

## Lung Cancer Patients Have Increased 8-Hydroxydeoxyguanosine Levels in Peripheral Lung Tissue DNA

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**The 8-hydroxydeoxyguanosine (8-OH-dG) levels in the peripheral parts of human lung tissues were compared between lung cancer patients (n=70) and non-cancer patient controls (n=15). An increased level of 8-OH-dG was observed in the lung cancer group, in both the adenocarcinoma and non-adenocarcinoma (mainly squamous cell carcinoma) groups, as compared to the non-cancer control group. This result suggests that reactive oxygen species are partly involved in the induction of lung carcinomas (both adenocarcinoma and non-adenocarcinoma).**

Key words: 8-Hydroxydeoxyguanosine — Oxidative DNA damage — Lung cancer

Among worldwide cancer mortality rates, that of lung cancer, which is caused by both exogenous and endogenous factors,<sup>1-7</sup> is one of the highest. Several studies have described how environmental mutagens and carcinogens act to induce lung cancer.<sup>4,8</sup> Genetic alterations in *K-ras* and *p53* have been shown to occur during the multi-step process of lung carcinogenesis (namely, in hyperplasia, metaplasia, dysplasia and carcinoma).<sup>3,9,10</sup> Microsatellite instability, as well as various forms of DNA damage, such as bulky adducts and strand breakage, has been reported in relation to human lung cancer.<sup>11-13</sup>

Reactive oxygen species (ROS) cause DNA damage and may induce genetic changes in all stages (initiation, promotion and progression) of carcinogenesis.<sup>14</sup> ROS are produced by ionizing radiation, mutagens and carcinogens, and also during endogenous oxygen metabolism in cells.<sup>15</sup> 8-Hydroxydeoxyguanosine (8-OH-dG) is one of the major forms of DNA damage induced by ROS.<sup>16,17</sup> Since 8-OH-dG causes G·C to T·A transversions *in vitro*<sup>18</sup> and *in vivo*,<sup>19-22</sup> as a result of 8-OH-dG·A mispairing, its levels in the DNAs of animal and human organs, as well as in human leukocyte DNA, have been measured as a sensitive biomarker of oxidative DNA damage.<sup>23</sup> G·C to T·A transversions have been detected in the *ras* oncogene and the *p53* tumor suppressor gene in human lung cancers.<sup>14,24</sup> It is suspected that ROS might be involved in the mechanism of lung carcinogenesis.<sup>14</sup> The 8-OH-dG levels have been analyzed in human peripheral blood,

stomach, liver, breast, and lung tissues.<sup>25-31</sup> However, there have only been a few reports about human lungs, and most of these describe the differences in the 8-OH-dG levels between cancerous and non-cancerous tissues. If oxidative stress is induced in the entire lung during lung carcinogenesis, either due to environmental factors or for genetic reasons, increased 8-OH-dG levels may be detected within the non-cancerous tissues of lung cancer patients. In this study, the 8-OH-dG levels in the DNA from the peripheral parts of human lung tissues, from both non-cancer patients and lung cancer patients, were measured by a high-performance liquid chromatography-electrochemical detector (HPLC-ECD) system and compared.

### MATERIALS AND METHODS

**Materials** The DNA Extractor WB Kit was purchased from Wako Biochemicals (Osaka). Nuclease P<sub>1</sub> (YA 7801) was from Yamasa Co., Choshi and acid phosphatase (type XA, P-1435) was from Sigma Chemical Co. (St. Louis, MO). Lung samples were obtained during surgery at the Hospital of the University of Occupational and Environmental Health, Kitakyushu, and the National Cancer Center Hospital, Tokyo. In the cancer group, patients with active inflammation in the lung or previous cancer, or who had received chemotherapy or radiation therapy for lung cancer, or had undergone an operation under general anesthesia within the previous six months were excluded. After resection, samples were taken from a peripheral part of the lung, which was most distant from the cancer in the same lobe. In the non-cancer group, patients who had either active inflammation in the lung or a history of any

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cancer were excluded. The samples were taken from lung resections of patients with either pneumothorax, giant bulla, tuberculosis, inflammatory pseudotumor, interstitial pneumonia, or bronchiectasis. All samples collected during the operations were divided on ice into portions of about 0.2–0.3 mg. The ice-cooled samples were transported to a laboratory and were immediately stored at –80°C until the 8-OH-dG analysis.

