Myh Deficiency Enhances Intestinal Tumorigenesis in Multiple Intestinal Neoplasia (Apc\textsuperscript{Min/−}) Mice

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ABSTRACT

Monoallelic APC and biallelic MYH (homolog of Escherichia coli mutY) germ-line mutations are independently associated with a strong predisposition to colorectal adenomas and carcinoma in humans. Whereas mice heterozygous for mutant Apc develop intestinal tumors, mice homozygous for mutant Myh do not show increased tumor susceptibility. We analyzed the phenotype of Apc\textsuperscript{Min/−}/Myh\textsuperscript{+/+} mice and found that they developed significantly more adenomas in the small intestine than did Apc\textsuperscript{Min/−}/Myh\textsuperscript{−/+} or Apc\textsuperscript{Min/−}/Myh\textsuperscript{−/−} mice (median 231 versus 151 versus 152). In the large bowel, Apc\textsuperscript{Min/−}/Myh\textsuperscript{−/+} mice showed significant increases in the number of aberrant crypt foci. In addition, Apc\textsuperscript{Min/−}/Myh\textsuperscript{−/−} mice developed an increased number of mammary tumors. Molecular analyses suggested that at least 19% of intestinal tumors from Apc\textsuperscript{Min/−}/Myh\textsuperscript{−/−} mice had acquired intragenic Apc mutations rather than allelic loss. Consistent with a defect in base excision repair, three intragenic Apc mutations in polyps without allelic loss from Apc\textsuperscript{Min/−}/Myh\textsuperscript{−/−} mice were shown to be G:C to T:A transversions which resulted in termination in the large bowel,

INTRODUCTION

Familial adenomatous polyposis is a rare, autosomal dominant disease characterized by hundreds or thousands of colorectal adenomas, resulting in a near 100% lifetime risk of colorectal cancer. In addition, familial adenomatous polyposis patients often develop extra-colonic features, including upper gastrointestinal tumors, congenital hypertrophy of the retinal pigment epithelium (CHRPE), desmoid tumors and dental abnormalities. Familial adenomatous polyposis is caused by germ-line mutations in the adenomatous polyposis coli (APC) gene, and somatic APC mutations are found in up to 80% of sporadic colorectal cancers. Both familial adenomatous polyposis and sporadic tumors usually harbor biallelic APC mutations, resulting in constitutive activation of the Wnt signaling pathway by up-regulating the level of β-catenin (1, 2).

Patients with MYH (homolog of Escherichia coli mutY)-associated polyposis have a phenotype similar to patients with mild familial adenomatous polyposis (3, 4). However, the disease shows autosomal recessive inheritance. MYH-associated polyposis accounts for ~30% of patients with 15 to 100 adenomas, and ~10% of APC mutation-negative patients with classical polyposis (>100 adenomas; ref. 4). Colorectal cancer associated with MYH-associated polyposis appears to have a prevalence similar to that associated with familial adenomatous polyposis (5).

MYH-associated polyposis is caused by biallelic germ-line mutations in the base excision repair gene MYH (6). Base excision repair is important in repairing oxidative DNA damage originating as a byproduct of normal cellular metabolism or from extrinsic sources. The most frequent mutagenic lesion is 7,8-dihydro-8-oxoguanine (8-oxo-G). 8-oxo-G can mispair with adenine during DNA replication and cause G:C to T:A transversions. The products of three human base excision repair genes, MTH1, OGG1, and MYH, act together to prevent 8-oxo-G-induced mutagenesis. In the nucleotide pool, MTH1 hydrolyzes 8-oxo-dGTP to 8-oxo-dGMP (7–9); OGG1 detects and removes 8-oxo-G generated in situ or incorporated into DNA during replication (10–13); and MYH, an adenine-specific DNA glycosylase, scans the daughter strand after DNA replication and removes adenosines mispaired with 8-oxo-G (14–16). MYH deficiency leads to an increased frequency of G:C to T:A mutations in genes such as APC and K-ras in MYH-associated polyposis tumors (17, 18).

