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SHORT REPORT

Mlh1 deficiency enhances several phenotypes of *Apc*$^{Min/+}$ mice

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Defects in *APC* and DNA mismatch repair genes are associated with a strong predisposition to colon cancer in humans, and numerous mouse strains with mutations in these genes have been generated. In this report we describe the phenotype of *Min*/+ *Mlh1*$^{-/-}$ mice. We find that these doubly mutant mice develop more than three times the number of intestinal adenomas compared to *Min*/+ *Mlh1*$^{+/+}$ or $^{+/-}$ mice but that these tumors do not show advanced progression in terms of tumor size or histological appearance. Full length Apc protein was not detected in the tumor cells from *Min*/+ *Mlh1*$^{-/-}$ mice. Molecular analyses indicated that in many tumors from *Min*/+ *Mlh1*$^{-/-}$ mice, *Apc* was inactivated by intragenic mutation. Mlh1 deficiency in *Min*/+ mice also led to an increase in cystic intestinal crypt multiplicity as well as enhancing desmoid tumorigenesis and epidermoid cyst development. Thus, Mlh1 deficiency influences the somatic events involved in the development of most of the phenotypes associated with the *Min* mutation. *Oncogene* (2000) **19,** 2774–2779.

Keywords: *Apc*; *Mlh1*; Min; mutation; tumor

Familial adenomatous polyposis (FAP) and hereditary nonpolyposis colorectal cancer (HNPCC) are two distinct forms of autosomal dominantly inherited intestinal cancer in humans. In addition to colon tumors, both syndromes predispose affected individuals to tumorigenesis in various other tissues (Peckham *et al*., 1995). FAP, less common than HNPCC, can lead to the formation of up to several thousand colonic polyps and has been shown to result from germline mutation of the *APC* gene (Groden *et al*., 1991; Kinzler *et al*., 1991). Individuals with HNPCC generally develop far fewer tumors with a later age of onset. However, HNPCC is believed to account for approximately 5% of all colorectal cancer in humans and this disease results primarily from germline mutations in the DNA mismatch repair (MMR) genes *MSH2* and *MLH1* (Bronner *et al*., 1994; Fishel *et al*., 1993; Kolodner, 1996).

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Although the precise cellular role(s) in suppressing neoplasia remain(s) to be elucidated, the APC protein has been implicated in apoptosis, cell adhesion and transcriptional regulation of gene expression (Polakis, 1997). Defects in MMR functions lead to an increased accumulation of mutations that result from unrepaired replication errors, or other forms of DNA damage (Kolodner, 1996). Elevated tumor predisposition in HNPCC individuals is thought to be at least in part a direct consequence of an increased frequency of mutations in tumor suppressor genes and/or oncogenes. Somatic *APC* mutations are frequently found in the tumors from HNPCC patients, as well as in the majority of sporadically occurring colonic tumors (Huang *et al*., 1996), suggesting that *APC* plays a central role in maintaining intestinal homeostasis.

Numerous mouse strains with mutations in *Apc* and several of the known DNA MMR genes are now being used to analyse experimentally the process of intestinal neoplasia (Baker *et al*., 1995; de Wind *et al*., 1995; Edelmann *et al*., 1996; Prolla *et al*., 1998; Shoemaker *et al*., 1997a). *Apc*$^{Min/+}$ (*Min*/+) is the most fully characterized of these mutants (Shoemaker *et al*., 1997a). *Min* is a germline nonsense mutation at codon 850 of *Apc* that predisposes heterozygotes to the development of dozens of neoplastic intestinal adenomatous tumors (primarily in the small intestine) as well as causing an increased susceptibility to mammary tumors, desmoid tumors and epidermoid cysts (Shoemaker *et al*., 1997a). Mice with targeted mutations in the MMR *mutS* homologs *Msh2*, *Msh3*, *Msh5*, and *Msh6*, as well as the *mutL* homologs *Pms1*, *Pms2*, and *Mlh1* have also been created (Baker *et al*., 1995, 1996; de Vries *et al*., 1999; de Wind *et al*., 1995; Edelmann *et al*., 1996, 1997; Prolla *et al*., 1998; Reitmair *et al*., 1995). No evidence for enhanced tumorigenesis has so far been reported for the *Msh3*, *Msh5*, or *Pms1* mutant strains (de Vries *et al*., 1999; Prolla *et al*., 1998; de Wind *et al*., 1999). Mice homozygous for a null allele of *Pms2* show an enhanced susceptibility to lymphoma and sarcoma, but not intestinal neoplasia (Baker *et al*., 1995). In contrast, *Msh2* and *Mlh1* homozygous null mice are predisposed to intestinal neoplasia as well as a variety of other tumor types (Baker *et al*., 1996; de Wind *et al*., 1995; Edelmann *et al*., 1996, 1997; Prolla *et al*., 1998). There is conflicting data on the tumor susceptibility of *Msh6* mutant mice that may reflect the effects of genetic background, but *Msh3*$^{-/-}$ *Msh6*$^{-/-}$ double mutants are predisposed to intestinal and other tumor types (de Wind *et al*., 1999; Edelmann *et al*., 1997).

