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Microsatellite Instability in the Adenoma-Carcinoma Sequence of the Stomach

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SUMMARY: Sixty-three cases of stomach resections harboring both adenoma and carcinoma were analyzed for microsatellite instability (MSI). The cases included 28 carcinomas arising from adenoma (Type I) and 35 carcinomas with separate adenoma (Type II). The results of MSI assessed by 49 markers were the same for *BAT-26* instability. The incidence of MSI was 21% in gastric adenoma and 30% in gastric carcinoma, which is significantly higher than gastric carcinoma without associated adenoma ($p < 0.01$). Five of eight (63%) cases of multiple carcinomas associated with adenoma showed MSI+ in adenoma and in one or more carcinoma lesion(s). Eight of thirteen (62%) MSI+ adenomas were associated with carcinoma, whereas 20 of 50 (40%) MSI− adenomas were associated with carcinoma. MSI+ adenomas of Type I showed a higher mutation rate of the *TGF-β* RII gene than Type II (88% versus 40%). Gastric adenoma with *TGF-β* RII gene mutation was more prone to transform into carcinoma ($p = 0.03$). This study revealed that gastric carcinoma arising from adenoma is frequently associated with a mismatch repair deficiency mechanism. In the gastric adenoma-carcinoma sequence, *TGF-β* RII gene mutation occurred early in the adenoma stage and it persisted after malignant transformation. (*Lab Invest 2000, 80:57–64*).

Gastric adenoma is a precancerous lesion, 11% to 40% of which transforms into carcinoma (Kamiya et al, 1982; Ming and Goldman, 1965). The intestinal type of gastric carcinoma is different from the diffuse type in patterns of histopathology and genetic alterations. The former is generally derived from the adenoma-carcinoma sequence in stomach carcinogenesis (Semba et al, 1996) and might develop through a cumulative series of gene alterations similar to that of colorectal cancer (Tahara, 1995).

The form of genomic instability associated with defective DNA mismatch repair in tumors is called microsatellite instability (MSI). Much confusion and many conflicting concepts regarding MSI were resolved after a workshop sponsored by the (US) National Cancer Institute (Boland et al, 1998). During the workshop, MSI-H was defined as a cancer demonstrating MSI in 40% of the markers. The remaining cases consisted of either MSI-L cases (MSI in less than 40% of the markers) or MSS cases (MSI in none of the markers). Clinicopathologic data revealed that MSS and MSI-L belong to the same group, but that MSI-H is a different entity. Although the workshop analyzed the results of colorectal carcinoma, subsequent studies proved that the definition of MSI could be applied in the same way to other carcinomas, such as gastric, endometrial, or biliary.

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At least six human genes involved in the mismatch repair have been identified (*hMSH2, hMSH3, hMSH6, hMLH1, hPMS1,* and *hPSM2*). Among them, aberrant methylation of the *hMLH1* gene promoter has been proven to be the major cause of MSI in sporadic colorectal carcinoma (Kane et al, 1997), and similar results have been demonstrated in gastric carcinoma (Leung et al, 1999). The mechanism by which MSI contributes to cancer has been assumed to be a mismatch repair deficiency that results in mutations of cancer-related genes. Such mutations are thought to be responsible for carcinogenesis or tumor progression, or both (Arnheim and Shibata, 1997). Known target molecules affected by mismatch repair deficiency include the transforming growth factor-β receptor Type II (*TGF-β* RII) gene (Chung et al, 1996, 1997; Markowitz et al, 1995; Myeroff et al, 1995), the *IGFIIR* gene (Chung et al, 1997; Ouyang et al, 1997; Souza et al, 1996), and the *BAX* gene (Chung et al, 1997; Rampino et al, 1997).

To study MSI and frameshift mutations of target genes in the gastric adenoma-carcinoma sequence, we retrieved 63 cases of stomach resections that harbored adenomas and carcinomas simultaneously, and analyzed them for MSI and mutations at coding mononucleotide repeats. Our results suggest that gastric carcinoma arising from the adenoma-carcinoma sequence showed a higher frequency of MSI and is frequently associated with frameshift mutation of the *TGF-β* RII gene.