In contrast to MYH-associated polyposis patients, Myh-deficient mice do not seem to show increased tumor susceptibility. Mice homozygous for a loss-of-function Myh allele (Myh\textsuperscript{−/−}) showed no difference from wild-type littermates in terms of survival after 14 months or of tumor incidence after 15 to 17 months (19). However, Myh and Ogg1 double knockout mice displayed a marked increase of lymphomas and lung and ovarian tumors. Myh\textsuperscript{−/−} mice have been shown to accumulate 8-oxo-G at an increased rate in the liver when compared with wild-type mice, although no age-dependent accumulation was detected in brain, small intestine, spleen, or kidney (20).

We aimed at developing a mouse model for MYH-associated polyposis. We reasoned that, despite the data above, Myh deficiency might have some subtle effect on the intrinsic mutation rate that was insufficient to cause macroscopic tumor development on an otherwise wild-type genetic background. Because most colorectal tumors from MYH-associated polyposis patients seem to harbor biallelic APC mutations (17, 18), we crossed Myh-deficient mice with multiple-intestinal-neoplasia (Apc\textsuperscript{Min/−}) mice, which carry a nonsense mutation at codon 850 of the Apc gene. Apc\textsuperscript{Min/−} mice are predisposed to multiple adenomas, primarily in the small intestine. In addition, these mice have an increased risk of developing mammary tumors, desmoids, and epidermoid cysts (21). Because homozygosity for the Min allele is embryonic-lethal, we analyzed animals of the six remaining genotypes (Apc\textsuperscript{Min/−}/Apc\textsuperscript{Min/−}, Apc\textsuperscript{Min/−}/Myh\textsuperscript{−/+}, Apc\textsuperscript{Min/−}/Myh\textsuperscript{−/−}, Apc\textsuperscript{Min/−}/Myh\textsuperscript{−/+}, Apc\textsuperscript{Min/−}/Myh\textsuperscript{−/−}, and Apc\textsuperscript{−/+}/Myh\textsuperscript{−/−}) for the development of intestinal tumors, cystic crypts of the small intestine and aberrant crypt foci (ACFs) of the colon.
MATERIALS AND METHODS

Mouse Breeding. Animals were bred at Cancer Research United Kingdom Laboratories (South Mimms, United Kingdom). Experiments were conducted in full accordance with the United Kingdom Animal Procedures Act 1986. The C57BL/6J-ApcMin/+ mice were originally a gift from A. R. Moser (McGard Laboratory, University of Wisconsin, Madison, WI). The 129/C57BL/6J-Myh+/− mice have been described previously (19). Male ApcMin/+ mice were bred with female Myh+/− mice. Of the F1 generation, male ApcMin/+/Myh+/− mice were intercrossed with Apc+/−/Myh+/− females. Only the F2 offspring were included in the analyses because these are expected to share equivalent contributions of the genetic background from the two strains, with the only systematic difference between the groups of mice being Myh itself and, inevitably, small regions flanking this gene. Our three study groups were ApcMin/+/Myh+/+, ApcMin+/Myh+/−, and ApcMin+/Myh−/− mice; our three control groups were Apc+/−/Myh+/+, Apc+/−/Myh+/−, and Apc+/−/Myh−/− mice. Animals of the six groups arose at the expected Mendelian frequencies (P = 1, χ2 test).

Genotyping. DNA was extracted from tail snips by using standard methods. All of the animals were genotyped for Apc and Myh with three-primer, allele-specific PCR assays (19, 22); all founder mice were homozygous for the sensitive allele of Momi1 (22).

Counts of Tumors, Cystic Crypts, and Aberrant Crypt Foci. Mice were sacrificed at 110 to 130 days by CO2 asphyxiation. The intestinal tract was removed and divided into four segments consisting of three equal lengths of small intestine (proximal, middle, and distal) and the whole of the large intestine. Each segment was flushed with PBS, opened longitudinally, and laid out on filter paper. The samples were fixed in Carnoy’s medium for 3 hours and stored in 70% EtOH. Before analysis, preparations were stained with 0.2% methylene blue for 3 minutes and were washed in PBS for 20 minutes. Tumors were counted with a dissecting microscope at ×3 magnification, and categorized according to size with an inoculating loop with an inner diameter of 1 mm and an outer diameter of 2 mm. Cystic crypts in the small intestine and ACFs in the large intestine were counted at ×3 and ×5 magnification, respectively. Criteria used to identify ACFs were dark-blue staining, increased crypt size, and flat appearance hidden in the surrounding mucosa. All of the counts were done in duplicate by an observer (O. M. S.) blinded to the genotype of the mice. A subset of cases was counted by a second observer (C. T.) to confirm the first observer’s results.