Analysis of variations in genetic background, as well as mutations in several known genes, has demonstrated that the tumor phenotype of Min mice can be dramatically modified. These modifiers include *Dnmt*, *p53*, *Matrilysin*, *Mom1* (which is believed to encode *Pla2g2a*), and two DNA MMR genes (Baker *et al*., 1998; Clarke *et al*., 1995; Cormier *et al*., 1997; Dietrich *et al*., 1993; Laird *et al*., 1995; Reitmair *et al*., 1996; Wilson *et al*., 1997). In this latter case, combining the *Min* mutation with homozygosity for a null mutation in either *Msh2* or *Pms2* increases tumor multiplicity 3–3.5-fold relative to *Min*/+ mice.

In this report we examine more broadly the effects of combining Mlh1 deficiency with the *Min* mutation. We present evidence for a strong enhancement of intestinal tumor multiplicity in these mice without an effect on tumor progression. In addition, *Min*/+ $Mlh1^{-/-}$ mice were more susceptible to the development of cystic intestinal crypts, desmoid tumors, and epidermoid cysts, but did not show elevated predisposition to mammary tumorigenesis. We also examined the status of *Apc* in intestinal tumors that arise in *Min*/+ $Mlh1^{-/-}$ mice. Our data indicate that elimination of normal Apc function is necessary for tumor formation and that an increase in somatic mutation frequency associated with loss of Mlh1 function largely accounts for the increase in tumor multiplicity observed in doubly mutant mice.

After backcrossing to generate a predominantly C57BL/6J (B6) genetic background, *Mlh1* mutant mice were crossed to *Min*/+ mice and the phenotype of the *Min*/+ $Mlh1^{+/+}$, *Min*/+ $Mlh1^{+/-}$, and *Min*/+ $Mlh1^{-/-}$ mice was assessed (Figure 1). The total average tumor number from the scored regions of the intestine was 139 ± 25 for *Min*/+ $Mlh1^{-/-}$ mice versus approximately 40 for each of the other two groups (Figure 1a). Thus, intestinal tumor multiplicity was increased more than threefold in *Min*/+ $Mlh1^{-/-}$ mice while *Mlh1* heterozygosity had no effect on tumor number. The increase in tumor number was seen throughout the length of the intestine and there was no difference between the genotypic groups in tumor distribution along the length of the tract (data not shown). Intestinal tumor sizes were also determined for each group (Figure 2). *Mlh1* genotype had no effect on tumor size; the average maximum tumor diameters were 1.05, 1.03, and 1.13 mm for the $Mlh1^{+/+}$, $Mlh1^{+/-}$, and $Mlh1^{-/-}$ mice, respectively. A set of 15–30 tumors from each genotypic class was also assessed histologically. Local invasion of the intestinal mucosa was present in one very large tumor from the small intestine of a *Min*/+ $Mlh1^{-/-}$ mouse that was sacrificed at 78 days of age. This extent of tumor progression has not been observed in B6 *Min*/+ mice. With the exception of this single tumor sample, no compelling evidence was found for enhanced tumor progression in the Mlh1 deficient mice by 80 days of age.

