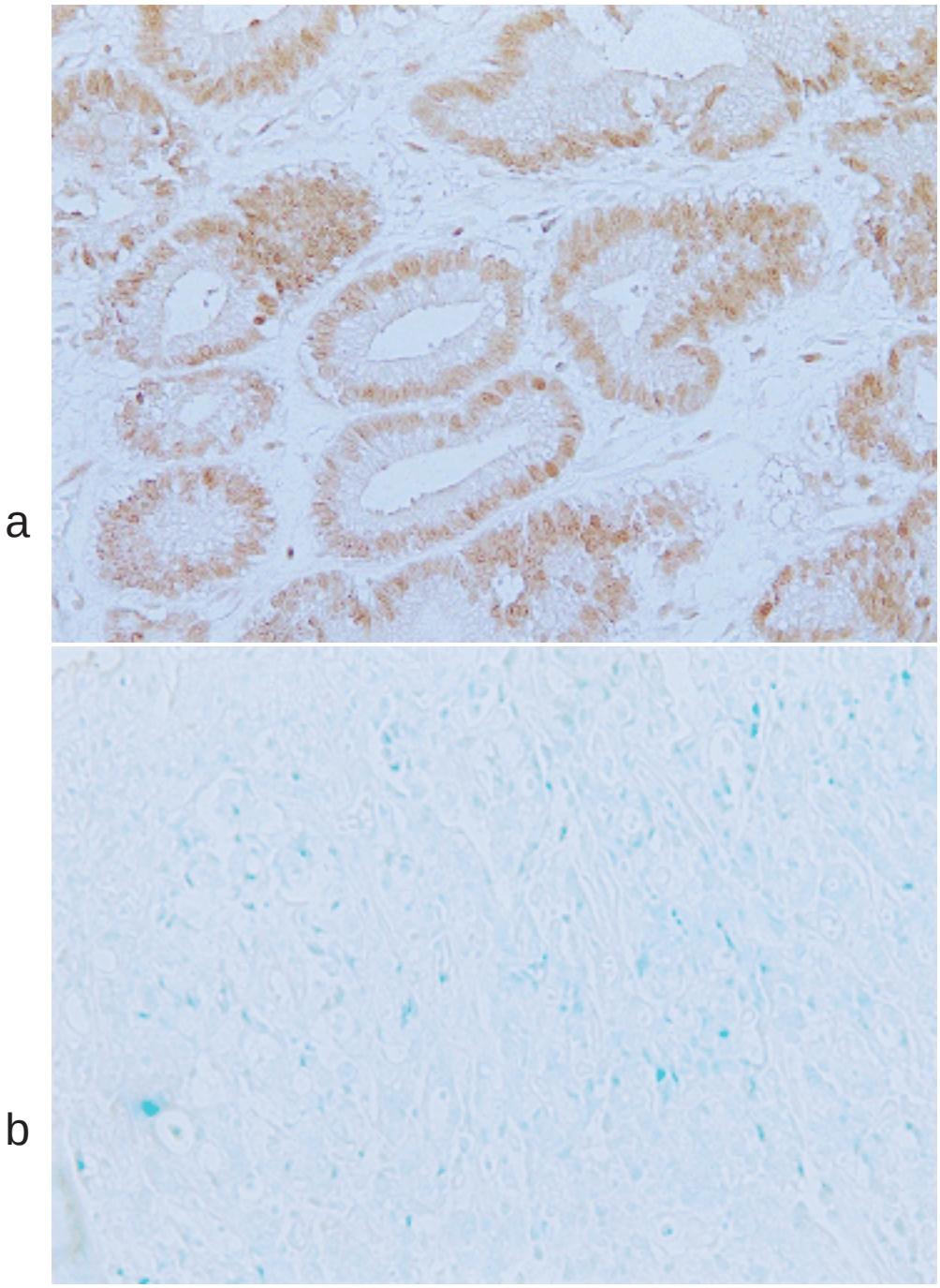

Fig. 2. Immunohistochemical staining of hMLH1 in gastric cancer. hMLH1 protein reveals abundant nuclear stainings in gastric cancer cells which are not deficient in mismatch repair (MMR) (**a**). MMR-deficient gastric cancer cells (**b**) show negative staining of hMLH1 protein (original magnification,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

1 Bronner CE, Baker SM, Morrison PT, Warren G, Smith LG, Lescoe MK, et al. Mutation in the DNA mismatch repair gene homologue hMLH1 is associated with hereditary non-polyposis colon cancer. Nature 1994;368:258–261.

2 Brown SR, Finan PJ, Hall NR, Bishop DT. Incidence of DNA replication errors in patients with multiple primary cancers. Dis Colon Rectum 1998; 41:765–769.