Results

Sixty-three cases of primary gastric carcinoma with coexisting adenoma were retrospectively identified

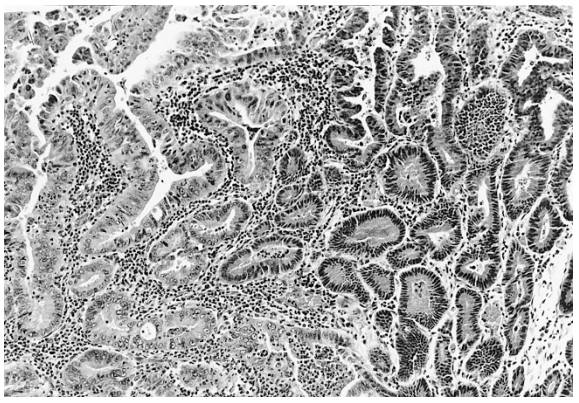

Figure 1.
Histopathology of Type I gastric adenoma with carcinomatous transformation (Case B5). Intestinal type adenocarcinoma (*left*) is surrounded by adenoma (*right*) (original magnification ×100, hematoxylin and eosin [HE]).

from the surgical pathology files of Seoul National University Hospital for this study. Pathologic slides were reviewed to analyze pathologic parameters, including tumor size, gross type, intestinal metaplasia of adjacent mucosa, and depth of invasion of the carcinoma. The carcinomas were classified histologically according to the World Health Organization classifications (Watanabe et al, 1990), Lauren (1965) and Ming (1977), and staged according to the criteria of the International Union Against Cancer (UICC) (1997). The adenomas consisted of 7 polypoid, 41 elevated, 10 flat, and 5 depressed types; 39 tubular, 17 tubulovillous, and 7 villous types; 63 intestinal types and no gastric types; and 22 high-grade and 41 low-grade adenomas. The carcinomas consisted of 43 early gastric carcinomas and 20 advanced gastric carcinomas; 44 intestinal types, 12 diffuse types, and 7 mixed types.

We classified these cases into two categories according to the association between carcinoma and adenoma. Type I was defined as carcinoma found directly adjacent to adenoma or admixed with adenoma (Fig. 1). Type II denoted cases in which carcinoma was located separately from adenoma. Type I provided the model for the gastric adenoma-carcinoma sequence and demonstrated significant associations with T1 stage, intestinal type, antral location, expanding growth, and histologically high-grade adenoma when compared to Type II (Table 1).

Assessment of MSI

We analyzed the MSI status using 49 microsatellite markers and *BAT-26* marker on 60 lesions from 30 cases. Fifteen samples showed high-frequency MSI (MSI-H or MSI in 40% or more of the tested markers), 43 samples showed low-frequency MSI (MSI-L or MSI in less than 40% of the tested markers), and 2 samples did not show alteration in any marker (microsatellite stable or MSS). When we compared these results with the alteration of the *BAT-26* marker, all of the MSI-H lesions showed a positive alteration on the *BAT-26* marker, regardless of adenoma or carcinoma in both Type I and II (Fig. 2). However, none of the

Table 1. Clinicopathologic Profiles of Gastric Carcinomas Associated with Adenomas Analyzed for MSI

	Type I[a] (n = 28)	Type II[b] (n = 35)	Total (n = 63)	p value
Mean age of patient	61.4 ± 7.1	59.3 ± 8.8	60.2 ± 8.1	NS
Ratio male:female	3.7:1	4.8:1	4.3:1	NS
Location of carcinoma				0.24
Cardia & body	11	19	30	
Antrum	17	16	33	
Size of carcinoma	4.3 ± 2.2	4.4 ± 2.6	4.3 ± 2.4	NS
Ming				<0.01
Expanding	28	18	46	
Infiltrative	0	17	17	
Lauren				0.01
Intestinal	25	19	44	
Diffuse	2	10	12	
Mixed	1	6	7	
Stage				<0.01
T1	26	17	43	
>T2	2	18	20	
LN metastasis				<0.05
Absent	25	22	47	
Present	3	13	16	
Adenoma grade				<0.05
High	14	8	22	
Low	14	27	41	

[a] Type I, carcinoma arising from adenoma.
[b] Type II, carcinoma with separate adenoma.

