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BRIEF COMMUNICATION

Somatic Genetic Events Linked to the *Apc* Locus in Intestinal Adenomas of the Min Mouse

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We have found previously that all spontaneous intestinal adenomas from Apc^{+}/Apc^{Min} mice lose the wild type *Apc* marker on two genetic backgrounds. On the (AKR × B6)F_1 background, this event involves loss of the entire homolog of mouse chromosome 18 carrying Apc^{+}. This chromosome carries both the *Mcc* and *Dcc* genes, which are homologs of genes that have been implicated in human colorectal cancer. To determine whether the loss of alleles of *Mcc* and/or *Dcc* is necessary for the formation of intestinal adenomas, subchromosomal somatic events were induced by γ-irradiation. The observed spectrum of intrachromosomal somatic genetic losses rules out a requirement for loss of heterozygosity at either locus during adenoma formation. Subchromosomal allelic losses linked to Apc^{+} occur spontaneously on other genetic backgrounds. In the majority of these events, the Apc^{+} allele itself was somatically lost, as judged by the wild type marker at the *Min* site. However, on the [*M. musculus castaneus* (CAST) × B6-Min]F_1 and (129/Sv × B6-Min)F_1 backgrounds, spontaneous adenomas were observed in which the wild type marker at the *Min* site was retained. Further analysis will be required to determine whether these exceptions involve intra-*Apc* mutations. If not, then these events would illustrate routes to intestinal neoplasia that do not require complete inactivation of wild type *Apc* function. *Genes Chromosom Cancer 17:194–198 (1996).*

Familial adenomatous polyposis (FAP) is one of the familial colon cancer syndromes of the human in which the genetic etiology is becoming understood. Germline mutations in the adenomatous polyposis coli (*APC*) gene are carried in heterozygous form in affected individuals of FAP families. The *APC* gene has been mapped to chromosomal region 5q21-22 of the human genome (Bodmer et al., 1987; Groden et al., 1991, 1995; Joslyn et al., 1991; Kinzler et al., 1991a; Nishisho et al., 1991). At the adenomatous polyp stage of intestinal neoplasia in FAP individuals and in sporadic cases, somatic genetic events are found to affect the wild type *APC* allele, often resulting in a truncated APC product (Solomon et al., 1987; Ashton-Rickardt et al., 1989, 1991; Miyaki et al., 1990; Miyoshi et al., 1992a,b; Powell et al., 1992; Nagase and Nakamura, 1993). To date, no case has been reported in which the entire homolog of human chromosome 5 carrying the normal *APC* allele is lost in intestinal polyps or tumors.

A mouse strain, Min (multiple intestinal neoplasia), has been produced by chemical mutagenesis of the mouse germline (Moser et al., 1990). This strain carries a nonsense mutation of *Apc*, the mouse homolog of *APC* (Su et al., 1992). The Min mouse matches human FAP families in the primary genetic lesion and in the predisposition to intestinal tumors and certain extracolonic manifestations (for review, see Dove et al., 1994, 1995); however, the Min mouse is not a perfect match to the corresponding human condition. Our goal in the work reported here was to study the patterns of somatic genetic events linked to Apc^{+} in the adenomas that form in Min mice both spontaneously and after γ-irradiation.

To determine whether it is necessary to lose the wild type Apc^{+} allele in the adenomas of Min mice, we previously analyzed intestinal adenomas from Apc^{+}/Apc^{Min} mice on two genetic backgrounds, C57BL6/J (B6) and (AKR × B6-Min)F_1. We performed these analyses by determining the ratio of wild type (Min^{+}) to mutant (*Min*) DNA fragments in polymerase chain reaction (PCR) products templated by DNA collected from sectioned tumor samples. The Min^{+}/Min ratio was reduced greatly in all tumor samples (Luongo et al., 1994). The loss of Apc^{+} in (AKR × B6-Min)F_1 mice was accompanied by allelic losses at loci along the entire chromosome 18 homolog carrying Apc^{+}. Loss of an entire chromosome is the most common mechanism of allelic loss in mouse tumors (Hegi et al., 1993; Dietrich et al., 1994; Luongo et al., 1994; Wiseman et al., 1994). This pattern of somatic events differs from that observed in human colorectal adenomas. One possible biological ex-

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planation for this difference is that adenoma formation in the mouse requires loss of function at more than the *Apc* locus. For example, *Mcc* and *Dcc*, the mouse homologs of two genes implicated in human colorectal cancer (Fearon et al., 1991; Kinzler et al., 1991b), also map to mouse chromosome 18 (Justice et al., 1992; Luongo et al., 1993). One or both of these loci may also need to lose function when tumors form in Min mice. In the human genome, by contrast, *DCC* is not syntenic with *APC* and *MCC*.