**Analysis of 8-OH-dG** In this study, the lung samples were cut with scissors into small particles of about 1 mm, and were homogenized in lysis solution with a Potter-type homogenizer. The DNA was isolated from lung tissues using the DNA Extractor WB Kit, according to the method of Nakae *et al.*<sup>32)</sup> with slight modifications. The extracted DNA was dissolved in 100 µl of 1 mM EDTA and was digested with 4 µl of nuclease P<sub>1</sub> (5 mg/ml) and 2 µl of acid phosphatase (47 mg/ml, suspension in 1.8 M (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>) in the presence of 20 mM sodium acetate buffer (pH 4.5). After an incubation at 37°C for 30 min, the mixture was treated with 10 µl of ion exchange resin, Muromac (Muromachi Kagaku Kogyo, Tokyo, suspension, 50 mg/ml), and centrifuged at 15,000g for 5 min. The supernatant was transferred to a filter tube (Millipore, Bedford, MA; Samprep C; 0.2 µm) and centrifuged at 5,000g for 5 min, then the filtrate was injected into an HPLC column. The 8-OH-dG analysis was performed using the HPLC-ECD method of Floyd *et al.*,<sup>33)</sup> modified as described previously.<sup>26)</sup>

**Statistical analysis** The differences between the two groups were tested for statistical significance by using Student's *t* test, and *P* values of <0.01 were regarded as significant.

RESULTS

The 8-OH-dG levels in the DNA of non-cancerous human lung tissues were compared between lung cancer patients (the cancer group) and non-cancer patients (the control group) by the method described above. The clinical characteristics of the 85 patients are summarized in Table I. The cancer group consisted of 46 males and 24 females, with a median age of 66.6 (range, 43 to 84). Adenocarcinoma was present in 34 cases, squamous cell carcinoma in 28 cases, large cell carcinoma in 4 cases, small cell carcinoma in 2 cases, and adenosquamous carcinoma and carcinoid in 1 case. The control group consisted of 9 males and 6 females, with a median age of 61.9 (range, 49 to 71), with pneumothorax in 5 cases, tuberculosis and inflammatory pseudotumor in 3 cases, giant bulla in 2 cases, and interstitial pneumonia and bronchiectasis in 1 case.

The 8-OH-dG levels in the lung tissues in the cancer group ranged from 0.25 to 0.99 8-OH-dG/10<sup>5</sup>dG. The 8-OH-dG content in the lung tissues in the control group

Table I. Patients' Characteristics and 8-OH-dG Levels

	Cancer group	Control group
Number	70	15
Age		
Mean±SD	66.6±9.0	61.9±8.8
Range	43–84	49–71
Sex		
Male	46	9
Female	24	6
8-OH-dG/10 <sup>5</sup> dG		
Mean±SD	0.52±0.17	0.37±0.08
Range	0.25–0.99	0.23–0.50
Brinkman Index <sup>a)</sup>		
Mean±SD	630±553	733±634

a) Brinkman Index: cigarettes/day×years.

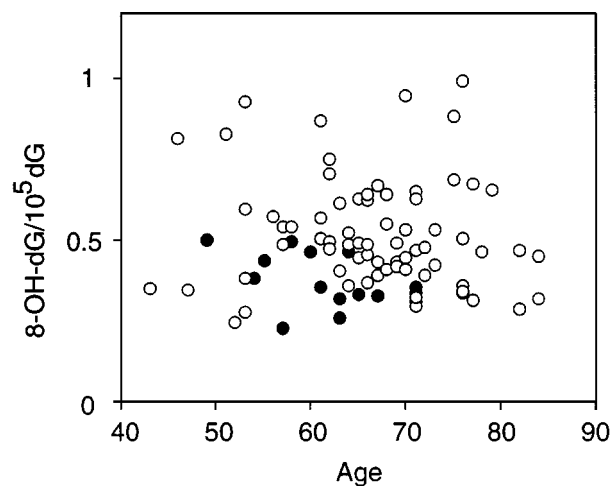


Fig. 1. Relation of age and the 8-OH-dG levels in the DNA from peripheral lung tissue: lung cancer (O); non-cancer control group (●).

ranged from 0.23 to 0.50 8-OH-dG/10<sup>5</sup>dG (Table I). In the cancer group, the 8-OH-dG levels were higher than those of the control group (Student's *t* test; *P*<0.01) (Figs. 1, 2). Histologically, there was no difference between the 8-OH-dG levels in adenocarcinoma and in squamous cell carcinoma, and the levels of both were higher than in the control group (Fig. 2). These data indicate that the lung cancer patients have more oxidative DNA damage as compared to the control. No correlation was found between the number of cigarettes smoked and the 8-OH-dG levels in the peripheral part of the lung.