Histology. After tumor counting, the proximal small-bowel segment from five animals per study group (ApcMin/+/Myh+/+, ApcMin+/Myh+/−, ApcMin+/Myh−/− Myh+/−) was rolled up, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E). In addition, selected large tumors of the small and large intestine were processed, as well as all detected mammary tumors. To examine the histology of colonic ACFs, serial transverse sections were prepared at 4 μm. All samples were analyzed by a histopathologist (G. S.) blinded to the genotype of the mice.

DNA Extraction from Tumor and Normal Tissue. Tumors were dissected from the methylene blue-stained, whole-mount preparations with a dissecting microscope at ×3 magnification. A fresh scalpel was used for each sample, and care was taken to avoid the surrounding normal epithelium. In total, 134 small-bowel and 6 colonic tumors from 12 ApcMin/+/Myh+/+ mice, 147 small-bowel and 21 colonic tumors from 16 ApcMin+/Myh+/− mice, and 167 small-bowel and 16 colonic tumors from 13 ApcMin+/Myh−/− mice were collected. Tail tissue and normal bowel epithelium were also sampled. DNA was extracted from these samples with standard methods.

Loss of Heterozygosity (Allelic Loss) Analysis at Apc and Myh. Loss of heterozygosity (LOH) at Apc was analyzed with a fluorescence–single-strand conformational polymorphism (F-SSCP) assay that distinguished the wild-type and Min alleles (Fig. 1) with dye-labeled primers: Apc LOH1 forward (5′-TACGGTATTGCCCAGCTCTT-3′) and Apc LOH1 reverse (5′-TGCAGACCTCGTTTTGATGA-3′). Each 25-μL PCR reaction contained 1× PCR buffer (Perkin-Elmer, Boston, MA), 2 mMol/L MgCl2, 200 μmol/L dNTPs, 200 μmol/L concentration of each primer, 30 ng of genomic DNA, and 1 unit of TaqDNA polymerase (Quagen). PCR conditions consisted of 95°C for 5 minutes, 35 cycles of 95°C/55°C/72°C for 1 minute each, and a final step at 72°C for 10 minutes. The resulting PCR products were run at 18°C through a 50-cm capillary on the ABI 3100 and analyzed with Genotyper 2.5 software (Perkin-Elmer Applied Biosystems). Allelic loss was considered present if the relative ratio of normal-to-tumor peak area was <0.5 or >2, thereby allowing for contaminating normal tissue. The validity of the F-SSCP-LOH assay had been established with serial dilutions of Apc wild-type and Min alleles, and results were confirmed for selected samples by sequencing tumor DNA alongside normal DNA (details not shown). Loss of the wild-type Myh allele was analyzed with the same allele-specific PCR assay as for the genotyping, in which a 262-bp product is diagnostic of the wild-type allele and a 376-bp product is diagnostic of the targeted allele. The common forward primer was fluorescently labeled. PCR products were run on the ABI 3100 and allelic loss scored as above.

Mutation Analysis. Primer pairs amplifying coding regions and exon-intron boundaries were designed for Apc exons 5 to 14, Myh exons 2 to 15, and K-ras exon 1. Apc codons 652 to 1648 (exon 15) were covered by 10 overlapping primer pairs. Details and PCR conditions are available from the authors. Apc and Myh were screened by F-SSCP analysis. In addition, areas of Apc rich in T/AGAA sequences that are particularly susceptible to G:C→T:A mutation to chain termination codons in humans (codons 894-1357 and 1545–1648 encompassing 44 such sites) were analyzed by direct DNA sequencing. For F-SSCP analysis, PCR products were run at 18°C and 24°C on the ABI 3100 and were analyzed with Genotyper 2.5 software. For tumor samples showing aberrant SSCP bands, the relevant region of the gene was sequenced directly from a new PCR product alongside normal DNA.

