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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; Nicolaides 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 extracolonic 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.

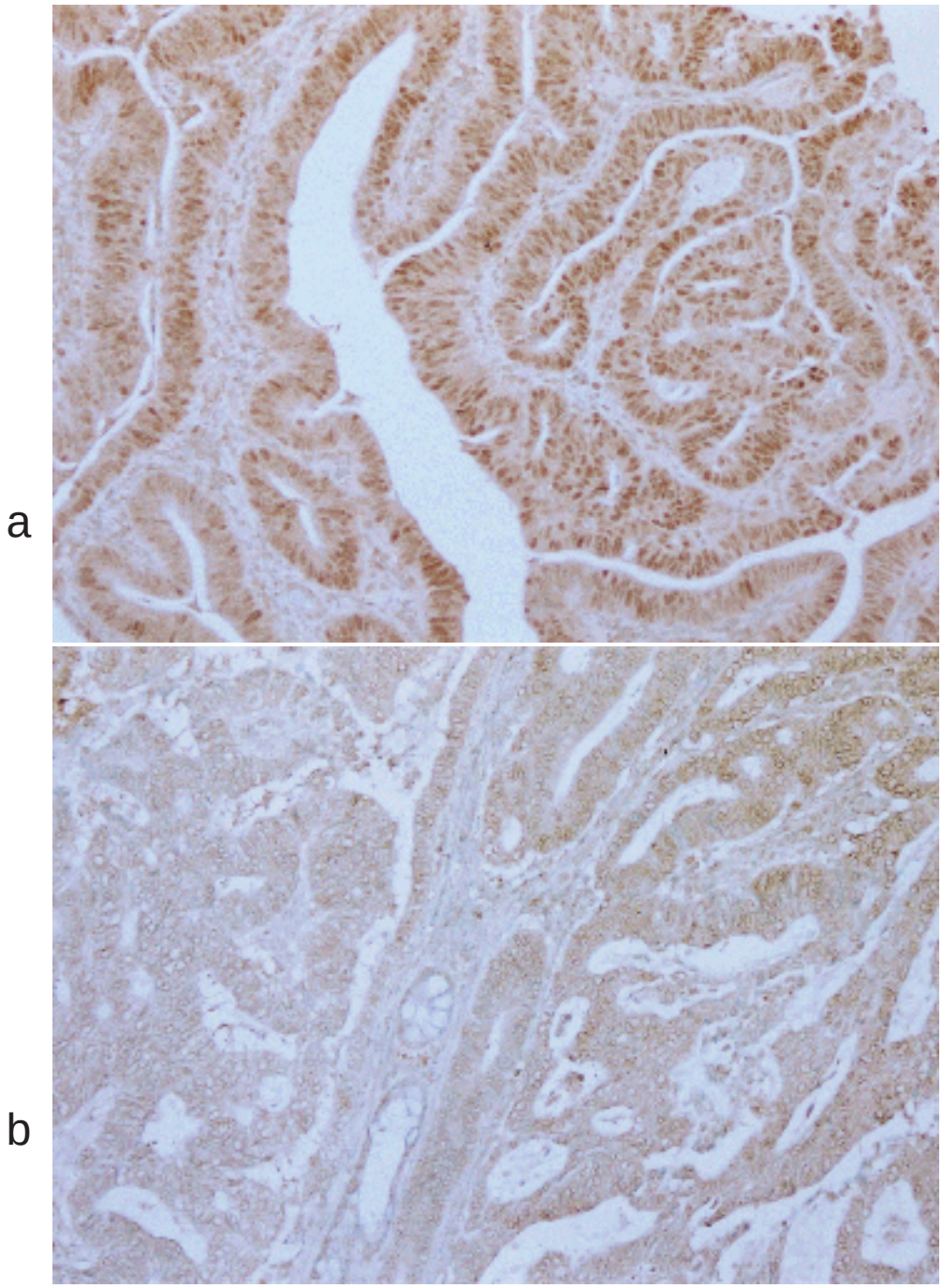

Fig. 1. Immunohistochemical staining of hMLH1 in colorectal cancer (CRC). hMLH1 protein reveals abundant nuclear stainings in CRC cells which are not deficient in mismatch repair (MMR) (**a**). MMR-deficient CRC cells (**b**) show negative staining of hMLH1 protein (original magnification, × 200).

