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Frequent Loss of Heterozygosity at the *MCC* Locus on Chromosome 5q21-22 in Sporadic Colorectal Carcinomas

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Recent studies have identified a gene on chromosome 5q, designated *MCC* (mutated in colorectal cancers), as a candidate for the putative colorectal tumor suppressor gene that is located at 5q21. We examined loss of heterozygosity (LOH) at the *MCC* locus and its vicinity in sporadic colorectal carcinomas, using 12 RFLP (restriction fragment length polymorphism) markers. One clone, L5.71, had been used to identify the *MCC* gene; all 12 markers also had tight linkage to the gene responsible for adenomatous polyposis coli. All 40 cases studied were informative with at least one marker, and 22 of them (55%) showed LOH at one or more loci. LOH in the tumors was more frequent in the immediate vicinity of L5.71 than in distant parts of the chromosome, and a common region of deletion was detected between markers L5.62 and 15A6. In one case, alleles were retained at L5.71 and at loci proximal to L5.71, but alleles were lost at loci distal to L5.71. In another case, both alleles were retained at L5.71 but alleles were lost at loci proximal and distal to L5.71. These results support the conclusion that a tumor suppressor gene for colorectal carcinoma is located within or around locus L5.71.

Key words: Loss of heterozygosity — *MCC* locus — Tumor suppressor gene — Sporadic colorectal carcinoma

The allelic losses reported at specific chromosomal loci in several types of human cancer[1–6] have implied the existence of a tumor suppressor gene on each chromosome in which a deletion was detected. Fearon and Vogelstein[7] have reported a genetic model for tumorigenesis in colorectal carcinoma which involves loss of heterozygosity (LOH) on chromosomes 5q, 17p, and 18q, and point mutation of the *ras* gene. They have also identified genes designated p*53* and *DCC* (deleted in colorectal carcinomas) as candidates for the tumor suppressor genes on chromosomes 17p and 18q, respectively.[8,9]

Many groups have reported LOH on chromosome 5q in colorectal carcinomas.[10–14] Early estimates suggested that 5q alleles were lost in at least 20% of colorectal tumors. However, additional restriction fragment length polymorphism (RFLP) markers located at chromosome 5q21-22 have revealed allelic losses in 50–60% of colorectal carcinomas. These data have suggested the presence of a tumor suppressor gene in this region, and recently a gene at 5q21, designated *MCC*, was identified as a candidate tumor suppressor for colorectal carcinoma.[15] This region of chromosome 5 also harbors the gene responsible for adenomatous polyposis coli (APC), an autosomal dominant disorder characterized by the presence of hundreds to thousands of adenomatous polyps in the colon, some of which progress to malignancy. The gene responsible for APC was mapped to chromosome 5q21-22 by linkage studies.[16,17] A map with relatively high resolution for the location of the *APC* gene was subsequently constructed by employing additional polymorphic markers, and a physical distance of $<3{,}000$ kb could be estimated between the *APC* locus and the closest marker, YN5.48.[18] However, whether the *MCC* gene is involved in APC is not yet known.

To confirm the location of a tumor suppressor gene for colorectal carcinoma on chromosome 5q, we undertook detailed deletion mapping by examining LOH at the *MCC* locus and in the surrounding region in 40 cases of sporadic colorectal carcinoma. Of the 12 RFLP markers used in our study, one (L5.71) contains two regions, each highly conserved in rodent DNA, that were used to identify the *MCC* gene. All 12 markers are tightly linked to APC.

MATERIALS AND METHODS

Tumor specimens Tumors and their corresponding normal mucosal tissues were obtained at surgery from 40 patients with colorectal carcinoma. Tissue specimens were rinsed in phosphate-buffered saline and frozen in liquid nitrogen immediately after removal. Routine histopathological diagnoses were performed for all tumors.

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Table I. Twelve RFLP Markers Used for the Analysis of LOH on Chromosome 5q

Marker	Restriction enzyme	Sizes of polymorphic alleles (kb)
E5.55	*Taq*I	8.5/7.1
YN5.64	*Taq*I	6.8/5.8
L5.62	*Bgl*II	10.5/6.2
L5.79	*Msp*I	1.3/1.0
L5.71	*Msp*I	4.4/4.3
	*Taq*I	8.0/4.9+3.1
EF5.44	*Msp*I	2.7/2.1
15A6	*Pvu*II	4.2/2.6+1.6
YN5.48	*Taq*I	7.4/5.1
L5.69	*Msp*I	7.2/6.4
LS5.3	*Msp*I	2.4/1.5
E5.57	*Pvu*II	3.2/2.9
KK5.97	*Taq*I	6.9/6.5