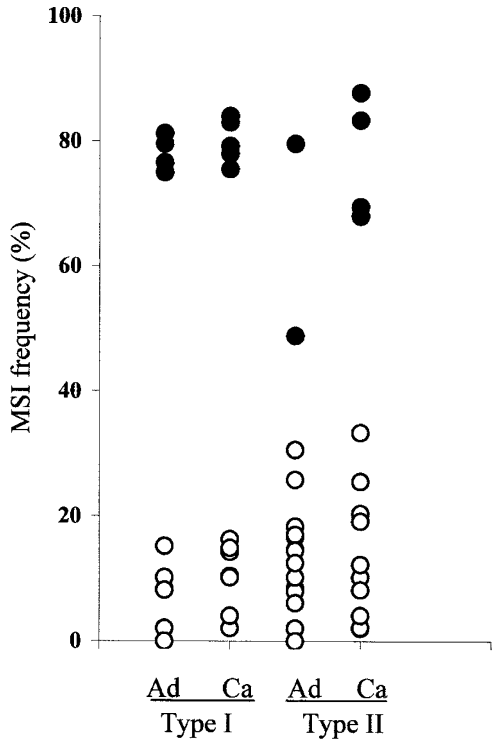

Figure 2.

Correlation between high-frequency MSI (MSI-H) and *BAT-26* instability in four subgroups of adenoma and carcinoma of Type I and Type II. ○ *BAT-26* stable; ● *BAT-26* unstable; Ad, adenoma; Ca, carcinoma. Fifteen samples with high-frequency MSI (MSI-H) are MSI+ in *BAT-26*. Forty-five samples showing low-frequency MSI (MSI-L) or microsatellite stable (MSS) are MSI− in *BAT-26*.

MSI-L or MSS lesions showed this alteration. This proved the high sensitivity and high specificity of the *BAT-26* marker in predicting MSI-H tumors (Hoang et al, 1997; Zhou et al, 1998; Perucho, 1999). These results also proved that most MSS tumors would be categorized as MSI-L if we increased the number of tested markers. Based on these results, we evaluated the MSI status of the remaining 76 lesions from 33 patients using *BAT-26* as an indicator of MSI-H. Accordingly, we designated the cases that showed alteration of *BAT-26* marker as MSI+ (Fig. 3). The rest of the cases were described as MSI−, consisting of either MSI-L or MSS.

MSI in Gastric Carcinoma and Adenoma

Of the 63 cases, 13 of the adenomas (21%) and 19 of the carcinomas (30%) were MSI+. The incidence of MSI+ was higher in Type I adenoma than in Type II adenoma (29% versus 14%), and higher in Type I carcinoma than in Type II carcinoma (39% versus 23%) (Fig. 4). Eight cases of Type I adenoma were MSI+, and of those cases, all carcinomas were also MSI+. In three cases of Type I, the carcinoma lesions were MSI+, whereas the adenoma lesions were MSI−. In contrast, only 3 of the 35 Type II cases were MSI+ in both the adenoma and carcinoma (Table 2). It is obvious that MSI is a phenomenon that is maintained during the adenoma-carcinoma sequence, and that MSI could develop during the carcinomatous transformation step. The MSI+ carcinomas were associated with an antral location ($p < 0.01$), expanding growth, intestinal type, T1 stage, and no lymph node metastases. However, no significant association was found between MSI+ adenoma and size, histologic type, histologic grade, residual intestinal metaplasia, or location (data not shown).

Frameshift-Mutations in the Coding Regions

No mutations were identified in the MSI− cases in any of the examined genes. We observed frameshift mutations in the poly$(A)_{10}$ tract of *TGF-β RII* gene in 9 (69%) of the MSI+ adenomas and 17 (90%) of the MSI+ carcinomas. The incidence of *TGF-β RII* gene mutation in Type I adenoma was significantly higher than in Type II adenoma (Fig. 4). This suggested that adenoma with *TGF-β RII* gene mutation is frequently associated with malignant transformation. Two adenomas of Type II with *TGF-β RII* gene mutation were 1.3-cm low-grade villotubular adenoma (Case B8) and 0.5-cm low-grade tubular adenoma (Case B4).

Mutations were also found in the poly$(G)_8$ tract of the *BAX* gene in four (31%) MSI+ adenomas and 13 (68%) MSI+ carcinomas. This suggested that *BAX* gene mutation usually occurs later than *TGF-β RII* mutation. Two (15%) of the MSI+ adenomas and one (5%) of the MSI+ carcinomas showed frameshift mutations of the poly$(G)_8$ tract of the *IGFIIR* gene. Mutation of the poly$(A)_8$ tract of the *hMSH3* gene was found in five (39%) MSI+ adenomas and six (33%) MSI+ carcinomas.

