Table 2 Patient characteristics

	D1	D2	Total
No. of patients	76	86	162
Median age (range)	64 (34-81)	61.46 (25-80)	62.01 (29-81)
≥70	22	18	40
Sex (m/f)	39/37	48/38	87/75
Location			
Upper	9	11	20
Medium	18	22	40
Distal	49	52	101
Diffuse	-	1	1
Tumour stage			
pT1	27	27	54
pT2	23	28	51
pT3	26	31	57
Type of resection			
Total gastrectomy	19	22	41
Distal gastrectomy	57	64	121
Splenectomy (S)	3	9	12
Distal pancr. + S	1	3	4

tumour. The mean number of nodes removed was 27.0 during a D1 gastrectomy and 36.6 during a D2.

Post-operative course

Table 3 gives data on post-operative course. Overall, the post-operative hospital morbidity was 13.6%. The rate was higher in the D2 group (16.3%) than in D1 group (10.5%), but this difference was not statistically significant. In both groups there were more complications after total than after distal gastrectomy, but again this difference was not significant.

As regards major abdominal infections, no anastomotic dehiscence occurred and only one case of duodenal stump leakage was registered, while two pancreatic leakages and two cases of acute pancreatitis were observed.

Reoperation was necessary after five major surgical complications (Table 3). The overall hospitality mortality was 1/163. This death occurred after a D1 gastrectomy (1/76) and was due to an intraoperative stroke; obviously no significant difference could be observed between D1 and D2 group as concerns mortality.

Post-operative hospital stay

The data on hospital stay excluded the early death (intraoperative), and consequently were based upon 161 patients. The median time of hospital stay was 12 days for D1 groups (mean 13.75, range 8-78) and 12 days for D2 group (mean 13.15, range 8-27). The effect of splenectomy on duration of hospital stay was not clear: patients having received splenectomy stayed in hospital half-a-day more (12.5 days, mean 13.49, range 9-17) than patients without splenectomy 12 days, (mean 12.87, range 8-78, see Table 4).

Discussion

Despite its recent decline, gastric cancer is still a common lethal disease in western countries. For apparently resectable cancers, surgery offers the best loco regional control; but unfortunately, average 5-year survival rates for treated patients remain low in the western world, ranging from 15 to 30%.[11,13] Over the years, Japanese surgeons have performed radical procedures involving extended lymphadenectomy, and have reported impressive survival figures with extremely low morbidity and mortality.[1,2,14] Two recent European randomised trials, however, failed to demonstrate a significant

Table 3 Post-operative complications and mortality

	D1 (76)	D2 (86)	Global (162)
Non-surgical complications	4 (5.26%) Cardiac 2 Pulmonary 2	7 (8.13%) Pulmonary 4 Pleural 3	11 (6.79%)
Surgical complications	4 (5.26%) Pancreatic leakage 1 Intraperit. haemorrhage 2 Colonic perforation 1[a]	7 (8.13%) Pancreatic leakage 1 Intraperit. haemorrhage 1[a] Duodenal leakage 1[a] Acute pancreatitis 2[a] Abdominal abscess 2	11 (6.79%)
Total morbidity	8 (10.52%)	14 (16.27%)	22 (13.58%)
Mortality	1 (1.31%)		1 (0.61%)

[a] Requiring reoperation.

Table 4 Lengths of hospital stay

	D1	D2	S0	S +
Days median (range)	12 (8-78)	12 (8-27)	12 (8-78)	12.5 (9-17)
Days mean	13.75	13.15	12.87	13.49

S0, splenectomy not performed; S +, splenectomy performed.

survival benefit of radical D2 gastrectomy over standard D1 resection.[5,6] The benefit of D2 gastrectomy's potential for reducing loco regional recurrence may be nullified by the significant increase of post-operative morbidity and mortality. These unfavourable results have been attributed to many factors, including the lack of technical experience of surgeons dealing with extended gastrectomy, the large number of elderly patients presenting with associated vascular and cardio respiratory diseases, the large number of centres involved in randomised trials with consequent low quality control, and particularly the distal pancreatico-splenectomy routinely performed during total gastrectomy in the D2 arms of randomised trials. Subset analysis of the MRC and Dutch randomised trials has recently indicated that the poorer outcomes in D2 resections are largely due to pancreas and spleen removal.[7,15]

We performed a previous prospective multicentre phase 1-2 study on feasibility and safety of D2 gastrectomy with pancreas preserving technique, involving only a few surgeons. In this study, distal pancreatico-splenectomy was not performed unless the pancreas was suspected of being involved by the tumour. We observed that, when performed in specialized centres, with a strict quality control system, by experienced surgeons, D2 gastrectomy with pancreas preservation could be safe in Western countries. Our morbidity and mortality rates were not only absolutely comparable to those observed after standard resections but also very close to those shown by Japanese surgeons.[8]

Compared to the patients in the Dutch and British trials our patients were younger, and had a higher proportion of early and distal cancers, and these factors may help to partially explain the striking difference between our morbidity and mortality results and those in these trials.

Having reached a good standard of experience in D2 procedures, we planned a new trial, randomising patient to either D1 or D2 gastrectomy.

To maintain a homogenous level of acquired technical experience in D2 procedures, only surgeons already involved in our previous study were allowed to participate in this new trial; this should avoid bias associated with new surgeons who have not yet completed their learning curve. After careful review of the safety results obtained in the first trial, four out of the nine surgical teams did not join this new randomised trial because completion of their learning curve could not be proven (see above).

These preliminary data seem to confirm our previous reports. Overall morbidity is around 14%; although this figure is a slight underestimate due to the fact that the majority of centres have registered in their database major and minor non-surgical but only major surgical complications, it is very low, and comparable to the best results shown by Japanese authors.[14] The overall morbidity is higher in D2 gastrectomy, but the difference between the two groups of patients is not statistically significant. Moreover, the rate of complications after D2 gastrectomy (16.35%) is considerably better than the rates of both arms (D1 and D2) in the English and Dutch trials.[5,6]

The ASA grade is a fairly crude and subjective measure of patient fitness, and it is not possible to make realistic comparisons of comorbid pathology and organ functional reserve between our patients and those in the Dutch and British trials. We cannot exclude the possibility that difference between these populations contributed to the difference in morbidity and mortality results. In support of our belief that proper surgical training and quality control played the leading part in our low morbidity, we observed very few 'technical' complications requiring re-operation, such as anastomotic leakage (seen in only one duodenal stump leak).

The importance of pancreatic complications after extended gastric surgery, was confirmed by our data. Although the pancreas was not removed routinely during D2 total gastrectomies, three out of the seven complications registered after a D2 procedure were related to the pancreas (two acute pancreatitis and one pancreatic leakage), and two of these required a reoperation.

Overall mortality was very low, at 0.6%. This rate is comparable to those shown by eastern authors in series from experienced centres, and is strikingly different from the rates of both arms reported in MRC and Dutch trials. Our study was powered to detect a difference in 5 year survival between D1 and D2 surgery: detecting a morbidity or mortality difference would require a larger number of

patients, and it is therefore, possible that a small difference exists. Our preliminary results are sufficient to indicate that any such difference is likely to be too small to be clinically important.

These preliminary results confirm that the radical technique of extended lymph node removal can be performed in Western centres without an increase in post-operative morbidity and mortality, if some conditions are respected. First, surgeons involved in these procedures should have completed their learning curve under strict quality control, possibly by a Japanese instructor; second, this procedure should be performed only in selected patients, suitable for extended surgery and with a potentially curable cancer; third, a policy of removing the spleen only when oncologically necessary, with preservation of the tail of the pancreas is associated with low morbidity and mortality, and routine pancreatico-splenectomy is absolutely to be avoided during total gastrectomy.

We found that after an adequate learning period, D2 gastrectomy can offer morbidity and mortality results comparable to those reported in Japanese series.

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Extensive but Hemiallelic Methylation of the h*MLH1* Promoter Region in Early-Onset Sporadic Colon Cancers With Microsatellite Instability

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Background & Aims: Methylation of the h*MLH1* promoter region is frequently observed in microsatellite instability (MSI)–positive sporadic colorectal carcinomas. We studied h*MLH1* promoter methylation in peripheral blood lymphocytes of 87 index patients representing 29 cases of hereditary nonpolyposis colorectal cancers (HNPCCs), 28 cases of atypical HNPCCs, and 30 sporadic cases of the development of early-onset colorectal carcinomas or multiple primary cancers. *Methods:* Methylation of the h*MLH1* promoter region was analyzed by Na-bisulfite polymerase chain reaction/single-strand conformation polymorphism analysis or methylation-specific polymerase chain reaction. MSI, allelic status of the h*MLH1* locus, and loss of hMLH1 protein expression were examined in cases for which tumor tissues were available. *Results:* Extensive methylation of the h*MLH1* promoter was detected in peripheral blood lymphocytes of 4 of 30 patients with sporadic early-onset colon cancer, among whom multiple primary cancers (1 colon and 1 endometrial cancer) developed in 2 cases. This methylation was not detected in analyses of HNPCC or atypical HNPCC groups or healthy control subjects. MSI was positive, and extensive methylation was detected in both cancers (colon and endometrial cancer) and normal tissues (colon, gastric mucosa, endometrium, and bone marrow) in all of the examined cases (3 of 3). Analysis of a polymorphic site in the h*MLH1* promoter in 2 informative cases showed that methylation was hemiallelic. In 1 case, the unmethylated allele was lost in the colon cancer but not in the metachronous endometrial cancer. *Conclusions:* Constitutive, hemiallelic methylation of the h*MLH1* promoter region was shown to be associated with carcinogenesis in sporadic, early-onset MSI-positive colon cancers.