To determine what region of chromosome 18 must undergo somatic loss, we induced intestinal adenomas by exposing neonatal Min mice to a single dose of γ-rays. Double-strand breaks in DNA caused by γ-rays can lead to interstitial deletions and chromosomal rearrangements (for review, see Breimer, 1988). Mice were irradiated sublethally with a single dose of 0.80 Gy at 9–12 days of age. This age span was chosen because carcinogenic treatment of mice prior to weaning has been shown to result in a higher rate of survival and intestinal adenoma induction compared with treatment at older ages (Abrams, 1951; Shoemaker et al., 1995).

The irradiated group consisted of 25 (AKR × B6-Min)F_1 mice (10 Apc^+/Apc^{Min} and 15 Apc^+/Apc^+). For controls, 15 nonirradiated (AKR × B6-Min)F_1 mice were analyzed in parallel (nine Apc^+/Apc^{Min} and six Apc^+/Apc^+). At 150 days of age, the mice were killed by CO_2 asphyxiation, and the intestinal tract from the duodenum to the colon was excised and processed as described in detail previously (Luongo et al., 1994). First, tumors from the entire intestinal tract were counted under a dissecting microscope at ×5 magnification; then, individual tumor samples were collected. The average tumor multiplicity of the irradiated Apc^+/Apc^{Min} mice was 28, whereas that of the untreated Apc^+/Apc^{Min} mice was 8 (Fig. 1a). This increase in intestinal adenoma multiplicity is significant ($P = 0.002$; Wilcoxon rank sum test). Because no tumors were observed in irradiated or untreated (AKR × B6)F_1 Apc^+/Apc^+ mice, the majority, if not all, of the adenomas in the irradiated mice depend on the germline *Min* mutation.

To determine whether these tumors remain heterozygous for loci on chromosome 18, we analyzed 55 intestinal adenomas and 23 normal intestinal tissue samples from nine irradiated mice by quantitative PCR, as described previously (Luongo et al., 1994). DNA was isolated from each tissue sample and analyzed for the ratio of wild type to mutant PCR product at the *Min* site and for the ratio of AKR to B6 product at three polymorphic loci on chromosome 18: *D18Mit19*, *D18Mit14*, and *D18Mit33* (Fig. 2). The AKR alleles at these loci lie *cis* to the Apc^+ allele in the F_1 animal. The unlinked marker *D7Mit38* was included in the *D18Mit33* PCRs as an internal control for the allelic loss analysis.