RESULTS

Myh Deficiency Enhances Tumor Formation in the Small Intestine of ApcMin/+ Mice. In the small intestine, ApcMin+/Myh+/+, ApcMin+/Myh−/−, and ApcMin+/Myh+/− F2 offspring developed median numbers of 151, 152, and 231 polyps, respectively (Table 1; Fig. 2). Thus, tumor multiplicity in the small intestine was increased by...
No adenomas or cystic crypts were found in the small intestine of any of 15 animals from each of the three control groups (Apc\(^{+/+}\)/Myh\(^{+/+}\), Apc\(^{+/+}\)/Myh\(^{-/-}\), and Apc\(^{+/+}\)/Myh\(^{-/-}\) mice).

**Myh Deficiency Increases ACF Number in the Colon of Apc\(^{Min/+}\) Mice.** Apc\(^{Min/+}\)/Myh\(^{+/+}\), Apc\(^{Min/+}\)/Myh\(^{-/-}\), and Apc\(^{Min/+}\)/Myh\(^{-/-}\) mice all developed a median of one polyp in the colon (P = 0.18 for Apc\(^{Min/+}\)/Myh\(^{+/+}\) versus Apc\(^{Min/+}\)/Myh\(^{-/-}\); P = 0.16 for Apc\(^{Min/+}\)/Myh\(^{+/+}\) versus Apc\(^{Min/+}\)/Myh\(^{-/-}\), Mann–Whitney U test; Table 1; Fig. 3). In addition, adenoma sizes did not differ significantly between Apc\(^{Min/+}\)/Myh\(^{+/+}\), Apc\(^{Min/+}\)/Myh\(^{-/-}\), and Apc\(^{Min/+}\)/Myh\(^{-/-}\) mice, with 82% (19 of 23), 60% (28 of 47), and 88% (21 of 24), of lesions being >2 mm in size, respectively (P = 0.06 for Apc\(^{Min/+}\)/Myh\(^{+/+}\) versus Apc\(^{Min/+}\)/Myh\(^{-/-}\); P = 0.70 for Apc\(^{Min/+}\)/Myh\(^{+/+}\) versus Apc\(^{Min/+}\)/Myh\(^{-/-}\), Fisher’s Exact test).

ACFs are putative premalignant lesions that develop in the colon of Apc\(^{Min/+}\) mice (25–27) and that can be induced by chemical carcinogens (28, 29). ACFs were counted for the same samples that were also scored for colonic adenomas. Apc\(^{Min/+}\)/Myh\(^{+/+}\), Apc\(^{Min/+}\)/Myh\(^{-/-}\), and Apc\(^{Min/+}\)/Myh\(^{-/-}\) had a median number of 20, 15, and 43 ACFs, respectively (Table 1). Thus, Myh-deficient mice showed an approximately 2-fold increase in ACF number compared with Myh wild-type mice (P = 0.001, Mann–Whitney U test), whereas heterozygous Myh deficiency had no effect (P = 0.10, Mann–Whitney U test). In all of the animals, ACFs occurred predominantly in the distal colon. Histopathological analysis of selected ACFs revealed mild dysplasia in all of the cases (Fig. 3). Surprisingly, ACF numbers were not associated with colonic tumor numbers, which suggested that

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**Table 1** Effect of heterozygous and homozygous Myh deficiency on adenoma, cystic crypt, and ACF numbers in the intestine of Apc\(^{Min/+}\) mice

<table>
<thead>
<tr>
<th>Segment</th>
<th>Adenomas</th>
<th>Cystic crypts</th>
<th>ACFs</th>
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<tbody>
<tr>
<td>Small bowel</td>
<td></td>
<td></td>
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<tr>
<td>Proximal</td>
<td>151, 62–285 (161 ± 58)</td>
<td>84, 30–150 (78 ± 33)</td>
<td>13, 3–90 (20 ± 24)</td>
</tr>
<tr>
<td>Middle</td>
<td>65, 22–105 (62 ± 23)</td>
<td>84, 30–150 (78 ± 33)</td>
<td>13, 3–90 (20 ± 24)</td>
</tr>
<tr>
<td>Distal</td>
<td>62, 5–128 (60 ± 34)</td>
<td>68, 13–184 (79 ± 47)</td>
<td>13, 0–6 (2 ± 2)</td>
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<tr>
<td>Large bowel</td>
<td></td>
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<tr>
<td>Adenomas</td>
<td>Apc(^{Min/+})/Myh(^{+/+}) (n = 15)</td>
<td>Apc(^{Min/+})/Myh(^{-/-}) (n = 25)</td>
<td>Apc(^{Min/+})/Myh(^{-/-}) (n = 15)</td>
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<tr>
<td>Cystic crypts</td>
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<td>ACFs</td>
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**NOTE.** Values are medians and ranges. Means ± SD are given in parentheses. The three study groups had similar male-to-female ratios (8:7, 11:14, and 6:9 for Apc\(^{Min/+}\)/Myh\(^{+/+}\), Apc\(^{Min/+}\)/Myh\(^{-/-}\), and Apc\(^{Min/+}\)/Myh\(^{-/-}\) mice, respectively).