Hereditary nonpolyposis colorectal cancer (HNPCC) is an autosomal dominantly inherited syndrome predisposing to cancers of the colorectum, endometrium, ovary, small intestine, and upper urinary tract.[1] The majority (85%–95%) of HNPCC tumors show microsatellite instability (MSI), which leads to the accumulation of deletion and insertion mutations at simple repeated sequences. In HNPCC, MSI is caused by germline mutations of mismatch repair genes (*MMR* genes) such as h*MSH2*, h*MLH1*, h*PMS1*, h*PMS2*, and h*MSH6*.[2–7] Among these *MMR* genes, mutations of h*MSH2* and h*MLH1* are known to be responsible for up to 45%–64% of HNPCCs.[8,9] HNPCCs are characterized phenotypically by early-onset colorectal carcinoma (CRC), prevalent tumor location in the proximal colon, and an increased risk of developing multiple CRCs and other malignancies.[10–13] On the other hand, some (10%–15%) sporadic CRCs also show MSI,[14–16] and methylation of the h*MLH1* promoter region has been suggested to be the major mechanism in these cases.[17–19] Methylation of the h*MLH1* promoter region and subsequent transcriptional silencing have been demonstrated in the formation of MSI-positive cancers.[17–21] In a previous study, methylation of the h*MLH1* promoter region induced transcriptional silencing of both of the h*MLH1* alleles in cell lines showing MSI[22] and this epigenetic mechanism of gene inactivation is in line with Knudson's two-hit hypothesis.[23] The proximal region of the h*MLH1* promoter contains cis-elements important for regulating gene expression.[24] Methylation of an adjacent CpG site inhibits binding of the core binding

Abbreviations used in this paper: BiPS, Na-bisulfite treatment and PCR single-strand conformation polymorphism; CRC, colorectal carcinoma; HNPCC, hereditary nonpolyposis colorectal cancer; LOH, loss of heterozygosity; *MMR* gene, mismatch repair gene; MSI, microsatellite instability; MSI-H, high-frequency MSI; MSP, methylation-specific PCR; PBL, peripheral blood lymphocyte; PCR, polymerase chain reaction; RT-PCR, reverse-transcription PCR; SSCP, single-strand conformation polymorphism.

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factor to the CCAAT box in this region and is one of the causes of h*MLH1* gene silencing in colon cancer cells.[25] We reported that extensive methylation (designated as full methylation) of the h*MLH1* promoter region played a crucial role in h*MLH1* gene inactivation,[26] and that full methylation occurred in both alleles of the h*MLH1* promoter region in high-frequency MSI (MSI-H) colon cancers.[27] In one third of the CRCs showing full methylation, methylation was also detected in the adjacent normal colonic mucosa, although it was confined to the most upstream region of the h*MLH1* promoter sequences (designated as partial methylation).[27] Sporadic MSI-positive CRCs show different clinicopathological characteristics from those of HNPCC in that they are preferentially associated with late-onset proximal colon cancer in female patients,[26,28] suggesting that changes of hormonal status might be related to the development of the h*MLH1* promoter methylation. Recently, Gazzoli et al.[29] examined 14 cases of suspected HNPCC with MSI-H but no detectable germline mutations of h*MSH2*, h*MLH1*, and h*MSH6* for hypermethylation of the h*MLH1* promoter region, and they reported a case in which 1 allele of h*MLH1* was methylated in DNA isolated from blood, and biallelic inactivation of the h*MLH1* gene in the tumor was caused by a loss of heterozygosity (LOH) of the other allele. They suggested that this was a novel mode of germline inactivation of a cancer susceptibility gene.

In this study we analyzed the methylation status of the h*MLH1* promoter region in peripheral blood lymphocytes (PBLs) of patients referred to genetic counseling clinics because of the suspicion of an HNPCC. We detected constitutive methylation of the h*MLH1* promoter region in 4 cases of early-onset sporadic MSI-H CRCs. They displayed hemiallelic but full methylation of the h*MLH1* promoter region in normal tissues such as PBLs, normal colonic mucosa, endometrium, gastric mucosa, and bone marrow, exhibiting distinctly different clinical characteristics from both cases of HNPCC and cases of sporadic MSI-H CRC.

Materials and Methods

Patients

The study protocol was carried out after receiving institutional review board approval and written informed consent for the study from 87 index patients. PBLs were obtained from the 87 index patients, who visited genetic counseling clinics because of suspicion of HNPCC. All of these patients developed CRCs, and 29 of them fulfilled 1 of the 2 HNPCC criteria, i.e., the Amsterdam's minimum criteria or the modified Amsterdam criteria.[30–32] Twenty-eight kindred were classified as having atypical HNPCC, because they had at least 1 first-degree relative with CRC but did not fulfill the above-mentioned criteria. Of the kindred with atypical HNPCC, 13 kindred fulfilled the second (B-2) and/or fourth (B-4) criteria of the Bethesda guidelines,[33] i.e., individuals with 2 HNPCC-related cancers, including synchronous and metachronous CRCs or associated extracolonic cancers (5 cases) (B-2), individuals with CRC or endometrial cancer diagnosed at age younger than 45 years (6 cases) (B-4), and 2 cases fulfilled both of these 2 criteria (B-2 + B-4). Thirty kindred fulfilled neither the criteria for HNPCC nor atypical HNPCC. They developed early-onset CRCs when younger than the age of 50 years or multiple CRCs and/or extracolonic cancers, without showing familial predisposition to HNPCC-related tumors in their first-degree relatives. As to the relation with the Bethesda guidelines, the number of cases fulfilling the second or fourth criteria of the Bethesda guidelines was 4 (B-2), 20 (B-4), and 2 (B-2 + B-4), respectively. Regarding case H403, a case of sporadic CRC showing constitutive methylation of the h*MLH1* promoter region, the proband's sister visited the clinic for genetic counseling, and her PBLs were examined for methylation. The methylation status of the h*MLH1* promoter region was also examined in PBLs from 100 normal healthy control subjects older than 50 years undergoing routine health checkups. Before the analysis, samples were made unlinkable as to their personal information.

Analysis of MSI

In 4 cases showing aberrant methylation of the h*MLH1* promoter region, the MSI status was examined in all available samples, including tumor tissues and normal tissues such as PBLs, colonic mucosa, gastric mucosa, endometrium, and bone marrow aspirate. Genomic DNAs were subjected to polymerase chain reaction (PCR) amplification at 9 microsatellite repeat loci (*D2S123, D5S346, D17S250, BAT26, BAT25, MSH3, MSH6, TGFBR2,* and *BAX*). Analysis of MSI was performed as described previously.[26] The definition of MSI status was as follows: high-frequency MSI (MSI-H), when 30% or greater of the 9 markers showed MSI, in accordance with the recommendation of the National Cancer Institute Workshop.[34]

Methylation Analysis of the h*MLH1* Promoter Region

Na-bisulfite PCR/single-strand conformation polymorphism (SSCP) (BiPS) analysis was performed as described previously[26,35] (Figure 1). With the adenine residue at the start codon numbered as +1nt, the h*MLH1* promoter (−755 to +86) was divided into 5 regions (region A [from −755 to −574, containing 23 CpG sites], B [from −597 to −393, 12 CpG sites], C [from −420 to −188, 16 CpG sites], D [from −286 to −53, 13 CpG sites], and E [from −73 to +86, 13 CpG sites]) and was amplified with 5 sets of PCR primers. Each primer set was designed to anneal to both methylated and unmethylated DNAs, of which the amplicons could be separated by SSCP analysis. Amplified DNA fragments were visualized by using SYBR Gold nucleic acid gel stain (Cosmo Bio

