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Research Paper

Expression of 8-hydroxy-2'-deoxyguanosine in gastric carcinomas☆

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Abstract

Objective: Reactive oxygen species may be involved in the progression of gastric carcinomas. To clarify whether the pathology of gastric carcinoma are related to oxidative DNA damage, the expression of 8-hydroxy-2'-deoxyguanosine (8-OHdG) was examined in 30 patients with gastric carcinomas. **Methods**: The expression of 8-OHdG and apoptosis in the gastric carcinoma were measured using the methods of immunocytochemistry and deoxynucleartididyl transferase-mediated dUTP-biotin nick end labeling (TUNEL), respectively. **Results**: Of the 30 cases, 25(83%) showed stronger immunoreactivity than normal control. The patients with poorly differentiated gastric carcinoma had a larger tumor size and higher labeling indices of TUNEL- and 8-OHdG-positive cells than those with well and moderately differentiated gastric carcinoma. **Conclusion**: Our findings suggest that oxidative DNA damage is increased in association with necroinflammation in chronic gastric injuries and determination of 8-OHdG is useful in assessing high-grade malignancy in gastric carcinomas.

Keywords: 8-hydroxy-2'-deoxyguanosine (8-OHdG); DNA damage; Reactive oxygen species (ROS); carcinoma; apoptosis; stomach

INTRODUCTION

Reactive oxygen species (ROS) are involved in a number of pathological conditions such as inflammation and cancer [1]. ROS have been reported to be involved in chronic gastric diseases and carcinomas[2-3]. The formation of 8-dydroxy-2'-deoxyguanosine (8-OHdG) reflects oxidative DNA damage by oxidative stress, mainly by hydroxyl radicals [4]. This DNA damage causes specific types of mutation and may involve in Helicobacter pylori gastritis. Chronic infection with Helicobacter pylori causes progression of gastric carcinomas [5-6]. Recently, it has been reported that 8-OHdG content in gastric carcinomas is increased as compared to that of normal individuals[2]. These findings are very intriguing as 8-OHdG may be a useful marker for assessing gastric damage. To clarify the association of the pathology with gastric 8-OHdG level in gastric carcinoma, 8-OHdG expression in stomach tissues was detected by immunohistochemical techniques.

MATERIALS AND METHODS

Patients

All specimens were obtained from Gulou hospital of Nanjing city, Jiangsu Province. This study included 30 patients (aged 60.3 ± 11.4 years, 39-72 years, M/F:18/12), 10 with good, 9 with moderate and 11 with poor differentiation. The differentiation, clinical and pathological findings were assessed according to the criteria of the International Union against Cancer. Specimens from tumor site and adjacent non-tumor site were obtained from surgical department and fixed in buffered formaldehyde and processed for histological examination. Adjacent

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non-tumor site was used as normal control.

Immunohistochemistry

De-waxed paraffin sections were stained using avidin-biotin-peroxidase complex(ABC). The sections were incubated with 0.3% H_2O_2 in methanol for 30 min. Then they were heated in 10 mmol/l sodium citrated buffer (pH 6.0) at 100℃ for 15 min. After being washed, they were stained using the anti-8-OHdG antibody (NOF Corp., Tokyo, Japan) diluted 1:100 at 4℃ overnight. Then they were incubated with bio-conjudgated anti-mouse IgG (Vecter Laboratories, Burlingame, CA) for 60 min. Immunoreactive cells were visualized using a Vectastain ABC-HRP (mouse IgG)kit (Vecter Laboratories, Burlingame, CA).

In situ nick labeling

For gastric carcinoma tissues, terminal deoxynucleartididyl transferase-mediated dUTP-biotin nick end labeling (TUNEL) was performed using an Apo Tag plus peroxidase In situ Apoptosis Detection Kit (Intergen, USA) according to the manufacturer's instructions. In brief, paraffin-sections were dewaxed, rehydrated through a graded alcohol series and washed in distilled water. After digestion in 20 ? g/ml of proteinase K for 10-20min at room temperature, the sections were washed in tap water. The sections were treated with 2% H_2O_2/methanol and washed in distilled water. Then the terminal deoxynucleotidyl transferase (TdT) buffer (100 mmol/L potassium cacodylate, 2 mmol/L cobalt chloride, 0.2 mmol/L dithiothreitol, pH7.2) containing 0.3 U/l TdT and 0.04 nmol/l biotinylated dUTP was added to cover the sections, which were incubated in a humidified atmosphere for 90 min at 37 ℃. The sections were washed in TB buffer (300mM sodium chloride, 300mM sodium citrate) for 15 min at room temperature. After being washed with phosphate buffer, they were subsequently incubated with peroxidase-labeled streptavidin-H_2O_2.

Statistical analysis

The 8-OHdG positive staining and TUNEL labeling in 1 mm^2 of tissue sections were counted in five randomly selected high-magnification fields per case, the staining sensitive were expressed as positive cells/mm^2. The data were expressed as mean ± SD. The data were analyzed by student's t-test. $P < 0.05$ was considered statistically significant.

RESULTS

Of the 30 cases, 25 cases showed positive 8-OHdG immunoreactivity and TUNEL-labeling, including 6 with good, 8 with moderate and 11 with poor differentiation. The 8-OHdG-positive cells were observed in the cancerous lesions, and not localized in the intestinal metaplastic glands and normal gastric glands (***Fig. 1***). The patients with poorly differentiated gastric carcinoma had a larger tumor size and higher labeling indices of 8-OHdG-positive cells than those with well and moderately differentiated gastric carcinoma. The number of 8-OHdG-positive cell in poorly differentiated cancers were significantly higher than that in moderately and well differentiated cancer. The number of 8-OHdG-positive cell in moderately differentiated cancer was also higher than that in well differentiated cancer(***Table 1***). The nuber of TUNEL-positive cell was positively correlated with that of 8-OHdG. Many 8-OHdG-positive cells also stained positive for TUNEL labeling (***Fig. 2***). The TUNEL-positive cells in poorly differentiated gastric carcinomas were also increased as compared to those of moderately and well differentiated gastric carcinoma. As the same, TUNEL-positive cells in moderately differentiated gastric carcinomas were increased than those in well differentiated patients. 8-OHdG positivity was not increased in a stage-dependent manner.