Cystic crypts are intestinal lesions of unknown neoplastic potential that are morphologically and histologically distinct from adenomas. Found predominantly in the proximal small intestine, the multiplicity of these lesions is increased dramatically following somatic ethylnitrosourea (ENU) mutagenesis of B6 *Min*/+ mice (Shoemaker *et al*., 1995). The effects of *Mlh1* deficiency on cystic crypt multiplicity was determined using the same intestinal samples scored for intestinal adenomas (Figure 1b). An average of 70 ± 22 cystic crypts was found for the *Min*/+ $Mlh1^{-/-}$ mice compared to 20 ± 11 and 24 ± 10 for the *Min*/+ $Mlh1^{+/-}$ and *Min*/+ $Mlh1^{+/+}$ mice, respectively. Thus, as for intestinal adenomas, *Mlh1* deficiency led to an approximately threefold increase in cystic crypt multiplicity.

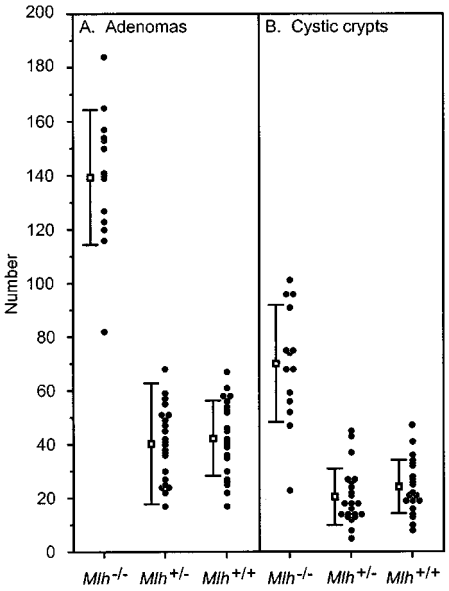

Figure 1 (**a**) Intestinal tumors in *Min*/+ mice of different *Mlh1* gentoypes. Each black dot indicates the tumor multiplicity for an individual animal. Open boxes and vertical bars represent the means and standard deviations for each group. The numerical values for the means and standard deviations for each group are as follows: *Min*/+ $Mlh1^{-/-}$, 139 ± 25; *Min*/+ $Mlh1^{+/-}$, 40 ± 10; *Min*/+ $Mlh1^{+/+}$, 42 ± 14. The average age at sacrifice (in days) for each group was as follows: *Min*/+ $Mlh1^{-/-}$, 65 ± 6; *Min*/+ $Mlh1^{+/-}$, 76 ± 10; *Min*/+ $Mlh1^{+/+}$, 74 ± 6. The difference in tumor number between the *Min*/+ $Mlh1^{-/-}$ mice and the other two classes is highly significant ($P < 6 \times 10^{-7}$, Wilcoxon Rank Sum test). (**b**) Cystic intestinal crypts in *Min*/+ mice of different *Mlh1* genotypes. The symbols are the same as indicated for intestinal tumors. The numerical values for the means and standard deviations for each group are as follows: *Min*/+ $Mlh1^{-/-}$, 70 ± 22; *Min*/+ $Mlh1^{+/-}$, 20 ± 11; *Min*/+ $Mlh1^{+/+}$, 24 ± 10. The difference between the *Min*/+ $Mlh1^{-/-}$ mice and the other two classes is highly significant ($P < 3 \times 10^{-6}$). Tumor and cystic crypt scorings were conducted as described (Shoemaker *et al*., 1998). The animals used to generate the data described here were produced as follows. Mice on a mixed C57BL/6J (B6) × 129/Sv genetic background and heterozygous for the *Mlh1* null mutation (Baker *et al*., 1996) were bred to B6 for two generations. These N3 $Mlh1^{+/-}$ mice were then crossed to congenic B6 *Min*/+ mice to generate double heterozygotes. The double heterozygotes were crossed to $Apc^{+/+}$ $Mlh1^{+/-}$ siblings to generate $Apc^{+/+}$ and $Apc^{Min/+}$ mice that were also either wild type, heterozygous, or homozygous null at the *Mlh1* locus. Thus, the mice used here were, on average, more than 93% B6 in genetic background. For *Min* and *Mlh1* genotyping, DNA was prepared from blood using the QIAamp Blood Kit (QIAGEN Inc., Valencia, CA, USA) and PCR amplified using assays described previously (Baker *et al*., 1996; Dietrich *et al*., 1993)

