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Primary Gastric Carcinoma Cells Frequently Lose Heterozygosity at the *APC* and *MCC* Genetic Loci

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Loss of heterozygosity (LOH) at *APC* and *MCC* gene loci (both mapped to 5q21) was investigated in 24 surgical specimens of primary gastric carcinomas using the polymerase chain reaction after tumor cell enrichment by cell sorting based on differences in DNA content. LOH at *APC* and/or *MCC* was detected in 87% (13/15) of the cases; at the *APC* in 86% (12/14) and at the *MCC* locus in 100% (7/7). LOH at the *APC* locus was always accompanied by LOH at the *MCC* locus. LOH at the *APC* and/or *MCC* was found in both differentiated and undifferentiated types in both early and advanced stages of gastric carcinoma. Thus, LOH at *APC* and/or *MCC* is considered to be one of the most prevalent genetic alterations in human gastric carcinoma and occurs at an early stage of the carcinogenesis.

Key words: Loss of heterozygosity — *APC* gene — *MCC* gene — Gastric carcinoma — Polymerase chain reaction

Investigators have isolated the *APC* (adenomatous polyposis coli) gene[1,2] responsible for familial adenomatous polyposis coli (FAP) and the *MCC* (mutated in colon cancer) gene,[3] which are located close to each other at 5q21. Somatic mutations of both genes have been identified in sporadic colon carcinomas[3–5] and germline mutations of the *APC* gene have been found in FAP patients.[4,6] These data indicate that the *APC* and *MCC* genes are involved in the pathogenesis of colon carcinoma.

The recent identification of a polymorphic *Rsa*I site in the *APC* gene and a variable insertion polymorphism in the *MCC* gene has made it possible to evaluate the loss of heterozygosity using the polymerase chain reaction (PCR-LOH assay).[7] Studies using the PCR-LOH assay have shown that there is frequent LOH of *APC* and *MCC* genes not only in colon carcinomas but also in esophageal[8] and small cell lung carcinomas.[9] Mutations of the *APC* gene were also found in gastric[10] and pancreatic carcinomas.[11] These observations suggest that *APC* and/or *MCC* genes, like the p53 tumor suppressor gene,[12] are involved in the development or progression of common human malignancies.

Recently, we[13,14] and other investigators[8,15] have shown that the combination of flow cytometric cell sorting and molecular genetic analysis is an extremely sensitive method for analyzing genetic abnormalities even when considerable amounts of non-tumor cells are present in the tumor samples. We have used this approach to detect LOH at *APC* and *MCC* gene loci in surgically resected specimens of gastric carcinoma.

Tumor specimens and their surrounding normal tissues were obtained at the time of surgery from 24 patients with gastric carcinoma. Each specimen was frozen immediately and stored at −80°C until analyzed. Tumor DNA was isolated from $1\text{–}5\times10^4$ nuclei which were sorted using a FACStar Plus (Becton Dickinson, Mountain View, CA). Aneuploid populations were isolated when found (Fig. 1), and cells at the $S+G_2M$ phases were isolated in diploid tumors as previously described.[13,14] The corresponding normal DNA was extracted in a standard manner. Oligonucleotide primers were synthesized using a DNA synthesizer (MilliGen/Biosearch, Bedford, MA) and purified on Oligo-pak columns (MilliGen/Biosearch). To analyze the *APC* gene, primers flanking an *Rsa*I restriction fragment length polymorphism within exon 11 were synthesized. Oligonucleotide primer sequences were 5′-GGACTACAGGCCATTGCAGAA-3′ and 5′-GGCTACATCTCCAAAAGTCAA-3′. To analyze *MCC*, primers flanking a variable insertion polymorphism within exon 10 were synthesized. Primer sequences used were 5′-TACGAATCCAATGCCACA-3′ and 5′-CTGAAGTAGCTCCAAACA-3′. Extracted DNA was amplified in 20 μl of a buffer recommended by Perkin-Elmer Cetus (Norwalk, CT), which contained 0.75 to 1 m*M* $MgCl_2$ and 1 unit of *Taq* DNA polymerase (Perkin-Elmer Cetus). PCR was performed under the conditions described by Boynton *et al.*[8] with a thermal cycler (Perkin-Elmer Cetus). For *APC* analysis, the PCR products were digested with 30 units of *Rsa*I (Toyobo,

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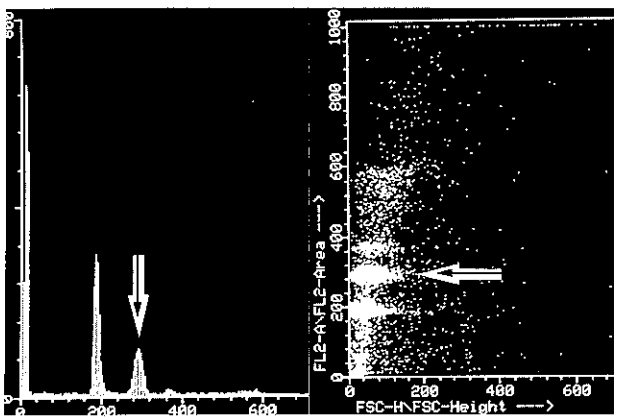

Fig. 1. DNA histogram (left) and cytogram (right) of an aneuploid tumor. The aneuploid population (arrow) was isolated and assayed by PCR-LOH.

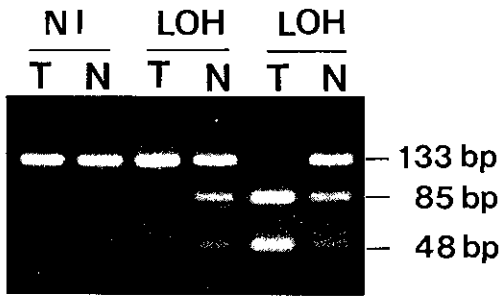

Fig. 2. PCR-LOH assay of *APC* gene exon 11. Lanes: T, tumor DNA; N, normal DNA. Each tumor DNA lane is followed by its corresponding normal DNA lane. NI, not informative; LOH, loss of heterozygosity.