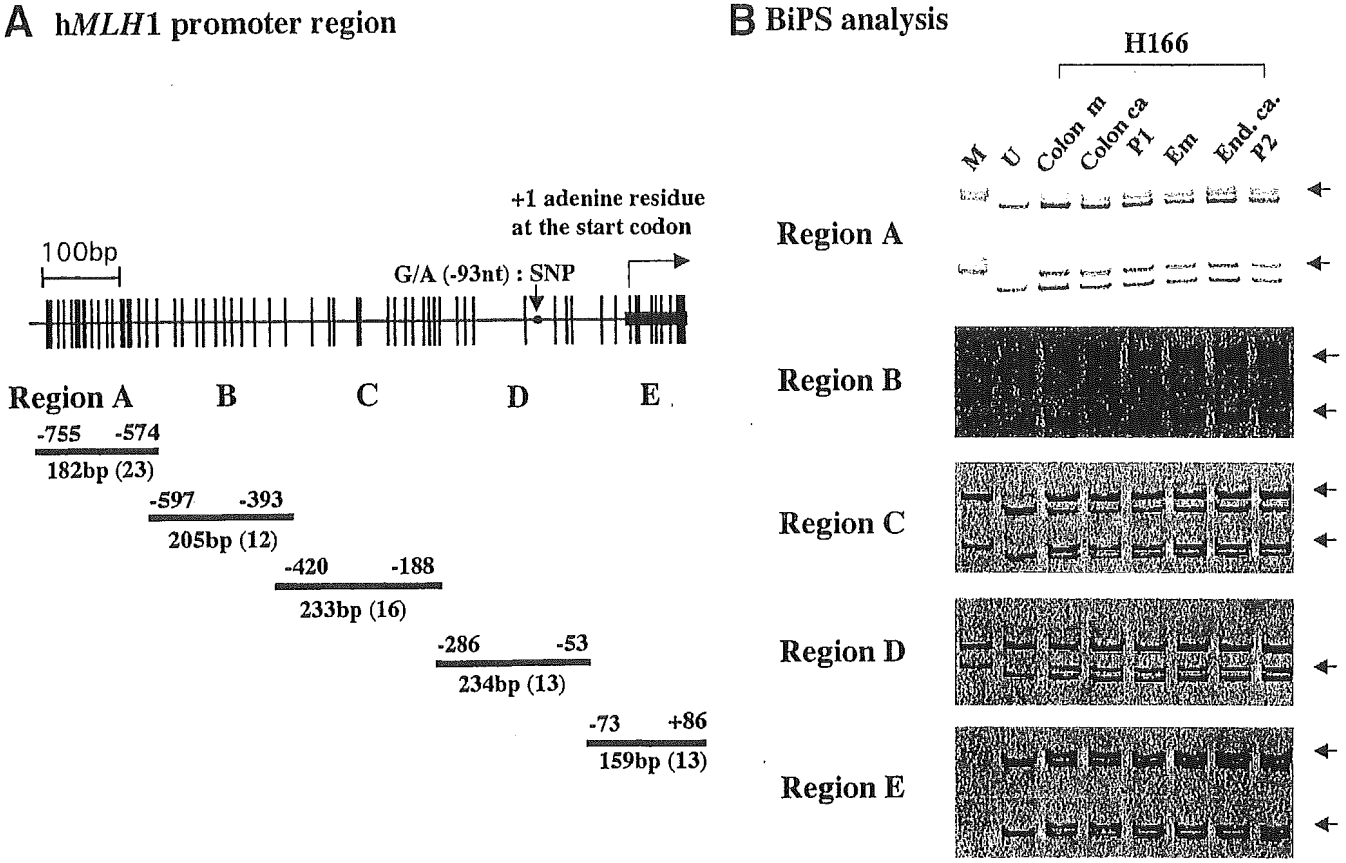

Figure 1. BiPS analysis of h*MLH1* promoter region and methylation profiles of various tissues in case H166. (*A*) Map of the 5′ CpG islands of the h*MLH1* gene. CpG sites are indicated by vertical lines. The arrow indicates G/A polymorphism at position −93nt in the h*MLH1* promoter region. The expected PCR products for regions A, B, C, D, and E are shown. Their positions relative to the adenine residue at the start codon and the sizes of the amplified DNA fragments are indicated. Figures in the parentheses indicate the numbers of CpG sites in each region. (*B*) Na-bisulfite treatment and PCR-SSCP (BiPS) analysis of the h*MLH1* promoter region in each tissue of case H166 (M, control methylated DNA; U, control unmethylated DNA; Colon m, colon normal mucosa; Colon ca, colon cancer; P1, PBLs obtained at 34 years of age (diagnosed with colon cancer); Em, endometrium; Eca, endometrial cancer; P2, PBLs obtained at 44 years of age (diagnosed with endometrial cancer). We divided the h*MLH1* promoter into 5 regions (regions A–E) and examined the methylation status. DNAs from all samples in case H166 showed methylated bands in all regions, indicating full methylation of the h*MLH1* promoter region, which was confirmed by direct sequencing of the mutated bands (data not shown).

Co., Tokyo, Japan) and scanned with a Fluorescent Image Analyzer Model FLA-3000G (Fuji Photo Film Co., Tokyo, Japan). When the bands showed mobility shifts, they were cut from the gel, reamplified, and directly sequenced without subcloning by using an ABI 310 PRISM sequencer (Perkin-Elmer Co., Branchburg, NJ) with a Big-Dye Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems, Foster City, CA). Full methylation was defined as the state in which all CpG sites from regions A through E were methylated.[26,27] The allelic status of methylation was examined by direct sequencing of the G/A polymorphic site at −93nt in region D.[36] Furthermore, the methylation status of the h*MLH1* promoter region D was also analyzed by methylation-specific PCR (MSP) as described previously[27] (Figure 2*B* and *C*). The PCR product was mixed with 5× loading buffer, electrophoresed on a nondenaturing 8% polyacrylamide gel, stained with ethidium bromide, and scanned with a Fluorescent Image Analyzer Model FLA-3000G. DNA fragments amplified by MSP were subjected to direct sequencing, and G/A polymorphism was examined to determine whether the methylation was a biallelic or hemiallelic event.

Mutation Analysis of the h*MSH2* and h*MLH1* Genes

Total RNA was extracted from the PBLs treated with puromycin by using the acid guanidine phenol chloroform method.[37] Long reverse-transcription (RT)–PCR was carried out from RNAs extracted from PBLs incubated in the presence of puromycin, according to the method we reported previously.[38,39] Signals from mutated alleles are enhanced after puromycin treatment as a result of the suppression of nonsense-mediated mRNA decay and easily distinguishable from signals from the wild-type allele. This approach is a sensitive method to screen deleterious mutations such as nonsense or frameshift mutations and large genomic disorganizations resulting in genomic deletion or partial duplication of the h*MLH1* gene.[39] Sequencing reactions were performed by using a Big-Dye Terminator Cycle Sequencing Reaction kit. Elec-

