

Mismatch Repair Deficiency in Patients with Double Primary Cancer of the Colorectum and Stomach

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We examined the correlation between the expression of mismatch repair (MMR) gene proteins and the development of double primary cancer, and studied clinical implications of an MMR deficiency in 15 patients with double primary cancer of the colorectum and stomach, immunohistochemically. The results were compared between the double primary cancer group of 15 patients and the control group consisting of 155 colorectal cancer (CRC) patients who had never developed other malignant diseases. Patients with hereditary nonpolyposis colorectal cancer and familial adenomatous polyiposis were excluded from both groups. The MMR deficiency in CRC was significantly more frequently detected in the double primary cancer group than in the control group (46.7% versus 20.6%, $P < 0.05$). Patients with MMR-deficient CRC of the double primary cancer group were significantly older, more frequently had poorly differentiated lesions, had less metastases to the liver and lymph node, and were more advanced in depth of invasion than those of the control group. We concluded that MMR deficiency might correlate with the development of double primary cancer of the colorectum and stomach. Patients with MMR-deficient CRC need periodical and intensive follow-up against the development of double primary cancer.

Key words: colorectum; double primary cancer; mismatch repair deficiency; stomach

Hereditary nonpolyposis colorectal cancer (HNPCC) is one of the most common predisposition syndromes for cancer caused by germ-line mutations in DNA mismatch repair (MMR) genes (Leach et al., 1993; Bronner et al., 1994; Nicolaidis et al., 1994). This syndrome is characterized by development at an early age, frequent occurrence on the right side, histology of poorly differentiated and mucinous adenocarcinomas and a favorable prognosis (Vasen et al., 1991; Kunitomo et al., 1992; Lynch et al., 1993, 1996). Another feature of HNPCC is the development of multiple primary colorectal cancer (CRC) (Horii et al., 1994; Brown

et al., 1998; Masubuchi et al., 1999; Pedroni et al., 1999). Nearly 5% to 10% of CRC patients develop a 2nd primary CRC within 10 years after surgical removal of the 1st tumor (Tsukuma et al., 1994), and genetic instability might play an important role in developing multiple primary CRC: synchronous tumors tend to have more frequent microsatellite instability (MSI) than metachronous ones (Horii et al., 1994). HNPCC is also characterized by frequently occurring extracolonic tumors, especially of the stomach, small intestine, upper urologic tract (renal pelvis and ureter) and endometrium (Vasen et al., 1991; Lynch et al., 1996). Women with endo-

Abbreviations: CRC, colorectal cancer; HNPCC, hereditary nonpolyposis colorectal cancer; MMR, mismatch repair; MSI, microsatellite instability

metrial cancer and CRC who had their 1st tumor diagnosed before age 50 demonstrated a high frequency of MSI (Planck et al., 2002).

Gastric cancer is the most prevalent extra-colonic malignancy in double primary cancer with colon cancer (Duval et al., 2002). Several studies have reported relations between MSI and gastric cancer: PCR analysis of primary gastric cancer detected one or more MSIs associated with an increased occurrence of remnant gastric cancer (Nakachi et al., 1999). According to Kim et al. (2002), *Helicobacter pylori* infection might lead to a deficiency of DNA MMR in gastric epithelial cells, which might increase the risk of mutation accumulation in gastric mucosa cells and the risk of gastric cancer. But reports were few on the contribution of MSI in double primary cancer of the colorectum and stomach.

In the present study, we have immunohistochemically detected an MMR deficiency in double primary cancer of the colorectum and stomach, and analyzed clinical implications of the deficiency in the cancer.

Materials and Methods

Patients and specimens

Specimens were 15 lesions each of CRC and gastric cancer removed from 15 patients with double primary cancer in surgery with curative intent between January 1990 and December 1996 at the First Department of Surgery, Tottori University Hospital. Lesions of HNPCC and familial adenomatous polyposis were excluded. All patients were followed-up until December 2001. CRC was clinicopathological determined according to the criteria of the Japanese Society for Cancer of the Colon and Rectum (1998); and gastric cancer, with the rules of the Japanese Research Society for Gastric Cancer (1999). Double primary cancers of the colorectum and stomach were synchronous in 5 patients and metachronous in 10 patients.

From patients who had undergone surgically curative resection in our hospital during the same

period, a total of 155 CRC patients exclusive of those with HNPCC and familial adenomatous polyposis, served as controls. The patients developed no other malignant diseases before the surgery or during the follow-up period.

After the resection, all specimens were fixed in 10% neutralized formalin and embedded in paraffin. A representative block of each tumor was selected and used to evaluate the expression of hMLH-1 and hMSH-2.

Immunohistochemistry

The paraffin-embedded sections were dewaxed, rehydrated through a graded alcohol series and washed with distilled water. Antigen retrieval was performed by microwave oven (700 W) for 12 min. Endogenous peroxidase was blocked by methanol containing 2% hydrogen peroxide. Tissues were further blocked by incubation for 30 min with 1% bovine serum albumin in phosphate-buffered saline. Sections were incubated overnight at 4°C with one of the primary antibodies, hMLH-1 (PharMingen, San Diego, CA) at a dilution of 1/100 and hMSH-2 (Oncogene Sciences, Cambridge, MA) at a dilution of 1/200. Antibody binding was detected using a Vectastain Elite ABC kit (Vector Laboratories Ltd, Burlingame, CA), based on the biotin-avidin system (manufacturer's protocol). Sections were stained with a solution of diaminobenzidine and hydrogen peroxide solution, and then counterstained with methyl green. Normal colorectal tissue adjacent to the cancer was used as a positive control. Loss of expression in the tumor was recorded when staining was negative in malignant cells, but positive in adjacent normal epithelial cells (Figs. 1 and 2).