Mutations involved deletion of one or two bases in *TGF-β RII*; deletion or addition of one base in *BAX*; deletion of one base or addition of two bases in *IGFIIR*; and deletion of one base in *hMSH3* genes (Fig. 5). In the adenoma-carcinoma sequence, the mutation pattern was usually preserved (Table 2).

Most of the mutations in the polytract genes were found within the cases with a mutated *TGF-β RII* gene. Only three samples showed mutations of other polytract genes without *TGF-β RII* gene mutation: one case of Type I carcinoma (Case 22) and two cases of Type II adenoma (Case 30, B10).

Analysis of Multiple Carcinomas Associated with Adenoma

Of 63 cases, eight had more than one carcinoma lesion; of those, five (63%) cases showed MSI+ in adenoma and in one or more carcinoma lesion(s). The incidence of MSI+ in cases with multiple carcinoma was higher than in cases with a single carcinoma, both in the carcinoma and the associated adenoma ($p < 0.05$) (Fig. 4). All three cases of Type I with multiple carcinomas were MSI+ in both adenoma and carcinomas.

Two cases exhibited different MSI status between the multiple carcinomas, but three cases showed co-occurrence of MSI in multiple carcinomas. Of nine

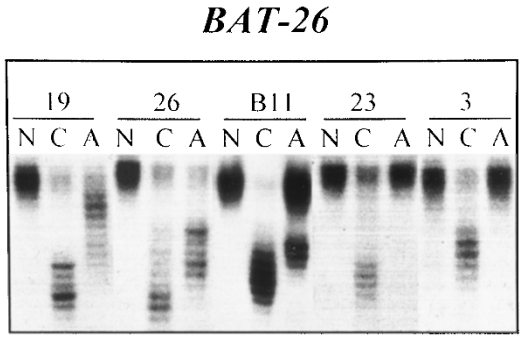

Figure 3.
Examples of *BAT-26* alteration. Adenoma or carcinoma with size variation in the *BAT-26* allele is defined as MSI+. N, normal; C, carcinoma; A, adenoma; Cases 19, 26, and B11 (Type I) were MSI+ in both adenoma and carcinoma. Case 23 (Type I) and Case 3 (Type II) were MSI+ in carcinoma and MSI− in adenoma.

MSI+ carcinoma lesions, *TGF-β RII* gene mutations were seen in seven (78%), *BAX* gene mutation in five, *IGFIIR* gene mutation in two, and *hMSH3* genes mutation in five (Table 3). All of the samples of one adenoma and two carcinomas from Case B8 showed mutations in all four examined genes. However, the mutation patterns of the *BAX* gene in the adenoma and carcinoma were slightly different.

Discussion

The MSI+ rate in gastric cancer among Koreans without co-occurrence of adenoma is approximately 10% (Kang et al, 1999). This figure is not much different from most of the recent data on Japanese (14.6%) (Yamamoto et al, 1999), Australians (9.9%) or Caucasian people (8.5%) (Halling et al, 1999). Our results on carcinoma arising from adenoma (Type I) showed an incidence of MSI+ that was 4 times the incidence in ordinary gastric cancer ($p < 0.01$). The above data suggest that gastric carcinoma arising from adenoma is more frequently associated with a mismatch repair-deficient carcinogenic pathway than de novo carcinoma. Furthermore, carcinoma with separate adenoma (Type II) showed an incidence of MSI+ 2-fold higher than in cases without adenoma. The cause of this higher incidence might be related to the higher frequency of intestinal type in this category, but the exact cause should be studied. Although one

