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Evidence for upregulated repair of oxidatively induced DNA damage in human colorectal cancer

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ABSTRACT

Carcinogenesis may involve overproduction of oxygen-derived species including free radicals, which are capable of damaging DNA and other biomolecules *in vivo*. Increased DNA damage contributes to genetic instability and promote the development of malignancy. We hypothesized that the repair of oxidatively induced DNA base damage may be modulated in colorectal malignant tumors, resulting in lower levels of DNA base lesions than in surrounding pathologically normal tissues. To test this hypothesis, we investigated oxidatively induced DNA damage in cancerous tissues and their surrounding normal tissues of patients with colorectal cancer. The levels of oxidatively induced DNA lesions such as 4,6-diamino-5-formamidopyrimidine, 2,6-diamino-4-hydroxy-5-formamidopyrimidine, 8-hydroxyguanine and (5′*S*)-8,5′-cyclo-2′-deoxyadenosine were measured by gas chromatography/isotope-dilution mass spectrometry and liquid chromatography/isotope-dilution tandem mass spectrometry. We found that the levels of these DNA lesions were significantly lower in cancerous colorectal tissues than those in surrounding non-cancerous tissues. In addition, the level of DNA lesions varied between colon and rectum tissues, being lower in the former than in the latter. The results strongly suggest upregulation of DNA repair in malignant colorectal tumors that may contribute to the resistance to therapeutic agents affecting the disease outcome and patient survival. The type of DNA base lesions identified in this work suggests the upregulation of both base excision and nucleotide excision pathways. Development of DNA repair inhibitors targeting both repair pathways may be considered for selective killing of malignant tumors in colorectal cancer.

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1. Introduction

Colorectal cancer is one of the principal causes of cancer-related deaths worldwide [1]. A genetic model for colorectal carcinogenesis has been developed and associated genetic events have been extensively studied [2]. It is now established that mutations in the genes controlling the cell cycle and/or in DNA repair genes lead to colorectal carcinogenesis. Defects in expression of DNA repair proteins may lead to the therapy resistance and affect overall survival in cancer patients [3,4]. Single-base substitutions such as C → T transversions dominate the majority of mutations found in colorectal cancer [5,6]. Most colorectal cancers are sporadic; however, 10–50% of incidents appear to involve an inherited predisposition [7]. Dietary habits such as high consumption of red meat, fat and alcohol may also play an important role [8]. In the past three decades, numerous studies have investigated the association of consumption of fruits and vegetables with risk of colorectal cancer. The results were often contentious and inconsistent in many cases (e.g., reviewed in [9,10]). Persistent oxidative stress has been shown in colorectal carcinoma patients [11–13]. Chronic inflammation, which is an important tumor promoter [14,15], may also contribute to colorectal carcinogenesis [16]. Oxidative stress and chronic inflammation cause oxidative damage to DNA and other biological molecules via free radicals such as the highly reactive hydroxyl radical (reviewed in [17]). Various DNA repair mechanisms exist in eukaryotes to repair oxidatively induced DNA damage, which is mainly repaired by base excision repair (BER) pathway and, to a lesser extent, by nucleotide excision repair (NER) [18]. A direct link has been discovered between colorectal cancer and mutations in the *myh* gene. The product of this gene, MYH is a DNA

Abbreviations: 8-OH-Gua, 8-hydroxyguanine; 8-OH-dG, 8-hydroxy-2′-deoxyguanosine; *R*-cdA, (5′*R*)-8,5′-cyclo-2′-deoxyadenosine; *S*-cdA, (5′*S*)-8,5′-cyclo-2′-deoxyadenosine; FapyAde, 4,6-diamino-5-formamidopyrimidine; FapyGua, 2,6-diamino-4-hydroxy-5-formamidopyrimidine; OGG1, 8-hydroxyguanine DNA glycosylase; CSB, Cockayne syndrome group B protein; GC/MS, gas chromatography/mass spectrometry; LC–MS/MS, liquid chromatography/tandem mass spectrometry; •OH, hydroxyl radical.

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glycosylase and removes adenine paired with 8-hydroxyguanine (8-OH-Gua) [19]. Mice deficient in *myh* alone or in combination with the *ogg1* gene deficiency have been found to be susceptible to spontaneous and oxidative stress-induced colorectal carcinogenesis [20]. The role of other BER genes in colorectal carcinogenesis is widely unknown. However, the study of BER genes may contribute to understanding of colorectal cancer risk [16]. DNA repair pathways as a whole may be used as targets for cancer therapy [21,22]. Polymorphic forms of human repair enzyme 8-hydroxyguanine DNA glycosylase (OGG1) such as OGG1-Cys^{326} have been found in human populations and several cancers [23–27]. In contrast, the presence of OGG1-Cys^{326} did not associate with the risk of colorectal cancer [28,29].

In the past two decades, numerous studies have shown elevated levels of oxidatively induced DNA base lesions in cancerous tissues when compared with surrounding normal tissues [30–36]. These findings are supported by the fact that persistent oxidative stress exists in cancer [37,38]. Moreover, low OGG1 activity has been shown to be a risk factor in several cancers [39,40]. In general, variations in DNA repair capacity may have an effect on cancer susceptibility, outcomes and treatment of cancer (reviewed in [21,22]). Greater over-expression of *ogg1* mRNA and normal repair activity for the excision of 8-OH-Gua has been observed in a number of lung cancer cell lines compared to control lung cell lines [41]. Similarly, lower levels of ethano-DNA adducts have been found in cancerous colon tissues than in surrounding non-cancerous tissues of colorectal cancer patients [42,43]. These findings agreed with the significantly greater excision activities for these DNA adducts in cancerous tissues than in surrounding non-cancerous tissues [43]. Greater levels of 8-OH-Gua have been found in DNA of leukocytes and in urine of colorectal cancer patients than in healthy individuals, accompanied with increased mRNA levels of DNA repair enzymes including OGG1 and with increased excision rate for 8-OH-Gua [44]. It has been concluded that oxidative stress may stimulate the 8-OH-Gua excision rate; however, this stimulation may not be sufficient to thwart oxidatively induced damage to DNA. Single base substitution mutations in colorectal cancer have been found to be substantially different from those in breast cancer [5,6]. For example, the most common C → T transitions were much more frequent in the former than in the latter. All these findings may reflect differences in DNA repair processes in different types of cancers.

