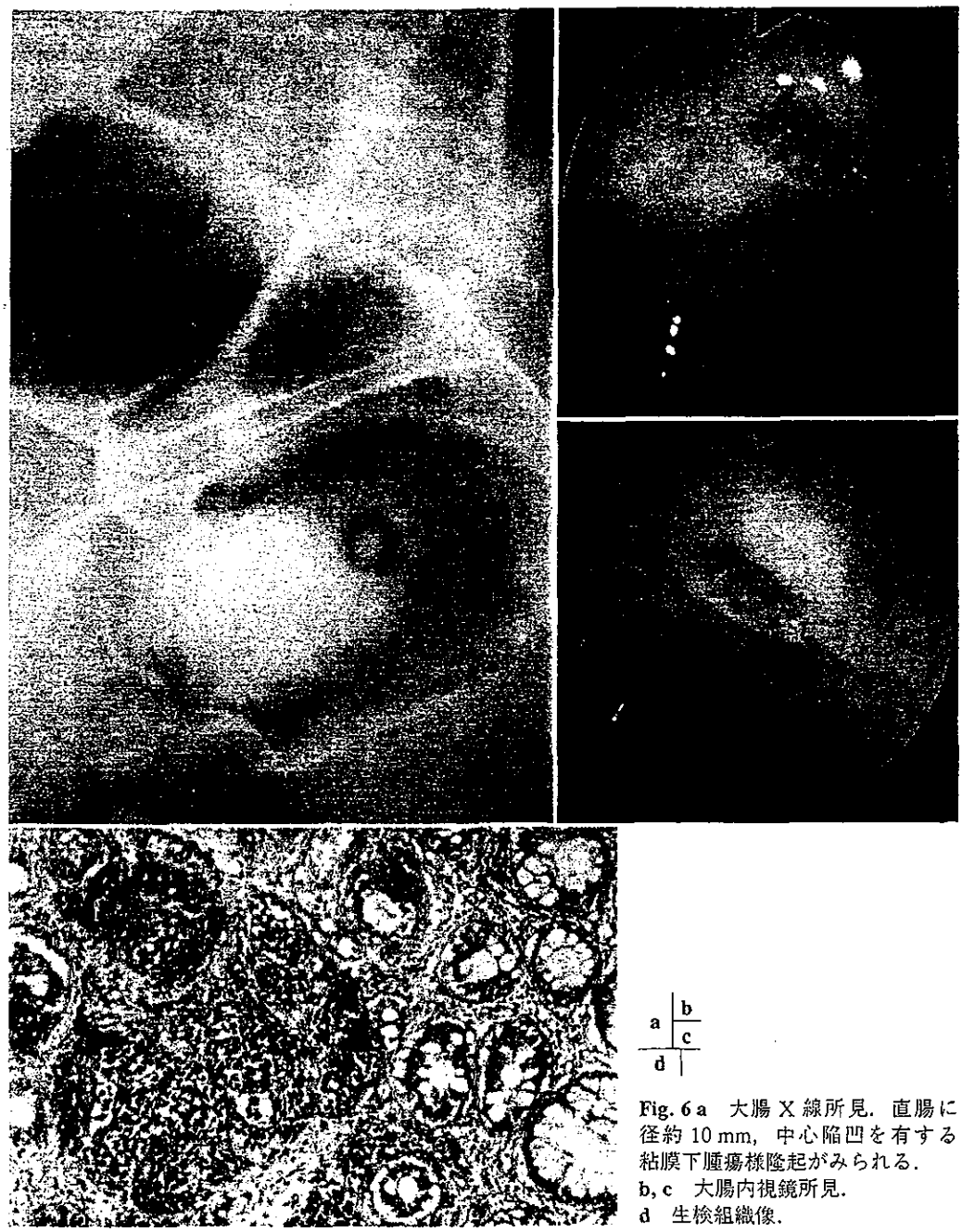

Fig. 6 a　大腸 X 線所見．直腸に径約 10 mm，中心陥凹を有する粘膜下腫瘍様隆起がみられる．
b, c　大腸内視鏡所見．
d　生検組織像．

度をみると 22.2％と少ない．一方，大腸でも，胃で述べたのと同様に，linitis plastica type の転移巣を形成する場合があり，胃癌，乳癌からの転移がその代表である[27)29)30)]．乳癌の場合の主な転移の経路は血行性転移であるが，胃癌の場合にはそれに加えて，胃結腸間膜を介しての，直接浸潤がある．linitis plastica type の転移では，primary の scirrhous carcinoma との鑑別が重要となってくる．直接浸潤の場合には部位的な特徴(横行結腸に認められる)があるが，血行性転移の場合，原発性のものとの鑑別は場合によっては困難なことがある．妹尾ら[29)]は両者の鑑別点について検討し，X 線上転移性大腸癌では狭窄部以外の腸間膜付着側と思われる部分にわずかにみられる縦じわ所見が 75％にみられたが，原発性には 1 例も存在しなかったとし，鑑別に有用としている．また，内視鏡上は，原発性では狭窄部に結節状変化および易出血性粘膜所見が多く認められるのに対して，転移性では狭窄部の粘膜が滑らかであることが鑑別のポイントになりうるとしている．一