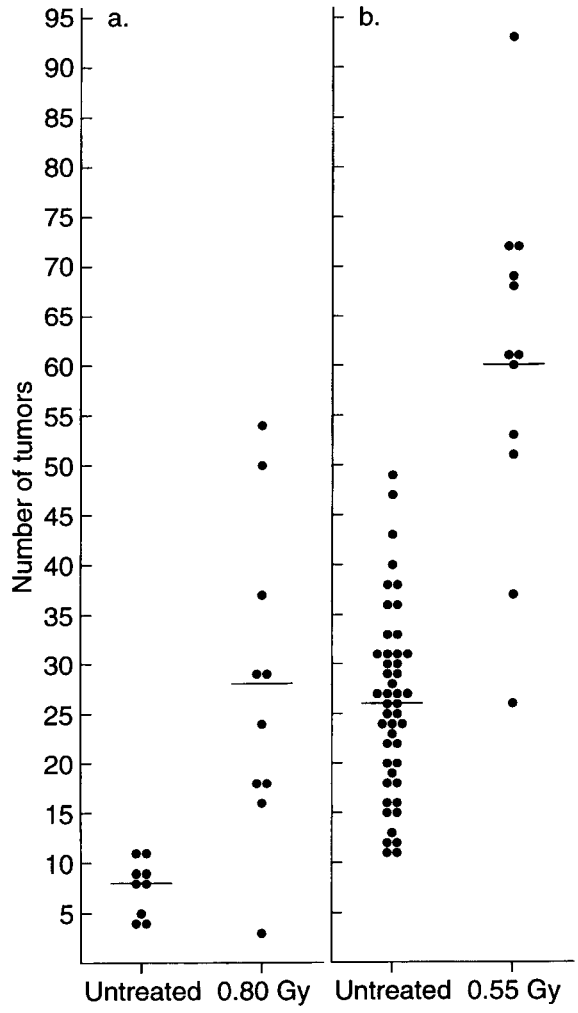

Figure 1. Distribution of tumor number in γ-irradiated and untreated Apc^+/Apc^{Min} mice. Tumor multiplicity is depicted by a scatter plot with each point representing the number of intestinal adenomas from a single animal. **a:** Distribution for (AKR × B6-Min)F_1 Apc^+/Apc^{Min} mice. Tumor numbers ranged from 4 to 11 (untreated) and from 3 to 54 (0.80 Gy). The mean tumor numbers, which are indicated by horizontal lines (± standard deviation), were 8 ± 3 (untreated) and 28 ± 16 (0.80 Gy). **b:** Distribution for B6-Apc^+/Apc^{Min} mice. Tumor numbers ranged from 11 to 49 (untreated) and from 26 to 93 (0.55 Gy). The mean tumor numbers were 26 ± 9 (untreated) and 60 ± 17 (0.55 Gy). *Apc*, adenomatous polyposis coli; *Min*, multiple intestinal neoplasia.

The predominant mechanism for Apc^+ loss in intestinal adenomas after neonatal γ-irradiation of (AKR × B6-Min)F_1 Apc^+/Apc^{Min} mice remains homolog loss (Fig. 2). For 9 of the 55 adenomas, however, interstitial loss of chromosome 18 AKR alleles surrounding Apc^+ was observed. Most, if

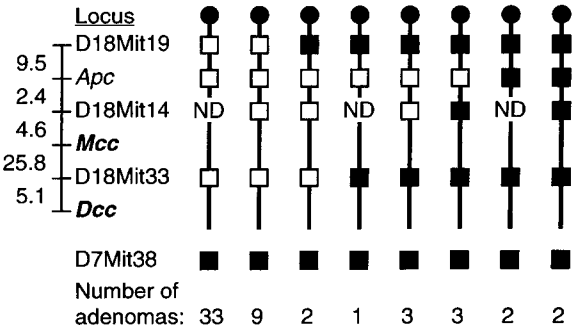

Figure 2. Allelic loss in γ-ray-induced intestinal adenomas from (AKR × B6-Min)F_1 mice. Allelic loss was considered to have occurred in a sample if the adenoma/control value of Apc^+/Apc^{Min} was ≤0.5 (AKR allele loss) or ≥2.0 (B6 loss; see Luongo et al., 1994). Open squares indicate loss, and solid squares indicate retention of the AKR allele. No B6 allele loss was detected in any adenoma. Loci are shown in map order with distances in cM (Luongo et al., 1993, 1994; C. Luongo, unpublished results). These loci span 42.3 cM of the 60 cM length of mouse chromosome 18 (Johnson and Davisson, 1994). Candidate suppressor genes *Mcc* and *Dcc* are indicated in boldface. ND, not done.

not all, of these nine cases of interstitial loss were caused by the neonatal γ-irradiation. Because γ-irradiation increased the intestinal adenoma multiplicity approximately 3.5-fold, 39 [95% confidence interval (CI), 31–47] of the 55 adenomas resulted from γ-irradiation.

Two specific loci on mouse chromosome 18, *Mcc* and *Dcc*, are candidates for controlling the progression of intestinal neoplasia. However, the marker *D18Mit33*, which lies between *Apc* and *Dcc*, remained heterozygous in 7 of the 51 adenomas in which the Min^+ marker was lost (of 55 total adenomas; Fig. 2). In the remaining four adenomas, the Min^+ marker was retained, and chromosome 18 remained heterozygous; these exceptions will be discussed below. Similarly, the marker *D18Mit14*, which lies between *Apc* and *Mcc*, remained heterozygous in three adenomas that had lost the Min^+ marker (Fig. 2). Thus, allelic loss of neither *Mcc* nor *Dcc* is required for *Min*-induced adenoma formation. Loss of *Mcc* or *Dcc* was never observed without Apc^+ loss. The *DCC* gene seems to be altered late in human colon cancer (see Fearon et al., 1991). Therefore, one may not expect *Dcc* to be involved in adenomagenesis in the Min mouse. To be completely certain that no somatic lesion has been incurred at the *Mcc* and *Dcc* loci in these cases of interstitial Apc^+ loss, sequencing will be necessary.