DNA analysis Frozen samples were ground to a very fine powder, transferred to a 15-ml tube and suspended in 4 ml of lysis buffer. High-molecular-weight DNAs were extracted in phenol/chloroform/isoamyl alcohol as described by Sato *et al.*[6] For analysis of LOH, DNA from each sample was digested with an appropriate restriction enzyme, electrophoresed in 0.8% agarose gel, and transferred to a nylon membrane (Pall Biodyne) with 0.1 *N* NaOH/0.1 *M* NaCl. The membrane was neutralized in 2×SSC and fixed by UV cross-linking. Prehybridization was performed in 7% PEG 8000/10% SDS containing 200 μg/ml of denatured human placental DNA or 100 μg/ml of denatured salmon sperm DNA, at 65°C overnight. Hybridization was conducted at 65°C for 16–24 h in the same solution, with a probe labeled with [^{32}P]dCTP by the random priming method.[19] After hybridization, the membrane was washed twice in 0.1× SSC/0.1% SDS at 65°C for 15 min and then exposed to Kodak XAR film at −70°C. The membrane was stripped in 0.4 *N* NaOH according to the method reported by Donis-Keller *et al.*,[20] and was repeatedly rehybridized.

LOH was recorded only if loss, or decrease in density, of one of a pair of bands on the autoradiogram was obvious. The probes used in this study, preferred restriction enzymes, and allele sizes are given in Table I.

RESULTS

LOH on chromosome 5q21-22 in sporadic colorectal carcinomas LOH was examined at 12 loci in all 40 cases. Figure 1 shows the linear order of these markers on the corresponding portion of chromosome 5q and the frequency of LOH for each of them. The order of these markers had been determined by physical and linkage mapping (unpublished data). All 40 cases were informative with at least one locus; 22 tumors (55%) showed LOH with at least one locus, and 13 of those 22 (59.1%) showed LOH at all informative loci. Because the number of informative cases for some probes was very small, the region was divided into 4 sections (A–D in Fig. 1) for presentation as overall percentages of LOH for all probes in each section. The most frequent losses were observed within section B (45.8%), where the *MCC* gene lies. The frequencies of losses were 28.6% in section A and 30.8% in section C. In section D, containing the most distal of the 12 markers, the frequency was 20.3%. These data indicated that loss is most consistent in the region surrounding L5.71.

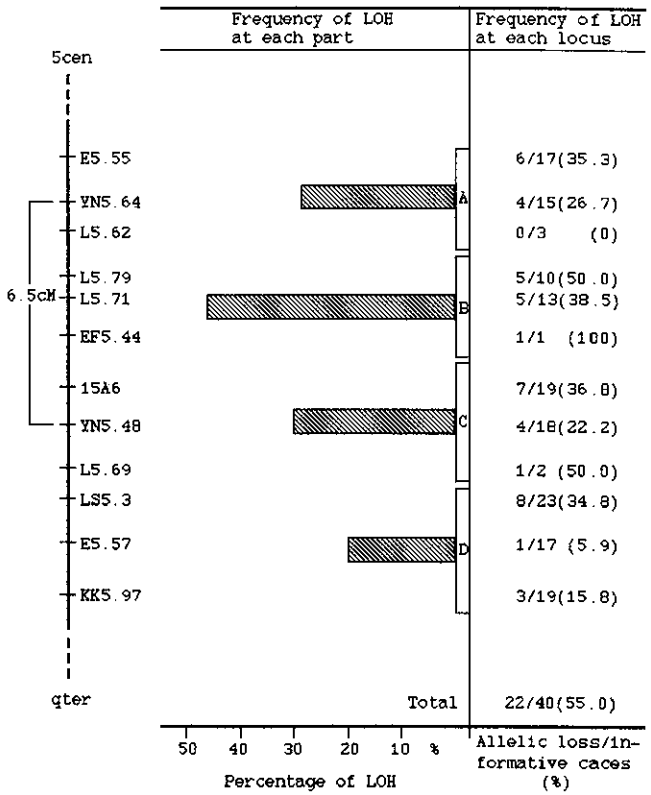

Fig. 1. Genetic map of markers on chromosome 5q21-22 and histogram showing the frequency of LOH at these loci. For convenience, the region was divided into four sections (A–D). A: E5.55, YN5.64, L5.62; B: L5.79, L5.71, EF5.44; C: 15A6, YN5.48, L5.69; D: LS5.3, E5.57, KK5.97.