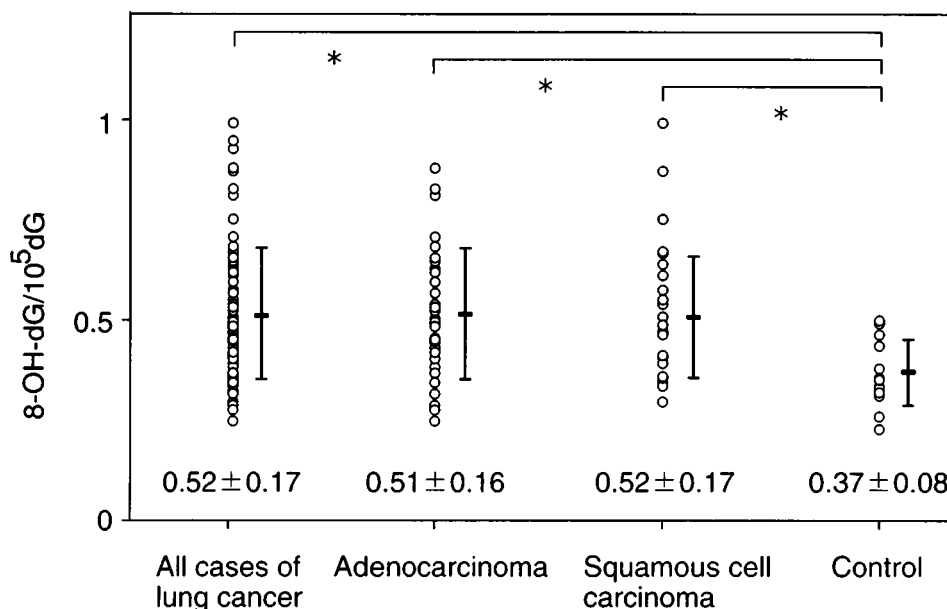


Fig. 2. Distribution of 8-OH-dG level in normal lung tissues of cancer, squamous cell carcinoma, adenocarcinoma and control patients. Each data point represents one individual. Bars and figures represent mean $\pm$ SD. \*  $P < 0.01$  for difference from the control.

## DISCUSSION

The 8-OH-dG levels in the DNA of human peripheral blood cells, as well as stomach, liver, breast and lung tissues, have been measured as a marker of oxidative DNA damage.<sup>25-31</sup> Some reports have mentioned an increase of 8-OH-dG in the DNA from cancerous tissues as compared to the cancer-free, normal tissues from the same patients.<sup>29-31</sup> However, oxidative DNA damage analysis in the cancerous tissue is not meaningful, because oxidative DNA damage is thought to cause genetic changes in oncogenes and tumor suppressor genes during carcinogenesis.<sup>14</sup> The measurement of the 8-OH-dG levels in non-cancerous tissues is more important, if the entire lung is equally exposed to oxidative stress during carcinogenesis. The 8-OH-dG level should be higher in the non-cancerous tissues of lung cancer patients than in those of non-cancer patients, if ROS are involved in lung carcinogenesis. In the present study, we found that the 8-OH-dG levels were increased in the non-cancerous tissues of lung cancer patients, as expected.

It is known that cellular oxidative stress is related not only to carcinogenesis, but also to aging. A few reports have shown a correlation between age and the 8-OH-dG level. Fraga *et al.*<sup>34</sup> analyzed the 8-OH-dG levels in Fischer 344 rats, and reported that the levels of 8-OH-dG in liver, kidney, and intestine DNA increased with age. Homma *et al.*<sup>35</sup> reported that the level of 8-OH-dG in

DNA increased with cellular aging of cultured human diploid fibroblasts. In the present study, however, there was no significant correlation between the 8-OH-dG level in the lung and age. The most frequent type of lung cancer to develop in the central part of the lung is squamous cell carcinoma, and its induction is related to the number of cigarettes smoked. In contrast, the most common type of lung cancer that develops in the peripheral part of the lung is adenocarcinoma, and its induction is not related to smoking.

A correlation between the number of cigarettes smoked and the 8-OH-dG levels has been observed in the central part of the lung.<sup>26</sup> However, in this study we found no significant correlation between these parameters in the peripheral part of the lung. These data suggest that ROS generated by smoking are involved in the induction of squamous cell carcinoma, but not of adenocarcinoma.

The reason why more oxidative DNA damage is induced in the peripheral part of the lungs of cancer patients is not known. However, two explanations are possible, namely, i) the lung tissue was attacked by more ROS, and was exposed to more environmental mutagens and carcinogens, such as diesel exhaust particles,<sup>36,37</sup> ii) in lung cancer patients, the lung tissue has increased sensitivity to ROS, and perhaps decreased ROS scavenging ability or repair activity for oxidized DNA. In our lung cancer patients, a higher level of 8-OH-dG was detected in the peripheral part of the lung, even in the non-adeno-

carcinoma group (mainly squamous cell carcinoma), suggesting that oxidative stress is induced in the entire lung, either by environmental agents or under the influence of genetic factors. The higher level of urinary 8-OH-dG excretion in human cancer patients<sup>38)</sup> supports this hypothesis.

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