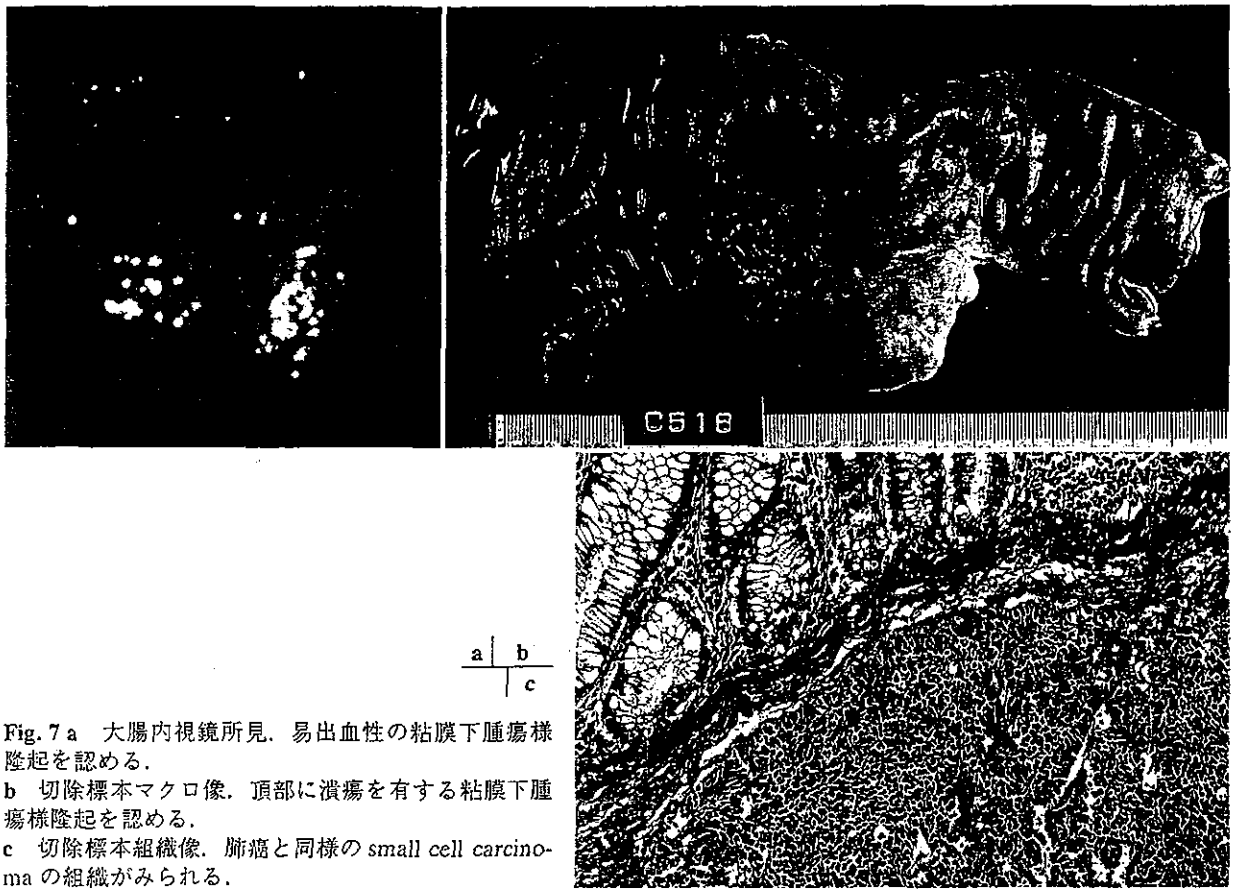

Fig. 7 a　大腸内視鏡所見．易出血性の粘膜下腫瘍様隆起を認める．
b　切除標本マクロ像．頂部に潰瘍を有する粘膜下腫瘍様隆起を認める．
c　切除標本組織像．肺癌と同様の *small cell carcinoma* の組織がみられる．

方，近年では大腸内視鏡技術の進歩に伴って，IIa＋IIc 病変に類似した形態を呈するものの報告がみられるようになってきた．**Table 5** は，IIa＋IIc 様を呈した大腸転移の本邦報告例であるが，原発臓器は胃が 9 例と 75％ を占め，他には肺癌，乳癌がみられる．室ら[31]は胃癌の大腸転移で多発性に IIa＋IIc 様病変を認めた症例を報告し，腹膜の播種性転移を伴わず，原発巣の病理組織学的検索にて，v0，ly3 であったことより，リンパ行性の転移経路が最も考えられるとしている．リンパ管侵襲の著明な胃癌では，症状がなくても大腸のスクリーニングを行う必要性があると考えられる．報告された症例の 83.3％ は多発性で，7 割近くは他の部位の転移を合併しており，小病変が必ずしも，転移進展の初期像ではないことがわかる．このような病変はほとんど症状を表すことがないため，転移巣発見のスクリーニングに消化管の検索を加える必要があると思われた．

転移性大腸癌の治療は，原則として手術となるが，他の部位に転移を合併していることも多く，手術を施行しても姑息的に終わることが多い．しかし，大塚らの報告では，転移巣を切除できた 9 症例中 3 例が 2 年以上生存したとされており，転移巣の早期発見と積極的治療によって，予後の改善が期待できると思われた．

〔**症例 6**〕 69 歳，男性．皮膚筋炎にて経過観察中に CEA の上昇を認めたためスクリーニングで施行した大腸 X 線検査にて，直腸に径約 10 mm，中心陥凹を有する粘膜下腫瘍様隆起を認めた（**Fig. 6 a**）．内視鏡検査でも同様の所見がみられた（**Fig. 6 b, c**）．同時期に施行された胸部 CT 検査にて，右肺に腫瘍を認め，生検にて small cell carcinoma の診断となった．上記直腸病変の組織像（**Fig. 6 d**）は肺癌と同様の様相を呈し，肺癌の直腸転移と診断された．脾，副腎，骨にも転移を認め，化学療法を施行されたが，診断から 6 か月後に死亡された．

〔**症例 7**〕 62 歳，男性．肺癌術後 14 か月目．CEA の上昇がみられたため，下部消化管内視鏡検査を受け，S 状結腸に頂部に易出血性の深い潰瘍を有する粘膜下腫瘍様の隆起を認めた（**Fig. 7 a**）．生検にて，肺癌と同様の small cell carcinoma が認められ，肺癌の大腸転移と

