Multiple Intestinal Neoplasia Caused by a Mutation in the Murine Homolog of the APC Gene

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Germ-line mutations of the APC gene are responsible for familial adenomatous polyposis (FAP), an autosomal dominantly inherited disease in humans. Patients with FAP develop multiple benign colorectal tumors. Recently, a mouse lineage that exhibits an autosomal dominantly inherited predisposition to multiple intestinal neoplasia (Min) was described. Linkage analysis showed that the murine homolog of the APC gene (mAPc) was tightly linked to the Min locus. Sequence comparison of mAPc between normal and Min-affected mice identified a nonsense mutation, which cosegregated with the Min phenotype. This mutation is analogous to those found in FAP kindreds and in sporadic colorectal cancers.

One or more tumor suppressor genes located on chromosome 5q are important in the development of colorectal cancer (1). Two candidate tumor suppressor genes (APC and MCO), on chromosome 5q21 (2–4), are somatically mutated in sporadic colorectal cancers (2, 5). The APC gene is also mutated in the germ line of patients with FAP, an autosomal dominantly inherited disease that predisposes to colorectal cancer (3, 6). Patients affected with FAP develop hundreds to thousands of benign colorectal tumors during their second and third decades of life. Some of these tumors will progress to cancer if they are not removed. Extracolonic manifestations, such as gastric and small intestinal polyps, osteomas, and desmoid tumors, have been observed in some FAP patients (7).

A previously described mutant mouse lineage that is predisposed to Min may provide a model for colorectal tumorigenesis. This lineage was established from an ethynitrosourea-treated C57BL/6 (B6) male mouse (8). The Min phenotype is a fully penetrant autosomal dominant trait. Young adult Min mice develop numerous adenomas throughout their intestinal tract. Although the distribution of tumors along the intestine is different from that in human FAP, the fact that both phenotypes are autosomal dominantly inherited and involve multiple intestinal tract tumors raises the possibility that Min results from a mutation in the murine homolog of the APC gene (mAPc).

To test this possibility, we determined whether mAPc was linked to the Min locus. We screened an adult BALB/c mouse brain cDNA library at reduced stringency with fragments of the human APC cDNA (9). One murine cDNA clone, mAPc-51a, corresponding to nucleotides 135 to 1412 of the human APC sequence (10), was then used to investigate linkage between mAPc and Min in segregating backcrosses. Min-affected B6 male mice were crossed to AKR/J (AKR) or CAST/Ei (CAST) females. The Min-affected F1 mice were then backcrossed to normal B6 mice in each parental orientation. The resulting backcross mice were scored (8) for the Min phenotype (the presence of intestinal tumors). If Min is a mutation in mAPc, then mice in the backcross expressing the Min phenotype would be homozygous for the B6 alleles of mAPc. Conversely, backcross mice that do not express the Min phenotype would be heterozygous (B6/AKR or B6/CAST).

A Pst I restriction fragment length polymorphism (RFLP) between the B6 and AKR or CAST strains was identified by Southern (DNA) blot hybridization to the mAPc-51a probe (Fig. 1). This polymorphism was used to analyze 74 mice from the CAST backcross, and no discordance between the inheritance of the B6 mAPc alleles and the Min phenotype was observed (Table 1). Analysis of 95 mice from the AKR backcross revealed a single discordant animal that was heterozygous but had tumors (Table 1). These results suggest that Min resulted from a mutation in the mAPc or a closely linked gene.

More complete examination of this discordant mouse revealed that it did not reflect fully the Min phenotype; it had lived much longer than most Min mice and had carried only two duodenal tumors, whereas Min-affected AKR backcross animals had an average of 16 tumors (SD, 12; range, 2 to 50). Sporadic intestinal tumors in mice, especially of the duodenum, have been documented (11) and are the probable source of tumors in this mouse.

We sequenced the entire coding region of mAPc to identify the mutation in Min mice. To maximize the information obtained in this study, we sequenced the mAPc cDNA from five mice: a normal B6 mouse, a Min B6 mouse, a normal (B6 × CAST) F1 mouse, a Min (B6 × CAST) F1 mouse, and a normal CAST mouse. Two different Min mice were used to identify and confirm any mutation responsible for the Min phenotype. However, it was possible that the Min mutation would only affect the amount of mAPc expression. The three normal mice were included to facilitate identification of nucleotide sequence polymorphisms between B6 and CAST. These polymorphisms could then be used to determine whether both wild-type and mutant alleles of mAPc were expressed. Total RNA was isolated from brains of the five mice, and randomly primed first strand cDNA was prepared with the use of reverse transcriptase (RT). The polymerase chain reaction (PCR) was then used to amplify (3) the coding region of the cDNA for mAPc. Fifteen pairs of primers were used to amplify the coding region of mAPc from the cDNA (12). Each PCR product was then cloned, and pools of clones were sequenced with internal primers (13). The entire coding region of mAPc and 27 base pairs (bp) of the 3' untranslated region was sequenced.