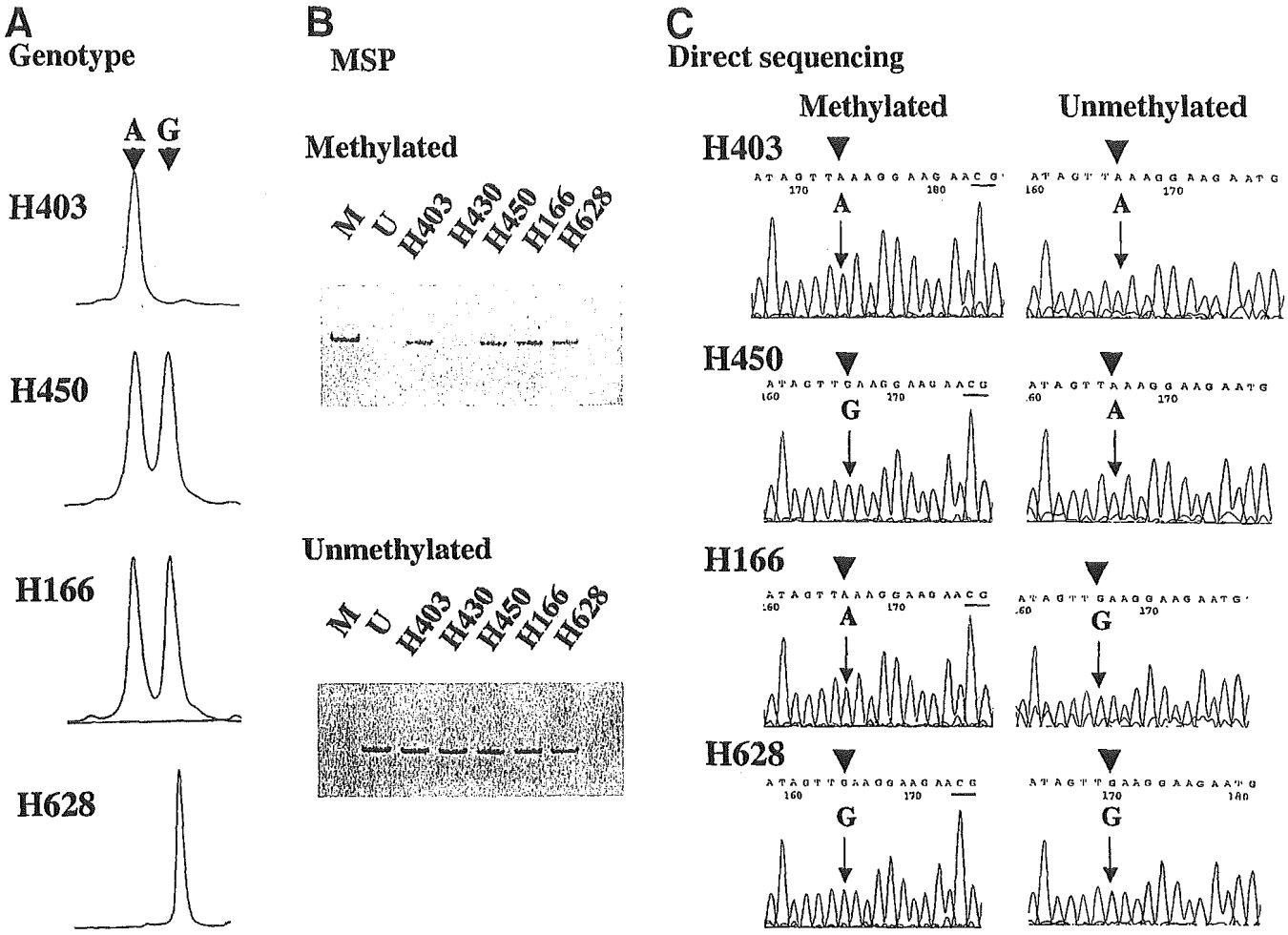

Figure 2. Uniparental methylation of the h*MLH1* promoter region. (*A*) PCR/SSCP analysis of the SNP at position −93nt was used to determine the genotype of 4 cases, i.e., A/A for H403, A/G for H450 and H166, and G/G for H628. (*B*) MSP analysis of the h*MLH1* promoter region D. M, control methylated DNA; U, control unmethylated normal DNA. DNA derived from H403, H450, H166, and H628 showed a methylated band in the promoter region D. DNA derived from H430 (unaffected sister of H403) did not show a methylated band. In addition, DNA derived from all cases showed an unmethylated band in the same region. (*C*) Direct sequencing of the PCR products derived from the methylated and unmethylated fragments in MSP analysis. The arrow indicates G/A polymorphism at position −93nt in the h*MLH1* promoter region. One allele (allele G in H450, allele A in H166) was observed to be a methylated fragment, and the other allele (allele A in H450, allele G in H166) was observed to be an unmethylated fragment.

trophoresis was carried out by using an ABI 310 PRISM sequencer. Primers used for direct sequencing were described in a previous report.[38] All mutations detected by direct sequencing were confirmed by PCR-based sequencing of the corresponding region of genomic DNA.

Analysis of Allelic Loss of h*MLH1*

Analysis of LOH of h*MLH1* was performed as described previously[27,40] (Figure 3). Briefly, an ALFexpress DNA sequencer (Pharmacia, Tokyo, Japan) was used for SSCP analysis. Electrophoresis was performed at 20W for 1500 minutes with a 15% polyacrylamide gel. During electrophoresis, the gel was kept at a constant temperature of 16°C by using a circulating water bath. The data were analyzed by using the software package Fragment Manager (Pharmacia, Tokyo, Japan). LOH was defined when the peak height of the signal from either allele was decreased more than 50% as compared with that of the normal control.

Immunohistochemical Examination of h*MLH1*

Immunohistochemistry was performed as described previously[26] (Figure 4). Briefly, tissue sections were deparaffinized with xylene and dehydrated by using a graded series of ethanol. Antigen retrieval was performed in citrate buffer by using a heat-induced microwave oven. The avidin-biotin–conjugated immunoperoxidase technique was performed by using a DAKO LSAB2 Kit (DAKO, Carpinteria, CA). Endogenous peroxidase activity was blocked by methanol supplemented with 0.02% H_2O_2. Sections were immersed in 4% commercial nonfat skim milk powder to inhibit nonspecific antibody binding. The sections were then incubated overnight

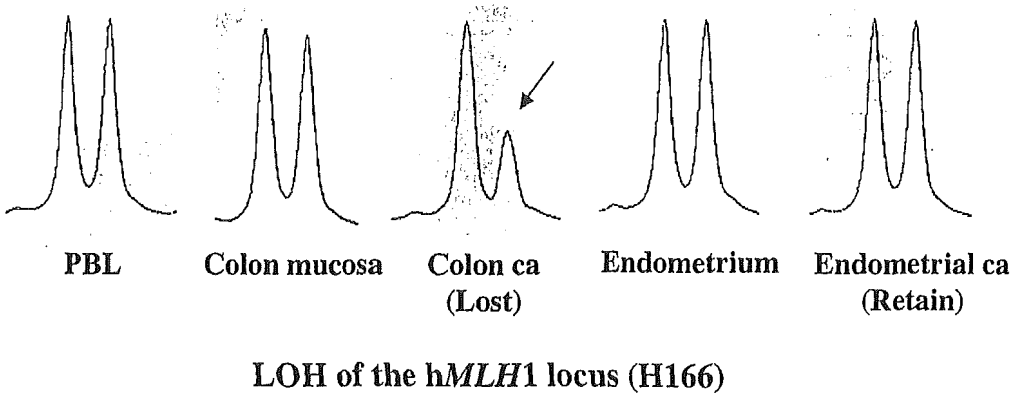

Figure 3. Electropherograms of SSCP analysis showing allelic loss of h*MLH1* in colon and endometrial tissues of case H166. Allelic loss was detected only in the colon cancer, and the position of the lost allele is indicated by an *arrow*.

with mouse monoclonal antibody to the h*MLH1* gene product (clone G168-15; PharMingen, San Diego, CA) (at a 1:50 dilution) and then with biotinylated secondary antibody and peroxidase-labeled avidin-biotin complex for 30 minutes, and staining was visualized by incubating the sections with 0.02% H_2O_2 and 0.02% diaminobenzidine in methanol for 10 minutes.

Results

Characteristics of Four Cases With Extensive Methylation of h*MLH1* Promoter Region in PBLs

Analysis of PBLs from 87 index patients in whom HNPCC was suspected revealed extensive methylation of the h*MLH1* promoter region in 4 cases (H166, H403, H450, and H628), whose characteristics are shown in Table 1. They were characterized by early-onset colon cancer and absence of family history of CRC in their first-degree relatives. Case H166 developed ascending colon cancer and endometrial cancer at the ages of 38 and 44 years, respectively, and PBL samples taken after the onset of each cancer showed extensive methylation of the h*MLH1* promoter region. Case H628 developed descending colon cancer at 29 years of age and had a history of left colectomy as a result of descending colon cancer at 17 years of age.

We examined MSI and methylation status of the h*MLH1* promoter region in colon cancer (H403, H166, and H628), endometrial cancer (H166) tissues, and in their normal counterparts (Figure 1*B*, Table 1). All of the

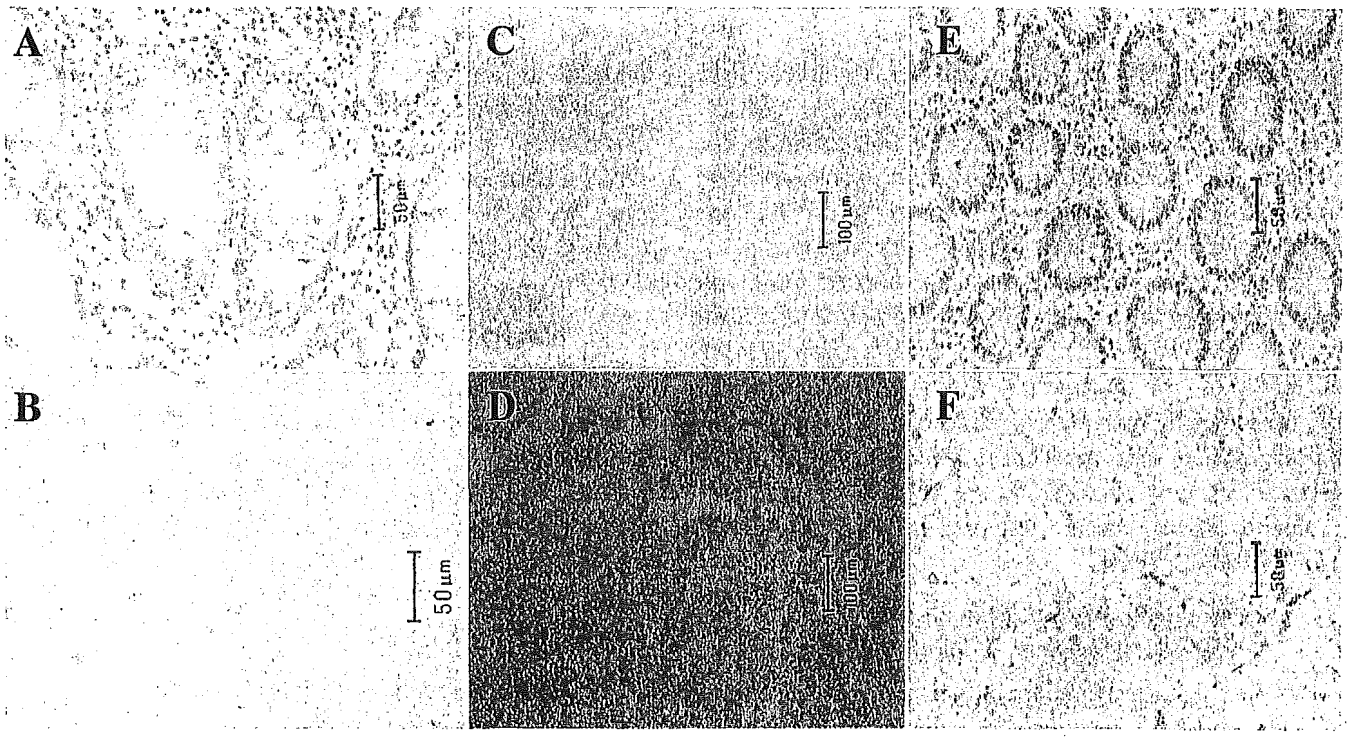

Figure 4. Immunohistochemical staining for h*MLH1* expression in colon tissues of case H166 (*A*, *B*) and H628 (*E*, *F*) and endometrial tissues of case H166 (*C*, *D*). Positive nuclear staining was observed in normal colonic mucosa (*A*, *E*) and endometrium (*C*), whereas a lack of positive nuclear staining was observed in carcinomas of the colon (*B*, *F*) and endometrium (*D*).