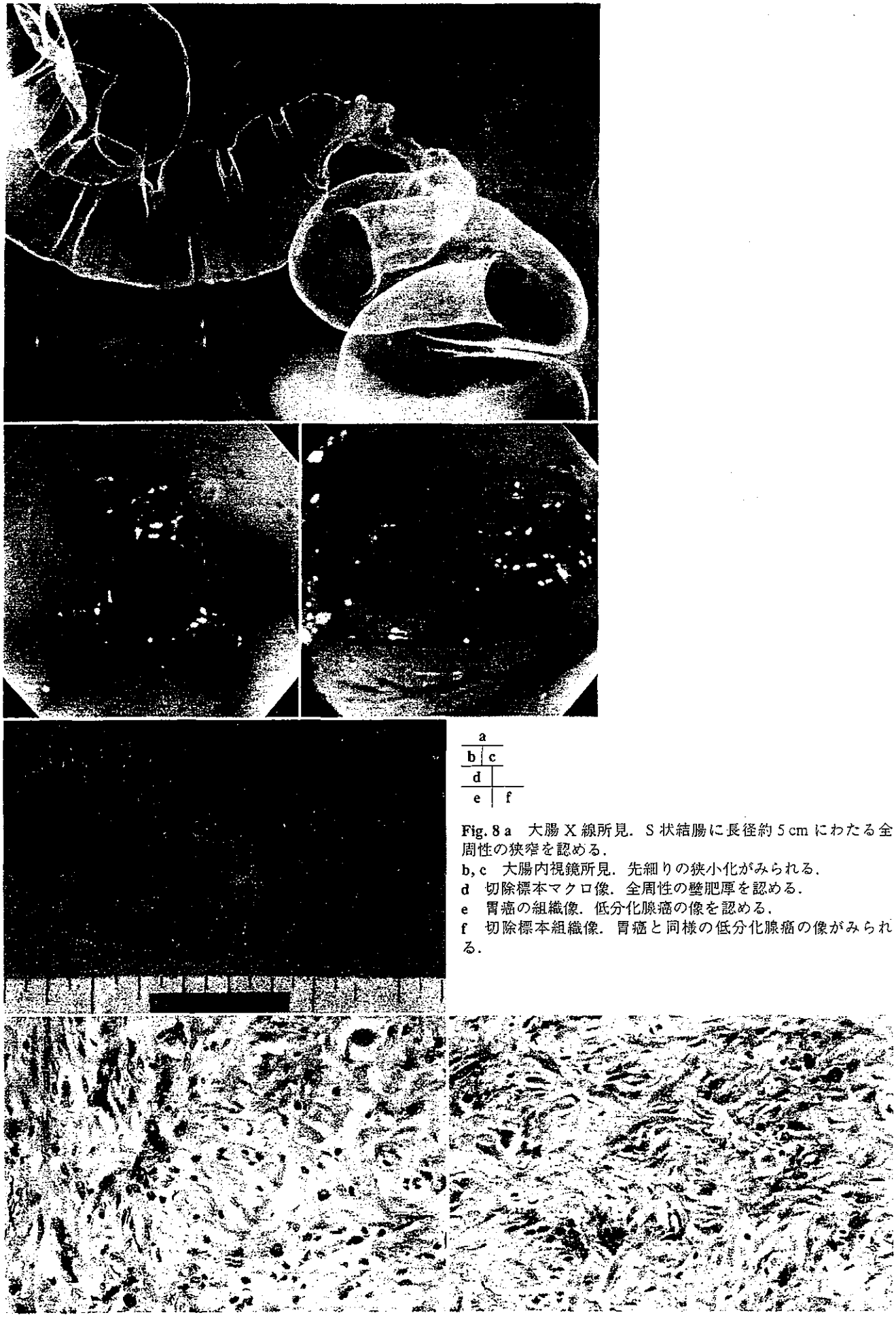

Fig. 8 a　大腸 X 線所見．S 状結腸に長径約 5 cm にわたる全周性の狭窄を認める．
b, c　大腸内視鏡所見．先細りの狭小化がみられる．
d　切除標本マクロ像．全周性の壁肥厚を認める．
e　胃癌の組織像．低分化腺癌の像を認める．
f　切除標本組織像．胃癌と同様の低分化腺癌の像がみられる．

診断され，手術が施行された．マクロ像(Fig. 7 b)では，頂部に潰瘍を有する粘膜下腫瘍様隆起が認められ，ミクロ像(Fig. 7 c)では，small cell carcinoma の像が認められた．

〔症例 8〕 53 歳，女性．胃癌術後 8 年目の大腸 X 線検査にて，S 状結腸に長径約 5 cm にわたる全周性の狭窄を認めた．狭窄の両端に明らかな隆起はみられず，狭窄部の辺縁は比較的スムーズであった(Fig. 8 a)．内視鏡では，同部位は先細りに狭小化しており，壁の伸展は不良であった(Fig. 8 b, c)．イレウス症状が出現したため，手術が施行された．術中所見では腹膜播種や腹水は認められず，切除標本のマクロ像では，病変部は全周性に著明に壁が肥厚し，一部結節状を呈していた(Fig. 8 d)．ミクロ像では，胃癌(Fig. 8 e)と同様の低分化腺癌が認められ(Fig. 8 f)，胃癌の大腸転移と診断された．

おわりに

消化管の転移性腫瘍ついて，血行性，リンパ行性転移を中心に概説し，画像所見を中心として自験例を呈示した．

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Summary

Imaging Diagnosis of Metastatic Gastrointestinal Tumors

Ikuko Iwashita [1], *Kyousuke Ushio*, *Akinori Iwashita* [2], *et al*

Among metastatic tumors, characteristics of true embolic metastasis (hamatoganeous and lymphatic metastasis) were mainly reviewed. Frequency of metastasis of malignant tumors to the gastrointestinal tract is as follows; esophagus: 1%, stomach: 2.3~6%, small intestine: 1.14~2.9%. Metastasis to the esophagus seemed to the least frequent. As for the origin of metastatic tumor, lung cancer and breast cancer were origins comparatively more frequent in every organ. In addition, gastric cancer, uterine cervical cancer and lingual cancer were origins frequent in the esophagus. Esophageal cancer and malignant melanoma in the stomach, malignant melanoma and renal cell carcinoma in the small intestine, and cervical cancer and esophageal cancer in the colon were frequent. As for clinical manifestation, dysphagea followed by anemia was the most frequent in the esophagus. Hematomesis and melena, weight loss, nausea and vomiting were frequent in the stomach. Melena and intestinal obstruction in the small intestine, and abdominal pain, abdominal distention and dyschezia were frequent in the colon. In a word, various manifestations characteristic to each organ were seen. Less than half of the cases had multiple lesions (esophagus: 40%, stomach: 47%, small intestine: 34~37%, colon 22%), however, 83.3% of colonic lesions of superficial type had multiple lesions. As for macroscopic features, there were not only submucosal tumor-like lesions but lesions resembling the original cancer or malignant lymphoma. Frequency of submucosal tumor-like lesions was less than 50% (esophagus: 40%, stomach: 43%, small intestine 33.3%, colon: 22.2~28.6%). The principal therapeutic procedure is an operation, but, as there are many cases having metastasis to other organs, chemotherapy is also frequently employed. Recently, less invasive treatment such as polypectomy or EMR has been employed for solitary metastatic lesions superficial in type, and cases in which progression has been delayed due to such treatment have been reported. Although the prognosis is generally poor, reports of cases who have survived for more than two years have recently increased. Therefore, delay of progression can be expected by early detection and intensive treatment.