Can neonatal γ-irradiation generate adenomas by a route that retains the wild type Apc^+ function? Irradiated Apc^+/Apc^+ wild type controls did not develop intestinal adenomas. Strikingly, 4 of the 55 adenomas analyzed from irradiated (AKR × B6-Min)F_1 Apc^+/Apc^{Min} heterozygotes retained the wild type *Apc* marker at the *Min* site (Fig. 2). It is possible that γ-irradiation can generate adenomas by a route that retains one wild type copy of *Apc*. For example, it may produce a lesion in a separate locus that interacts with the heterozygous *Min*/+ state of the *Apc* locus. Alternatively, γ-irradiation may cause intragenic lesions that inactivate the wild type allele but that were not detected in our assay, which examined only the region of *Apc* surrounding the *Min* site.

To determine whether neonatal γ-irradiation of B6-Apc^+/Apc^{Min} mice also can generate tumors retaining the Min^+ marker, we exposed 12 B6-Apc^+/Apc^{Min} and 14 Apc^+/Apc^+ mice at 10 days of age to a single dose of 0.55 Gy. The irradiated mice and their untreated litter mates (47 Apc^+/Apc^{Min} and 61 Apc^+/Apc^+) were scored for intestinal adenomas at 90 days of age. Treatment with γ-rays caused a 2.3-fold increase in intestinal adenoma multiplicity in Apc^+/Apc^{Min} mice (Fig. 1b; $P = 3.4 \times 10^{-6}$; Wilcoxon rank sum test). One of the irradiated Apc^+/Apc^+ mice developed a single colonic adenoma, whereas none of the untreated Apc^+/Apc^+ mice developed tumors.

Twenty-one colonic adenomas and three control tissue samples from three of the irradiated B6-Apc^+/Apc^{Min} mice were analyzed for the presence of the Min^+ marker by quantitative PCR (Luongo et al., 1994). Of these 21 adenomas, 9 were radiation induced (95% CI, 3–15). The ratio of Min^+ to *Min* markers was calculated for each DNA sample. The individual Min^+/Min ratios did not exceed 0.33 for the adenomas (average, 0.15 ± 0.08), whereas the ratios for the normal tissue controls were 0.51 or greater (average, 0.70 ± 0.26). This difference is highly significant ($P = 9.68 \times 10^{-4}$; Wilcoxon rank sum test), indicating that all of the adenomas showed extensive loss of the Min^+ marker. Thus, in this limited set of tumors on the B6 genetic background, no instance of *Min*-induced adenomagenesis has yet been found involving an intragenic lesion in the Apc^+ gene or mutation of another locus that interacts with the Apc^+/Apc^{Min} state.

On the (AKR × B6-Min)F_1 genetic background, spontaneous adenomas lost the alleles of all scored loci on chromosome 18 linked to Apc^+ in each of 50 tumors (Luongo et al., 1994). To determine whether certain heterozygous genetic backgrounds would show a different pattern of spontaneous somatic genetic events, we surveyed F_1 hybrids in which the AKR parent was replaced either by DBA/2 (DBA), 129/Sv (129), or *M. musculus castaneus* (CAST). Spontaneous intestinal adenomas