Epidermoid cysts are pigmented lesions of the subdermal layer of the skin that are a common extracolonic manifestation of FAP. We have shown previously that ENU treatment dramatically increases the incidence of these lesions in B6 *Min*/+ mice (Shoemaker *et al*., 1995). Epidermoid cysts were found in 7/14 *Min*/+ $Mlh1^{-/-}$ mice but were not observed in mice of the other two genotypic classes (22 animals of each genotype). Another extracolonic lesion seen in FAP patients, desmoid tumors, are fibromatoses that occur in the peritoneal wall (Peckham *et al*., 1995). In *Min*/+

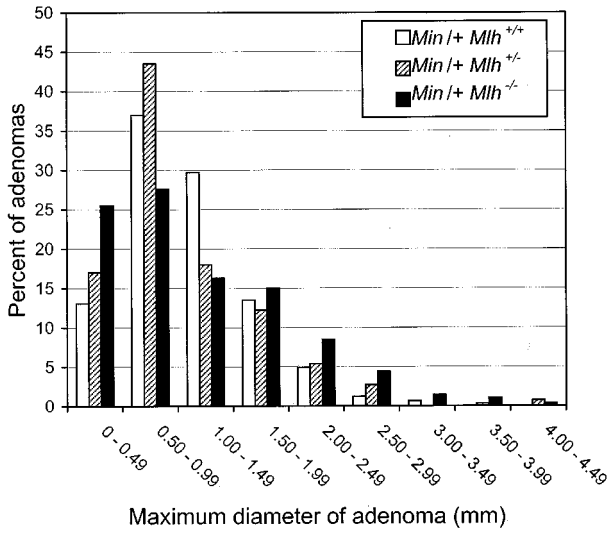

Figure 2 Intestinal adenoma sizes in *Min*/+ *Mlh1*$^{-/-}$, *Min*/+ *Mlh1*$^{+/-}$, and *Min*/+ *Mlh1*$^{+/+}$ mice. The percentage of total intestinal adenomas within the indicated range of tumor sizes is shown for each genotypic class. Tumor sizes and multiplicities were obtained from the same intestinal tissue samples. The maximum tumor diameter was determined for every tumor from nine mice from each genotypic class. These nine mice consisted of three mice with the lowest, the median, and the highest tumor number for each genotypic class. Tumor diameters were determined using a calibrated eye piece reticle on a dissection microscope. There is no statistically significant difference in tumor sizes between the three groups ($P > 0.05$, Wilcoxon Rank Sum test)