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ORIGINAL ARTICLE

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Biological features of sporadic colorectal carcinoma with high-frequency microsatellite instability: special reference to tumor proliferation and apoptosis

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Abstract
Background. We evaluated the relationship between biological behavior and microsatellite instability (MSI) status, with or without p53 status, in sporadic colorectal carcinoma.
Methods. MSI was analyzed with regard to biological features such as cellular proliferation and apoptotic cell death, in addition to clinicopathological features, in 87 patients with sporadic colorectal carcinoma.
Results. Fourteen (16.1%) of 87 tumors showed instability at two or more of the five loci examined (high-frequency MSI [MSI-H]). Four demonstrated instability at one locus (low-frequency MSI [MSI-L]), and 69 showed no instability (microsatellite-stable [MSS]). The MSI-H tumors tended to be located in the proximal colon and more often were mucinous carcinoma. The MSI-H tumors also tended to be in patients with multiple colorectal carcinomas and to demonstrate, rarely, an infiltrating growth pattern or venous invasion. The incidence of p53 protein overexpression in the MSI-H tumors was significantly lower than that in the MSI-L/MSS tumors (21% vs 54%). There was no significant difference in the proliferating-cell nuclear antigen (PCNA) labeling index (PI) or apoptotic index (AI) between the MSI-H and MSI-L/MSS tumors. The AI in the MSI-H tumors with p53 overexpression was significantly lower than that in the MSI-H tumors without p53 overexpression, and was also significantly lower than that in the MSI-L/MSS tumors with p53 overexpression. In the MSI-H tumors with p53 overexpression, no expression of BAX protein was found, and there was high expression of bcl-2 protein, resulting in a low BAX/bcl-2 ratio.
Conclusion. In sporadic colorectal carcinoma, an MSI-H tumor with p53 protein overexpression may display aggressive biological features.

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Key words Sporadic colorectal carcinoma · Microsatellite instability · Cellular proliferation · Apoptosis · PCNA · p53

Introduction

Two main genetic pathways leading to colorectal adenocarcinoma can be distinguished.[1,2] Tumors generated by the classic "suppressor" pathway display marked chromosomal instability, with frequent cytogenetic abnormalities and alleic losses.[3] The second genetic pathway is involved in the development of tumors in patients with hereditary nonpolyposis colorectal cancer (HNPCC). The hallmark of this alternative "mutator" pathway is widespread microsatellite instability (MSI), which is characterized by an accumulation of somatic alterations in the length of simple, repeated sequences called microsatellites. High-frequency microsatellite instability (MSI-H) results from DNA repair error.[4,5] The MSI that is typically seen in HNPCC is also reportedly observed in sporadic colorectal carcinoma.[6] The transforming growth factor (TGF) β receptor type II gene, the insulin-like growth factor II receptor gene, and the *BAX* gene, which encode molecules related to tumor proliferation and apoptosis, have been found to be targets for MSI.[7–9]

Colorectal adenocarcinomas originating through the suppressor pathway differ in pathological features from those originating through the mutator pathway.[10,11] Furthermore, patients with an MSI-H tumor have a more favorable survival than patients with low-frequency MSI (MSI-L) or microsatellite-stable (MSS) tumor.[12–14] However, reasons for the favorable survival in patients with an MSI-H tumor are still unclear. In general, the high mutational load in MSI-H tumors is suspected to be detrimental to their growth and metastatic potential. Thus, biological features related to the functions of the target genes for MSI in sporadic colorectal carcinoma should be particularly evaluated and compared with those in colorectal carcinomas derived from the suppressor pathway.

In the present study, to investigate any correlation between malignant biological behavior and MSI status, we analyzed MSI with regard to biological features such as cellular proliferation and apoptosis, in addition to clinicopathological features. We also discuss the prognosis of patients with sporadic colorectal carcinoma according to MSI status. We demonstrated a significant relationship between MSI status, combined with p53 protein expression status, and apoptosis in sporadic colorectal carcinoma, using immunohistochemical techniques and terminal deoxy-nucleotidyl transferase-mediated dUTP-biotin nick end-labeling (TUNEL) staining.

Patients and methods

Patients and specimens

A total of 87 patients with sporadic colorectal carcinomas who underwent surgery between 1995 and 1998 at Kurume University Hospital were reviewed. The tumors were defined as sporadic colorectal carcinoma by excluding familial adenomatous polyposis and HNPCC according to the Amsterdam criteria II.[15] The patients had had no preoperative chemotherapy or radiation therapy. Fresh surgical specimens and formalin-fixed specimens were used for MSI analysis, histopathological examination, immunostaining, and TUNEL staining. Fifty-five patients were male, and 32 were female. The patients ranged in age from 38 to 86 years, and their average age was 63.8 ± 10.5 years. The series consisted of 23 patients with cancer in the proximal colon, 35 patients with cancer in the distal colon, and 29 patients with rectal cancer. Pathological factors and staging for colorectal cancer were defined according to the International Union Against Cancer TNM classification.[16] The grading of lymphatic permeation and venous invasion was defined according to our previous reports.[17,18]

Follow-up investigations were performed through outpatient visits, by letter, or by telephone, and the most recent date of contact for each patient was regarded as the final date of survival. The maximum patient follow-up period was 78 months, and the mean follow-up period was 38 months.

MSI analysis

Representative tumor samples and fragments of corresponding normal mucosa were obtained from fresh surgical specimens, and stored in liquid nitrogen until DNA extraction. Genomic DNA was extracted from tissue using a standard phenol-chloroform procedure.[19] For MSI evaluation, we amplified five microsatellite regions, using the following primer sets: for the short arm of chromosome 2 (*D2S123*), for the short arm of chromosome 3 (*D3S1067*), for the short arm of chromosome 17 (*TP53*), for the long arm of chromosome 18 (*D18S51*), and for the short arm of chromosome (*BAT26*). These primers were obtained with a 380A DNA synthesizer (Applied Biosystems; Perkin-Elmer, Foster City, CA, USA), using a previously described protocol.[20] We developed a simple method, using fluorescence-based polymerase chain reaction (PCR) with an autosequencer for amplication of the five microsatellite loci. The PCR reaction was performed in one tube, using a RoboCycler 40 PCR machine (Stratagene, La Jolla, CA, USA) over 29 cycles with 0.2–1 μl of primers and a Gene Amp kit (Roche Molecular Systems, Branchburg, NJ, USA) under the conditions recommended by the kit supplier. In brief, we used a 50-μl aliquot containing 100 ng of DNA, 10 mM Tris-HCl (pH 8.3), 75 mM KCl, and 1.5 mM $MgCl_2$. PCR products were denatured for 5 min at 95°C in formamide dye, and electrophoresed in a 5% polyacrylamide gel containing 6 M urea, using a PRISM377 DNA sequencer (Applied Biosystems; Perkin-Elmer). The electrophoresis was performed using Tris-Borate-EDTA (TBE) buffer at 3000 V, and the interval time to collect fluorescence signals on the autosequencer was 2400 scans per hour.

