Aberrant methylation of p16 predicts candidates for 5-fluorouracil-based adjuvant therapy in gastric cancer patients

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Background. Aberrant methylation of some cancer-related genes has been reported to correlate with sensitivity to chemotherapeutic agents. The present study was designed to determine whether DNA methylation in six cancer-related genes affects recurrence of gastric cancer in patients who received 5-fluorouracil-based adjuvant chemotherapy.

Methods. The methylation status of six genes, MGMT, CHFR, hMLH1, p16INK4a, E-cadherin, and Runx3, was analyzed in 56 surgically resected gastric cancer tissue specimens by methylation-specific polymerase chain reaction. Of the 56 patients who underwent surgical resection, 38 received 5-fluorouracil (5-FU)-based adjuvant chemotherapy postoperatively (adjuvant group), whereas the other 18 (32%) did not (surgery group).

Results. There were no significant differences between the two groups with respect to sex, cancer differentiation, depth of tumor invasion, lymph node metastasis, lymphatic invasion, vascular invasion and tumor stage. Among the genes, methylation of p16INK4a showed a significant correlation with longer survival in the 38 patients of the adjuvant group, but not in the 18 patients of the surgery group. A multivariate analysis identified p16INK4a methylation to be an independent factor predicting a longer recurrence-free period under 5-FU-based adjuvant chemotherapy.

Conclusions. The present study demonstrated for the first time that gastric cancer patients with p16INK4a methylation specifically benefit from 5-FU-based adjuvant chemotherapy.

Key words: p16INK4a, methylation, 5-FU, adjuvant chemotherapy, advanced gastric cancer

Introduction

Gastric cancer is one of the most common malignancies in the world. Although surgical resection is a major modality to cure this malignancy, adjuvant (postoperative) chemotherapy also plays an important role in achieving a lower cancer recurrence rate. Among several chemotherapeutic agents, 5-fluorouracil (5-FU) has been widely used for adjuvant chemotherapy for gastric cancer. According to a meta-analysis of centrally randomized studies, Japanese groups currently report that adjuvant chemotherapy with oral fluorinated pyrimidine (o-FP) as long-term maintenance therapy appears to be effective in gastric cancer patients after curative resection. Recently, S-1, a modified o-FP prodrug, was developed, and it is now frequently used for chemotherapy for gastric cancer in Japan and Korea. S-1 consists of an oral formation of tegafur (FT), 5-chloro-2, 4-dihydroxypyridine (CDHP), and potassium oxonate. CDHP is an inhibitor of dihydopyrimidine dehydrogenase, which catabolizes 5-FU in the liver and cancer cells. S-1 has been proposed to have a stronger effect than previously used 5-FU prodrugs.

Changes in the DNA methylation status are known to be epigenetic events and the most common molecular alterations in human neoplasia. It has been increasingly recognized over the last decade that the CpG islands in many genes, which are mostly unmethylated in normal tissue, are methylated to varying degrees in human cancers, and thus represent tumor-specific alterations. Hypermethylation of CpG islands is associated with the silencing of the genes. Previous studies have demonstrated that aberrant methylation of tumor-related genes, including tumor-suppressor and DNA-repair genes, is correlated with carcinogenesis and tumor progression in human neoplasia. One study reported that reduced expression of DNA repair genes such as O6-methylguanine-DNA methyltransferase (MGMT) and human Mut L homolog-1 (hMLH1) is caused by...
aberrant CpG methylation, and that its reduced expression is correlated with a poor prognosis in patients with gastric, hepatocellular, and biliary tract carcinomas. In addition, several studies have demonstrated that aberrant methylation of genes related to DNA repair, the cell cycle, and drug metabolism often predicts the response of patients to chemotherapeutic treatment.