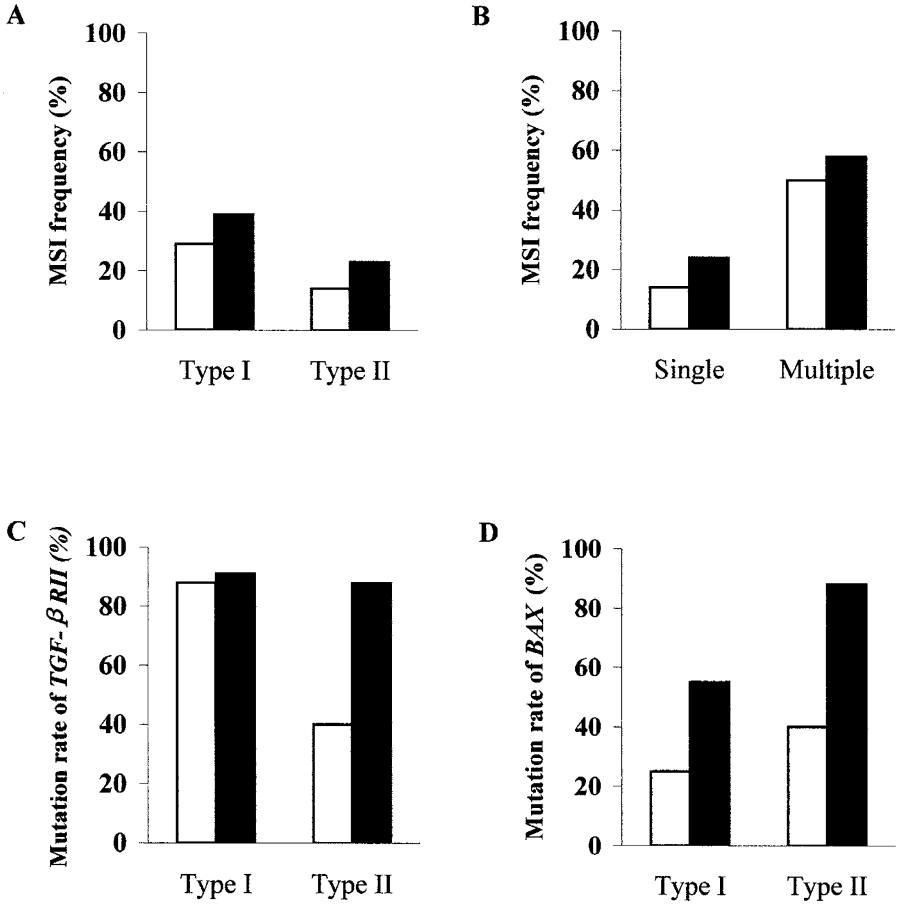

Figure 4.
Comparison of MSI+ frequency and gene mutation rate in gastric adenoma and carcinoma; □, adenoma and ■, carcinoma. *A*, Type I adenoma and carcinoma showed higher MSI+ incidence than those of Type II. *B*, MSI+ incidence is higher in the cases of multiple carcinomas associated with adenoma than cases of single carcinoma. *C*, *TGF-β RII* gene mutation is more frequent in Type I adenoma and carcinoma than Type II. (D), *BAX* gene mutation is more frequent in carcinoma than in adenoma in both Type I and Type II.

Table 2. Histopathologic Profiles, Status of MSI, and Coding Mononucleotide Repeats within the TGF-β RII, BAX, IGFIIR, and hMSH3 genes in 21 MSI+ cases

Case	Stage	Location[a]	Lauren[b]	Adenoma Grade[c]	Type[d]	MSI[e] Ad	MSI[e] Ca	TGF-βRII[f] Ad	TGF-βRII[f] Ca	BAX[f] Ad	BAX[f] Ca	IGFIIR[f] Ad	IGFIIR[f] Ca	hMSH3[f] Ad	hMSH3[f] Ca
23	T1	B	I	H	I	○	●	−	−1/−2	−	−1/wt	−	−	−	−
B30	T1	A	I	H	I	○	●	−	−1/wt	−	−1/wt	−	−	−	−
B31	T1	A	I	L	I	○	●	−	−1/−2	−	−	−	−	−	nd
19	T1	A	I	L	I	●	●	−1/wt	−1/wt	−	−	−	−	−1/wt	−1/wt
20	T3	A	D	L	I	●	●	−1/wt	−1/wt	+1/wt	+1/wt	−	−	−	−
22	T2	A	I	L	I	●	●	−1/wt	−	−	+1/wt	−	−	−	−1/wt
26	T1	B	I	H	I	●	●	−1/wt	−1/wt	−1/wt	−	−	−	−1/wt	−
B11	T1	A	I	L	I	●	●	−	−1/wt	−	−1/wt	−	−	−	−
B19	T1	A	I	L	I	●	●	−1/−2	−1/−2	−	−	−	−	−	−
B20	T1	A	I	H	I	●	●	−1/wt	−1/wt	−	−	−	−	−	−
B24	T1	B	I	H	I	●	●	−1/wt	−1/wt	−	−1/wt	−	−	−	−1/wt
2	T3	B	M	H	II	●	○	−	−	−	−	−	−	−	−
B10	T1	A	D	L	II	●	○	−	−	−	−	+1/wt	−	−1/wt	−
3	T2	A	M	H	II	○	●	−	−1/−2	−	+1/wt	−	−	−	−1/wt
6	T2	A	D	L	II	○	●	−	−1/wt	−	−1/wt	−	−	−	−
15	T1	A	I	H	II	○	●	−	−1/wt	−	−1/wt	−	−	−	−1/wt
B6	T4	B	M	L	II	○	●	−	−1/wt	−	+1/wt	−	−	−	−
B25	T1	A	I	L	II	○	●	−	−1/wt	−	−1/wt	−	−	−	−
30	T1	A	I	L	II	●	●	−	−	−1/wt	−	−	−	−1/wt	−
B4	T2	A	I	L	II	●	●	−	−1/wt	−	−1/wt	−	−	−	−
B8	T1	A	I	L	II	●	●	−1/wt	−1/wt	−1/−1	−1/+1	−2/wt	−2/wt	−1/wt	−1/wt