The MSIs of the five genes were assessed. In principle, detection of PCR product peaks was performed using Gene Scan software[21] (Applied Biosystems). When PCR products of abnormal size or novel alleles compared with corresponding normal tissue were recognized, the genes were defined as showing MSI. Tumors exhibiting MSI with at least two of the five microsatellite markers were classified as MSI-H.[22] Tumors demonstrating instability at a single locus were classified as MSI-L, and tumors without instability at any of the five loci examined were classified as MSS.[22] In all the analyses associated with MSI, MSI-L and MSS tumors were grouped together and are indicated as MSI-L/MSS.

Immunostaining

Immunohistochemical staining was performed using a labeled streptavidin-biotin technique (SLAB kit: DAKO Cytomation, Carpinteria, CA, USA) on 4-μm thick, formalin-fixed, and paraffin-embedded sections. In brief, after deparaffinization, the sections were autoclaved at 120°C for 10 min in citrate buffer. Nonspecific binding activity was inhibited with blocking serum at room temperature for 20 min. Sections were then incubated with primary monoclonal antibody against p53 (DO7; DAKO Cytomation, Glostrup, Denmark), diluted at 1:50; monoclonal antibody against p21 (WAF1; Novocastra Laboratories, Newcastle, UK), diluted at 1:30; monoclonal antibody against proliferating-cell nuclear antigen (PCNA) (PC-10; DAKO Cytomation, Glostrup, Denmark); monoclonal antibody against BAX (Anti-BAX; Upstate, Waltham, NY, USA); and monoclonal antibody against bcl-2 (clone124; DAKO Cytomation) at 4°C overnight, respectively, followed by incubation with biotinylated link antibody and peroxidase-labeled streptavidin at room temperature for 20 min. Staining was detected using 3-amino-9-ethylcarbazol (DAKO Cytomation, Carpinteria, CA, USA) for p53 and 3′,3′-diaminobenzidine (DAKO Cytomation, Carpinteria, CA,

USA) for p21, and then counterstaining was done with hematoxylin.

Labeling of apoptotic cells by TUNEL assay

TUNEL staining allows the in-situ detection of apoptotic cells. After deparaffinization and rehydration, the tissue sections were incubated in 20μg/ml of proteinase K for 15min at room temperature. After a rinsing in phosphate-buffered saline (PBS) for 5min, endogenous peroxidase activity was blocked by incubating the sections in 2% H_2O_2 for 5min at room temperature. Following the rinsing in PBS, the slides were covered with equilibration buffer (ApopTag Plus in situ apoptosis detection kit: Serologicals, Norcross, GA, USA), and incubated for 15min at room temperature. Apoptotic cells were then identified using an immunoperoxidase detection system.

Statistical analysis

Differences in distributions between the different variables were calculated by the χ^2 test or Fisher's exact test when appropriate. In comparisons with continuous distribution, a two-sample *t*-test was used. Survival rates based on the MSI status were calculated using the Kaplan-Meier method. Comparison of the survival rates was performed using the log-rank test.

Results

MSI status

A tumor was classified as MSI-H when instability at two or more microsatellite loci was detected (more than or equal to 40%). Fourteen (16.1%) of the 87 tumors were classified as MSI-H. Of these 14 MSI-H tumors, 1 displayed instability with all five microsatellite markers, 2 displayed instability with four microsatellite markers, 2 displayed instability with three microsatellite markers, and 9 displayed instability with two microsatellite markers. Four tumors (4.6%) demonstrated instability at one locus (MSI-L), and 69 (79.3%) showed no instability at any of the five loci examined (MSS). The *BAT26* marker exhibited MSI in 14 (100%) of the 14 MSI-H tumors.

Clinicopathological features according to MSI status

Correlations between MSI status and clinicopathological factors are summarized in Table 1. The incidence (43%) of a proximal colon tumor in MSI-H cases was higher than that (23%) in MSI-L/MSS cases. The incidence (29%) of mucinous adenocarcinoma in MSI-H cases was higher than that (3%) in MSI-L/MSS cases. The MSI-H cancers had a significantly higher incidence (21%) of multiple colorectal cancers (synchronous, 2 cases; metachronous, 1 case). MSI-H tumors rarely demonstrated an infiltrating growth pattern or severe venous invasion, although these differences from MSI-L/MSS were not significant. There was no difference in age, sex, tumor infiltration, lymphatic invasion, or lymph node involvement between the patients according to MSI status.

The cumulative 5-year-survival rates of patients with MSI-H tumors and MSI-L/MSS tumors were 82% and 62%, respectively, but the difference did not reach significance (Fig. 1).

The incidence of p53 and p21 immunoexpression according to MSI status

Immunostaining of p53 was observed in the tumor cell nuclei (Fig. 2a). When p53 staining was seen in 10% or more tumor cells, the case was defined as positive for p53,[12] in consideration of what was shown to correlate closely with p53 mutation. Positivity of p53 protein expression was seen in 49% of the patients (42 of 86). Immunoreactivity for p21 was also detected in the tumor cell nuclei (Fig. 2b). When positive tumor staining for p21 was seen in 10% or more tumor cells, the case was considered as positive for p21. Positivity of p21 protein expression was seen in 28% of the patients (24 of 86). Positivity of p53 protein expression in MSI-H tumors was 21.4% (3 of 14); this was significantly lower than that (54.1%; 39 of 72) in MSI-L/MSS tumors. No significant difference in the incidence of p21 protein overexpression was found between the MSI-H and MSI-L/MSS tumors (35.7% vs 26.4%). When the tumors were classified according to both the MSI status and p53 protein expression, 39, 33, 11, and 3 tumors were defined as MSI-L/MSS and p53-positive, MSI-L/MSS and p53-negative, MSI-H and p53-negative, and MSI-H and p53-positive, respectively.