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![Figure 2](image-url) Adenomatous polyps and cystic crypts in the small intestine of Apc\(^{Min/+}\)/Myh\(^{-/-}\) mice. Methylene blue-stained whole-mount preparations showing adenomatous polyps (A) and cystic crypts (B, arrowheads). H&E-stained transverse sections showing cystic crypts with normal pathology (C) and dysplastic change (D).

![Figure 3](image-url) Adenomatous polyps and ACFs in the colons of Apc\(^{Min/+}\)/Myh\(^{-/-}\) mice. Methylene blue-stained, whole-mount preparations showing adenomatous polyps (A) and ACFs (B, arrowheads). H&E-stained transverse sections showing mononuclear (C) and polycystic (D) ACFs. Scale bars, 500 μm.
there is no simple stochastic progression of ACF to adenoma (R² = 0.0054, P = 0.61, linear regression analysis).

No tumors or ACFs were found in the colons of any of 15 animals from each of the three control groups (Apc+/+/Myh+/+, Apc+/+/, Myh+/−, and Apc+/−/Myh+/−).

Adenomas from Myh-Deficient ApcMin/+ Mice Show No Evidence of Progression to Carcinoma. Histopathological analysis of 8 small-bowel and 8 large-bowel tumors from ApcMin/+/Myh+/+, 21 small-bowel and 17 large-bowel tumors from ApcMin/+/Myh+/−, and 30 small-bowel and 17 large-bowel tumors from ApcMin/+/Myh−/− mice revealed that all were adenomatous polyps and none displayed evidence of malignant change. Thus, similar to tumors from Myh wild-type and heterozygous mice, tumors from Myh-deficient ApcMin/+ mice did not appear to progress to carcinoma by 120 to 130 days of age. In addition, no differences were apparent in the histology of normal intestinal mucosa between the three study groups.

Myh-Deficient ApcMin/+ Mice Develop an Increased Frequency of Mammary Gland Tumors. Mammary gland tumors were detected in 0 (0%) of 16 ApcMin/+/Myh+/+, 3 (7%) of 43 ApcMin/+/Myh+/−, and 4 (27%) of 15 ApcMin/+/Myh−/− mice. The increase in mammary gland tumors was significant in Myh-deficient mice as compared with Myh wild-type and heterozygous mice (P = 0.028, Fisher’s Exact test). All mammary gland tumors showed squamous cell differentiation, and one, from a Myh-deficient mouse, had invaded into the surrounding fat.

Small-Bowel Tumors from ApcMin/+/Myh−/− but Not from ApcMin/+/Myh+/− Mice Show a Reduced Frequency of Allelic Loss at Apc. Tumor formation in ApcMin/+ mice has been shown to involve somatic inactivation of the wild-type Apc allele (30, 31). On a C57BL/6J background, this generally occurs by allelic loss. If the increased number of small-bowel tumors in ApcMin/+/Myh−/− mice reflected a failure to repair oxidative DNA damage, a corresponding proportion of the tumors from these mice were expected to harbor truncating somatic mutations rather than allelic loss at Apc. We found that 118 (93%) of 127 tumors from ApcMin/+/Myh+/+ mice and 87 (89%) of 98 tumors from ApcMin/+/Myh+/− mice had lost the wild-type Apc allele, compared with 121 (81%) of 150 tumors from ApcMin/+/Myh−/− mice (Fig. 1; P < 0.001, for Myh-deficient mice versus Myh wild-type and heterozygous mice, Fisher’s Exact test). These results are consistent with the notion that whereas the growth of most polyps in ApcMin/+/Myh+/− mice is initiated because of loss of wild-type Apc, as in ApcMin/+/Myh+/+ mice, the significant excess of tumors occurs because absent Myh activity leads to “second hits” that are distinct from allelic loss. The fraction of tumors that retained the Apc wild-type allele in Myh-deficient mice (~19%) was, however, lower than the observed increase in tumor number (~53%). This difference may be the result of chance but may also reflect a requirement for mutation in one or more additional genes for macroscopic polyp development. Alternatively, Myh deficiency may have some other effect in addition to increasing the intrinsic mutation rate.