[a] A, antrum; B, body.
[b] I, intestinal; D, diffuse; M, mixed
[c] H, high; L, low.
[d] Type I and II; same as Table 1.
[e] Ad, adenoma; Ca, carcinoma; ○, MSI−; ●, MSI+.
[f] nd: not done; wt: wild type. Mutation pattern denotes the description of the band pattern, but not the exact status of the allele.

paper describes the incidence of MSI+ in gastric adenoma, comparison with our results is impossible due to the different criteria used for MSI assessment (Semba et al, 1996).

In this study, we proved that *BAT-26* is a useful marker for the identification of the MSI status of gastric adenoma and carcinoma. Fifteen samples showing MSI in more than 40% of the 49 markers tested demonstrated alterations of *BAT-26*, whereas the 43 samples showing MSI in less than 40% of the markers demonstrated intact *BAT-26*. Similar results have already been published regarding colon carcinoma (Hoang et al, 1997) and gastric carcinoma (Zhou et al, 1998; Perucho, 1999).

The clinicopathologic characteristics of MSI+ carcinoma reported by several researchers are: older age, antral or body location, intestinal type, Borrmann Type II, negative lymph node metastasis, and better survival. In this study, we could add that the carcinoma arising from adenoma is a subgroup that demonstrates a high frequency of the MSI+ phenotype.

Compared with a large series of colon adenomas (Boland et al, 1998), the overall MSI incidence in gastric adenoma (21%) is 7 times higher than that of colonic adenoma (3%). Furthermore, the *TGF-β RII* gene mutation rate in the stomach (69%) is much higher than in the colon (35%) (Grady et al, 1998). Although this might be related to the fact that our cases consisted of larger adenomas than in nonresected cases, it also might be related to the higher risk of carcinomatous transformation in gastric adenoma than in colonic adenoma.

Our results indicate that cells do not lose the MSI characteristic during carcinomatous transformation, but gain MSI continuously during the gastric adenoma-carcinoma sequence, as shown in colorectal tumorigenesis (Shibata et al, 1994). In our study, nine of the adenomas demonstrated a mutation of *TGF-β RII* gene. Among them, seven adenomas showed carcinomatous transformation ($p < 0.01$). In contrast, *IGFRII* mutation was found in adenomas without carcinomatous transformation, and *BAX* or *hMSH3* gene mutation was not more frequently seen in Type I than in Type II adenomas. This suggests that MSI+ adenoma, especially with *TGF-β RII* gene mutation, is prone to cancerous change. In addition, MSI and *TGF-β RII* gene mutation are early somatic events in the gastric adenoma-carcinoma sequence. Increased cancer risk of MSI+ adenoma in the colon has been suggested (Jacoby et al, 1995; Lothe et al, 1995), and our data support an increased risk in gastric adenoma.