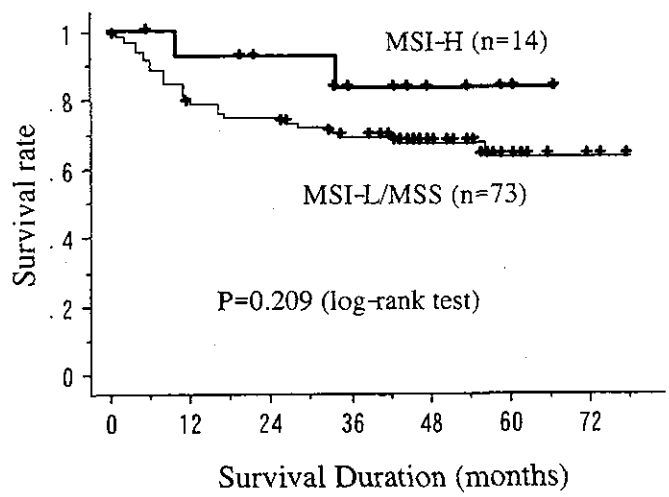

Fig. 1. Survival curves according to microsatellite instability MSI status. Cumulative survival curves of the patients with high-frequency MSI (*MSI-H*) and low-frequency MSI/microsatellite stable (*MSI-L/MSS*) tumors were prepared using the Kaplan-Meier method. Statistical comparison was performed using the log-rank test

Table 1. Clinicopathological factors and MSI status

Clinicopathological factors	MSI-H (%)	MSI-L/MSS (%)	*P* value
Age (mean ± SD)	63.6 ± 10.7	63.8 ± 10.5	0.935
Tumor size (mm; mean ± SD)	58.4 ± 27.1	51.9 ± 20.9	0.309
Sex			0.239
Male	11 (79)	44 (60)	
Female	3 (21)	29 (40)	
Location			0.063
Proximal colon	6 (43)	17 (23)	
Distal colon	7 (50)	28 (38)	
Rectum	1 (7)	28 (38)	
Histological type			0.012
Well	6 (43)	39 (53)	
Mod	4 (29)	26 (36)	
Por	0	5 (7)	
Muc	4 (29)	2 (3)	
pT-factor			0.451
pT1-2	1 (7)	13 (18)	
pT3-4	13 (93)	60 (82)	
pN-factor			0.799
pN0	6 (43)	34 (47)	
pN1-2	8 (57)	39 (53)	
M-factor			0.535
M0	11 (79)	49 (67)	
M1	3 (21)	24 (33)	
Grade of lymphatic permeation[16]			0.766
ly 0-1	10 (71)	48 (66)	
ly 2-3	4 (29)	25 (34)	
Grade of venous invasion[17]			0.128
v 0-1	12 (86)	46 (63)	
v 2-3	2 (14)	27 (37)	
Growth pattern			0.044
Expanding type	10 (71)	31 (42)	
Infiltrating type	4 (29)	42 (58)	
Multiple cancer			0.028
Negative	11 (79)	71 (97)	
Positive	3 (21)	2 (3)	

well, well-differentiated adenocarcinoma; mod, moderately differentiated adenocarcinoma; por, poorly differentiated adenocarcinoma; muc, mucinous adenocarcinoma; MSI, microsatellite instability; MSI-H, high-frequency MSI; MSI-L, low-frequency MSI; MSS, microsatellite stable

PCNA labeling index (PI) and apoptosis index (AI) according to MSI status

The PI was scored as the fraction of positively stained tumor cell nuclei among 500 to 1000 tumor cells (Fig. 2d). The PI of 87 patients available for PCNA scoring was 57.0 ± 1.6. On the other hand, apoptotic tumor cells were scattered in the tumor tissues (Fig. 2c). The AI was scored as the mean value of apoptotic tumor cells per one field under 200× light microscopy in five random fields. The AI of 87 patients available was 2.18 ± 0.25.

There was no significant difference in PI or AI between the MSI-H and MSI-L/MSS tumors. When compared in combination with p53 protein status, the AI in the MSI-H tumors with p53 protein overexpression was significantly lower than that in the MSI-H tumors without p53 protein overexpression as well as being significantly lower than that in the MSI-L/MSS tumors with p53 protein overexpression. No difference in PI was found according to the combined status of p53 expression and MSI (Table 2).

Immunoexpression of BAX and bcl-2 according to MSI status

Both BAX and bcl-2 protein were expressed in the cytoplasm of the tumor cells (Fig. 2e,f). When positive staining of BAX and bcl-2 occurred in more than 10% of tumor cells, the case was considered as positive for BAX and bcl-2. The positive ratios for BAX and bcl-2 expression were compared according to MSI status. The ratio of BAX expression in the MSI-H tumors appeared to be lower than that in the MSI-L/MSS tumors, although the difference did not reach significance. The positive ratio of BAX expression was stratified according to the combined status of MSI and p53 protein expression. No BAX expression was found in the MSI-H tumors with p53 protein overexpression; the incidence of BAX expression was 45% in the MSI-H tumors without p53 overexpression, 62% in the MSI-L/MSS tumors with p53 overexpression, and 67% in the MSI-L/MSS tumors without p53 overexpression, with no significant differences seen between these groups. When the tumors were classified into three groups and scored 1 to 3 for the concomitant expression of bcl-2 and BAX (score 1, bcl-2-positive and BAX-negative; score 2, bcl-2-positive and

