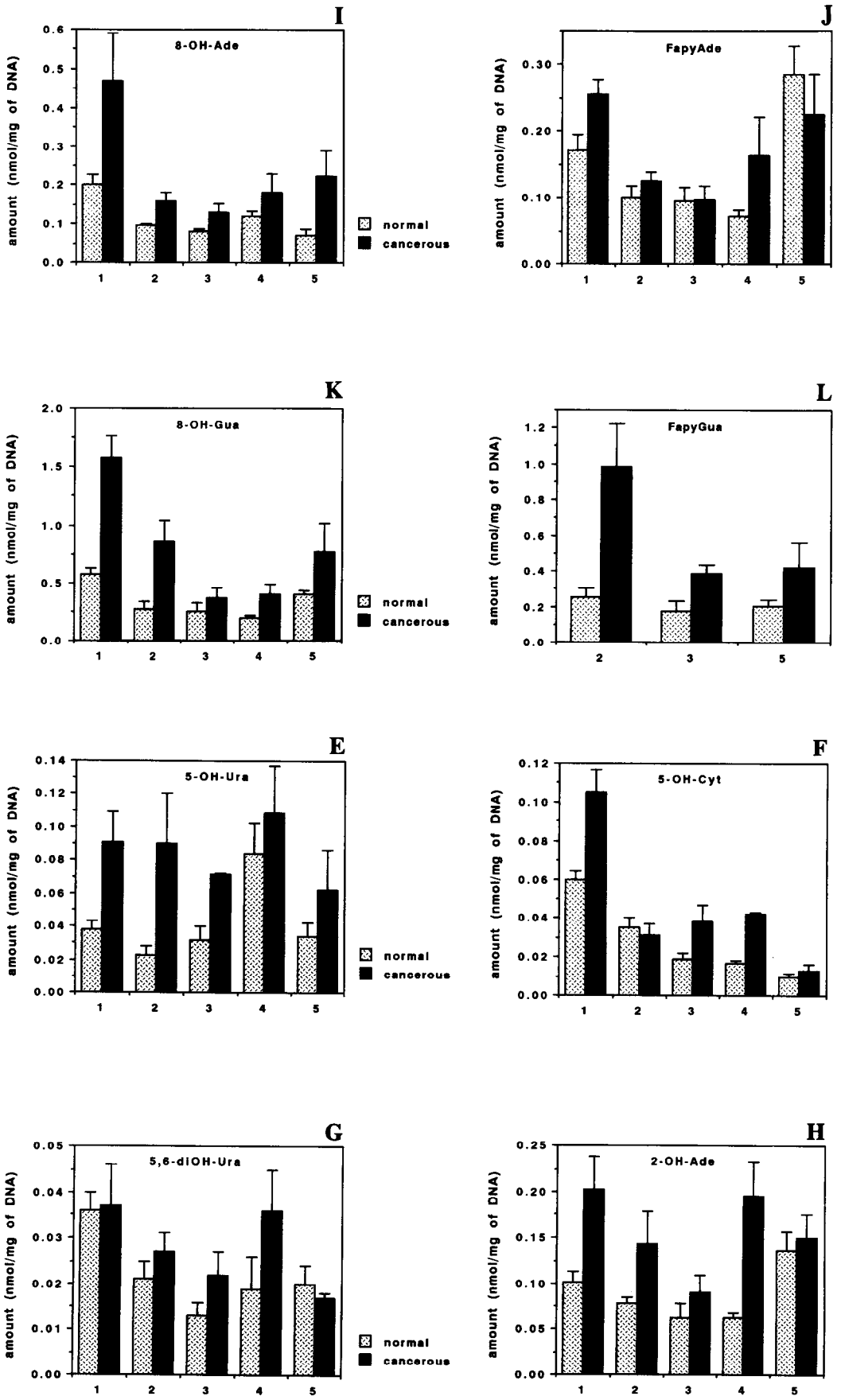

Fig. 1. (*Continued*).