Table 1. Characteristics of Patients With Extensive Methylation of h*MLH1* Promoter Region in Lymphocyte Cells

				Family history						h*MLH1*			h*MSH2*
Case	Age[a]	Sex	Site	CRC[b]	Other cancer	Genotype[c]	Specimen		MSI	Methylation	Mutation[d]	IHC	mutation
H166	38	F	A	–	—	A/G	PBL	38 yr	MSS	Full	–		–
							Colon mucosa		MSS	Full		+	
							Colon cancer		MSI-H	Full		–	
							PBL	44 yr	MSS	Full	–		–
							Endometrium		MSS	Full		+	
							Endometrial		MSI-H	Full		–	
							cancer		MSS	Full			
							Colon mucosa	45 yr (biopsy)	MSS	Full			
							Gastric mucosa		MSS	Full			
							Bone marrow						
H403	28	M	T	–	Gastric cancer (grandfather)	A/A			MSS	Full	–		–
							PBL		MSS	Full		N.D.	
							Colon mucosa		MSI-H	Full		N.D.	
							Colon cancer						
H450	23	F	A	–	Pancreas cancer (grandmother)	A/G	PBL		MSS	Full	–		–
H628	29	M	D (17 yr)	–	Gastric cancer (grandfather)	G/G			MSS	Full	–		–
			A (29 yr)				PBL		MSS	N.D.		+	
					Breast cancer (aunt)		Colon mucosa		MSI-H	N.D.		–	
							Colon cancer	(biopsy)	MSS	Full			
					Breast cancer (aunt)		Colon mucosa	(biopsy)	MSS	Full			
							Gastric mucosa						

IHC, immunohistochemical analysis; A, ascending colon; MSS, MSI-stable; T, transverse colon; N.D., not done; D, descending colon; PBL, peripheral blood lymphocyte; MSI-H, high-frequency MSI; +, positive staining; –, negative staining.
[a]CRC onset age.
[b]No family history of CRC.
[c]h*MLH1* promoter genotype (−93 nt from translation start site).
[d]Mutation negative.

tumors showed MSI-H, and extensive methylation of the h*MLH1* promoter region was demonstrated in both tumors and normal mucosa. In cases H166 and H628, the patients underwent further examinations postoperatively such as digestive endoscopy (H166 and H628) and bone marrow aspiration (H166) for persistent leukopenia.

In both cases, methylation of the h*MLH1* promoter region was shown to be constitutive and hemiallelic in all samples examined. PBLs of case H403's sister (H430) did not show the methylation (Figure 2*B*). The PBLs of the other family members were not available. No germline mutations were detected in the h*MLH1* or h*MSH2* genes of these 4 patients. Methylation of the h*MLH1* promoter region was not detected in the PBLs of 100 healthy blood donors.

Hemiallelic Methylation of h*MLH1* Promoter Region in Normal Tissues

We previously reported that methylation of the h*MLH1* promoter region was a biallelic event in MSI-positive CRCs.[27] To determine whether methylation of the h*MLH1* promoter region in PBL is a biallelic epigenetic event, we examined the methylation status of this region by using G/A polymorphism at position −93nt in the h*MLH1* promoter by use of MSP combined with DNA sequencing (Figures 1 and 2*A*). In the 2 informative cases, we could confirm that methylation was hemiallelic (allele G in H450, allele A in H166) in all specimens.

Immunohistochemical Assessment of hMLH1 Protein Expression

To determine whether h*MLH1* gene inactivation was caused by extensive methylation of the h*MLH1* promoter region, we investigated hMLH1 protein expression in colon (cases H166 and H628) and endometrial (case H166) tissues by immunohistochemistry (Figure 4). hMLH1 protein expression was not detected in colon or endometrial cancer, but it was detected in normal colonic mucosa and endometrium.

Cause of Lack of hMLH1 Protein Expression in Cancer Tissues

To determine how the hemiallelic methylation of the h*MLH1* promoter region induced silencing of

hMLH1 protein expression in cancer tissues, we investigated the LOH of h*MLH1* in case H166 (Figure 3). Analysis of the colon cancer showed somatic loss of the G allele at the h*MLH1* locus, and biallelic inactivation of the h*MLH1* gene was caused by extensive methylation of allele A, followed by loss of the opposite allele. However, analysis of the endometrial cancer did not show LOH, and thus we could not identify the cause of the reduced expression of hMLH1 protein in endometrial cancer.

Discussion

In the present study we examined the methylation status of the h*MLH1* promoter region in 87 index patients in whom HNPCC was suspected. The 87 index cases included 30 cases that were sporadic but had developed early-onset CRCs or multiple primary cancers. We identified 4 of 30 sporadic cases with extensive methylation of the h*MLH1* promoter region in PBLs. They all developed CRCs at a very young age (the age at onset for a first cancer varied from 17 through 38 years of age), and there were no HNPCC-related cancers in their first-degree relatives. Analysis of 2 cases heterozygous for a G/A polymorphism at position −93nt showed that the methylation was hemiallelic (Figure 2*C*). These findings were in accord with those of a case reported by Gazzoli et al.[29] Those authors reported hypermethylation of the h*MLH1* promoter region in 1 allele in the DNA from PBLs of a CRC patient with young age (25 years) at onset and without family history of CRC. We examined the methylation status of the h*MLH1* promoter region in DNAs from various tissues, including normal mucosa of the colon, stomach, and endometrium and bone marrow, and the methylation was invariably detected in all tissues examined. Methylation occurred as a constitutive, hemiallelic event. All of these 4 cases were early-onset, and they were also sporadic without family history of HNPCC-related tumors in their first-degree relatives. PBLs of case H403's sister (H430) did not show the methylation (Figure 2*B*). The PBLs of the other family members were not available. Constitutive methylation of the h*MLH1* promoter region was not detected in analyses of HNPCC or atypical HNPCC groups or healthy control subjects. Taken together, these findings suggest that hemiallelic methylation was not heritable, and that it was inconsistent with the mode of autosomal dominant mendelian inheritance, although aberrant methylation might be due to other unknown genetic mechanisms.

In MSI-H CRCs, methylation of the h*MLH1* promoter region has been reported to be extensive, usually occurring in both alleles of the h*MLH1* promoter, and strong association has been observed between the methylation profile of the h*MLH1* promoter region and the clinicopathologic background of the cases, i.e., preferential occurrence in the proximal colon, female predominance, and older age at onset.[26,28] The 4 cases studied here showed different characteristics from ordinary MSI-H tumors in that the methylation was a constitutive but hemiallelic event, preferentially observed in early-onset CRC and without gender specificity (2 male and 2 female patients). The frequency of constitutive methylation of the h*MLH1* promoter region was 13.3% (4 of 30 cases) in the cases of sporadic CRCs we examined, suggesting that hemiallelic methylation of the h*MLH1* promoter region accounts for a subset of early-onset sporadic CRCs with MSI-H. Liu et al.[41] identified 1 case of germline mutation in early-onset CRC showing MSI, but the previously reported rates of detection of mutations in the *MMR* genes in early-onset CRCs were low.[42–44] A study of 31 patients younger than 35 years of age and not fulfilling the Amsterdam minimum criteria, in which MSI was exhibited in 18 cases (58%), was also reported.[45] Twelve of those cases were evaluated for alterations of *MMR* genes, and 5 (42%) were found to harbor germline mutations of either h*MSH2* or h*MLH1*. Germline mutations of *MMR* genes might account for a part of early-onset CRCs, and some of them are suspected to be de novo mutations.

In our analysis of 30 sporadic cases, we detected 3 cases of germline mutations of the *MMR* genes (data not shown), whereas no germline mutations of h*MSH2* or h*MLH1* were detected in analyses of the 4 patients described here. Genomic disorganizations such as large deletions or duplications of the *MMR* genes have been thought to occur in a considerable proportion of HNPCC cases.[46,47] Previously, we reported 2 cases of genomic deletion and 1 case of partial duplication of the h*MLH1* gene that were detected by using long RT-PCR from puromycin-treated samples, and this method is sensitive enough to screen large genomic disorganizations of the *MMR* genes.[39] Recently, several genes were reported to be involved in familial predisposition to CRC.[48–50] In the case of h*MSH6*, many of the mutation carriers develop carcinomas of the distal colon and endometrium, and analysis of tumor tissues showed that half of them were MSI-negative.[48] As for MYH, the mutation carriers showed autosomal recessive inheritance, whereas their phenotypes were characterized by the presence of multiple colorectal adenomas.[49,50] The clinical characteristics of our cases seem to be incompatible with mutations of these 2 genes.