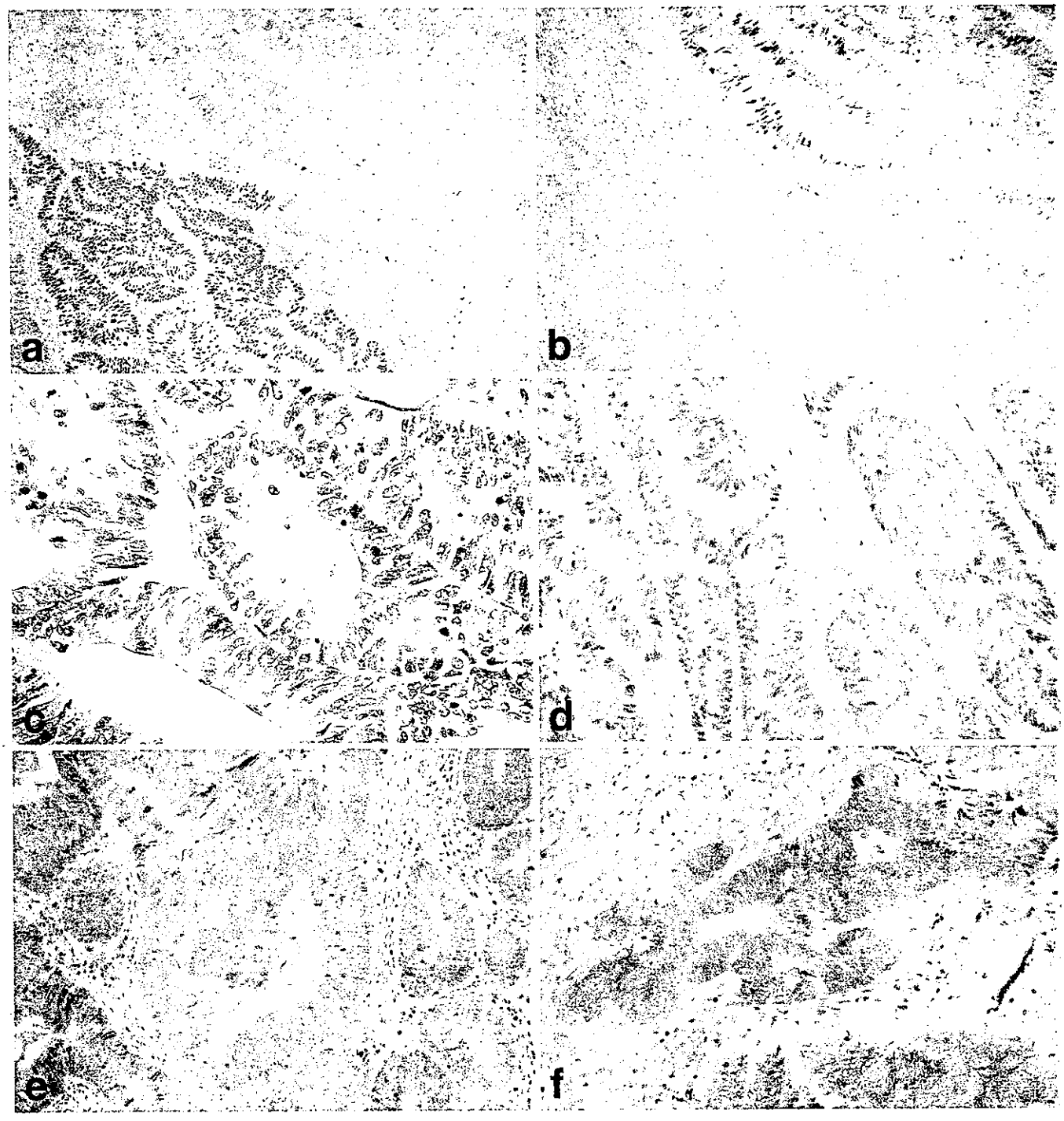

Fig. 2a–f. Immunostaining and terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick-end labeling (TUNEL) staining. Immunostaining for p53, p21, proliferating-cell nuclear antigen (PCNA), BAX, and bcl-2, and TUNEL staining, were performed as described in "Materials and methods". **a** Nuclear staining of p53, **b** p21, and **d** PCNA, and **e** cytoplasmic staining of BAX and **f** bcl-2 were observed. p53 and p21 expressions tended to differ at the cellular level. **c** Apoptotic cells detected by TUNEL staining were scattered. **a**, **b** ×40; **d**, **e**, **f** ×100; **c** ×200

BAX-negative; score 3, bcl-2-negative and BAX-positive), the score in the MSI-H tumors with p53 overexpression was significantly lower than that in the MSI-L/MSS tumors with p53 overexpression or that in the MSI-L/MSS tumors without p53 overexpression (Table 3).

Discussion

An international workshop in Bethesda, Maryland, sponsored by the National Cancer Institute, has formulated guidelines for the evaluation of MSI in colorectal carci-

Table 2. Apoptosis index (AI) and PCNA labeling index (PI) in colorectal cancer tissues

Category	Number	AI (mean ± SE)	*P* value	PI (mean ± SE)	*P* value
MSI			0.327		0.347
MSI-L/MSS	$n = 73$	2.39 ± 0.24		56.8 ± 1.6	
MSI-H	$n = 14$	1.84 ± 0.33		60.6 ± 3.7	
MSI and p53					
MSI-L/MSS, 0p53(−)	$n = 33$	2.35 ± 0.42	0.167*	55.5 ± 2.4	0.345*
MSI-L/MSS, p53(+)	$n = 39$	2.43 ± 0.27	0.042*	57.9 ± 2.2	0.512*
MSI-H, p53(−)	$n = 11$	2.24 ± 0.32	0.012*	59.9 ± 4.6	0.720*
MSI-H, p53(+)	$n = 3$	0.37 ± 0.12		63.4 ± 5.8	
MSI and p21					
MSI-L/MSS, p21(−)	$n = 52$	2.26 ± 0.23	0.103**	55.4 ± 2.0	0.724**
MSI-L/MSS, p21(+)	$n = 19$	2.67 ± 0.65	0.177*	60.5 ± 3.0	0.572**
MSI-H, p21(−)	$n = 9$	1.30 ± 0.37		57.2 ± 5.4	
MSI-H, p21(+)	$n = 5$	2.80 ± 0.34	0.021**	66.8 ± 2.5	0.234**

*Compared with MSI-H, p53(+); **compared with MSI-H, p21(−)
PCNA, proliferating-cell nuclear antigen

Table 3. Positivity of BAX and bcl-2 protein expression and the concomitant expression score