3 Connolly DT, Heuvelman DM, Nelson R, Olander JV, Eppley BL, Delfino JJ, et al. Tumor vascular permeability factor stimulates endothelial cell growth and angiogenesis. J Clin Invest 1989;84: 1470–1478.

4 Duval A, Reperant M, Compoint A, Seruca R, Ranzani NG, Iacopetta B, et al. Target gene mutation profile differs between gastrointestinal and endometrial tumors with mismatch repair deficiency. Cancer Res 2002;62:1609–1612.

5 Enker WE, Dragacevic S. Multiple carcinomas of large bowel: a natural experiment in etiology and pathogenesis. Ann Surg 1978;187:8–11.

6 Horii A, Han HJ, Shimada M, Yanagisawa A, Kato Y, Ohta H, et al. Frequent replication errors at microsatellite loci in tumors of patients with multiple primary cancers. Cancer Res 1994;54:3373–3375.

7 Japanese Research Society for Gastric Cancer. The general rules for the gastric cancer study. Tokyo: Kanehara; 1999.

8 Japanese Society for Cancer of the Colon and Rectum. General rules for clinical and pathological studies on cancer of the colon, rectum, and anus. Tokyo: Kanehara; 1998.

9 Karnes WE, Shattuck-Brandt R, Burgart LJ, Dubois RN, Tester DJ, Cunningham JM, et al. Reduced COX-2 protein in colorectal cancer with defective mismatch repair. Cancer Res 1998;58:5473–5477.

10 Kim HS, Cho NB, Yoo JH, Shin KH, Park JG, Kim Y, et al. Microsatellite instability in double primary cancers of the colorectum and stomach. Mod Pathol 2001;14:543–548.

11 Kim JJ, Tao H, Carloni E, Leung WK, Graham DY, Sequlveda AR. Helicobacter pylori impairs DNA mismatch repair in gastric epithelial cells. Gastro-

enterology 2002;123:542–553.
12 Kunitomo K, Terashima Y, Sasaki K. HNPCC in Japan. Anticancer Res 1992;12:1856–1857.
13 Leach FS, Nicolaides NC, Papadopoulos N, Liu B, Jen J, Parsons R, et al. Mutations of a mutS homolog in hereditary nonpolyposis colorectal cancer. Cell 1993;75:1215–1225.
14 Leung DW, Cachianes G, Huang WJ, Goeddel DV, Ferrara N. Vascular endothelial growth factor is a secreted angiogenenic mitogen. Science 1989;246: 1306–1309.
15 Lynch HT, Smyrk T. Hereditary nonpolyposis colorectal cancer (Lynch syndrome) [An updated review]. Cancer 1996;78:1149–1167.
16 Lynch HT, Smyrk TC, Watson P, Lanspa SJ, Lynch JF, Lynch PM, et al. Genetics, natural history, tumor spectrum, and pathology of hereditary nonpolyposis colorectal cancer. Gastroenterology 1993;104: 1535–1549.
17 Masubuchi S, Konishi F, Togashi K, Okamoto T, Senda S, Shitoh K, et al. The significance of microsatellite instability in predicting the development of metachronous multiple colorectal carcinomas in patients with nonfamilial colorectal carcinoma. Cancer 1999;85:1917–1924.
18 Nakachi A, Miyazato H, Shimoji H, Hiroyasu S, Isa T, Shiraishi M, et al. Microsatellite instability in patients with gastric remnant cancer. Gastric Cancer 1999;2:210–214.
19 Nicolaides NC, Papadopoulos N, Lie B, Wei VF, Carter KC, Ruben SM, et al. Mutations of two PMS homologues in hereditary nonpolyposis colon cancer. Nature 1994;371:75–80.
20 Pedroni M, Tamassia MG, Percesepe A, Roncucci L, Benatti P, Lanza GJ, et al. Microsatellite instability in multiple colorectal tumors. Int J Cancer 1999;81: 1–5.
21 Planck M, Rambeck E, Moslein G, Muller W, Olsson H, Nilbert M. High frequency of microsatellite instability and loss of mismatch-repair protein expression in patients with double primary tumors of the endometrium and colorectum. Cancer 2002;94: 2502–2510.
22 Rennert G, Robinson E, Rennert HS, Neugut AI. Clinical characteristics of metachronous colorectal tumors. Int J Cancer 1995;60:743–747.
23 Tsukuma H, Fujimoto I, Hanai A, Hiyama T, Kitagawa T, Kinoshita N. Incidence of second primary cancers in Osaka residents, Japan, with special reference to cumulative and relative risks. Jpn J Cancer Res 1994;85:339–345.
24 Vasen HF, Mecklin JP, Khan PM, Lynch HT. The international collaborative group on hereditary nonpolyposis colorectal cancer (ICG-HNPCC). Colon Rectum 1991;34:424–425.

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