Oxidatively induced DNA lesions in cancerous tissues of colorectal cancer patients have not been investigated in detail. In view of differences in excision rates of DNA lesions and in mutations between cancer types, we hypothesized that repair of oxidatively induced DNA base damage may be increased in colorectal tumors, resulting in lower levels of DNA base lesions than in surrounding normal tissues. To test this hypothesis, we investigated the levels of several DNA bases modifications in tumors of colorectal patients and in non-cancerous tissues surrounding the tumors. We also checked whether tumor location, tumor stage and perineural invasion had an effect on the levels of DNA lesions.

2. Materials and methods

2.1. Materials

Nuclease P1 (from *Penicillium citrinum*) and alkaline phosphatase were purchased from United States Biological (Swampscott, MA) and Roche Applied Science (Indianapolis, IN), respectively. Snake venom phosphodiesterase and acetonitrile (HPLC grade) were from Sigma (St. Louis, MO). Biomax5 ultrafiltration membranes (5 kDa molecular mass cutoff) from Millipore (Bedford, MA) were used to filter hydrolyzed DNA samples. Water (HPLC-grade) for analysis by liquid chromatography/isotope-dilution tandem mass spectrometry (LC–MS/MS) was from Sigma (St. Louis, MO). Water purified through a Milli-Q system (Millipore, Bedford, MA) was used for all other applications. N,O-bis(trimethylsilyl)trifluororacetamide containing 1% trimethylcholorosilane was purchased from Pierce Chemicals (Rockford, IL).

2.2. Patients and control individuals

This study included 55 randomly selected patients with colorectal cancer (median age 57 years; range 32–78 years; 37 men, 18 women) treated surgically in the Division of Colorectal Surgery, Department of General Surgery, School of Medicine, Dokuz Eylul University. The tissue samples were collected over a period of two years. They comprised cancerous tumor tissues removed during the resection and the surrounding histologically normal mucosa. Ethical approval was granted by the School of Medicine's Ethics and Medical Research Committee of Dokuz Eylul University to conduct this study.

2.3. DNA isolation

DNA was isolated from tissues as soon as possible after collection. Colon and rectum tissue samples were minced and rinsed free of blood with 5 mL PBS buffer (pH 7.4). The tissue was then homogenized with 1 mL PBS buffer and then centrifuged at 10,000 g for 15 min. The pellet was suspended in 3 mL of lysis buffer (10 mM Tris HCl, 0.4 M NaCl and 2 mM EDTA, pH 8.2) and then incubated at 37 °C for 60 min. The sample was centrifuged and the pellet was digested with 0.2 mL of 10% SDS and 0.5 mL of protease K solution (1 mg protease K in 1% SDS and 2 mM EDTA) overnight at 37 °C. After digestion, 1 mL of saturated NaCl (6 M) was added. Tubes were shaken vigorously until foam from protein appeared. The sample was then incubated for 10 min at 56 °C followed by centrifugation at 5000 g for 30 min at room temperature. The supernatant fraction containing the DNA was transferred to a 15 mL polypropylene tube. Two volumes of absolute ethanol kept at room temperature were added. After centrifugation and removal of the supernatant fraction, the DNA pellet was washed twice with 70% ethanol and centrifuged. After the removal of ethanol, and air-drying for 1 h, DNA was dissolved in water for 24 h at 4 °C. The UV spectrum of each DNA sample was recorded between 230 nm and 350 nm by an absorption spectrophotometer. The absorbance at 260 nm was used to measure the DNA concentration of each sample (absorbance of 1 = 50 μg of DNA/mL). Aliquots (50 μg) of DNA samples were dried in a SpeedVac under vacuum.

2.4. Analysis of DNA samples

LC–MS/MS was used to identify and quantify (5′*R*)-8,5′cyclo-2′-deoxyadenosine (*R*-cdA) and (5′*S*)-8,5′cyclo-2′-deoxyadenosines (*S*-cdA). Internal standards *R*-cdA-$^{15}N_5$ and *S*-cdA-$^{15}N_5$ were prepared using dATP-$^{15}N_5$ [Medical Isotopes, Inc. (Pelham, NH)] as described [45]. Aliquots (50 μg) of DNA samples were supplemented with aliquots of *R*-cdA-$^{15}N_5$ and *S*-cdA-$^{15}N_5$, and hydrolyzed with nuclease P1, snake venom phosphodiesterase and alkaline phosphatase and then analyzed by LC–MS/MS [46]. 4,6-Diamino-5-formamidopyrimidine (FapyAde), 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyGua) and 8-OH-Gua were identified and quantified using gas chromatography/isotope-dilution mass spectrometry (GC/MS), following hydrolysis of DNA samples with *E. coli* Fpg protein and using FapyAde-^{13}C,$^{15}N_2$, FapyGua-^{13}C,$^{15}N_2$ and 8-OH-Gua-$^{15}N_5$ as internal standards [47].