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

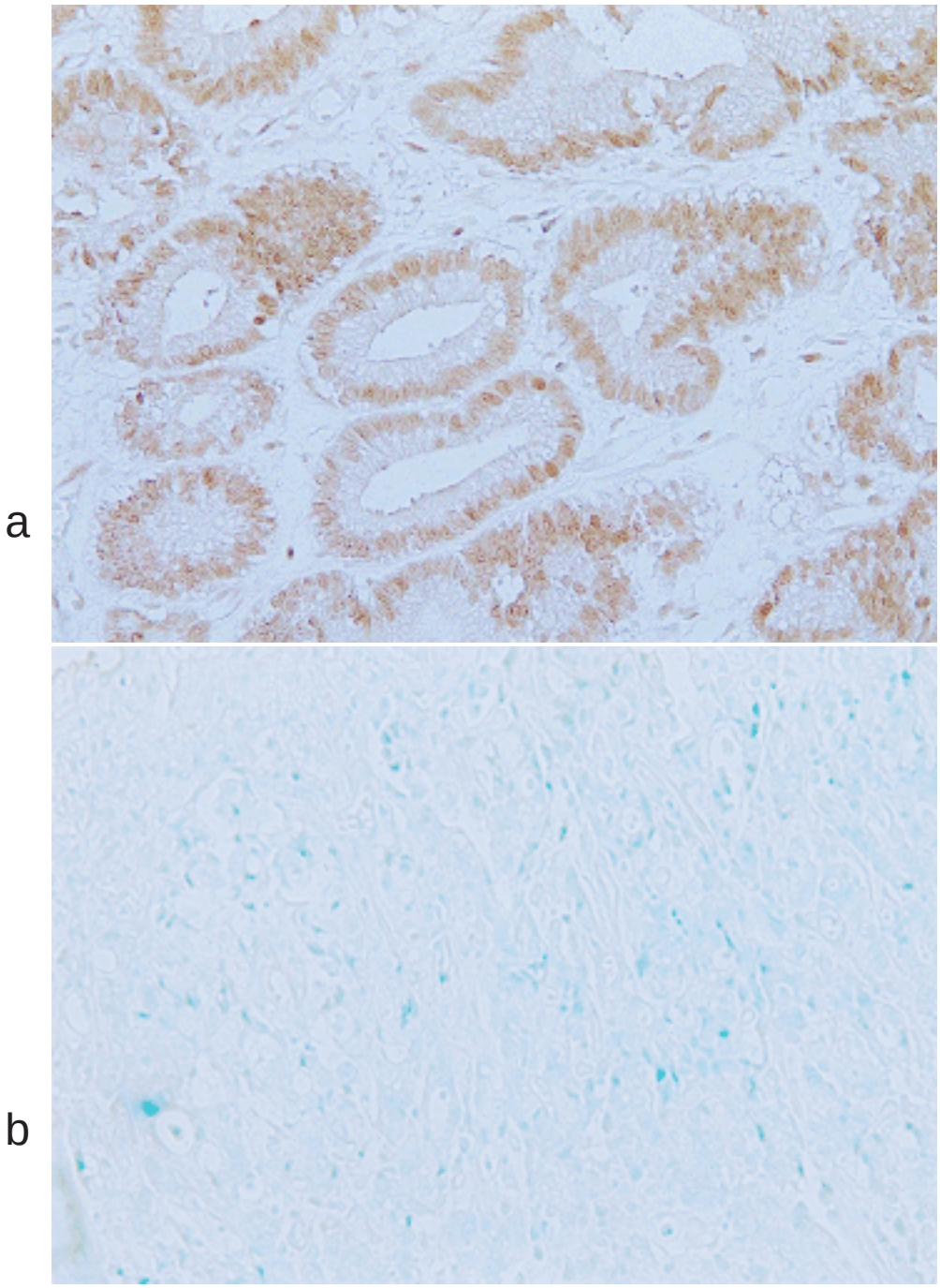

Fig. 2. Immunohistochemical staining of hMLH1 in gastric cancer. hMLH1 protein reveals abundant nuclear stainings in gastric cancer cells which are not deficient in mismatch repair (MMR) (**a**). MMR-deficient gastric cancer cells (**b**) show negative staining of hMLH1 protein (original magnification, × 200).