Deletion mapping on chromosome 5q We constructed a detailed deletion map of chromosome 5q in each tumor. In 13 of the 22 tumors showing LOH, heterozygosity was lost at all informative loci. Figure 2 presents the results for the nine cases that showed loss of a small portion on chromosome 5q. Five tumors (Nos. 1–5) had interstitial

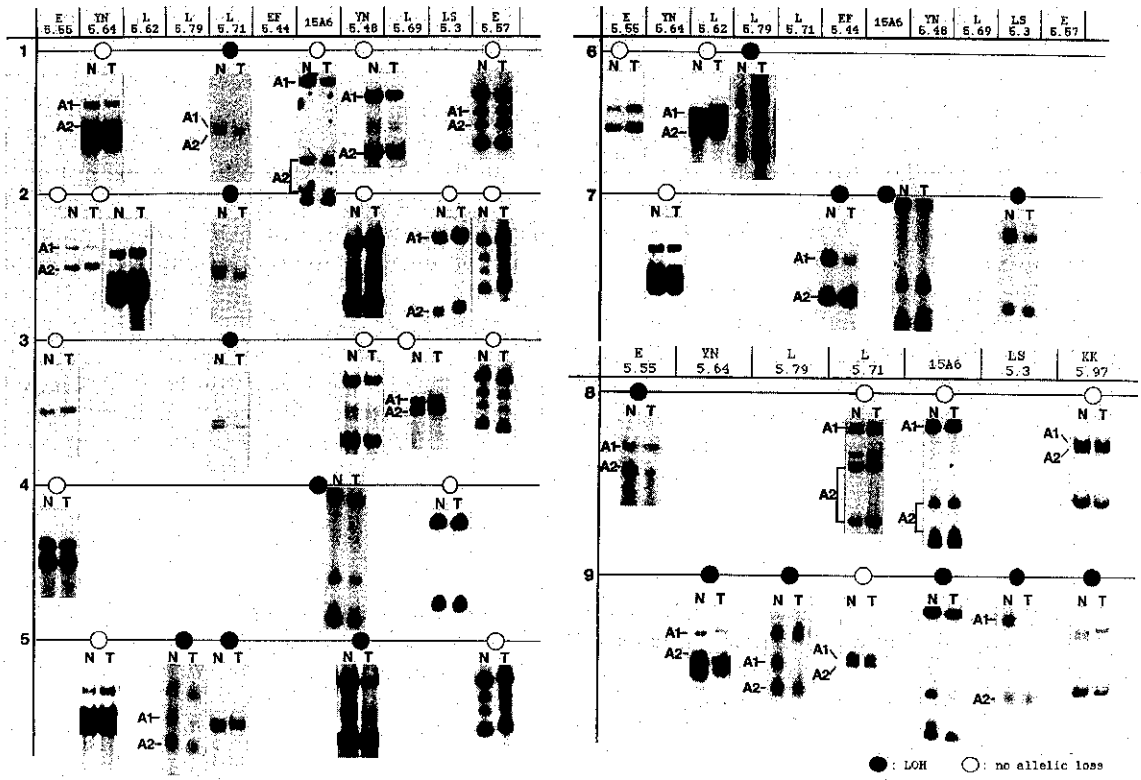

Fig. 2. Detailed deletion mapping of 12 markers on chromosome 5q21-22 in nine sporadic colorectal carcinomas. The loci are indicated above each map. Closed circles (●) indicate LOH; open circles (○) and horizontal lines (—) indicate no allelic loss and uninformative cases, respectively. Southern hybridizations of DNAs from tumor (T) and normal tissue (N) are shown under each circle. A1, A2 are polymorphic fragments. The probes and preferred restriction enzymes and allele sizes are described in Table I.

deletions including L5.71. The deletions in tumors No. 1 and No. 2 were between YN5.64 and 15A6, and between YN5.64 and YN5.48, respectively. A common region of deletion in these five tumors was identified in the region between YN5.64 and 15A6. Although it is not certain that the additional three tumors (Nos. 6–8) had interstitial deletions, the results from these tumors also provided information for a regional assignment. Combining the results from the eight tumors, a common region was further localized to the region between L5.62 and L5.71, the size of which is estimated to be nearly 3,000 kb. Tumor No. 9 showed an interesting pattern of LOH; L5.71 was retained but both distal and proximal loci were lost. As DNA of parents of this patient was not available, it is unclear whether chromosomal losses had occurred in only one chromosome, or whether the proximal region to L5.71 was lost in one allele and the distal region was lost in the other allele. If the latter is true, and in view of the results from the other eight tumors, L5.71 may contain the DNA sequence of a tumor suppressor gene associated with colorectal cancer.