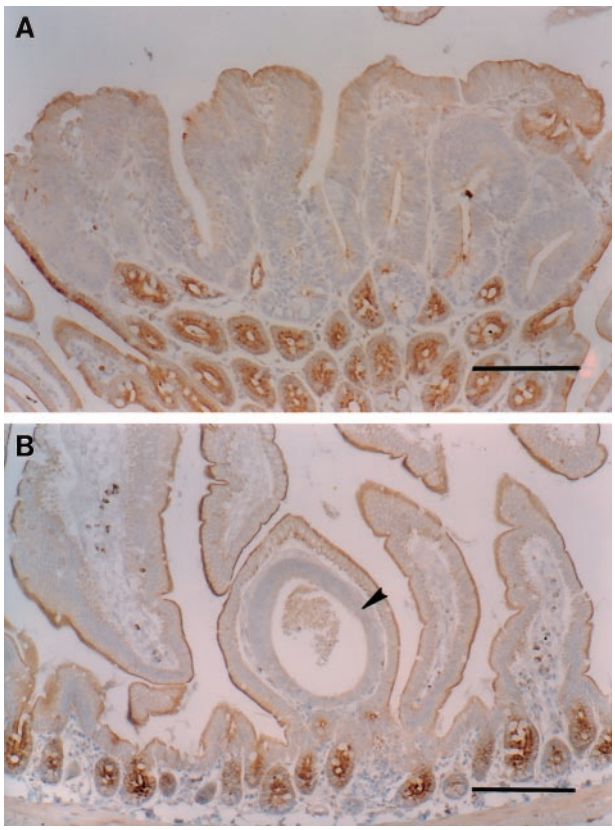

Figure 3 Analysis of Apc expression in intestinal tissue samples from *Min*/+ *Mlh1*$^{-/-}$ mice. Periodate-lysine-paraformaldehyde (PLP) fixed tissues were stained with rabbit polyclonal antibody 3122, raised against amino acids 8–347 of human APC, at a dilution of 1 : 500 (Shoemaker *et al*., 1998). (**a**) An intestinal adenoma and surrounding tissue from a *Min*/+ *Mlh1*$^{-/-}$ mouse. Brown Apc staining can be seen in the normal intestinal crypts and no Apc expression is detected in the neoplastic cells of the adenoma. (Scale bar = 100 μm) (**b**) A cystic crypt and surrounding tissue from a *Min*/+ *Mlh1*$^{-/-}$ mouse. The arrowhead indicates the Apc-negative anaplastic cells. (Scale bar = 100 μm)

mice, desmoid tumorigenesis is strongly enhanced by the absence of p53 function (Halberg *et al*., 2000). Desmoids were observed in 5/14 *Min*/+ *Mlh1*$^{-/-}$ mice, and in only one animal (*Min*/+ *Mlh1*$^{+/+}$) of the other two classes.

Increased intestinal tumor multiplicity in *Min*/+ *Mlh1*$^{-/-}$ mice may reflect an increased frequency of somatic inactivation of *Apc* and/or other loci important for tumor initiation. It has been demonstrated previously that tumor formation in *Min*/+ mice invariably involves somatic inactivation of the wild type *Apc* allele (Levy *et al*., 1994; Luongo *et al*., 1994). On the B6 genetic background, *Apc* inactivation occurs by allele loss, most likely by a chromosomal event. However, several studies have shown that the mechanism of *Apc* inactivation can be influenced by genetic background or by treatment with mutagens (Luongo and Dove, 1996; Shoemaker *et al*., 1997b, 1998). We used several methods to examine *Apc* status in tumors from mice of each genotypic class. Immunohistochemical analysis indicated a lack of normal Apc expression in cells from all tumors examined from mice of each class (Figure 3a). Apc expression was also absent in the anaplastic cells found in the cystic intestinal crypts (Figure 3b). These results are consistent with numerous studies suggesting that inactivation of *APC*/*Apc* is commonly an early and necessary event for adenoma formation. In order to examine the mechanism of *Apc* inactivation in more detail, we first employed a quantitative PCR assay to examine allele loss. A total of 36 tumor and seven normal intestinal tissue samples were examined (Table 1). Seven tumors (19%) showed loss of the wild type *Apc* allele (average Apc^{+}/Apc^{Min} ratio = $0.15 \pm .07$). However, loss of the Apc^{+} allele was not observed in the remaining 29 tumors (81%; average Apc^{+}/Apc^{Min} ratio of $0.67 \pm .14$). The average Apc^{+}/Apc^{Min} ratio for the normal tissue controls was $0.74 \pm .06$. The fraction of tumors reported here that retained the Apc^{+} allele is roughly consistent with the increase in tumor number caused by Mlh1 deficiency. These results suggest that Mlh1 deficiency leads to inactivation of Apc^{+} expression by mechanisms other than allelic loss, such as by intragenic mutation. We have shown previously that treatment of B6-*Min*/+ mice with ENU leads to a significant increase in tumor number and that many of these tumors have acquired somatic *Apc* truncation mutations, similar to the types of *APC* mutations often observed in human intestinal tumors (Shoemaker *et al*., 1997b). Tumors from the *Min*/+ *Mlh1*$^{-/-}$ mice that failed to show Apc^{+} allelic loss were examined for *Apc* truncation mutations by the *in vitro* synthesized protein (IVSP) assay. Truncation mutations were found in six of these 29 tumors in a region of the gene spanning approximately codons 680–1230 (Figure 4). These six truncations occurred in the same region of *Apc* and at a similar frequency as previously identified ENU-induced truncating mutations (Shoemaker *et al*., 1997b). This segment of the gene is slightly upstream from the corresponding somatic mutation cluster region of human *APC*. Due to limiting amounts of tumor sample, we were not able to identify the nature of these mutations by sequence analysis.