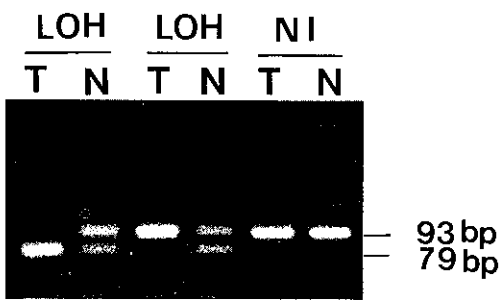

Fig. 3. PCR-LOH assay of *MCC* gene exon 10. Lanes: T, tumor DNA; N, normal DNA. Each tumor DNA lane is followed by its corresponding normal DNA lane. NI, not informative; LOH, loss of heterozygosity.

Osaka). For *MCC* analysis, no restriction enzyme digestion was necessary. Products were then electrophoresed on a 3% agarose gel and stained with ethidium bromide. LOH was defined as a visible change in the allele:allele ratio in the tumor DNA relative to the ratio in corresponding normal DNA.[7]

Fifteen cases were heterozygous for at least one locus. Among these, aneuploid populations were used in 12 cases and cells at the $S+G_2M$ phases were used in 3 cases. LOH at the *APC* and/or *MCC* locus was detected in 87% (13/15); at the *APC* in 86% (12/14) (Fig. 2) and at the *MCC* in 100% (7/7) (Fig. 3). The remaining 9 cases were homozygous for both loci. The results of PCR-LOH assay are summarized in Table I. LOH at the *APC* locus was always accompanied by LOH at the *MCC* locus. LOH at the *APC* and/or *MCC* was found in both differentiated and undifferentiated types[16] and in both early (including a carcinoma confined to the mucosa) and advanced stages of gastric carcinoma.

Recent studies of *APC* and/or *MCC* gene aberrations have suggested that these genes may be involved in the genesis of common human malignancies. LOH on chromosome 5q, where the *APC* and *MCC* genes are located, has been detected in 42% of differentiated and in none of undifferentiated types of gastric carcinoma.[17] More recently, Nakamura *et al.* and Tahara have identified mutations of the *APC* gene frequently in differentiated type and less frequently in undifferentiated type of gastric carcinoma.[10, 18] These results suggest that aberrations of the *APC* gene play a crucial role in the genesis of differentiated gastric carcinomas. However, it is also possible that such aberrations were underestimated in undifferentiated gastric carcinomas, because considerable amounts of normal stromal and inflammatory cells usually contaminate such specimens. In fact, LOH at the *APC* and/or *MCC* genetic loci was very frequently detected in the present study with the PCR-LOH assay in both differentiated and undifferentiated gastric carcinomas after tumor cell enrichment by cell sorting. However, it could be detected in only a few cases using ordinary extracted DNAs (data not shown). In addition, LOH at these loci was detected in both early (including a carcinoma confined to the mucosa) and advanced stages, and therefore, it may be an early event in gastric carcinogenesis, as in colon carcinoma. It has also been suggested that LOH at the *APC* or *MCC* gene locus is a late event in the genesis of small cell lung carcinoma because LOH is seen frequently at advanced stages in these tumors.[9]

In carcinomas in which LOH has been observed at both gene loci, it is not known whether one or both *APC* and *MCC* genes are important in carcinogenesis. Since

Table I. Results of PCR-LOH Assay at the *APC* and *MCC* Genetic Loci in 24 Primary Gastric Carcinomas

Patients number	Stage[a]	Histological type[b]	Depth of invasion[c]	Lymph node metastasis[d]	LOH[e]	
					APC exon 11	*MCC* exon 10
1	II	D	pm	N	LOH	ND
2	III	U	ss	N	NI	NI
3	III	D	ss	N	HET	NI
4	IV	U	se	P	LOH	LOH
5	II	U	ss	P	NI	NI
6	III	D	ss	P	LOH	NI
7	I	D	m	N	NI	NI
8	IV	D	si	P	NI	NI
9	IV	D	ss	P	LOH	NI
10	IV	U	si	P	LOH	LOH
11	IV	D	pm	P	LOH	LOH
12	IV	D	ss	P	NI	LOH
13	III	U	ss	P	LOH	LOH
14	III	U	ss	P	LOH	LOH
15	III	U	ss	N	NI	NI
16	IV	U	se	P	NI	NI
17	I	D	sm	N	NI	NI
18	I	D	m	N	LOH	ND
19	I	D	sm	N	LOH	NI
20	I	U	sm	N	LOH	NI
21	II	D	pm	P	NI	NI
22	I	D	m	N	NI	NI
23	III	D	ss	P	LOH	LOH
24	III	D	ss	N	HET	NI

a) According to the clinicopathological staging system of the Japanese Research Society for Gastric Cancer (1985).
b) D, differentiated type; U, undifferentiated type.
c) m, mucosa; sm, submucosa, pm, muscularis propria; ss, subserosa; se, exposed serosal invasion; si, exposed serosal invasion with infiltration of other organs.
d) P, positive; N, negative.
e) NI, not informative; HET, retained heterozygosity; LOH, loss of heterozygosity; ND, not determined because the amount of DNA was limited.

the genes are located close to each other, one of them may be deleted inconsequentially.[4] Another tumor suppressor gene may also exist on 5q.[19] Mutation analysis together with LOH detection of *APC* and *MCC* genes in gastric carcinoma should eventually resolve this issue.

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