DISCUSSION

Recent studies of LOH have shown that one or more tumor suppressor gene(s) for colorectal carcinoma might exist on chromosome 5q21-22, and the *MCC* gene at 5q21 has been identified as a candidate for this function. This chromosomal region, which includes the mapped position of the *APC* gene, is often deleted from the adenomas and carcinomas of patients with and without APC.[9–14] Therefore, a putative tumor suppressor gene on chromosome 5q21 appears to play a significant role in both sporadic and familial cases of colorectal tumors, but there is as yet no evidence that the *MCC* gene is involved in APC. In the work reported here, we detected a high incidence of LOH in the region immediately surrounding the *MCC* locus and showed that the frequencies of LOH

increased according to the proximity of the markers to *MCC*. Moreover, we constructed a detailed deletion map and were able to detect a common region of deletion between L5.62 and L5.71. This means that at least one tumor suppressor gene for colorectal carcinoma is located in this region. The data from tumors No. 8 and No. 9 imply that an allele was deleted in each of them proximal to L5.71; in tumor No. 9 an allele was deleted both distal and proximal to L5.71. On the basis of this information, L5.71 may detect sequences inside a tumor suppressor gene itself. Such critical cases are rare, but they can greatly assist the definitive identification of an intragenic sequence. Thus, the present LOH data support the possibility that the *MCC* gene located at L5.71 is a tumor suppressor gene for colorectal carcinoma, but the relationship between the *MCC* gene and the putative *APC* gene remains unclear.

Although colorectal carcinomas in the present study demonstrated a high incidence of LOH in the region surrounding the *MCC* locus, the frequency of LOH fell to 20–30% in the region 5–10 cM away from the *MCC* locus. Accordingly, a tumor suppressor gene may exist near the region in which LOH is not so frequent, and it is important to evaluate LOH data with great care.