In case H166, biallelic inactivation of the h*MLH1* gene in colon cancer was caused by an LOH of the

unmethylated allele (Figure 3). Gazzoli et al.[29] reported that biallelic inactivation resulted in loss of hMLH1 protein expression in the tumor and suggested a novel mode of germline inactivation of a cancer susceptibility gene. These results were inconsistent with our previous study showing that allelic loss of the h*MLH*1 locus was infrequent, and methylation was biallelic in the majority of the ordinary MSI-H sporadic CRCs.[27] All of the 4 cases examined here were postoperative, and it remains unclear when the methylation of the h*MLH* gene occurred.

In case H166, the patient developed ascending colon cancer at the age of 38 years and endometrial cancer at the age of 44 years. In case H628, the patient developed descending colon cancer at the age of 17 years and ascending colon cancer at the age of 29 years (Table 1). In retrospective analysis, MSI-positive sporadic CRC patients have been reported to be at risk for developing extracolonic cancers and metachronous multiple CRCs.[51–54] Full methylation of the h*MLH*1 promoter region in PBLs might have a significant influence on the carcinogenesis of these multiple primary cancers and might be a potent diagnostic marker for identifying individuals at high risk of developing cancer.

In conclusion, we have tentatively identified a rare group of patients who have the MSI-H phenotype, show early-onset colon cancers without a family history of CRC, and exhibit extensive but hemiallelic methylation of the h*MLH*1 promoter region in PBLs and other normal tissues.

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Original Paper

Clinical and Pathological Prognostic Indicators with Colorectal Mucinous Carcinomas

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KEY WORDS:
Colorectal neoplasms; Adenocarcinoma; Mucinous; Signet-ring cell; Prognosis

ABBREVIATIONS:
Colorectal Mucinous Carcinomas (CMC); Carcinoembryonic Antigen (CEA); Signet-Ring Cell Carcinomas (SRCC)

ABSTRACT

Background/Aims: Colorectal mucinous carcinomas are considered to have a worse prognosis than typical adenocarcinomas. To evaluate the prognostic relevance of a series of clinical and pathological variables, patients with colorectal mucinous carcinomas were studied retrospectively.

Methodology: Ninety-eight patients who underwent surgery for colorectal mucinous carcinomas were included in this study. We firstly examined whether signet-ring cell carcinomas exhibited worse prognosis than the other mucinous carcinomas. Prognostic factors were then analyzed by both univariate and multivariate analysis for 70 patients who underwent complete resection.

Results: The overall five-year survival rate was 44%. Amount of signet-ring cells was a non-significant indicator of poor prognosis. For the cases whose cancers were completely resected, four parameters (liver metastasis, lymph node involvement, vessel involvement, spread beyond the bowel wall) were significantly related to prognosis on univariate analysis. With the multivariate analysis, liver metastasis and spread beyond the bowel wall were independent variables.

Conclusions: This study reaffirmed the importance of liver metastasis and spread beyond the bowel wall for prediction of prognosis with colorectal mucinous carcinomas for cases who undergo complete resection. In addition, the presence of signet-ring cells is a non-significant indicator of a poor prognosis.

INTRODUCTION

Primary colorectal mucinous carcinomas (CMC) including signet-ring cell carcinomas (SRCC) are generally thought to exhibit a more aggressive clinical course and to have a less favorable prognosis as compared with typical colorectal adenocarcinomas (1-6). However, there are CMC patients who survive for long periods without recurrence.

Therefore, prediction of prognosis is important for deciding whether adjuvant therapy should be given. The purpose of the present study was to review medical records and pathological specimens for 98 patients with CMC and evaluate the prognostic relevance of clinical and morphological parameters.

METHODOLOGY

Between 1975 and 1990, 1875 patients with primary colorectal carcinomas whose tumors invaded beyond the mucosal layer underwent surgery at the National Cancer Center Hospital, Tokyo, Japan. Among them, CMC was identified in 98 cases (5.2%). Medical records and pathological sections of these cases with primary CMC were reviewed. Informed consent was obtained from all patients prior to surgery. All of the patients were followed for at least 5 years or until death. In line with the 1989 WHO criteria (7), histological diagnosis of CMC was made when more than 50% of the tumor was composed of extracellular mucin. The tumor was defined as SRCC when more than 50% of the tumor cells were composed of signet-ring cells, based on examination of all available sections (2).

Clinical variables tested included gender, age, tumor site, gross appearance, tumor size, preoperative serum carcinoembryonic antigen level, status of liver metastases and peritoneal dissemination, and macroscopic completeness of resection, obtained from the medical records. The criteria for grading each clinical variable are summarized in **Table 1**. For gross appearance, the classification defined by Borrmann for advanced gastric cancers was used (8): polypoid or fungating (type 1), excavating (type 2), ulcerated and infiltrating (type 3) and infiltrating (type 4). The size of the tumor was determined by measuring the largest diameter. Cases of cancers considered to have been completely resected were defined as curative, and those with remnants as non-curative. Patients with liver metastasis, peritoneal dissemination or direct invasion to other organs were placed in the curative group when these were completely resected macroscopically.

Histological variables evaluated included Dukes' stage (9) modified by Turnbull *et al.* (10), depth of transmural invasion, lymph node involvement, distant

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TABLE 1 The Criteria for Grading Each Variable

Clinical Variables	
Gender	: male; female
Age (years)	: ≤59; 60≤
Site of tumor	: colon; rectum
Gross appearance	: type 1; 2; 3; 4
Size of tumor (mm)	: ≤49; 50≤ - ≤79; 80≤
CEA level (ng/dL)	: ≤4.9; 5.0≤
Liver metastasis	: absent; present
Peritoneal dissemination	: absent; present
Macroscopic completeness of resection	: curative; non-curative
Morphologic Variables	
Dukes stage	: A; B; C; D
Spread beyond the bowel wall	: t2; t3; t4
Lymph node involvement	: n0; n1; n2; n3
Distant metastasis	: m0; m1
Vessel involvement	: absent; present
Structure of tumor cells	: trabecular; scattered
Pattern of growth	: expanding; infiltrating
Cytological atypia	: mild; severe
Percentage volume of signet-ring cells	: ≤49%; 50%≤

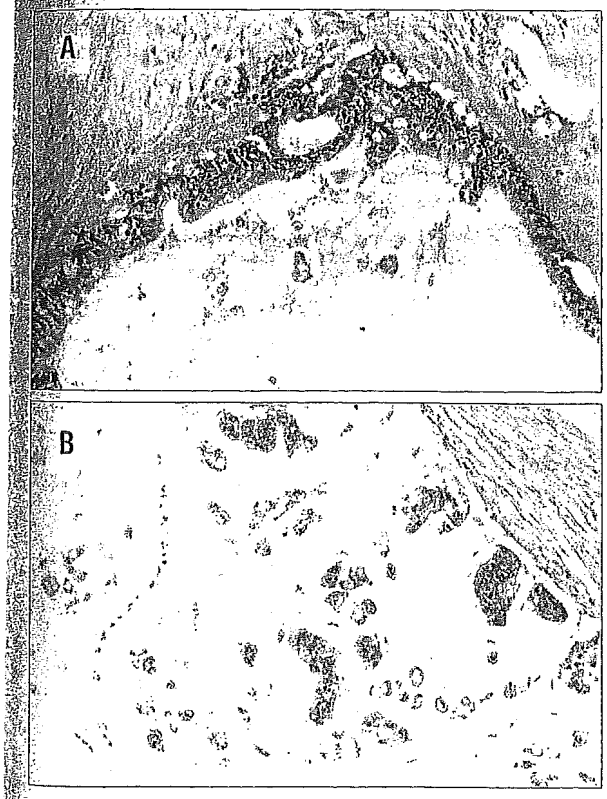

FIGURE 1 (A) Trabecular type showed marked intraluminal growth, as opposed to outpouching, producing a pseudocribriform pattern. (B) Scattered type was recorded either when cells were single or arranged in small clusters.

organ metastasis, vascular invasion, tumor structure (tubular configuration), pattern of growth, cytological atypia and % volume of signet-ring cells. The pathologic sections examined were stained with hematoxylin and eosin. Each slide was examined and the tumors were graded by one pathologist, who was unaware of the clinical outcome. The criteria for grading each morphologic variable are summarized in **Table 1**. Spread beyond the bowel wall, lymph node involvement and distant metastasis were all defined according to TNM clinical classification (11). There were no carcinomas *in situ* or tumors within the submucosa. Trabecular type showed marked intraluminal growth, as opposed to outpouching, producing a pseudocribriform pattern (**Figure 1A**). Scattered type was recorded either when cells were single or arranged in small clusters (**Figure 1B**) (12). As suggested by Jass *et al.* (13), tumors were defined as expanding or infiltrating following the morphologic guidelines previously defined by Ming for gastric carcinomas (14). Tumors were classified as having mild or severe atypia according to the grade of cytological atypia of the tumor cells in infiltrating portions. With mild atypia, the nucleocytoplasmic ratio was low, but the nuclei were elongated, crowded and appeared stratified. Mucus secretion was usually preserved (**Figure 2A**). With severe atypia, the nuclei were greatly enlarged, ovoid or round, hyperchromatic and often contained a prominent nucleolus. Mitoses were numerous, with occasional abnormal mitotic figures. Mucus production appeared absent (**Figure 2B**).

