Age-Associated Increase of 8-Hydroxydeoxyguanosine in Human Colorectal Tissue DNA

Yosuke Tsurudome,1,2 Takeshi Hirano,1 Keiji Hirata,2 Aiichiro Higure,2 Naoki Nagata,2 Ken Takahashi,3 Hideaki Itoh,2 and Hiroshi Kasai1

Departments of 1Environmental Oncology, 2Surgery, and 3Environmental Epidemiology, University of Occupational and Environmental Health, Kitakyushu, Japan.

To clarify the role of oxidative stress in aging of colorectal tissue, we analyzed the 8-hydroxydeoxyguanosine (8-OH-dG) levels in colorectal biopsy samples from normal tissue of patients with either colorectal cancer (n = 15) or benign colorectal polyps (n = 40). An age-associated increase of 8-OH-dG was observed (p = .002), although the 8-OH-dG levels were not significantly different between the patients with cancer and those with polyps. These results suggest an increased level of 8-OH-dG formation in human colorectal tissue with age.

In this paper, we report that (a) the level of 8-OH-dG in the colon increases with age and (b) the colorectal 8-OH-dG levels are not significantly different between patients with colon cancer and those with polyps, based on the data obtained by the most reliable DNA isolation method using NaI, coupled with the HPLC-ECD method.

METHODS

Preparation of Tissue Samples
Normal colorectal tissue was collected from patients with colorectal cancer (n = 15; 12 colon adenocarcinoma, and 3 rectal adenocarcinoma) and those with benign polyps (n = 40; 26 colon adenoma, 5 colon hyperplastic polyp, 4 rectal adenoma, 1 colon melanosis, 2 colon adenoma with gastric cancer, 1 colon adenoma with hepatocellular carcinoma, and 1 rectal melanosis and rectal adenoma), with informed consent. Approximately 10 colorectal biopsy samples per person were removed with forceps at a position approximately 10 cm distant from the anal verge, avoiding the tumor lesions macropathologically, during total colorectal endoscopy using a colonoscope (Olympus Optical Co., Tokyo, Japan). The samples were frozen at −80°C, and they were kept at −80°C until their 8-OH-dG levels were measured.

Measurement of 8-OH-dG Levels in Colorectal Tissues
The method for determination of 8-OH-dG used in the present study could reduce the background as described in a previous report (5–7). The biopsy samples were homogenized by a potter-type homogenizer. The nuclear DNA was extracted by using the DNA Extractor WB kit (Wako, Japan), which contains NaI, an OH radical scavenger. After the digestion using nuclease P1 and acid phosphatase, 100 μl of the samples were analyzed once by the use of an HPLC-ECD per each sample. As standard samples, 20 μl
each of deoxyguanosine (0.5 mg/ml) and 8-hydroxydeoxyguanosine (5 ng/ml) solutions were injected. The 8-OH-dG value was calculated as the number per 10^5 of guanine residues.

**Statistical Analysis**

Differences between groups were tested by the analysis of variance factorial with Fisher’s protected least significant difference (PLSD) at a 5% significance level. The lines’ correlation of the paired data was calculated by means of Pearson’s correlation coefficient \( r \). All analyses were carried out by using the Stat View 4.5 program (Berkeley, CA) and PC-SAS version 4.

**RESULTS AND DISCUSSION**

Many studies have suggested that oxidative stresses are responsible for the mechanisms of aging (8,9). 8-OH-dG is known to be one of the most abundant forms of oxidative DNA damage and is increased with age in animal models (10,11) or cultured human cells (12). However, as far as we know, very few studies have reported the relationship in human organs. Thus, in the present study, we analyzed the relationship by measuring the 8-OH-dG levels in human colorectal biopsy samples.

We used 55 human biopsy samples of colorectal tissues resected under endoscopic observation. Out of the 55 samples, 15 samples were taken from patients with colorectal cancer and the other 40 samples were from patients with benign colorectal polyps. The levels of 8-OH-dG in the colorectal tissues from both groups of patients increased with age. As shown in Figure 1, we observed a significant relationship between them, suggesting that oxidative DNA damage in human colorectal tissue is increased during the aging process.

Next, we addressed the relationship between 8-OH-dG levels and cancer. Because free radical formation is known as a causal factor for tumorigenesis, we expected increased 8-OH-dG formation in colorectal tissues of patients with cancer (13). However, unexpectedly, no significant difference between these two groups was observed. The mean levels of 8-OH-dG in the colorectal tissue of patients with cancer and those with polyps were 0.525 \( \pm \) 0.217 and 0.450 \( \pm \) 0.183, respectively. When we compared the 8-OH-dG levels of persons over 60 years of age (0.527 \( \pm \) 0.201) with those of persons under 60 years of age (0.357 \( \pm \) 0.114), the former level was significantly increased in comparison to the latter (\( p = .0016 \)). The positive relationship between 8-OH-dG and age was maintained in a multiple-regression model including potential confounding factors, that is, smoking status and sex (data not shown). In this model (adjusted \( R^2 = .19, p = .003 \)), age was the only significant predictor (\( \beta = .006, p = .003 \)) of 8-OH-dG. Thus, we conclude that the present status of 8-OH-dG in these patients was not directly responsible for the development of cancer in colorectal tissue.

Additionally, we measured the 8-OH-dG levels in tumor and normal tissues of patients with cancer. The 8-OH-dG level was 0.506 \( \pm \) 0.351/10^5 dG in the tumor tissues and 0.536 \( \pm \) 0.195/10^5 dG in the normal tissues. No significant difference between these two groups (\( p = .804 \)) was observed by paired t test.

In conclusion, 8-OH-dG formation is associated with increased age in patients with colorectal cancer and benign polyps.

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Address correspondence to H. Kasai, Department of Environmental Oncology, University of Occupational and Environmental Health, 1-1 Iseigaoka, Yahatanishi-ku, Kitakyushu, 807-8555 Japan. E-mail: h-kasai@med.uoeh-u.ac.jp

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