Analysis of large-bowel polyps from each genotypic class revealed no significant difference in the frequency of allelic loss at Apc. We found that 6 (100%) of 6 tumors from ApcMin/+/Myh+/+, 18 (100%) of 18 tumors from ApcMin/+/Myh+/−, and 15 (94%) of 16 tumors from ApcMin/+/Myh−/− harbored loss of the wild-type Apc allele (P = 1.0 for ApcMin/+/Myh+/− versus ApcMin/+/Myh−/−, Fisher’s Exact test).

G:C → T:A Truncating Somatic Mutations Occur in Adenomas from Myh-Deficient ApcMin/+ Mice. All 29 small-bowel tumors without allelic loss at Apc from ApcMin/+/Myh−/− mice were examined for somatic mutations in Apc. Mutations were detected in 3 (10%) polyps. All three changes were G:C to T:A transversions (2× nucleotide (nt) 3919G→T, nt4754C→A) resulting in “stop” codons (2× E1307X, S1585X). The sequence context around the E1307X mutations was AAAGAGA (Fig. 4), the reverse of the consensus motif (underlined) identified in human MYH-associated polyposis tumors. No somatic Apc mutations were detected in eight and nine small-bowel tumors without allelic loss from ApcMin/+/Myh+/+ and ApcMin/+/Myh−/− mice, respectively.

Adenomas from Myh-Deficient ApcMin/+ Mice Do Not Harbor K-ras Mutations. Activating K-ras mutations occur in ~50% of sporadic colorectal adenomas in humans (32, 33), and tumors from MYH-associated polyposis patients show an increased frequency of K-ras mutations as compared with tumors from familial adenomatous polyposis patients (18, 34). In addition, about 75% of lung tumors from Myh and Ogg1 double-knockout mice harbor G to T mutations in K-ras codon 12 (19). However, we found no K-ras mutation in 20 intestinal tumors from ApcMin/+/Myh+/+ or in 40 tumors from ApcMin/+/Myh−/− mice (35).

Adenomas from Myh-Heterozygous ApcMin/+ Mice Do Not Show Somatic Mutations or Allelic Loss at Myh. In our study, ApcMin/+/Myh+/− mice did not show an increase in polyp number or size as compared with ApcMin/+/Myh−/− mice, providing little evidence for a role of Myh heterozygosity in tumor development. However, it has been suggested in humans that inactivation of the wild-type Myh allele in heterozygotes is selected in intestinal tumorogenesis (36). We, therefore, screened adenomas from ApcMin/+/Myh+/− mice for somatic mutations and allelic loss at Myh. Only 2 (1%) of 137 tumors showed allelic loss at Myh, but in one of these cases, LOH targeted the inherited mutant rather than the wild-type allele. A total of 82 tumors were subsequently screened for somatic mutations in Myh exons 2 to 15, but no changes were detected. Taken together, these data do not support the hypothesis that inactivation of the wild-type Myh allele is selected in tumors from ApcMin/+/Myh+/− mice.

DISCUSSION

In this study, we have demonstrated that Myh deficiency has a significant effect on both intestinal and extraintestinal tumor development in ApcMin/+ mice. ApcMin/+/Myh−/− mice showed an in-
increased adenomatous polyp number in the small bowel. In addition, Myh deficiency increased the frequency of mammary tumors. The magnitude of the increase in tumor number in the small intestine was correlated with the fraction of tumors that failed to show loss of the wild-type Apc allele. This association is consistent with studies on tumors from ApcMin/+ mice with deficiencies in the base excision repair protein Mbd4 or the MMR proteins Mlh1, Msh2, or Pms2 (24, 37–40). In addition, G:C→T:A somatic Apc mutations were found in polyps from ApcMin/+/Myh−/− mice, as expected for defective base excision repair. Taken together, these data strongly suggest that the loss of Myh function enhances tumorigenesis in ApcMin/+ mice.