During the first step, we examined whether SRCC exhibited a worse prognosis than the other mucinous carcinomas. The Kaplan-Meier method was used to obtain overall survival curves (15). Deaths from other causes were treated as events at the time of death. Dif-

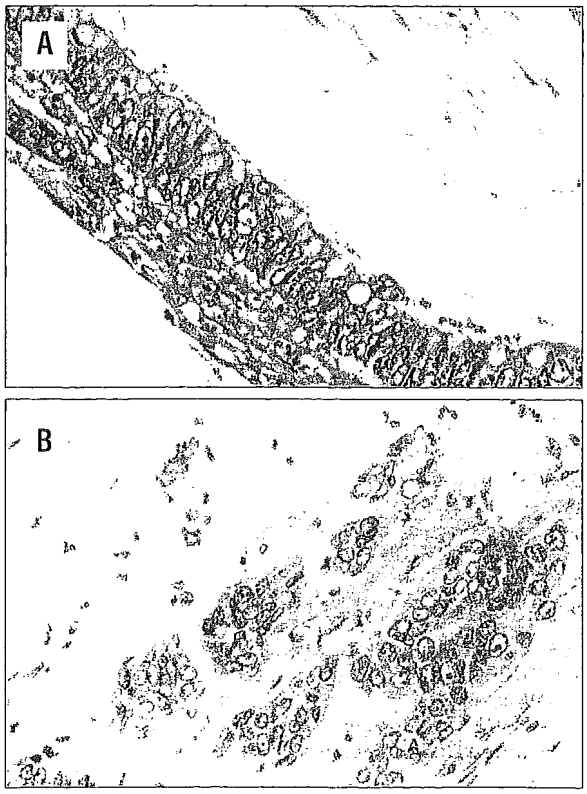

FIGURE 2 (A) In mild atypia, the nucleocytoplasmic ratio was low, but the nuclei were elongated, crowded and appeared stratified. (B) In severe atypia, the nuclei were greatly enlarged, ovoid or round, hyperchromatic and often contained a prominent nucleolus. Mitoses were numerous, and there might be abnormal mitotic figures.

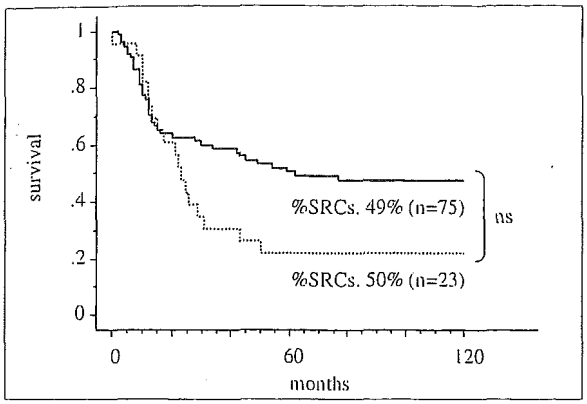

FIGURE 3 Comparison of survivals of SRCC and other typical CMC. There was no statistically significant difference between the two. %SRCs: percentage of signet-ring cells.

ferences were compared using the log-rank test. This method was used for all univariate analyses.

During the second step, univariate and multivariate analyses were conducted to find prognostic factors for the patients who underwent macroscopically complete resection. Multivariate analyses were performed by the Cox regression model (16).

RESULTS

The patients comprised 56 men and 42 women. The median age was 60 years (range 29 to 90 years). Thirty-three tumors were located in the right colon (cecum, ascending colon), 10 in the left colon (transverse, descending, sigmoid colon) and 55 in the rectum or rectosigmoid junction, according to the International Classification of Diseases (17). Six were Dukes' A cancers; 21 Dukes' B, 41 Dukes' C and 30 Dukes' D. Curative surgery was performed on 70 (71%) patients. Overall 5-year survival was 44%. None of the patients were suffering from risk factor disease such as ulcerative colitis, Crohn's disease, familial adenomatous polyposis or hereditary non-polypotic colon cancer.

SRCC was found in 23 cases. Amount of signet-ring cells was a non-significant indicator of poor prognosis. Survival curves with respect to this variable are shown in **Figure 3**. None of the SRCC were Dukes' A; 2 were Dukes' B, 13 were Dukes' C and 8 were Dukes' D.

The results of univariate analyses for the cases where cancers were completely resected are summarized in **Table 2**. Prognosis was strongly related to liver metastasis, lymph node involvement and vessel involvement. Spread beyond the bowel wall exhibited significant association with prognosis. On multivariate analysis, liver metastasis and spread beyond the bowel wall were significant variables after adjusting other prognostic factors **(Table 3)**.

DISCUSSION

In any series of colorectal cancers, mucus production will range from trace to a considerable abun-

TABLE 2 Univariate Analysis for the 70 Curative Cases

Factor	n	5-yr survival	*P* value
Gender			
male	39	64.1	ns
female	31	58.1	
Age			
≤59	38	60.5	ns
60≤	32	62.5	
Site of tumor			
colon	29	69.0	ns
rectum	41	56.1	
Gross appearance			
1	11	81.8	ns
2	47	61.7	
3	11	45.5	
4	1	0.0	
Size of tumor			
≤49	18	50.0	ns
50≤ - ≤79	37	62.2	
80≤	15	73.3	
CEA level			
≤4.9	32	68.8	ns
5.0≤	37	56.8	
Liver metastasis			
absent	64	63.2	<0.01
present	6	0.0	
Peritoneal dissemination			
absent	68	62.1	ns
present	2	50.0	
Dukes stage			
A	6	100.0	ns
B	21	71.4	
C	35	54.1	
D	8	33.3	
Spread beyond bowel wall			
t2	9	88.9	0.02
t3	19	63.3	
t4	42	33.3	
Lymph node involvement			
n0	29	75.9	<0.01
n1	15	60.0	
n2	9	11.1	
n3	17	64.7	
Distant metastasis			
m0	62	62.7	ns
m1	8	33.3	
Vessel involvement			
absent	35	77.1	<0.01
present	35	45.7	
Structure of tumor cells			
trabecular	60	66.7	ns
scattered	10	30.0	
Pattern of growth			
expanding	23	73.9	ns
infiltrating	47	55.3	
Cytological atypia			
mild	15	86.7	ns
severe	55	54.6	

ns: not significant ($p>0.05$).

TABLE 5. Summary of Results of Multivariate Analysis

Factor	Hazard ratio	p value
Liver metastasis	13.5	0.0007
Spread beyond the bowel wall	2.95	0.0054

dance, contributing to the greater part of the tumor size. In the literature, there is no clear agreement as to the minimum percentage of extracellular mucin required to define a carcinoma as mucinous (1-4;18,19). Since the WHO classification provides uniform, simple guidelines that are particularly useful clinically, it was employed in the present study (7).

The prognostic value of various histologic and grade-related parameters for CMC has remained unclear (20), but Jass and coworkers suggested that at least nine morphologic parameters (in addition to stage) had significant prognostic relevance from their univariate analysis (13). Among these, lymphocytic infiltration, tubular configuration and pattern of growth had independent prognostic value on multivariate analysis. In contrast, Leon *et al.* found that TNM staging was the only parameter with independent prognostic importance (21).

The main purpose of this study was to determine whether signet-ring cells exert an influence on prognosis which reflects their amount. There are several reports suggesting that SRCC show a worse prognosis than other mucinous carcinomas and typical non-mucinous adenocarcinomas (3,4). However, some authors have reported no clinical differences and there is a possibility that the poor prognosis may be due to a delay in diagnosis (22-25). In our series, the proportion occupied by signet-ring cells was not a significant indicator of poor prognosis. SRCC tend to be discovered at an advanced stage, although this is also the case for mucinous carcinomas as a whole.

Metastases from mucinous carcinomas and SRCC tend to develop in the lymph nodes and peritoneal surfaces rather than the liver (5). In our series, lymph node involvement was strongly related to prognosis on univariate analysis but was not an independent factor on multivariate analysis. Peritoneal dissemination was not related to prognosis on univariate analysis. They were independent of the amount of signet-ring cells using the χ^2 test.

A second aim was to identify clinical and morphologic parameters that may be of prognostic relevance in patients with CMC undergoing curative operation. Four variables (liver metastasis, lymph node involvement, vessel involvement, spread beyond the bowel wall) were significantly related to prognosis on univariate analysis. However, using multiregression models, only liver metastasis and spread beyond the bowel wall were independent prognostic factors and thus these appear to be the most important for predicting clinical outcome. This finding seems to be almost the same for ordinary non-mucinous carcinomas (1,24,26).

This may allow us to determine the plan of adjuvant therapy and follow-up. Our study indicated that patients who have liver metastasis, even if the tumors are completely resected macroscopically, only have a poor prognosis. Six such patients all died within 13 months. Spread beyond the bowel wall also has significant importance. Adjuvant chemotherapy using intraperitoneal injection may play a positive role for patients with tumors perforating the visceral peritoneum, because peritoneal dissemination was here found to be more frequent (8 patients) than other patterns of recurrence, including local recurrence (2 patients), liver metastasis (3 patients), and distant metastasis (2 patients).