In our study, the incidence of *BAX* gene mutation was 68% in MSI+ carcinomas, similar to the previously published data of 64% in the Western population (Yamamoto et al, 1997). In contrast, the incidence

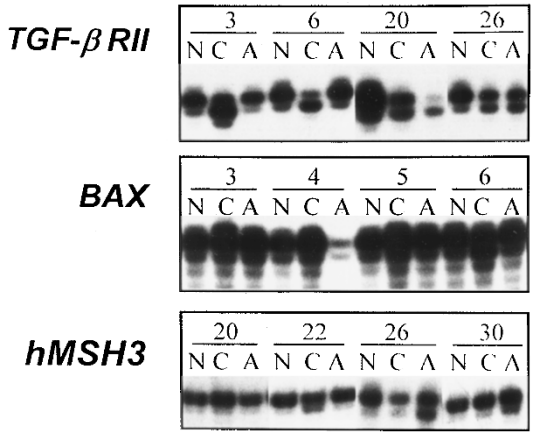

Figure 5.
Examples of frameshift mutation in MSI+ adenoma and carcinoma of the stomach. The genes are indicated at the left of each panel. Mutation was identified by the presence of bands in tumor DNA that are not present in the corresponding normal DNA. N, normal; C, carcinoma; A, adenoma. Top panel, Cases 3 (C), 6 (C), 20 (A), and 26 (C and A) showed deletion of one nucleotide in the poly(A)$_{10}$ tract of the *TGF-β RII* gene. Middle panel, Case 3 (C) showed gain of one nucleotide, and Case 6 (C) showed one nucleotide deletion in the poly(G)$_8$ tract of the *BAX* gene. Bottom panel, Cases 22 (C), 26 (A), and 30 (A) showed deletion of one nucleotide in poly(A)$_8$ tract in the *hMSH3* gene.

of *BAX* gene mutation is much lower in adenoma (31%). This suggests that although MSI is an early change, mutations of specific genes could be accumulated during the carcinomatous transformation. In contrast to *TGF-β RII* gene mutation, *BAX* mutation might develop during the carcinomatous transformation. We observed that 38% of MSI+ adenomas showed *hMSH3* gene mutation, which is higher than the incidence in carcinomas (33%). Our data could not prove the previous theory that *hMSH3* mutation increased the mutation rate of other genes with microsatellites.

In our study, two of the Type II cases showed mutations in the *IGFIIR* gene, but none of the Type I cases were associated with *IGFIIR* gene mutation. The absence of *IGFIIR* gene mutation in adenoma-associated carcinoma should draw our attention, but the rarity of MSI+ cases in Type II carcinoma or adenoma prohibits drawing any conclusion.

The incidence of MSI+ in the cases with multiple carcinomas and adenoma is higher than in the patients with single carcinoma. We also identified heterogeneity of the MSI and frameshift mutations, in which the carcinogenic process of each tumor may have diverse microsatellite alterations even under the same genetic background. In contrast, multiple synchronous gastric carcinomas without adenoma do not show a higher rate of MSI+ (manuscript in preparation). Although several reports have shown that multiple gastric carcinomas are frequently associated with MSI+, it remains to be proven whether this is related to the association of adenoma, or to the multiplicity itself.

In summary, our data showed that carcinoma-associated adenomas either in Type I or in Type II are frequently associated with MSI+. Multiple synchronous carcinomas associated with adenoma showed an even higher frequency of MSI+. We also proved that MSI and mutation of *TGF-β RII* gene are changes that occur early in the adenoma-carcinoma sequence and that they persist after malignant transformation, whereas *BAX* gene mutation seems to occur at a later stage. Research regarding the molecular mechanism of MSI in adenoma and during adenoma-carcinoma sequence is needed.

Materials and Methods

DNA Extraction

DNA from 63 cases was obtained from formalin-fixed, paraffin embedded surgical sections. To reduce the possibility of genetic abnormalities in tumor cells compromised by the presence of normal cells, the neoplastic areas were selected microscopically and microdissected from hematoxylin and eosin-stained slides. This microdissection procedure harvests more than 60% of tumor cell populations. DNA was extracted from microdissected tissues in lysis buffer (40 μg/ml proteinase K, 0.5% Tween-20, 50 mM Tris, 25 mM EDTA, pH 8.5) and incubated for 24 to 48 hours at 55° C. After boiling for 10 minutes to inactivate proteinase K and centrifugation for 5 minutes at 15,000 rpm, the extracted DNA was stored at −20° C until use.