Statistical Analysis

The differences of MMR deficiency between groups and the associations between clinicopathological factors and hMLH-1 and/or hMSH-2 expression were evaluated by chi-square test. Survival curves were constructed by Kaplan-Meier's method and the differences were examined by log rank test.

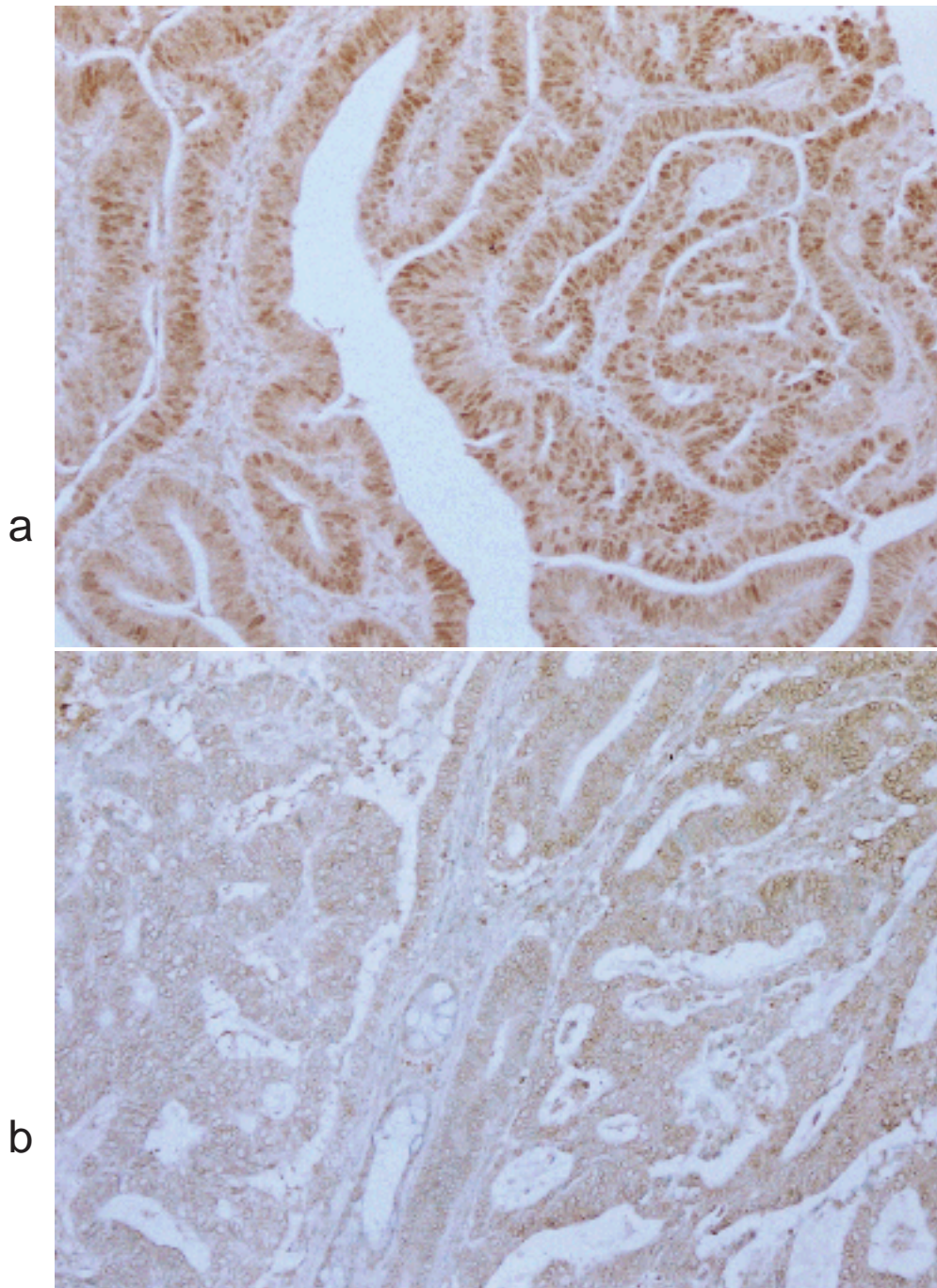


Fig. 1. Immunohistochemical staining of hMLH1 in colorectal cancer (CRC). hMLH1 protein reveals abundant nuclear stainings in CRC cells which are not deficient in mismatch repair (MMR) (a). MMR-deficient CRC cells (b) show negative staining of hMLH1 protein (original magnification, $\times 200$).

P values less than 0.05 were considered statistically significant.

Results

Clinicopathological features of 15 patients with double primary cancer of the colorectum and stomach are shown in Table 1. The double primary