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REFERENCES

1) Zbar, B., Brauch, H., Talmadge, C. and Linehan, M. Loss of alleles of loci on the short arm of chromosome 3 in renal cell carcinoma. *Nature,* **327,** 721–724 (1987).
2) Kok, K., Osigna, J., Carritt, B., Davis, M. B., van der Hout, A. H., van der Veen, A. Y., Landsvater, R. M., de Leij, L. F. M. H., Berendsen, H. H., Postmus, P. E., Poppema, S. and Buys, C. M. H. Deletion of a DNA sequence at chromosomal region 3p21 in all major types of lung cancer. *Nature,* **330,** 578–581 (1987).
3) Tsai, Y. C., Nichols, P. W., Hiti, A. L., Williams, Z., Skinner, D. G. and Jones, P. A. Allelic losses of chromosome 9, 11, and 17 in human bladder cancer. *Cancer Res.,* **50,** 44–47 (1990).
4) Fearon, E. R., Vogelstein, B. and Freinberg, A. P. Somatic deletion and duplication of genes on chromosome 11 in Wilms' tumor. *Nature,* **309,** 176–178 (1984).
5) Lee, W., Bookstein, R., Hong, F., Young, L., Shew, J. and Lee, E. Y. P. Human retinoblastoma susceptibility gene: cloning, identification, and sequence. *Science,* **235,** 1394–1399 (1987).
6) Sato, T., Tanigami, A., Yamakawa, K., Akiyama, F., Kasumi, F., Sakamoto, G. and Nakamura, Y. Allelotype of breast cancer: cumulative allele losses promote tumor progression in primary breast cancer. *Cancer Res.,* **50,** 7184–7189 (1990).
7) Fearon, E. R. and Vogelstein, B. A genetic model for colorectal tumorigenesis. *Cell,* **61,** 759–767 (1990).
8) Baker, S. J., Fearon, E. R., Nigro, J. M., Hamilton, S. R., Preisinger, A. C., Jessup, J. M., van Tuinen, P., Ledbetter, D. H., Barker, D. F., Nakamura, Y., White, R. and Vogelstein, B. Chromosome 17 deletions and p53 gene mutations in colorectal carcinomas. *Science,* **244,** 217–221 (1989).
9) Fearon, E. R., Cho, K. R., Nigro, K. M., Kern, S. E., Simons, J. W., Ruppert, J. M., Hamilton, S. R., Preisinger, A. C., Thomas, G. Kinzler, K. W. and Vogelstein, B. Identification of a chromosome 18q gene that is altered in colorectal cancers. *Science,* **247,** 49–56 (1990).
10) Solomon, E., Voss, R., Hall, V., Bodmer, W. F., Jass, J. R., Jeffreys, A. J., Lucibello, F. C., Patel, I. and Rider, S. H. Chromosome 5 allele loss in human colorectal carcinomas. *Nature,* **328,** 616–619 (1987).
11) Vogelstein, B., Fearon, E. R., Hamilton, S. R., Kern, S. E., Preisinger, A. C., Leppert, M., Nakamura, Y., White, R., Smith, A. M. M. and Bos, J. L. Genetic alterations during colorectal-tumor development. *N. Eng. J. Med.,* **319,** 525–532 (1988).
12) Law, D. J., Olschwang, S., Monpezat, J-P., Lefrancois, D., Jagelman, D., Petrelli, N. J., Thomas, G. and Feinberg, A. P. Concerted nonsyntenic allelic loss in human colorectal carcinoma. *Science,* **241,** 961–965 (1988).
13) Ashton-Rickardt, P. G., Dunlop, M. G., Nakamura, Y., Morris, R. G., Purdie, C. A., Steel, C. M., Evans, H. J., Bird, C. C. and Wyllie, A. H. High frequency of APC loss in sporadic colorectal carcinoma due to breaks clustered in 5q21-22. *Oncogene,* **4,** 1169–1174 (1989).
14) Miyaki, M., Seki, M., Okamoto, M., Yamanaka, A., Maeda, Y., Tanaka, K., Kikuchi, R., Iwama, T., Ikeuchi, T., Tonomura, A., Nakamura, Y., White, R., Miki, Y., Utsunomiya, J. and Koike, M. Genetic changes and histopathological types in colorectal tumors from familial adenomatous polyposis. *Cancer Res.,* **50,** 7166–7173 (1990).
15) Kinzler, K. W., Nilbert, M. C., Vogelstein, B., Bryan, T. M., Levy, D. B., Smith, K. J., Preisinger, A. C., Hamilton, S. R., Hedge, P., Markham, A., Carlson, M., Joslyn, G., Groden, J., White, R., Miki, Y., Miyoshi, Y., Nishisho, I. and Nakamura, Y. Identification of a chromosome 5q21 gene that is mutated in colorectal cancers. *Science,* **251,** 1366–1370 (1991).
16) Bodmer, W. F., Bailey, C. J., Bodmer, J., Bussey, H. J. R., Ellis, A., Gorman, P., Lucibello, F. C., Murday, V. A.,

Rider, S. H., Scambler, P., Sheer, D., Solomon, E. and Spurr, N. K. Localization of the gene for familial adenomatous polyposis on chromosome 5. *Nature,* **328,** 614–616 (1987).

17) Leppert, M., Dobbs, M., Scambler, P., O'Connell, P., Nakamura, Y., Stauffer, D., Woodward, S., Burt, R., Hughes, J., Gardner, E., Lathrop; M., Wasmuth, J., Lalouel, J-M. and White, R. The gene for familial polyposis coli maps to the long arm of chromosome 5. *Science,* **238,** 1411–1413 (1987).
18) Nakamura, Y., Lathrop, M., Leppert, M., Dobbs, M., Wasmuth, J., Wolff, E., Carlson, M., Fujimoto, E., Krapcho, K., Sears, T., Woodward, S., Hughes, J., Burt, R., Gardner, E., Lalouel, J-M. and White, R. Localization of the genetic defect in familial adenomatous polyposis within a small region of chromosome 5. *Am. J. Hum. Genet.,* **43,** 638–644 (1988).
19) Feinberg, A. P. and Vogelstein, B. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.,* **132,** 6–13 (1983).
20) Donis-Keller, H., Green, P., Helms, C., Cartinhour, S., Weiffenbach, B., Stephens, K., Keith, T. P., Bowden, D. W., Smith, D. R., Lander, E. S., Botstein, D., Akots, G., Rediker, K. S., Gravius, T., Brown, V. A., Rising, M. B., Parker, C., Powers, J. A., Watt, D. E., Kauffman, E. R., Bricker, A., Phipps, P., Muller-Kahle, H., Fulton, T. R., Ng, S., Schumm, J. W., Braman, J. C., Knowlton, R. G., Barker, D. F., Crooks, S. M., Lincoln, S. E., Daly, M. J. and Abrahamson, J. A genetic linkage map of the human genome. *Cell,* **51,** 319–337 (1987).