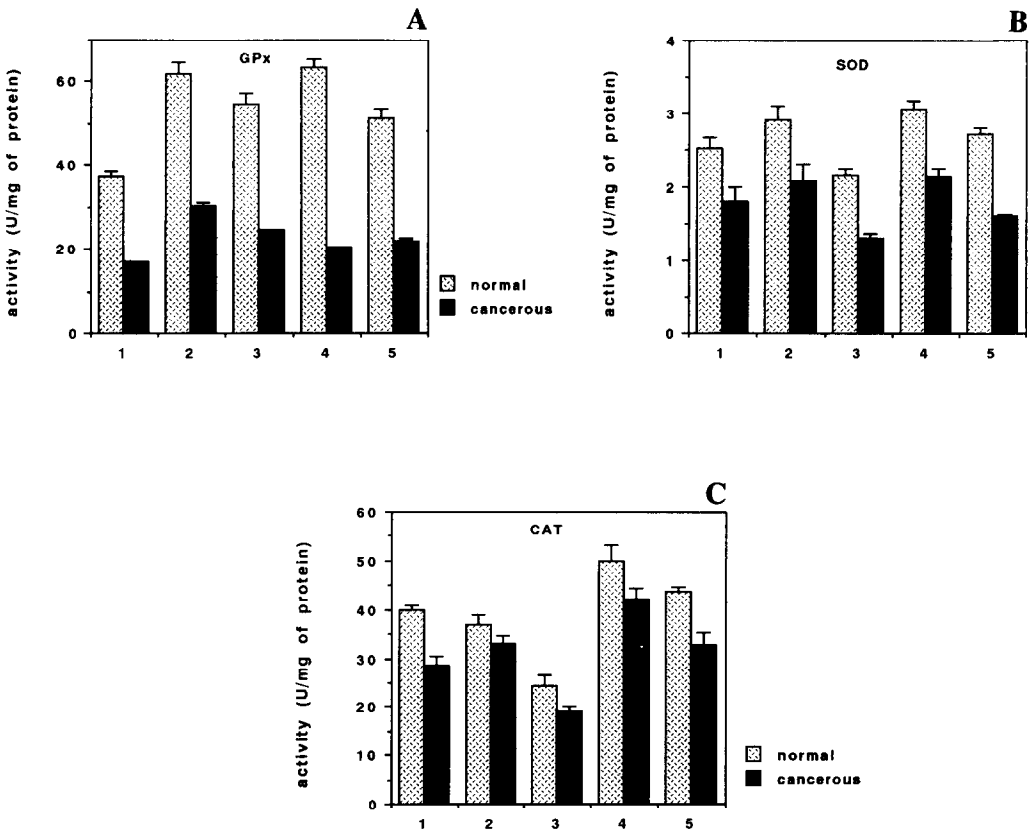

Fig. 2. Activities of GPx, SOD and CAT in human cancerous lung tissues and their surrounding cancer-free tissues. Each data point represents the mean ± S.D. from three independent measurements.

The amounts of modified bases that were measured in cancer-free lung tissues varied depending on the individuals (Fig. 1). Increased amounts of 8-OH-Ade were observed in cancerous tissues of all five patients (Fig. 1I), whereas four patients had increased amounts of 8-OH-Gua in their cancerous tissues (Fig. 1K). FapyGua could be quantified in tissues of three patients only, and its amount was higher in cancerous tissues than in cancer-free tissues in all cases (Fig. 1L). The amounts of 5-OHMe-Ura, 5-OH-Ura, 5-OH-Cyt and 2-OH-Ade were higher in cancerous tissues of three patients (Fig. 1D, E, F and H). Increased amounts of 5-OH-Hyd, Thy glycol, 5,6-diOH-Ura and FapyAde were observed in cancerous tissues of one or two patients only (Fig. 1A, C, G and J).

In the case of all five patients, the activities of the enzymes GPx, SOD and CAT were found to be decreased in cancerous tissues with respect to their surrounding cancer-free tissues (Fig. 2). The highest decrease was observed in the activity of GPx, which amounted to ≈ 2- to 3-fold (Fig. 2A). The activity of SOD in cancerous tissues was also significantly lower than in normal tissues (Fig. 2B). Only a modest decrease in the CAT activity was observed (Fig. 2C).