The mAPc transcript had an open reading frame of 8535 bp that could encode a protein of 2845 amino acids if translation was initiated at the first methionine of the open

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Fig. 1. Linkage between mAPc and the Min phenotype. Genomic DNA (10 to 15 μg) isolated from parental strains and backcross animals was digested with the restriction enzyme Pst I, and Southern blot analysis was performed with mAPc-51a as the probe. Animals with intestinal tumors were scored as Min, and tumor-free animals were scored as normal. (A) The probe identified a unique 3.4-kb fragment in the CAST DNA. This 3.4-kb fragment was present in the normal but not in the Min animals of the backcross generation. (B) The probe identified a unique 4.3-kb fragment in the AKR DNA. This 4.3-kb AKR fragment was present in the normal but not in the Min animals of the backcross generation.

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reading frame (14). The murine and human APC coding sequences were 86 and 90% identical at the nucleotide and amino acid levels, respectively. All the amino acid motifs previously identified in the human APC gene product are conserved in the mAPc gene product. These include the heptad repeats, n-2 homology, masurine acetylcholine receptor (mAChR)-G protein coupling homology, and the 20-amino acid repeats (3, 6). A shorter, alternatively spliced transcript of mAPc, corresponding to a previously described alternatively spliced human APC transcript (4, 6), was also identified by RT-PCR (15).

There were 32 nucleotide polymorphisms in the coding region and 1 nucleotide polymorphism in the 3′ untranslated region between B6 and CAST (14). Because all of the B6-specific polymorphisms were present in the mAPc cDNA from the Min-affected (B6 × CAST) F1 mouse, we concluded that the entire coding region of both alleles was expressed in Min mice.

A transversion from T to C at nucleotide 2549 (16) was found in both Min-affected mice but not in any of the three normal mice (Fig. 2A). This Min-specific mutation converts codon 850 from a leucine (TTG) to a stop (TAG) codon. Nonsense mutations such as this one are frequently observed in FAP kindreds (5, 6). To confirm the cosegregation of the mutation with the B6 allele of the mAPc gene, we sequenced several individual clones of RT-PCR product from the Min-affected (B6 × CAST) F1 mouse. The results show that the nonsense mutation was present only in the B6 allele (Fig. 2B).

We confirmed the cosegregation of the nonsense mutation with the Min phenotype by allele-specific hybridization. The region of the genome spanning the mutation was amplified by PCR from 107 mice, including parental, F1, and the backcross animals from Table 1. The PCR products were blotted onto duplicate filters and hybridized with either a mutant-specific oligonucleotide or a control oligonucleotide (Fig. 3). Only the DNA from those animals with the typical Min phenotype hybridized with the mutant-specific oligonucleotide. The discordant mouse described above did not carry the Min-specific mutation. This result is consistent with the suggestion that this animal had sporadic, rather than Min-induced, tumors (17).

Thus, a germ-line nonsense mutation in the mAPc gene is responsible for the Min phenotype. The phenotypic and genetic similarities between murine Min and human FAP suggest that the Min mouse is an excellent animal model for the human disease FAP. Because mutation of the APC gene occurs somatically during the development of sporadic colorectal tumors in humans (5), Min mice should also be a suitable model for human colorectal cancer in