Microsatellite Markers

Primers for the 49 microsatellite loci (containing di-, tri-, and tetranucleotide repeat sequences) were purchased from Research Genetics (MapPair, Huntsville, Alabama). The markers were D1S162, D1S237, D2S119, D2S104, D3S1274, D3S1300, D3S1766, D3S1216, D4S174, D4S1652, D5S299, APC2, D5S346, D5S409, D6S271, D6S105, D6S310, D7S507, D7S1805, D8S254, D8S555, D9S104, D9S103, D10S183, D10S109, D11S875 (MISC), D11S897, D12S95, D12S375, D13S120, D14S51, CYP19, D16S403, D16S413, TP53, D17S796, D17S786, D17S520, D17S791, D18S53, D18S34, D18S386, D19S177, D19S416, D20S66, D20S17, D21S411, D21S258, and IL2RB. Primers for the mononucleotide repeat microsatellite sequences were: *BAT-26* (Hoang et al, 1997; Zhou et al, 1998), located within intron 5 of the *hMSH2* gene; *BAT-25*, located in the introns of the c-kit oncogene; poly(A)$_{10}$ tract of *TGF-β RII* (Markowitz et al, 1995); poly(G)$_8$ tract of *BAX* (Rampino et al, 1997); poly(G)$_8$ tract of *IGFIIR* (Souza et al, 1996); and poly(A)$_8$ tract of *hMSH3* (Malkhosyan et al, 1996) were made according to the procedures described in previously published papers.

PCR Amplification and Microsatellite Analysis

PCR amplification with MapPair primers was performed with 5 pmol/μl of each primer, 1.5 mM $MgCl_2$, 0.2 mM deoxynecleotide triphosphate, 0.5 unit *Taq* polymerase, 0.1 μl [α-^{32} P] dCTP (3,000 Ci mmol^{-1}, NEN, Dupoint, Boston, Massachusetts), and 1–2 μl DNA in a total volume of 10 μl. The PCR conditions

Table 3. Microsatellite Instability and Mutations in Coding Mononucleotide Repeats in Multiple Gastric Carcinomas Associated with Adenoma

Case	Type[a]	Lesion	MSI[b]	TGF-β RII	BAX	IGFIIR	hMSH3
B11		A	●	–	–	–	–
	I	C1	●	−1/wt	−1/wt	–	–
	II	C2	●	−1/wt	–	–	–
	II	C3	○	–	–	–	–
B20		A	●	−1/wt	–	–	–
	I	C1	●	−1/wt	−1/wt	–	−1/wt
	II	C2	○	–	–	–	–
B24		A	●	−1/wt	–	–	–
	I	C1	●	−1/wt	−1/wt	–	−1/wt
	II	C2	●	−1/wt	–	–	−1/wt
30		A	●	–	−1/wt	–	−1/wt
	II	C1	●	–	–	–	–
	II	C2	●	–	–	–	–
B8		A	●	−1/wt	+1/−1	−2/wt	−1/wt
	II	C1	●	−1/wt	−1/+1	−2/wt	−1/wt
	II	C2	●	−1/wt	−1/+1	−2/wt	−1/wt
B12		A	○	–	–	–	–
	II	C1	○	–	–	–	–
	II	C2	○	–	–	–	–
B27		A	○	–	–	–	–
	II	C1	○	–	–	–	–
	II	C2	○	–	–	–	–
B28		A	○	–	–	–	–
	II	C1	○	–	–	–	–
	II	C2	○	–	–	–	–
	II	C3	○	–	–	–	–

[a] Type I and II, same as Table 1. The type denotes the relationship between adenoma and each carcinoma.
[b] ○ and ●, same as Table 2.

were 95° C for 5 minutes, followed by 33 cycles (94° C for 30 seconds, 45° C to 58° C for 30 seconds, 72° C for 40 seconds) and a final elongation step at 72° C for 10 minutes. PCR products were diluted to a concentration of 1:4 with loading buffer, heated at 100° C for 5 minutes, and stored on ice until analysis. Then 1.5 μl aliquots of each sample were separated on denatured 6% polyacrylamide gel. The gel was dried on a vacuum slab gel dryer at 80° C for 1–1.5 hours and exposed to X-ray film at −70° C for 12–72 hours.

To detect frameshift mutations in the coding regions, the reaction involved 32 cycles at 94° C for 1 minute, at 53° C to 60° C for 1 minute, and at 72° C for 1 minute, using 3.7×10^4 Bq of ^{32}P-dCTP in 10 μl of reaction mixture. The subsequent procedures were the same as for the MSI analysis.

Statistical Analysis

The association between MSI and the clinicopathologic profiles was analyzed using χ^2 test or Fisher's exact test. Statistical significance was defined as $p < 0.05$.

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