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Apc^{Min}: A Mouse Model for Intestinal and Mammary Tumorigenesis

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***Min* (multiple intestinal neoplasia) is a mutant allele of the murine *Apc* (adenomatous polyposis coli) locus, encoding a nonsense mutation at codon 850. Like humans with germline mutations in *APC*, *Min*/+ mice are predisposed to intestinal adenoma formation. The number of adenomas is influenced by modifier loci carried by different inbred strains. One modifier locus, *Mom-1* (modifier of *Min-1*), maps to distal chromosome 4. Intestinal tumours from both B6 (C57BL/6J) and hybrid Min/+ mice show extensive loss of the wild-type allele at *Apc*. B6 *Min*/+ female mice are predisposed to spontaneous mammary tumours. The incidence of both intestinal and mammary tumours can be increased in an age-specific manner by treatment with ethylnitrosourea (ENU). *Min* mice provide a good animal model for studying the role of *Apc* and interacting genes in the initiation and progression of intestinal and mammary tumorigenesis.**

Key words: *Min* (multiple intestinal neoplasia), *Apc* (adenomatous polyposis coli), mouse, intestinal adenomas, mammary tumours, animal model

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Min (MULTIPLE INTESTINAL neoplasia) is an ethylnitrosourea (ENU)-induced mutation in the murine *Apc* (adenomatous polyposis coli) gene [1, 2]. *Min* encodes a stop codon at codon 850 resulting in premature truncation of the polypeptide. This is similar to germline mutations in the *APC* gene in humans with familial adenomatous polyposis (FAP) or Gardner syndrome (GS), two inherited colon cancer syndromes [3, 4]. *APC* is also the most frequently mutated gene in sporadic colon tumours in humans (reviewed in [4–6]).

On the C57BL/6J (B6) background, *Min*/+ mice develop more than 50 adenomas throughout the intestinal tract and rarely survive beyond 120 days [1]. In the mouse, the small intestine is the site of most of these tumours, unlike the human where the colon is more heavily involved. On certain genetic backgrounds, the number of tumours is reduced and the tumours can progress to become locally invasive [7]. *Min*/+ mice also develop a variety of lesions in other tissues including desmoid tumours, epidermoid cysts and mammary tumours. With the exception of mammary tumours, this reflects part of the spectrum of lesions seen in humans with mutations in the *APC* gene (Table 1).

The APC protein is expressed in most tissues, not all of which are common sites of tumour formation in humans carrying germline mutations in *APC* [8, 9]. This discordance between expression pattern and tumour susceptibility implies that there may be specific cell types or developmental stages of these tissues that are more susceptible to the effect of mutations in *APC*. At present, the function of the *APC* gene product is not well understood. Recent studies have indicated that APC interacts to form homodimers [10, 11] and can bind to the catenins [12, 13]. APC competes for catenin binding with E-cadherin, the Ca^{2+}-dependent cell adhesion molecule [14]. A carboxy terminal region of APC associates with the microtubules of mammalian cells [15, 16].

Although all cells in the intestine of a *Min*/+ mouse, or a human with FAP, carry a mutant copy of *Apc* (*APC*) only a relatively small number of tumours form. This indicates that further somatic events are required before tumours can develop. Loss or mutation of the wild-type allele at *APC* has been detected in a majority of tumours from FAP patients [7, 17, 18]. The inability to detect mutations in all tumours may reflect the difficulty in detecting all mutational events in a very large gene in genetically heterogeneous human tumours. Alternatively, it

Table 1. Heterozygous phenotypes associated with heterozygosity for germline mutations in APC/Apc

Human	Mouse
Multiple colon adenomas (100–1000s)	Multiple colon adenomas
Small intestine adenomas	Small intestine adenomas
Desmoid tumours	Desmoid tumours
Epidermoid cysts	Epidermoid cysts
Gastric polyps	Mammary adenocarcinomas
Osteomas of skull and mandible	Mammary keratoacanthomas
Hypertrophy of retinal pigment epithelium	
Abnormal dentition	

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may indicate that mutations in other loci can also lead to tumour development.

We have tested intestinal tumours from *Min*/+ mice for loss of the wild-type allele at *Apc* [19]. Analysis of tumours from inbred and F1 hybrid mice has two advantages: a large number of tumours can be collected from genetically identical individuals and mice with fully informative genetic differences in the *Apc* region can be analysed. To minimise the contamination from normal tissue, material for analysis must be harvested from sectioned tumours. Tumours were first tested for the presence or absence of the wild-type nucleotide at the site of the *Min* mutation in the *Apc* gene. When tumours from B6 *Min*/+ mice were analysed, all 50 tumours showed loss of the wild-type allele at *Apc*. This analysis included tumours of both the small intestine and the colon.