Table 1 Analysis of *Apc* allelic status in intestinal tumors for *Min*/+ *Mlh1*$^{-/-}$ and *Min*/+ mice

Genotype	*Tumor multiplicity*	Apc^+/Apc^{Min} *ratio [mean*±*s.d. (N/total) for tumors; mean*±*s.d. (n) for normal tissue]*		
		Tumors that maintained Apc^+	*Tumors that lost* Apc^+	*Normal intestinal tissue*
Min/+ *Mlh1*$^{-/-}$	139.4±25.0	0.67±0.14 (29/36)	0.15±0.07 (7/36)	0.74±0.06 (7)
B6-*Min*/+[a]	29.2±9.4	NA	0.17±0.07 (12/2)	0.70±0.15 (54)

[a]Data taken from Shoemaker *et al*., 1997b. The relative ratio of the Apc^+ to Apc^{Min} alleles was determined by a quantitative PCR assay (Luongo *et al*., 1994). This assay has been shown to be linear over a wide range of input DNA quantities. All samples were independently PCR amplified at least two times. Only samples that gave repeatable values (within 10%) with signals at least 10-fold above background were included in the results. Numerous comparisons of normal and intestinal tumor samples have allowed us to conclude that an average Apc^+/Apc^{Min} ratio of less than or equal to 0.30 is indicative of extensive Apc^+ loss. Examination of *Apc* for truncation mutations was carried out using the *in vitro* synthesized protein (IVSP: Figure 4). NA: not applicable

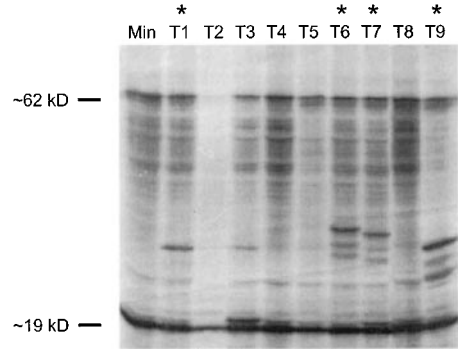

Figure 4 IVSP assay of a portion of *Apc* for truncation mutations in intestinal tumors from *Min*/+ *Mlh1*$^{-/-}$ mice. For these studies only the portion of *Apc* comprising codons 680–1230 was analysed. The *Min* lane represents analysis of control genomic DNA isolated from normal tissue from a B6 *Min*/+ animal. The lanes marked *T1–T9* represent analysis of intestinal tumors from *Min*/+ *Mlh1*$^{-/-}$ mice. The full-length product of a portion of Apc covering codons 680–1230 is approximately 62 000 (M_r). The polypeptide product resulting from truncation at the *Min* mutation (codon 850 of *Apc*) is approximately 19 000 (M_r). Lanes marked with an asterisk display truncated products

In this report we demonstrate that elimination of Mlh1 function leads to a 3.5-fold increase in intestinal tumor number in *Min*/+ mice. Furthermore, the magnitude of increase in tumor number is correlated with the fraction of these tumors that fail to show Apc^+ allelic loss. Many of the tumors that failed to show LOH had instead acquired somatic *Apc* truncation mutations. These results suggest that increased tumor multiplicity is a direct consequence of an elevated frequency of *Apc* mutation in MMR-deficient intestinal stem cells. Mlh1 deficiency also led to an increase in the number of cystic intestinal crypts, epidermoid cysts and desmoid tumors in *Min*/+ mice. These results demonstrate that defects associated with the loss of this *mutL* homolog have a significant effect on both intestinal and extra-intestinal phenotypes of *Min*/+ mice.