cancer group consisted of 5 patients with synchronous double primary cancer (median age 75.4 years, ranges 68–88 years) and 10 patients with metachronous double primary cancer. Among the 10 patients, 2 patients developed their 1st cancer in the colorectum at the age of 63 and 69 years, respectively, with the intervals between the 1st and 2nd onsets were 6 and 8 years, respectively. The re-

Table1. Clinicophathological features of 15 patients with double primary cancer of the colorectum and stomach

Patient number	Sex	Colorectal cancer			Gastric cancer						Stage
		Expression of		Age at diagnosis	Expression of		Age at diagnosis	Depth of	Lymph node	Histological differ-	
		MLH1	MSH2	(year)	MLH1	MSH2	(year)	invasion	metastasis	entiation	
1	M	–	+	72	–	+	49	t3	1	por	IIIA
2	M	–	+	70	–	+	49	t1	0	por	IA
3	M	+	+	61	+	+	50	t2	1	pap	II
4	M	+	+	72	+	+	68	t1	0	tub	IA
5†	M	–	+	68	+	+	68	t1	0	pap	IA
6†	M	–	+	88	+	+	88	t1	0	tub	IA
7†	M	+	+	72	+	+	72	t1	0	tub	IA
8	F	–	+	71	+	+	65	t3	1	pap	IIIA
9*	M	+	+	63	+	+	71	t1	0	tub	IA
10†	F	+	+	76	+	+	76	t3	3	muc	IV
11	M	–	+	63	+	+	61	t1	0	tub	IA
12*	M	+	+	69	+	+	75	t2	1	por	IIIA
13	M	+	+	73	+	+	61	t3	2	por	IIIB
14†	M	+	–	73	+	+	73	t1	0	tub	IA
15	F	+	+	58	+	+	47	t2	0	por	IB

t1, invasion within submucosa; t2, invasion from the muscularis propria to submucosa; t3, penetration to serosa; t4, invasion to adjacent organs.
F, female; M, male; muc, mucinous adenocarcinoma; pap, papillary adenocarcinoma; por, poorly differentiated adenocarcinoma; tub, tubular adenocarcinoma.
* Patients who developed colorectal cancer previous to gastric cancer.
† Patients who developed colorectal and gastric cancer synchronously.

maining 8 patients developed their 1st cancer in the stomach at the median age of 56.3 years (ranges 47–65 years), with the median interval between onsets of 11.3 years (ranges 2–23 years).

Table 2 compares the clinicopathological findings of CRCs between the double primary cancer and control groups. The incidence of MMR-deficient CRC was significantly higher ($P < 0.05$) in the double primary cancer group (7/15, 46.5%) than in the control group (32/155, 20.6%). In the double primary cancer group, MMR tended to be deficient in patients who were older, had less liver-and-lymph-node metastases and were advanced in depth of invasion than in patients of the control group.

In both groups, patients with MMR-deficient CRC tended to show localization proximal to the colon (60.0% versus 40.0% and 28.2% versus 17.5%, respectively) and poorly differentiated and mucinous adenocarcinomas in histology (100% versus 38.5% and 45.1% versus 17.0%, respectively); both features are characteristics of HNPCC. Furthermore, MMR-deficient CRCs tended to be less in venous invasion (22.2% versus 66.7% and 5.7% versus 51.0%, respectively) and liver metastasis (0.0% versus 50.0% and 11.1% versus 21.2%, respectively) than non-MMR-deficient CRCs (Table 2).

MMR-deficient gastric cancer was immunohistochemically detected in 2 (13.3%) of the 15 patients of the double primary cancer group. The 2 patients had the same deficient MMR gene protein (MLH1) in both CRC and gastric cancer. Furthermore, the 2 patients had several common clinicopathological features: both were male, their cancers were poorly differentiated in histology and the preceding gastric cancer developed at a younger age.