In an effort to determine the mechanism of this loss, tumours from (AKR × B6) *Min*/+ mice were analysed. In these tumours, markers were absent all along the entire wild-type chromosome (in this case from AKR). Comparison of the intensity of the PCR products for chromosome 18 with markers on other chromosomes indicated that only one copy, the B6 copy of chromosome 18, was present. Whole chromosome loss has not been observed as a mechanism for loss of the wild-type allele at *APC* in human adenomas; intragenic mutations are detected in these adenomas (reviewed in [5, 6, 17, 18]. Why is chromosome loss the predominant mechanism for allele loss in *Min*/+ mouse tumours? It could be that chromosome loss is a more likely event in mouse intestinal cells than in humans; or monosomy for chromosome 18 is tolerated in mouse cells while monosomy for chromosome 5 is not tolerated in human cells; or whole chromosome loss is selected for because it is necessary for other loci to become hemizygous. At this point, we cannot differentiate between these possibilities. Note, however, in the mouse, chromosome 18 carries not only *Apc* but also *Mcc* and *Dcc* [20, 21], two genes postulated to have a role in intestinal tumour formation in humans. In the human, *MCC* is linked to *APC* on chromosome 5 [22], but *DCC* is located on chromosome 18 [23].

The phenotype of humans with germline mutations in *APC* can be quite heterogeneous (reviewed in [24]). The number of intestinal polyps can range from the hundreds to the thousands, and the incidence of extracolonic lesions is also variable. Such variability could be due to either the phenotypic effects of different mutations within the *APC* gene or the interaction of other loci, or both. There is some evidence that the particular *APC* mutation may affect the severity of the intestinal phenotype [25–27]. However, in general, the incidence of extracolonic manifestations does not correlate well with the site of the mutation. One exception to this is the incidence of congenital hypertrophy of retinal pigment epithelium (CHRPE), which is predominantly observed in individuals with mutations in exon 9 [28]. We have been investigating the role of genetic background on intestinal tumour development in *Min*/+ mice. In these studies, we can test the phenotype of a specific mutation on different genetic backgrounds under controlled conditions.

In contrast to the intestinal phenotype on the B6 background, *Min*/+ hybrid mice show a reduced number of intestinal tumours [7, 29]. On the B6 background, *Min*/+ mice develop approximately 30 tumours in the regions of the intestine analysed (Figure 1, open bars). AKR × B6 *Min*/+ mice develop an average of five tumours (solid bars). When the F1 mice are backcrossed to the B6 strain, the backcross generation contain mice that resemble each parental population (stippled bars). We have mapped one of the loci responsible for this modified

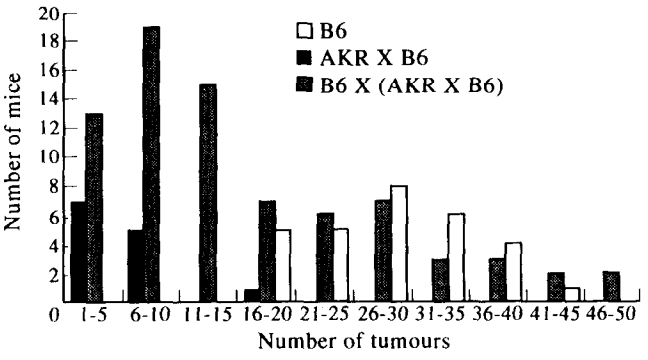

Figure 1.

phenotype, *Mom-1* (modifier of *Min-1*), to distal chromosome 4 [29]. Several inbred strains, including AKR/J, MA/MyJ and CAST/Ei, carry resistant alleles at the *Mom-1* locus. The *Mom-1* locus accounts for only approximately 50% of the decrease in intestinal tumour number in hybrid animals. Many of these strains carry alleles at other loci that also affect intestinal tumour number. However, we have been unable to determine the chromosomal location of any of these other modifier genes [29].

One strain, the DBA/2J (DBA) strain, appears to carry resistant alleles only at the *Mom-1* locus (M.K. McNeley, University of Wisconsin, U.S.A.). B6 *Min*/+ mice were crossed with females from the DBA strain, and the resulting F1 *Min*/+ mice were crossed with either B6 or DBA mates. Tumour counts were performed on all animals and then mice were genotyped to determine the source of the *Mom-1* region. The average tumour number (an average of 14) for animals heterozygous for the *Mom-1* region was approximately 50% of that for the animals that were homozygous B6 for the *Mom-1* region (an average of 29). All the mice heterozygous for *Mom-1* had the same phenotype, regardless of the DBA or B6 contribution to the background, indicating that no other loci were controlling tumour number. In the backcross to DBA, it was possible to determine the effect on the intestinal tumour number of homozygosity for two resistant alleles at *Mom-1*. While one copy of the resistant DBA allele results in approximately a 50% reduction in tumour number (to an average of 14), two copies of the DBA allele at *Mom-1* resulted in a further reduction to approximately 25% (an average of eight).