Inactivation of *APC*/*Apc* function has been shown to be a necessary event for the majority of intestinal tumorigenesis that occurs in both humans and mice (Polakis, 1997; Shoemaker *et al*., 1997a). In *Min*/+ mice, loss of normal *Apc* function appears to be necessary for tumor formation and multiple pathways for *Apc* inactivation have been discovered. In B6 *Min*/+ mice, this inactivation occurs exclusively by a chromosomal loss of heterozygosity event (Luongo *et al*., 1994). When additional tumors are induced in B6 *Min*/+ mice by treatment with ENU, many of these tumors do not show LOH at *Apc*. Instead, *Apc* inactivation occurs by other mechanisms, including intragenic mutation (Shoemaker *et al*., 1997b). In congenic AKR/J *Min*/+ mice, intestinal tumor multiplicity is decreased by a factor of 200 compared to B6 *Min*/+ mice (Shoemaker *et al*., 1998). The majority of both spontaneously arising and ENU-induced tumors from AKR *Min*/+ also do not show LOH. However, in contrast to ENU-induced tumors in B6 *Min*/+ mice, inactivation of *Apc* in tumors from AKR/J *Min*/+ mice does not seem to involve intragenic *Apc* truncation mutations. In this case, the inactivation scenario appears to involve an as yet uncharacterized silencing mechanism. Thus, depending on the genetic and environmental context, inactivation of *Apc* in *Min*/+ mice may involve LOH, maintenance of heterozygosity (MOH) with associated intragenic truncation mutation, or MOH with inactivation of *Apc* occurring by other mechanisms.

All intestinal tumors from *Min*/+ *Mlh1*$^{-/-}$ mice failed to show detectable levels of Apc expression (Figure 3). LOH was found to be the mechanism of *Apc* inactivation in approximately 20% of these tumors (Table 1). *Apc* truncation mutations were found in six of the 29 tumors (21%) with MOH at *Apc* (Figure 4). This frequency of truncation mutations is similar to the 25% truncation frequency previously reported for ENU-induced tumors in B6 *Min*/+ mice (Shoemaker *et al*., 1997b). For the remaining tumors with MOH, *Apc* inactivation must be the result of other types of *Apc* mutations and/or other somatic events that lead to silencing of Apc expression.

Importantly, although both FAP and HNPCC are autosomal dominantly inherited diseases in humans, mice heterozygous for mutations in MMR genes show no pronounced tumor phenotype, even on the sensitized *Min*/+ genetic background (Edelmann *et al*., 1996, 1999; Prolla *et al*., 1998). Our finding that *Min*/+ *Mlh1*$^{+/-}$ and *Min*/+ *Mlh1*$^{+/+}$ mice have similar tumor multiplicities is consistent with other studies involving *Apc* and MMR mutant mouse strains (Baker *et al*., 1998; Reitmair *et al*., 1996). The reason for this difference in tumor susceptibility between humans and mice is not understood but may reflect the relatively short life span and smaller size of mice. The total number of intestinal mitoses during an approximately 1.5 year mouse life span is estimated at 1×10^{11}. This is several orders of magnitude less than the number of mitoses that are expected to occur in the colon of humans by the average age of onset of HNPCC (approximately 41 years) (Potten and Morris, 1988). Alternatively, other factors such as environmental differences may result in a higher probability of somatic mutations occurring in human intestinal stem cells compared to that of laboratory mice.

Initial characterization of *Mlh1* and *Pms2* mutant mice indicates that $Mlh1^{-/-}$ but not $Pms2^{-/-}$ mice develop intestinal tumors (Prolla *et al*., 1998). This difference may reflect the different functions of these proteins in MMR. Genetic and biochemical analyses of MMR in yeast have shown that Mlh1 can form heterodimers with either Pms1 (mouse Pms2) or Mlh3 (mouse Pms1) and that these different protein complexes are each involved in the repair of distinct types of mismatches (Flores-Rozas and Kolodner, 1998; Kolodner, 1996). Indeed, detailed examination of mutation frequencies in mouse tissues has shown that the mononucleotide mutation frequency is 2–3-fold higher in $Mlh1^{-/-}$ mice compared to $Pms2^{-/-}$ mice (Yao *et al*., 1999). Interestingly, the effect of *Mlh1* deficiency on tumor multiplicity in *Min*/+ mice is only slightly greater than that previously reported for *Min*/+ $Pms2^{-/-}$ mice (3.5 *vs* 2.9-fold increase relative to MMR^+ *Min*/+ mice). These results suggest that there may be different pathways of intestinal tumorigenesis in MMR^- $Apc^{+/+}$ *vs* MMR^- $Apc^{Min/+}$ mice and that the mutator phenotype associated with *Mlh1* deficiency influences both pathways, whereas *Pms2* deficiency may have a stronger effect on the latter pathway. Rigorous analysis of this possibility will require the concurrent examination of intestinal tumor and mutator phenotypes of these strains on identical genetic backgrounds and environmental conditions.