![Fig. 2. Identification of a Min-specific nonsense mutation in mAPc. (A) A T-to-C mutation in Min mice. The mutation is indicated by an arrowhead. Cloned RT-PCR product (3, 13) derived from total RNA isolated from mice was sequenced. Lanes 1, a normal B6 mouse; lanes 2, a normal CAST mouse; lanes 3, a normal B6 mouse; lanes 4, a Min-affected (B6 × CAST) CAST mouse; lanes 5, a normal CAST mouse. The primers used for PCR were 5′-GCCATCCCTTCAGGGTGTAGG-3′ and 5′-TTCCACTTTGCGCATAGGCAC-3′, which amplified nucleotides 2241 to 2859 of the mAPc cDNA. The primer used for sequencing was 5′-ACAACGCTACCTTAGG-3′, corresponding to nucleotides 2365 to 2384 of the mAPc cDNA. (B) The cosegregation of the nonsense mutation with the B6 allele. RT-PCR product of the DNA from a Min-affected (B6 × CAST) CAST mouse was cloned and sequenced as described (13) except that individual clones were analyzed. B6 and CAST indicate the B6 and the CAST alleles, respectively. M indicates the sequence spanning the mutated region. The mutated nucleotide is indicated with an asterisk. P indicates the sequence spanning the polymorphic region. The primers used for PCR were 5′-ACAACGCTACCTTAGG-3′ and 5′-TTCCACTTTGCGCATAGGCAC-3′, which amplified nucleotides 1615 to 2859 of the mAPc cDNA. The primer used for sequencing the polymorphic region was 5′-TAGGAAACGAGAAGCTTAGG-3′, corresponding to nucleotides 2256 to 2275 of the mAPc cDNA.](image-url)

![Fig. 3. Identification of the Min-specific mutation by allele-specific hybridization. (A) Hybridization with the control oligonucleotide. (B) Hybridization with the mutant-specific oligonucleotide. (C) The pattern of samples blotted on the filters. These are PCR products unless otherwise indicated. Sources of DNA used in PCR are indicated as follows: 1, a normal B6 mouse; 2, a normal AKR mouse; 3, a normal AKR mouse; 4, a Min-affected (B6 × CAST) CAST mouse; 5, a normal AKR mouse; 6, a normal CAST mouse; 7, a Min-affected (B6 × CAST) F1 mouse; 8, a Min-affected (B6 × CAST) CAST mouse; 9, a Min-affected (B6 × CAST) CAST mouse; 10, a normal CAST mouse; 11, a normal B6 mouse; 12, a normal AKR mouse; 13, a Min-affected (B6 × CAST) F1 mouse; 14, a Min-affected (B6 × CAST) CAST mouse; 15, a normal AKR mouse. PCR reactions were performed as described (3) except that 100 to 200 ng of genomic DNA were used as template. Primers used for the PCR were the same as described in (A). Approximately 100 ng of the PCR product was blotted onto each filter. For plasmids, about 500 ng of DNA was blotted onto each filter. Hybridization was carried out as described in (9) except at 45°C and washing was carried out at 50°C in 450 mM NaCl, 18 mM sodium citrate, 1 mM tris, pH 7.2, and 0.1% SDS. The sequence for the mutant-specific oligonucleotide is 5′-ACAACGCTACCTTAGGAAAGCTTAGG-3′, which hybridizes to nucleotides 2540 to 2558 of the mAPc cDNA. The underlined A is the mutated nucleotide. The sequence for the control oligonucleotide is 5′-ACAACGCTACCTTAGGAAAGCTTAGG-3′, which hybridizes to nucleotides 2571 to 2588 of the mAPc cDNA.](image-url)

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Table 1. Linkage analysis demonstrating cosegregation of the Min phenotype with mAPc. The backcross cohort includes all mice crossed with the Min B6 mice to obtain the F1 generation. Cosegregation of the Min phenotype and the B6 allele of mAPc was determined by RFLP analysis using the mAPc-5′fl probe. Animals with intestinal tumors were scored as Min, and tumor-free animals were scored as normal. The non-B6 allele is either the AKR or the CAST allele, depending on the backcross set. Animals were randomly chosen from the Min and normal set in each backcross. Seventy-four animals were analyzed from the CAST backcross set, which consisted of 135 Min and 103 normal mice. Ninety-five animals were analyzed from the AKR backcross set, which consisted of 77 Min and 77 normal mice.
A similar analysis of mice from the CAST backcross yielded no evidence for sporadic tumors.


20. Supported in part by grants from the Clayton Fund, McAshan Fund, National Foundation for Cancer Research, Damon Runyon-Walter Winchell Cancer Fund (DRG-1058), and NIH grants CA-07175, CA-06973, CA-23076, CA-50585, and CA-35494. We thank M. Woch for care of the mouse genetic resource, E. Mattes for help with mice and with DNA preparations, N. Bornstein for numerous DNA preparations, and I. Riegel for help with the manuscript. This is publication #3230 from the Laboratory of Genetics.

30 December 1991; accepted 10 March 1992