Our finding of exclusively G:C→T:A somatic mutations in polyps from ApcMin/+/Myh−/− mice (and no such mutations in polyps from ApcMin/+/Myh+/+ and ApcMin/+/Myh+/− mice) provides good evidence to show that this is the mechanism that causes the excess of tumors, as it is in humans (6, 17). Previous studies have shown somatic G:C→T:A Apc mutations to be very rare in tumors from ApcMin/+ mice (40, 41), we detected truncating somatic Apc mutations in a minority of ApcMin/+/Myh−/− tumors without allelic loss, a similarly low mutation frequency to that reported in almost all previous studies in which ApcMin/+ mice have been crossed with animals defective in DNA repair (24, 37, 40). A highly plausible explanation for the low frequency of somatic, truncating Apc mutations is that adenomas from ApcMin/+ mice are polyclonal, the contribution of each mutant allele to the tumor DNA too small to be detected in most cases (42).

Detailed examination of both tumor size and histology failed to reveal any evidence of tumor progression in the small bowel of ApcMin/+/Myh−/− mice by 120 to 130 days of age. These data are consistent with studies on tumors from ApcMin/+ mice with deficiencies in Mlh1, Msh2, or Pms2 (24, 38, 39) and suggest that Myh deficiency has a larger effect on tumor initiation than on progression in the small bowel. ApcMin/+/Myh−/− mice showed a more pronounced increase in tumor number in the proximal and middle segments than in the distal segment of the small bowel. A similar pattern is observed in MYH-associated polyposis patients who sometimes develop duodenal polyps (4). MMR-deficient ApcMin/+ mice, by contrast, show a uniform increase in the number of small-bowel polyps (24). The finding in ApcMin/+/Myh−/− mice and MYH-associated polyposis patients may reflect variation in the level of oxidative stress throughout the small bowel, although it could also reflect variation in the level or activity of Myh protein or site-specific differences in functional redundancy.

Heterozygous Myh deficiency had no detectable effect on tumor development in ApcMin/+ mice or the frequency of allelic loss at Apc. Furthermore, inactivation of the wild-type Myh allele was very rare in these mice. Although these data must be interpreted with some caution because of the possible polyclonal origin of polyps in the mouse small intestine, they are consistent with our findings, in sporadic colorectal cancers from humans, that failed to detect somatic mutations or allelic loss at MYH (43).

Besides intestinal adenomas, ApcMin/+ mice are predisposed to two types of lesions of unknown neoplastic potential, cystic crypts of the small intestine and ACFs of the colon. Cystic crypt multiplicity is increased in ApcMin/+ mice deficient in Mlh1 or treated with chemical carcinogens (23, 24). Although our data were suggestive of a similar effect in Myh-deficient ApcMin/+ mice, the increase did not reach statistical significance. In contrast, ApcMin/+/Myh−/− mice showed a significant, ~2-fold, increase in the number of ACFs, supporting a role of Myh deficiency in promoting the development of these lesions.

In conclusion, our data suggest that Myh deficiency leads to an increase in the intrinsic mutation rate. This increase is insufficient to cause intestinal tumorigenesis in our Myh-deficient mice on an otherwise wild-type genetic background but is made manifest when mice who already harbor the first step of tumorigenesis (Apc mutation) are studied. Myh deficiency may have a more profound effect on the mutation rate in humans, who are subjected to greater levels of oxidative stress in the intestine than are the laboratory mouse. Although we did not detect somatic K-ras mutations, we believe that the excess adenomas in ApcMin/+/Myh−/− mice are a useful model of MYH-associated polyposis, allowing studies of molecular mechanisms or the evaluation of therapeutic agents. Furthermore, suppression of loss of the wild-type Apc allele, for example, by crossing ApcMin/+/Myh−/− mice onto the AKR genetic background (44), may produce a mouse model of MYH-associated polyposis in which most polyps develop as a result of defective base excision repair.

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