In conclusion, the present study reaffirmed the importance of liver metastasis and spread beyond the bowel wall along with staging and grading for CMC with curative surgery. This appears to be of extreme practical importance in defining the subgroups of patients who are at different risk of recurrence and who could be treated more or less intensively. Future studies should assess the prognostic significance of various biologic markers within each Dukes' class.

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SURGICAL ENDOSCOPY
and Other Interventional Techniques

A comparison of the complication rates between laparoscopic colectomy and laparoscopic low anterior resection

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Abstract
Background: This study compared the short-term outcomes, including the complication rate and minimum surgical invasiveness, between patients with colon and rectal carcinomas, who underwent laparoscopic surgery.
Methods: A review evaluated 151 patients who underwent laparoscopic colectomy (Lap-colectomy; $n = 120$) and laparoscopic low anterior resection (Lap-LAR; $n = 31$) between July 2001 and December 2003. The short-term outcomes were compared between the two groups.
Results: The mean operative time and blood loss were significantly greater in the Lap-LAR group. However, the complication rates and postoperative course between the two approaches were similar, and no anastomotic leakage was observed. There was no significant difference in the serum C-reactive protein level and white blood cell count between the two groups in the early postoperative period.
Conclusions: Lap-LAR for rectal carcinoma can be performed safely without increased morbidity or mortality, and its short-term benefits are comparable with those conferred by Lap-colectomy.

Key words: Laparoscopic colectomy — Laparoscopic low anterior resection — Complication — Colorectal cancer — Short-term outcome

More than 10 years have passed since laparoscopic surgery became the approach of choice for colorectal cancer, but its value still remains unestablished. One of the reasons for this is that oncologic safety, which is the most important factor in a cancer surgery, has not been well confirmed for LS as it has for conventional open surgery. Oncologic outcome is not compromised by the laparoscopic approach, at least in the short term [6, 7, 9, 19]. According to some reports, the treatment outcome for laparoscopec surgery is not inferior to that for open surgery in terms of 5-year survival. However, the safety of laparoscopic surgery should be evaluated and confirmed in prospective randomized controlled trials [8, 15].

Unfortunately, laparoscopic surgery as an approach to rectal cancer is a very difficult surgery from a technical standpoint. Consequently, many trials have excluded patients with middle and lower rectal carcinomas. Laparoscopic low anterior resection (Lap-LAR) reportedly involves a high rate of anastomotic leakage (5.7–21%), and some authors have recommended covering ileostomy routinely in Lap-LAR cases, a step that is not required in some open surgery cases [1, 3, 5, 10, 13, 20]. Technical difficulties may be overcome by the surgeon's proficiency, and by the improvement and development of instruments, but because of the high complication rate, it currently is controversial whether Lap-LAR can be regarded as a minimum invasive surgery for rectal cancer.

Since our first laparoscopic colectomy for colorectal carcinoma in 1993, approximately 280 laparoscopic resections for colorectal malignancies have been performed at our institution. In June 2001, we unified our surgical and postoperative management procedures, and began to expand the use of laparoscopic surgery to include middle and lower rectal carcinomas. As a consequence, the complication rate and mean length of hospitalization have been reduced at our institution.

In the current study, short-term outcomes, including the complication rate and minimum surgical invasiveness, were compared selected patients with colon carcinoma and those with rectal carcinoma who underwent laparoscopic surgery at our hospital after June 2001 to evaluate whether Lap-LAR is a surgical technique with benefits similar to those for laparoscopic colectomy (Lap-colectomy).

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Patients and methods

Patients

Between June 2001 and December 2003, we performed 151 continuous laparoscopic resections for selected patients with colorectal carcinoma. Because the safety of laparoscopic surgery patients with cancer remains to be established, candidates for radical surgery were patients who had a preoperative diagnosis of T1 or T2. Additionally, laparoscopic surgery cases also included patients with a preoperative diagnosis of T3 who nevertheless wished to undergo laparoscopic surgery and those with colon or upper rectal carcinoma for which palliative resection was considered necessary. We excluded the following groups of patients from laparoscopic resection: patients with tumors larger than 6 cm, patients with a history of extensive adhesions, patients with severe obesity (body mass index exceeding 32 kg/m2), patients with intestinal obstruction, and patients who did not consent to laparoscopic surgery.

All the patients were evaluated before surgery by clinical investigation including barium enema, total colonoscopy, chest x-ray, abdominal ultrasonography, and computed tomography. For the patients with rectal carcinoma, a primary rectal carcinoma was defined according to its distance from the anal verge, as determined by colonoscopy. The tumors were grouped into lower rectum (0–7 cm), middle rectum (7.1–12 cm), and upper rectum (12.1–17 cm). We defined conversion to open surgery as any incision larger than 7 cm, excluding cases in which the incision was enlarged because of a large specimen that could not be removed through a 7-cm incision.

Laparoscopic technique

The techniques of laparoscopic resections have previously been described thoroughly [6, 19, 20]. For right-sided lesions, the right colon was mobilized initially, and the vascular pedicles were divided at their origin, together with the draining lymph nodes intracorporeally. For patients with a preoperative diagnosis of T2–T3 lesions, the laparoscopic no-touch isolation technique was performed [12]. With this technique, after early proximal ligation of the tumor-feeding vessels and resection of the mesentery intracorporeally, mobilization of the right colon was performed. The bowel loop was delivered under a wound protector through a small incision. The division of the marginal vessels and the anastomosis were performed extracorporeally.

For transversecolon lesions, mobilization of hepatic, splenic, or both flexures was performed according to the tumor location. Proximal ligation of the right, left, or both branches of the middle colic vessels at their origins was performed intracorporeally or extracorporeally. The bowel loop was delivered, and anastomosis was performed in the same way.

For the descending colon and the proximal sigmoid colon lesions for which extracorporeal anastomosis was considered possible, the left colon was mobilized initially. After mobilization of the splenic flexure, intracorporeal ligation of the tumor-feeding vessels (left colic artery, sigmoid arteries, inferior mesenteric vessels) at their origins was performed. The bowel loop was delivered through a small incision, and the division of the mesenterium was performed extracorporeally, followed by extracorporeal anastomosis.

For the distal sigmoid colon and rectal lesions, after mobilization of the left colon and splenic flexure, if necessary, intracorporeal high ligation of the inferior mesenteric vessels followed by mobilization of the rectum and mesorectum was performed. For higher lesions, mesorectal tissue down to 5 cm below the tumor was excised routinely. Middle and lower rectal tumors were treated by total mesorectal excision. Rectal transection was performed with endolinear staplers (Endo GIA Universal; Auto Suture, U.S. Surgical Corp., Norwalk, CT, USA). A 4-cm incision then was made over the mid-lower port site, and the bowel was exteriorized under wound protection. The anastomosis was performed by the double stapling technique. For patients with lesions located within 2 cm of the dentate line, laparoscopic intersphincteric rectal resection and handsewn coloanal anastomosis were performed. This surgical technique has been described previously [18].

Study parameters

The parameters analyzed included gender, age, body mass index (BMI), prior abdominal surgery, operative time, operative blood loss, conversion rate, days to resume diet, length of postoperative hospital stay, and both intraoperative and postoperative complications within 30 days of surgery. Pathologic staging was performed according to Dukes' stage. White blood cell (WBC) count and C-reactive protein (CRP) in serum were measured preoperatively and on postoperative day 1 routinely, and on postoperative days 2, 3, and 4, if necessary.

Statistical analysis

Statistical analysis was performed using Student's t test, Fisher's exact test, and the chi-square test as appropriate. A p value less than 0.05 was considered significant.

Results

The patient demographics are summarized in Table 1. No significant differences were observed in baseline characteristics between the two groups, with the exception that mean BMI was significantly greater in the Lap-LAR group ($p = 0.0438$). In the Lap-LAR group, two patients underwent laparoscopic handsewn coloanal anastomosis, and a transverse-coloplasty pouch was constructed for two patients. All the patients with covering ileostomy underwent ileostomy closure. With regard to simultaneously performed surgical techniques, the Lap-colectomy group had two patients who underwent combined surgery: one had a laparoscopic cholecystectomy and the other had resection of a benign submandibular gland tumor. In the Lap-LAR group, two patients underwent concurrent laparoscopic cholecystectomy. Data on these combined surgical techniques all were included in the analyses of the colorectal cancer surgeries.

Operative and postoperative results are shown in Table 2. All the operations were completed laparoscopically in this study. The mean operative time and blood loss were significantly greater in the Lap-LAR group. We did not experience accidental intestinal perforation at or near the tumor site. Liquid and solid foods were started on median postoperative days 1 and 3 in both groups. The median length of postoperative hospitalization was 8 days in both groups. No significant differences were observed in the postoperative course between the two groups. All the patients were discharged to home.

The postoperative complications are listed in Table 3. There were no perioperative mortalities. The morbidity rate was 13.3% (16/120) in the Lap-colectomy group and 16.1% (5/31) in the Lap-LAR group. However, no anastomotic leakage occurred in this study. Reoperation of the laparoscopic division of an adhesive band for a postoperative small bowel obstruction was necessary for one patient in the Lap-colectomy group (0.8%). No significant differences in complication rates were observed between the two groups. No significant differences were found between the two groups in terms of CRP and WBC levels after surgery (Fig. 1). At the end of the study period, only one patient in the Lap-