4. Discussion

The results of this work show higher endogenous levels of typical •OH-induced products of all four DNA bases and lower activities of antioxidant enzymes in human cancerous lung tissues than in their respective surrounding cancer-free tissues. These results are consistent with our earlier observations of higher levels of pyrimidine- and purine-derived DNA lesions in different human cancerous tissues [9]. However, a possible association between increased levels of modified DNA bases and decreased levels of antioxidant enzymes in human cancerous tissues with respect to cancer-free tissues is reported here for the first time.

Patients whose tissues were examined in the present work were all smokers. Epidemiological studies have established smoking as the major cause of human lung cancer [20,21]. Cigarette smoke has been shown to cause DNA damage in cultured human lung cells [22]. Oxygen-derived species such as H_2O_2 and superoxide radical ($O^{\bullet}_2^-$) are known to be involved in causing DNA damage by cigarette smoke and tar, most likely by producing •OH in metal ion-catalyzed reactions (reviewed in [3,23,24]). H_2O_2 and $O^{\bullet}_2^-$ alone do not produce any DNA damage

(reviewed in [3]). Furthermore, cigarette smoking depletes intracellular antioxidants and increases the number of neutrophils in the lung, which are another source of oxygen-derived species (reviewed in [24]).

Elevated levels of modified DNA bases in cancerous tissues may be due in part to the presence of large amounts of leukocytes in human tumors [25]. Activated leukocytes are a source of H_2O_2, which can cross cellular and nuclear membranes, and reach the nucleus to cause site-specific DNA damage by producing $^{\bullet}OH$ in reactions with DNA-bound metal ions (reviewed in [1,26]). Moreover, human tumor cells have been shown to produce large amounts of H_2O_2 [27]. In fact, treatment of mammalian cells with H_2O_2 or with activated leukocytes causes modification of all four DNA bases with a pattern of modified bases similar to that observed in the present work [28,29].

In tumor cells, activities of antioxidant enzymes have been found to be generally lower than in normal cells, although GPx activities were found to be higher in some tumor cell lines with large interindividual variations (reviewed in [7]). Our results showing lower activities of GPx, SOD and CAT in cancerous lung tissues are consistent with the general tendency of previous findings. Lower levels of antioxidant enzymes may cause the accumulation of H_2O_2 and $O_2^{\bullet-}$ in tumor cells [7]. This may result in subsequent greater base damage to DNA in cancerous tissues as the findings of the present work indicate. Higher levels of typical $^{\bullet}OH$-induced DNA base lesions as observed here lends credence to the hypothesis that free radical reactions may be increased in malignant cells, concurrent with decreased antioxidant protection [30].

It is not known whether lower levels of antioxidant enzymes in cancerous lung tissues play a causative role in carcinogenesis or are merely the result of the disease. The same is true of the pyrimidine- and purine-derived DNA lesions that were identified in cancerous tissues at higher levels than in cancer-free surrounding tissues. However, treatment of laboratory animals with carcinogenic agents causes formation of a similar pattern of pyrimidine- and purine-derived DNA lesions in their target organs before tumor formation occurs [31–34]. Furthermore, several lesions identified in this work have been found to possess mutagenic properties (reviewed in [35–37]), although the majority of these lesions has not been investigated for their biological consequences. All these data may indicate an important role for these DNA lesions in carcinogenesis. On the other hand, in fully developed cancer, increased levels of modified DNA bases may contribute to the genetic instability and metastatic potential of tumor cells.

In conclusion, the present work provides evidence for a possible association between decreased activities of antioxidant enzymes and increased levels of pyrimidine- and purine-derived DNA lesions in human cancerous lung tissues. Higher levels of DNA lesions indicate that free radical reactions may be increased in malignant cells. Higher levels of these lesions may contribute to the genetic instability and metastatic potential of tumors, and may be necessary for maintaining the malignancy.

Acknowledgements: We gratefully acknowledge partial financial support from the US–Polish Maria Sklodowska-Curie Joint Fund II (No. MZ/NIST-93-140). We are grateful to Mrs. Teresa Olinska for excellent technical assistance. M.D. acknowledges partial financial support from the Office of Health and Environmental Research, Office of Energy Research, US Department of Energy, Washington, DC.

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