A striking increase in intestinal tumor multiplicity has also been reported in an independently derived $Mlh1^{-/-}$ mouse strain heterozygous for the *1638N* allele of *Apc* (Edelmann *et al*., 1999). Neither $Mlh1^{-/-}$ nor $Apc^{1638N/+}$ mice develop more than five tumors throughout the intestinal tract. However, the combination of these mutations results in an average tumor multiplicity of more than 30 per animal. The synergistic effect of this double mutant may reflect the unique nature of the *Apc* allele used in these studies (Smits *et al*., 1999). Although the fold increase in tumor number is greater than reported here for *Min*/+ $Mlh1^{-/-}$ mice (and for previous studies with *Min*/+ $Pms2^{-/-}$ and *Min*/+ $Msh2^{-/-}$ mice), the absolute increase in tumor number is still much smaller than found in any of the studies with the Min strain.

It has been proposed recently that MMR proteins may also function in apoptosis. Mammalian cells, including mouse intestinal crypt cells, deficient in either *Msh2* or *Mlh1* were shown to be defective in induction of apoptosis following treatment with several DNA damaging agents (Gong *et al*., 1999; Hickman and Samson, 1999; Toft *et al*., 1999). In addition, over expression of hMSH2 or hMLH1, but not hMSH3, hMSH6, or hPMS2, in human cell lines resulted in the induction of apoptosis (Zhang *et al*., 1999). Taken together, these results suggest that defective apoptosis could contribute to the enhanced tumor susceptibility observed in HNPCC patients. It is presently not known whether defective apoptosis contributes to the increased tumor multiplicity observed in MMR^- $Apc^{Min/+}$ mice.

Min/+ mice are predisposed to several types of extra-intestinal lesions. Simple haploinsufficiency at the *Apc* locus seems to be inadequate to explain the development of these lesions, which include cystic crypts, desmoid fibromas, and epidermoid cysts. Mlh1 deficiency caused an approximately threefold increase in the multiplicity of cystic intestinal crypts relative to $Mlh1^{+/+}$ *Min*/+ mice. This increase in cystic crypt multiplicity is similar to the effect previously observed for ENU treated *Min*/+ mice (Shoemaker *et al*., 1995). Cystic crypts are morphologically and histologically distinct from intestinal adenomas and thus may represent a non-neoplastic endpoint lesion. The absence of detectable Apc expression in the cells from cystic crypts suggests that events beyond elimination of normal Apc function are needed for full adenoma formation. The incidence of epidermoid cysts and desmoid tumors was also increased in *Min*/+ $Mlh1^{-/-}$ mice. Our results indicate that, as for the formation of intestinal adenomas, loss of MMR influences the somatic events necessary for the formation of lesions in tissues other than the intestinal epithelium in *Min*/+ mice. Recent studies have shown that $Apc^{+/\Delta 716}$ $Dpc4^{+/-}$ mice are also strongly predisposed to epidermoid cyst formation (Takaku *et al*., 1998) and that desmoid tumorigenesis is enhanced in *Min*/+ $p53^{-/-}$ mice (Halberg *et al*., 2000). These findings suggest that *Mlh1* deficiency may enhance the probability of acquiring *Dpc4* and/or *p53* mutations in these tissues.

Detailed examination of both tumor size and histology failed to reveal any significant evidence for tumor progression in *Min*/+ $Mlh1^{-/-}$ mice. These results are consistent with investigations of intestinal tumorigenesis in *Min*/+ $Msh2^{-/-}$ and *Min*/+ $Pms2^{-/-}$ mice (Baker *et al*., 1998; Reitmair *et al*., 1996). The lack of tumor progression may reflect the shortened life span that results from the high adenoma burden in these animals. However, it is worth noting that aggressive intestinal tumor progression is also relatively uncommon in $Mlh1^{-/-}$ (and other mismatch repair defective strains) that develop a small number of tumors and routinely live longer than 1 year. These results argue that the mutator phenotype associated with compromised mismatch repair may have a stronger effect on intestinal tumor initiation than on tumor progression.

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