Table 1 **8-OHdG expression and apoptosis in gastric cancer tissue**

Groups	Positive staining sensitive (cells/mm^2)	
	8-OHdG	TUNEL
Poorly differentiated (n=11)	1182 ± 102[a,b]	473 ± 102 [a,b]
Moderately differentiated (n=8)	1010 ± 82[c]	219 ± 48 [c]
Well differentiated (n=6)	698 ± 102	114 ± 27

Compared with moderately differentiate[a] $P < 0.05$, compared with well differentiated[b] $P < 0.05$, compared with well differentiated[c] $P < 0.05$

DISCUSSION

Gastric carcinogenesis is a complex, multistep, multifactorial process. Like many malignancies, gastric cancer results from genetic factors of the host and environmental factors. Although just how the biological mechanisms underlying these factors are involved in its development is not fully understood, one of the proposed mechanisms involves oxidative DNA damages caused by reactive oxygen species (ROS). Oxidative DNA damages occur in a cell when the production of ROS exceeds the cell's antioxidant-defense capacity, producing a mutation that in turn can activate oncogenes or inactive tumor suppressor genes and eventually lead to cancer [7-8]. Among the types of oxidative DNA damage, 8-OHdG residue is one of the most abundant oxidative

products of cellular DNA, and is a mutagenic agent causing GC to TA transversions, since 8-OHdG preferentially pairs with adenine instead of cytosine during DNA replication [1,9-10]. An increase in 8-OHdG content in DNA has been shown to elevate cancer risk [11]. Many studies suggest that antioxidants contribute to the prevention of carcinogenesis in various tissues. Vitamin C is the most popular antioxidant currently available. Several epidemiological studies have suggested that diminished dietary Vitamin C may contribute to a high incidence of gastric cancer [12].

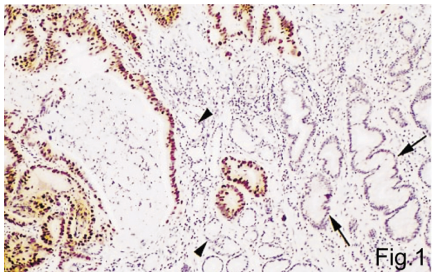

Fig. 1 8-OHdG-positive staining in the gastric carcinoma, note lack of nuclear staining in the intestinal metaplastic gland as well as the normal gastric glands (× 33)

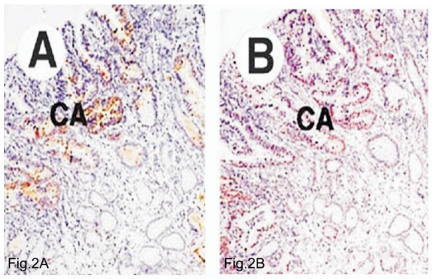

Fig. 2 8-OHdG (A) and TUNEL staining (B) in early stage gastric carcinoma, both 8-OHdG and TUNEL stains were strong in the cancerous lesion (CA,× 100)

In the present study, the results suggested that the number of 8-OHdG positive cell was significantly correlated with gastric carcinoma. Increased level of 8-OHdG in gastric mucosal tissues has been associated with chronic atrophic gastritis, and the level of 8-OHdG in tumor-adjacent and tumor tissues was significantly higher than that in the normal tissue of gastric cancer patients [3]. Exposure to tobacco smoke and Helicobacter pylori infection play a role in ROS generation and oxidative DNA damages, and both have been shown to be positively associated with gastric cancer risk[5,13-14].A large number of epidemiological studies have confirmed the effects of fresh fruits and vegetables containing micronutrients such as vitamin C and carotene that have antioxidative activities, and the consumption of these foods have been shown to be inversely associated with gastric cancer risk[12,15]. These may explain our observation.

Interestingly, many TUNEL positive cells also presented with positive 8-OHdG, suggesting the close relationship between cell apoptosis and hydroxyl radical formation. In the present study, both 8-OHdG and TUNEL-positive cells were increased significantly in poorly differentiated cancer than those in moderately differentiatiated and well differentiated cancer. The number of TUNEL-positive cells was correlated with that of 8-OHdG-positive cells. Indeed, the close association of cell proliferation with apoptosis in cancer cells is well known, and proliferation and apoptosis were reported to be prognostic factors for carcinoma [16]. The formation of 8-OHdG and various DNA strand breaks, as well as accelerated cell proliferation, were apparent which were consistent with previous reports on human gastric mucosa infected with Helicobacter pylori [17]. Hydroxyl radicals, the major cause of 8-OHdG production, stimulate cell proliferation by activating MAPK, where the Fenton reaction plays an important role. On the other hand, the oxidative DNA damages cause apoptosis of fibroblasts [18]. Taken together, oxidative DNA damages may be closely associated with the turnover of cancer cells. Therefore, the measurement of 8-OHdG in gastric carcinoma may be a marker of malignant potential.

In conclusion, the results of the present study suggest that 8-OHdG is related to necroinflammation of gastric carcinoma and is useful in assessing high-grade malignancy in gastric carcinomas.

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