One example of the extent of the effect of genetic background on intestinal phenotype in *Min*/+ mice can be demonstrated by examination of animals carrying *Min*/+ on the AKR genetic background (A.R. Moser and A.R. Shoemaker, University of Wisconsin, U.S.A.). These mice were generated by crossing the *Min* allele on to the AKR strain for multiple generations. As the AKR component of the genome is increased, the tumour number is reduced from five in the F1 hybrids to an average of 1.7 for the first backcross generation to AKR. After several further generations of backcrossing to AKR, six of seven *Min*/+ mice had no tumours in the entire intestine, and one had only two tumours in the entire intestinal tract.

Because of their decreased tumour number, hybrid animals have an increased life span. By counting tumours in genetically identical mice of different ages, we have determined that tumour numbers do not increase between 100 and 300 days of age [7]. This suggests that tumours are not equally likely to form at all ages in the life of the animal. To investigate further the timing of tumour development in *Min*/+ mice, we have begun to investigate the effects of carcinogen treatment on *Min*/+ mice of

various ages (A.R. Shoemaker and A.R. Moser, University of Wisconsin, U.S.A.). Treatment of 5–14 day-old B6 *Min*/+ mice with ENU results in a 3.8-fold increase in intestinal tumours as compared to untreated age-matched *Min*/+ mice. However, when mice are treated at older ages, between 20 and 35 days of age, the increase in intestinal tumours was only 1.6-fold. These data indicate that *Min*/+ mice are most susceptible to intestinal tumour initiation early in their lives. The mechanism for this age-specific sensitivity is not, as yet, clear.

Approximately 5% of B6 *Min*/+ females and 10% of longer lived hybrid *Min*/+ females develop mammary adenocarcinomas and acanthomas [30]. Although this incidence of mammary tumours is higher than in their +/+ siblings, it is still too low a frequency to allow a facile experimental study of the role of *Min* in spontaneous mammary tumour development. However, when B6 *Min*/+ females are treated with ENU many animals develop mammary tumours and focal alveolar hyperplasias within 65 days of a single ENU treatment. *Min*/+ females treated between 25 and 35 days of age show the highest response, with approximately 75% developing at least one tumour. No +/+ siblings develop mammary tumours or hyperplasia under this protocol (A.R. Shoemaker and A.R. Moser, University of Wisconsin, U.S.A.).

In order to study *Min*/+ mammary glands for a longer time period and to assess the effect of other treatment protocols, mammary tissue from *Min*/+ mice (and their +/+ siblings) was transplanted into healthy host animals. *Min* was shown to increase the frequency of tumour formation in transplants after treatment with either DMBA or ENU and to decrease the latency of tumour formation after treatment with DMBA [30]. Only *Min*/+ transplants developed tumours and hyperplasias spontaneously or after ENU treatment. These results demonstrate that the susceptibility to mammary tumour formation is intrinsic to the *Min*/+ mammary cells.

In summary, *Min*/+ mice develop a variety of tumours and lesions similar to those seen in humans carrying germline mutations in *APC*. The intestinal tumours in *Min*/+ mice show loss of the wild-type allele at *Apc*. In hybrid tumours, where the status of other loci on chromosome 18 could be analysed, this loss occurs through the loss of the entire chromosome carrying the wild-type allele at *Apc*. Although the mechanism of loss of the wild-type allele at *Apc*/*APC* may be different in humans and mice, it is clear that, in both cases, loss is an important and early event during tumour formation.

Investigation of the effects of genetic background on the intestinal phenotype in *Min*/+ mice has allowed us to map one modifier locus, *Mom-1*, to distal chromosome 4. Other background loci also have an effect on intestinal tumour number, as clearly demonstrated by the essentially complete suppression of intestinal tumour development when *Min* is carried on the AKR background. Understanding how these background genes can compensate for or suppress the effects of a mutation in *Apc* should lead to a further understanding of the factors that are involved in tumour formation.

ENU treatment of *Min*/+ and +/+ mice demonstrates that the intestine has an age-specific sensitivity to intestinal tumour induction. What is different about the cells of the intestine in the 5–15 day-old mice that make them more sensitive to ENU-induced tumour induction? Are these cells also more sensitive to spontaneous tumour formation? Knowing the time of maximal sensitivity to tumour induction by ENU may help us to answer these questions.

Min/+ female mice are also susceptible to both spontaneous and carcinogen-induced mammary tumours. The mammary gland also demonstrates an age-specific sensitivity to ENU-induced tumour development. Although breast cancer is not a common feature of FAP in humans, APC may still play a role in human breast cancer development. The incidence of spontaneous mammary tumours in *Min*/+ mice is low, even in older animals. It may be that in humans the susceptibility to breast tumours due to germline mutations in *APC* is not significantly increased above the incidence in the general population. The increased incidence of mammary tumour development in *Min*/+, but not +/+, mammary glands after ENU treatment is clear evidence for a role for *Apc* in mammary tumour development in the mouse.

Overall, these mice present a good molecular and biological model for studying the role of *Apc* in tumour development in a number of tissues.

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