Category	Number	BAX (%)	*P* value	bcl-2 (%)	*P* value	CES (mean ± SE)	*P* value
MSI			0.072		0.775		0.015
MSI-L/MSS	$n = 73$	64.4		48.6		2.39 ± 0.07	
MSI-H	$n = 14$	35.7		42.9		1.93 ± 0.20	
MSI and p53			0.109		0.377		
MSI-L/MSS, p53(−)	$n = 33$	66.7		39.4		2.39 ± 0.11	0.007*
MSI-L/MSS, p53(+)	$n = 39$	61.5		53.7		2.39 ± 0.10	0.008*
MSI-H, p53(−)	$n = 11$	45.5		36.4		2.09 ± 0.21	0.114*
MSI-H, p53(+)	$n = 3$	0		66.7		1.33 ± 0.33	

*compared with MSI-H, p53(+)
CES, concomitant expression score

noma.[22] According to the recommendations endorsed at the workshop, a minimum of five markers should be employed to assess MSI in these tumors. Furthermore, a panel of five microsatellites has been validated as a reference panel, and several alternative loci of established utility have also been indicated. When the tumors in our study were classified as MSI-H tumors and MSI-L/MSS tumors according to the criteria, the incidence of MSI-H tumors in sporadic colorectal carcinomas was 16%. This value is similar to that in previous studies.[12,13]

It has been reported that MSI-H colorectal tumors differ from MSI-L/MSS tumors in several pathological features. In agreement with most previous investigations,[10–12] we found that MSI-H tumors tended to be located in the proximal colon and were more often mucinous carcinoma than MSI-L/MSS tumors. The MSI-H tumors also tended to be in patients with multiple colorectal carcinomas and to, rarely, demonstrate an infiltrating growth pattern or venous invasion.

The survival rate in the patients with MSI-H tumors tended to be higher than that in patients with MSI-L/MSS tumors. Previous studies in large numbers of patients have shown a more favorable prognosis for patients with MSI-H colorectal carcinomas. However, the biological basis of the more favorable clinical course of patients with MSI-H colorectal carcinoma is still undetermined. As a suggested reason for the favorable outcome, it has been reported that the high mutational load in MSI-H tumors elicited a strong host immune response arising as a result of a high mutation rate in tumor-associated antigens.[23,24] In fact, intense lymphocytic infiltration and conspicuous Crohn-like lymphoid reaction in tumor tissues are significantly associated with MSI-H tumors.[12] It has also been indicated that diploid nuclear DNA content, determined using flow cytometry, and low rates of loss of heterozygosity in *DCC/18q*, *p53/17p*, and *K-ras* mutations represented relevant biological characteristics of MSI-H colorectal carcinomas.[25,26] In other words, tumors derived via the "suppressor" pathway, most of which display MSI-L/MSS, are thought to have more frequent cytogenetic abnormalities related to tumor growth and metastasis than tumors derived via the "mutator" pathway.

With regard to tumor cell proliferation, wild p53 protein has been implicated in controlling checkpoints during the G1 phase of the cell cycle that may monitor the state of the DNA before entry into the S phase.[27] Following DNA damage, p53 protein levels rise dramatically, and the p53 induces p21 protein,[28] which causes G1 growth arrest through inhibition of cyclin-dependent kinase,[29,30] and the entry into the S phase is delayed until the genomic lesions are fully repaired. Otherwise, p53 protein inhibits bcl-2 protein expression and induces BAX protein expression, leading to cell apoptosis.[31] When p53 function is lost, tumor cells enter the S phase without appropriate DNA repair or induction of apoptosis, leading to fixation and propagation of genetic alterations.[27] Our immunohistochemical results

showed a significantly higher incidence of abnormal p53 protein accumulation in the MSI-L/MSS tumors compared with the MSI-H tumors, as has been reported previously.[12,32,33] Because MSI-L/MSS tumors derived via the "suppressor" pathway have a high incidence of mutation of the *p53* suppressor gene, the tumors are thought to have aggressive biological features related to tumor proliferation and apoptosis. On the other hand, because the TGF β type II receptor gene, the insulin-like growth factor II receptor gene, and the *BAX* gene are targets for MSI,[7-9] we have proposed that MSI-H tumors also have a high activity of tumor proliferation and a low activity of apoptosis induction.

Our question is how the target genes for MSI, such as the TGF β type II receptor gene and the *BAX* gene, contribute to the determination of tumor biological activity in sporadic colorectal carcinomas. In our results, although there was no difference in AI or PI according to MSI status, the AI in MSI-H tumors with p53 protein overexpression was significantly lower than that in the MSI-H tumors without p53 protein overexpression, as well as being significantly lower than that in the MSI-L/MSS tumors with p53 protein overexpression. This result was explained by the concomitant expression of bcl-2 and BAX protein in the tumors (these being negative and positive regulators of apoptosis, respectively). Namely, in the MSI-H tumors with p53 protein overexpression, no expression of BAX protein was found and there was high expression of bcl-2 protein, resulting in a low BAX/bcl-2 ratio. The BAX/bcl-2 ratio is reportedly a good indicator of apoptosis.[34] In general, *p53* gene mutations, were identified in MSI-L/MSS tumors. Unexpectedly, *p53* gene mutations were identified in a distinct substantial subset of MSI-H tumors.[35] Our results suggested that, in MSI-H tumors with *p53* mutation, tumor apoptosis may be strongly inhibited by alterations of related genes which resulted from both MSI and p53 dysfunction. Eshleman et al.[35] reported that MSI-H cell lines bearing mutant *p53* demonstrated the same stability of chromosome number, and the same stability of chromosome structure, as MSI-H cell lines with wild-type *p53*. It is suggested that the apparent chromosomal stability in MSI-H sporadic colorectal carcinomas is a consequence of the presence of wild-type *p53* alleles; otherwise, mutation on the *p53* gene in MSI-H tumors may occur accidentally in a different manner from that in the "suppressor" pathway.

In conclusion, from the aspect of tumor growth activity, we could not find a reason for the favorable outcome in patients with an MSI-H tumor compared to those with an MSI-L/MSS tumor. However, in MSI-H tumors with p53 protein overexpression, genes such as *BAX* and *bcl-2* may be directly affected by the loss of p53 function and by a high mutational load of MSI-H, resulting in potentially high activity of tumor growth. There is a possibility that patients having an MSI-H tumor with p53 protein overexpression may show a poorer prognosis compared to those having an MSI-H tumor with wild-type *p53*. Unfortunately, we could not reach a conclusion on this point in the present study because of the small number of samples. In the future, studies using large sample numbers should confirm that our data are reproducible, and whether or not the prognosis of patients having an MSI-H tumor with *p53* mutation is poor compared to prognosis in the other subsets.

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