Table 2. Clinicophathological features and MMR deficiency in the double primary cancer group and control group

		Double cancer group		Control group		*P* value
		MMR deficiency		MMR deficiency		
		Positive		Positive		
		Number/Total	%	Number/Total	%	
Patients		7/15	46.7	32/155	20.6	0.022
Age (year)	60 ≥	7/14	50.0	27/118	22.9	0.028
	60 <	0/1	0.0	5/37	13.5	0.693
Sex	Male	6/12	50.0	18/75	24.0	0.061
	Female	1/3	33.3	14/80	17.5	0.484
Location of tumor	Proximal	3/5	60.0	11/39	28.2	0.151
	Distal	4/10	40.0	21/116	18.1	0.061
Histological type	Poorly diff. and mucinous adenocarcinomas	2/2	100	9/20	45.0	0.136
	Others	5/13	38.5	23/135	17.0	0.060
Depth of invasion	t1	0/2	0.0	1/13	7.7	0.685
	t2, t3, t4	7/13	53.8	31/142	21.8	0.010
Liver metastasis	Positive	0/1	0.0	1/9	11.1	0.725
	Negative	7/14	50.0	31/146	21.2	0.016
Lymph node metastasis	Positive	2/7	28.6	9/45	20.0	0.606
	Negative	5/8	62.5	23/110	20.9	0.008
Venous invasion	Positive	2/9	22.2	6/104	5.7	0.065
	Negative	4/6	66.7	26/51	51.0	0.467
Lymphatic invasion	Positive	3/5	60.0	14/63	22.2	0.060
	Negative	4/10	40.0	18/92	19.6	0.136

t1, invasion within submucosa; t2, invasion from the submucosa to the muscularis propria; t3, penetration to serosa; t4, invasion to adjacent organs.
diff., differentiated; MMR, mismatch repair.

Discussion

Multiple CRC occurs in 5 to 10% of CRC patients (Enker et al., 1978; Rennert et al., 1995), and over 85% of multiple CRC patients show MSI (Horii et al., 1994; Masubuchi et al., 1999). In our study, the incidence of double primary cancer was 8.8% (15/170) in CRC patients, and the incidence of an MMR deficiency in CRC of double primary cancer patients was 46.7% (7/15), consistently with a reported level (Kim et al., 2001). The incidence of multiple CRC and that of double primary cancer were similar in CRC patients; however, the incidence of an MMR deficiency was lower in double primary cancer patients than in multiple CRC patients. These findings indicate that MMR deficiency plays a more important role in carcinogenesis in the colorectum than in other extracolonic sites.

MMR-deficient CRC shows localization in the proximal colon and poor histological differentiation, which are characteristics of HNPCC. As reported, genetic instability might play an important role in developing multiple primary CRC (Horii et al., 1994); however, our data suggested that patients with MMR-deficient CRC may also have a risk of developing double primary cancer. Furthermore, MMR-deficient CRCs were more advanced in depth of invasion but less frequent in venous invasion and liver metastasis than non-MMR-deficient CRCs. This tendency was more marked in the double primary cancer group than in the control group. Tumors with an MMR deficiency might affect some factors and suppress spreading cancer cells.

MMR-deficient tumors reduce the expression of COX-2 protein (Karnes et al., 1998) and suppress the expression of vascular endothelialgrowth factor. This factor is very potent in tumor angiogenesis and plays an important role in the migration and growth of vascular endothelial cells, the promotion of vascular permeability and the formation of vascular canals (Connolly et al., 1989; Leung et al., 1989). These reports could explain the few vessel invasions in tumors with an MMR deficiency. Also, in another of our studies (in submission), we reported that MMR-deficient tumors tended to express β_2-microglobulin protein at a lower level than non-MMR-deficient tumors, and that MMR-deficient tumors might evoke an immune response. This also may be a reason for the favorable prognosis of patients with MMR-deficient CRC.

Of the 15 gastric cancer patients of the double primary cancer group, 2 patients (13.3%) had an MMR deficiency, and their deficient MMR gene protein (MLH1) was the same in CRC and gastric cancer, similarly as reported (Kim et al., 2001). An MMR deficiency could relate to carcinogenesis of double primary cancer in the colorectum and stomach, but the incidence of an MMR deficiency in gastric cancer was lower than that in CRC. Thus, an MMR deficiency seems to affect the development of gastric cancer to some extent, which is not so marked as in development of CRC.

In the present study, we observed that an MMR deficiency of CRC was more frequent in the double primary cancer group than in the controls. Patients with MMR-deficient CRC or gastric cancer need periodical and intensive follow-up focusing not only on metachronous multiple CRC but also on double primary cancer.

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