Esteller et al. reported that MGMT gene silencing by promoter methylation is significantly correlated with the clinical response of a glioma to alkylating agents. We also demonstrated that methylation of the mitotic checkpoint gene, checkpoint with FHA and RING finger (CHFR), correlates with responsiveness to treatment with microtubule inhibitors in gastric cancer cell lines and patients. Several studies have also indicated that gene methylation is involved in determining sensitivity to 5-FU. For instance, MGMT methylation is significantly correlated with a low risk of recurrence of colorectal cancer in patients receiving o-FP. Conversely, loss of hMLH1 by promoter methylation conferred resistance to various chemotherapeutic agents, including 5-FU, in colorectal cancer cells. In addition, CpG island methylator phenotype, which is defined as simultaneous hypermethylation of cancer-specific CpG loci, is an independent predictor of the survival benefit of 5-FU in colorectal cancer. However, few studies have addressed whether methylation of specific genes is correlated with sensitivity to 5-FU in gastric cancer.

This study analyzed DNA methylation of six cancer-related genes, hMLH1, CHFR, MGMT, p16INK4a (p16), E-cadherin (CDH), and Runx3, in resected gastric cancer tissue specimens from patients who received 5-FU-based chemotherapy following surgical resection. The methylation of these genes was also analyzed in a control group of patients who underwent surgery alone and did not receive chemotherapy. We retrospectively examined in which genes methylation contributed to the duration of the recurrence-free period in the patients receiving chemotherapy. The goal of the study was to identify gene methylation essential for predicting the drug effect of adjuvant chemotherapy with 5-FU.

Materials and methods

Patients and tissue samples

Cancer specimens were obtained from patients with gastric cancer who underwent curative resection in the Department of Surgery at Saga University Hospital (Saga, Japan) from June 2000 through July 2006. The median follow-up period was 32.3 months (range, 6.0 to 78.5 months). Informed consent for the use of the specimens, which was written in a form approved by the Ethics Committee, was obtained from all patients. A total of 56 cancer specimens were obtained and immediately frozen and stored at −80°C until use. The fresh-frozen tissues from gastric cancers were subjected to genomic DNA extraction. The 56 patients comprised 16 (29%) women and 40 (71%) men. Their mean age was 67.3 years (range, 26–86 years). The patients were followed for prognosis and recurrence for more than 6 months (median, 32.3 months; range, 6–78.5 month). Of the 56 patients who underwent the surgical resection, 38 received 5-FU-based adjuvant chemotherapy (adjuvant group) postoperatively, whereas the remaining 18 (32%) did not receive the treatment (surgery group). The 5-FU-based drugs were 5-FU, FT, S-1, and doxifluridine (5′-DFUR). S-1 alone was orally administered to 31 of 38 (81.6%) patients in the adjuvant group. Oral FT treatment was administered to three patients (7.9%), and oral 5′-DFUR was used for one patient (2.6%). 5-FU with cisplatin (CDDP) was intravenously administered to one patient (2.6%). Oral S-1 along with intravenous CDDP was given to one patient (2.6%), and oral S-1 with intravenous paclitaxel treatment was administered to one patient (2.6%). The median duration of drug administration was 4.3 months (range, 1–28 months). Clinical stage, curability of the operation, histological classification, depth of tumor invasion (T), and lymph node metastasis (N) were determined on the basis of criteria of the Japanese Classification of Gastric Carcinoma guidelines. The curative potential of gastric resection was evaluated by surgical and histologic observation as follows: Cur A (no residual disease with a high probability of cure); Cur B (no residual disease but not fulfilling the criteria for Cur A); and Cur C (definite residual disease). In the 38 patients of the adjuvant group, 35 were diagnosed as Cur B and the remaining three as Cur A. The entire surgery group was categorized into Cur B.

DNA extraction and modification

The genomic DNA from tissue samples was isolated using a DNA extraction kit (EZ1 DNA tissue kit, Qiagen, Hilden, Germany). The DNA was then chemically modified by urea/bisulfite treatment according to the method of Paulin et al., in which unmethylated cytosines are converted to uracils. Briefly, 1 μg of genomic DNA was denatured with sodium hydroxide and modified with sodium bisulfite. The samples were then purified with Wizard SV Gel and the PCR Clean-Up System (Promega, Madison, WI, USA), treated with NaOH, and precipitated in ethanol. Finally, each modified DNA pellet was resuspended in 20 μl of TE buffer.