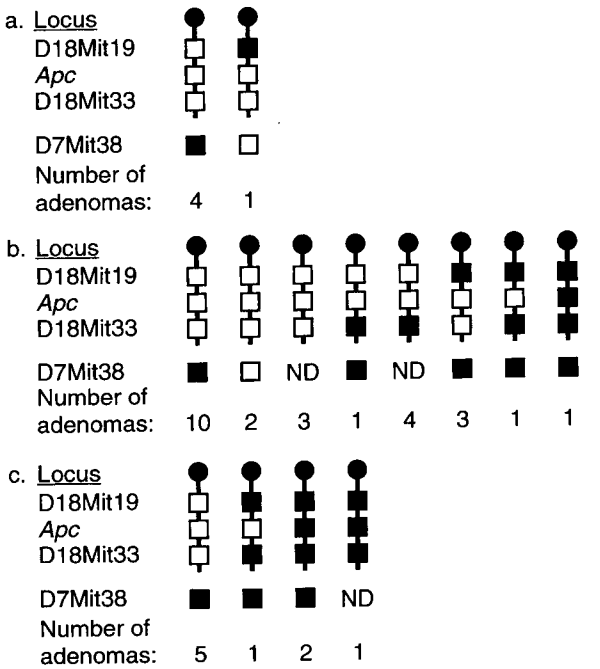

Figure 3. Allelic loss in spontaneous intestinal adenomas from F_1 Apc^+/Apc^{Min} mice. The genetic backgrounds are (DBA × B6-Min; **a**), (129 × B6-Min; **b**), and [*M. musculus castaneus* (CAST) × B6-Min; **c**). Open squares indicate loss of the non-B6 allele (adenoma/control value of $Apc^+/Apc^{Min} \leq 0.5$). Solid squares indicate retention of the non-B6 allele. No B6 allele loss was seen in any adenoma (adenoma/control value of $Apc^+/Apc^{Min} \geq 2$). The chromosome 18 loci are shown in map order. ND, not done.

were analyzed for allelic loss of the markers *D18Mit19*, Min^+, *D18Mit33*, and *D7Mit38* (as a control). Allelic loss of the control marker *D7Mit38* occurred in 3.5% (3/86, including those studied by Luongo et al., 1994) of all adenomas analyzed, independent of genetic background. This loss frequency is within the range (0–4%) seen for non-chromosome 18 markers (Luongo et al., 1994). The results of these studies are summarized in Figure 3.

Among the small set of five spontaneous adenomas analyzed on the (DBA × B6)F_1 background, one instance of subchromosomal allelic loss was found that can be explained either by mitotic recombination between the *D18Mit19* and *Apc* loci or by terminal deletion (Fig. 3a). Among 25 adenomas analyzed on the (129 × B6)F_1 background, we found nine cases of subchromosomal allelic loss (Fig. 3b). These data differ very significantly from those observed on the (AKR × B6-Min)F_1 background ($P = 4 \times 10^{-6}$; Fisher's exact test). One case of retention of the Min^+ marker was noted on this F_1 hybrid background; such a situation has also been observed by Laird and his colleagues (1995).

On the (CAST × B6)F_1 genetic background, one of nine spontaneous adenomas showed subchromosomal losses of markers linked to Apc^+. Strikingly, in three other cases, the wild type fragment at the *Min* site was retained (Fig. 3c). This pattern of retention of at least the Min^+ fragment of *Apc* in the (CAST × B6-Min)F_1 hybrid differs significantly from the spontaneous loss of the entire Apc^+ chromosome observed in (AKR × B6-Min)F_1 tumors ($P = 0.003$; Fisher's exact test). We do not know the genetic basis for the difference between F_1 hybrids involving AKR and those involving CAST in the pattern of somatic events leading to *Min*-induced adenomas. The *Mom1* locus that affects the multiplicity of adenomas in Min mice carries a resistance allele in both AKR and CAST (Dietrich et al., 1993). Thus, this locus cannot provide a simple explanation for this difference.

All of the intestinal tumors analyzed were classified histologically as adenomas. No correlation was detected between subchromosomal allelic loss and adenoma histology or size (data not shown). Thus, there is no evidence that loss of alleles at loci on chromosome 18 other than *Apc* affects intestinal tumorigenesis.

In summary, we have found conditions of γ-irradiation and of genetic background in which somatic allelic losses linked to the *Apc* locus in the intestinal adenomas of Min mice are subchromosomal. The *Mcc* and *Dcc* loci can each remain heterozygous in these tumors. In a few cases, even the site of the *Min* mutation in *Apc* remains heterozygous. In these few cases, clearly, it is important to determine now whether mutations have arisen within the Apc^+ allele, inactivating its function while retaining the Min^+ marker.

The Min mouse strain is being developed as a model of human FAP colon cancer families and, by inference, sporadic colon cancer in humans. To this end, the observations reported here indicate that it may be possible to place the Apc^{Min} mutation on a genetic background on which the major somatic pathways for loss of the wild type Apc^+ allele would include intragenic mutation, interstitial deletion, or mitotic recombination in addition to loss of the wild type chromosome. Such a second-generation Min strain would represent a model that would share more fully both the germline and somatic genetic features of FAP in the human.

We have demonstrated that the somatic genetic events linked to *Apc* do not need to include losses of either *Mcc* or *Dcc*. Further studies can now address whether, under any circumstances, adenomas can form in Apc^+/Apc^{Min} heterozygotes by a path-

way that retains one functional wild type *Apc* allele (see Solomon et al., 1987; D'Abaco et al., 1996).

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