DOI: 10.1534/genetics.108.091967

The Pleiotropic Phenotype of *Apc* Mutations in the Mouse: Allele Specificity and Effects of the Genetic Background

Richard B. Halberg,* Xiaodi Chen,* James M. Amos-Landgraf,* Alanna White,* Kristin Rasmussen,* Linda Clipson,* Cheri Pasch,* Ruth Sullivan,† Henry C. Pitot* and William F. Dove*,‡,[1]

**McArdle Laboratory for Cancer Research,* †*Research Animal Resources Center and Comprehensive Cancer Center and* ‡*Laboratory of Genetics, University of Wisconsin, Madison, Wisconsin 53706*

Manuscript received June 27, 2008
Accepted for publication July 6, 2008

ABSTRACT

Familial adenomatous polyposis (FAP) is a human cancer syndrome characterized by the development of hundreds to thousands of colonic polyps and extracolonic lesions including desmoid fibromas, osteomas, epidermoid cysts, and congenital hypertrophy of the pigmented retinal epithelium. Afflicted individuals are heterozygous for mutations in the *APC* gene. Detailed investigations of mice heterozygous for mutations in the ortholog *Apc* have shown that other genetic factors strongly influence the phenotype. Here we report qualitative and quantitative modifications of the phenotype of *Apc* mutants as a function of three genetic variables: *Apc* allele, *p53* allele, and genetic background. We have found major differences between the *Apc* alleles *Min* and *1638N* in multiplicity and regionality of intestinal tumors, as well as in incidence of extracolonic lesions. By contrast, Min mice homozygous for either of two different knockout alleles of *p53* show similar phenotypic effects. These studies illustrate the classic principle that functional genetics is enriched by assessing penetrance and expressivity with allelic series. The mouse permits study of an allelic gene series on multiple genetic backgrounds, thereby leading to a better understanding of gene action in a range of biological processes.

HIGHLY penetrant familial cancers, including 200 variants, represent 5–10% of the total cancer burden (Fearon 1997; Nagy *et al.* 2004; Garber and Offit 2005). Individuals in such families inherit a germline mutation that predisposes them to cancer of one or more tissues. Identification of the mutated gene is only the first step in understanding the molecular basis of a particular familial cancer. Patients carrying the same mutation can have quite different clinical outcomes because other genes affect both penetrance (the fraction of mutation carriers exhibiting one or another of the neoplastic phenotypes) and expressivity (tumor multiplicity and progression). Thus, a familial cancer is not a fixed consequence of an alteration in a single gene, but involves the effect of such an alteration in the context of the alleles of other genes.

Patients afflicted with familial adenomatous polyposis (FAP) carry mutations in the *APC* gene (Groden *et al.* 1991; Kinzler *et al.* 1991). They are predisposed to the development of colorectal tumors by the third decade of life (Croner *et al.* 2005), with tumor multiplicities from 10 to 2000. FAP is a rare disease, whereas sporadic colorectal cancer is common. The vast majority of tumors from these patients carry somatic mutations in *APC* (Nishisho *et al.* 1991)—a gene essential to homeostasis in the intestine. Consequently, a full characterization of the molecular basis of this familial colon cancer syndrome may facilitate the understanding and management of the more prevalent sporadic disease.

The *APC* gene is broadly expressed in mammals and has been shown to be necessary not only for growth of the early postimplantation embryo (Moser *et al.* 1995), but also for skin and thymus (Kuraguchi *et al.* 2006). This may explain the observation that FAP patients are predisposed to a number of extracolonic neoplasms. The relative risks vary dramatically by site: from 852 for desmoid fibromas to 4.46 for pancreatic carcinomas (Giardiello *et al.* 1993; Gurbuz *et al.* 1994). Multiplicity of colorectal polyps and incidence of extracolonic manifestations are at least partially affected by the particular *APC* germline mutation (Enomoto *et al.* 2000; Bertario *et al.* 2001, 2003): mutations in the 5′ end of the gene result in an attenuated form of FAP that is characterized by the development of few colonic polyps, while mutations in the 3′ end increase the tendency to develop desmoid fibromas. Apc has been linked to several different biological processes through its interactions with other proteins. The binding of APC to β-catenin and consequent degradation of the latter is essential for maintaining homeostasis in the mammalian intestine (reviewed in Matushansky *et al.* 2008).

[1]*Corresponding author:* University of Wisconsin, 1400 University Ave., Madison, WI 53706. E-mail: dove@oncology.wisc.edu

Several mouse models of FAP have been generated over the past decade (Moser *et al.* 1990; Oshima *et al.* 1997; Shibata *et al.* 1997; Smits *et al.* 1998, 1999). The *Min* allele of *Apc*, induced by germline mutagenesis with ethylnitrosourea (Moser *et al.* 1990), is a T-to-A transversion that creates a translational stop at codon 850 (Su *et al.* 1992). C57BL/6J (B6) $Apc^{Min/+}$ mice develop on average 100 adenomas along the entire length of the intestinal tract (Cormier and Dove 2000). Tumorigenesis in these mice is initiated when function of the wild-type allele of *Apc* is lost, commonly by somatic recombination (Haigis *et al.* 2004). Two alleles of *Apc*, *1638N* and *1638T*, were generated by inserting a selectable Neomycin resistance cassette at *Apc* codon 1638. B6 $Apc^{1638N/+}$ mice develop on average one tumor near the duct of Vader in the proximal region of the small intestine (Smits *et al.* 1998), while B6 $Apc^{1638T/+}$ mice are tumor free (Smits *et al.* 1999). These insertion alleles differ in the orientation of transcription from the insertion relative to transcription from the *Apc* promoter; they also differ in expression of a protein fragment from the *1638T* but not the *1638N* mutant allele (Smits *et al.* 1999). Smits *et al.* (1998) reported that only 17% of intestinal adenomas from $Apc^{1638N/+}$ mice with a mixed genetic background exhibited maintenance of heterozygosity (MOH) at the *Apc* locus. By contrast, Haigis *et al.* (2004) found that 100% of the intestinal adenomas from $Apc^{1638N/+}$ mice on a congenic B6 genetic background exhibited MOH at *Apc*. They interpreted this observation and the regionality of tumorigenesis in these mice to be consistent with a second hit involving intragenic mutation in the wild-type *Apc* allele. This interpretation has found support from the demonstration of intragenic *Apc* truncation mutations in 16 of 50 tumors from B6 Apc^{1638N} mice (Kucherlapati *et al.* 2007).

The phenotype of a mouse model clearly depends on the particular mutant allele of *Apc*. The multiplicity of intestinal adenomas in $Apc^{Min/+}$ is also profoundly affected by genetic background. AKR/J $Apc^{Min/+}$ mice develop on average less than one adenoma per mouse (Shoemaker *et al.* 1998). One genetic factor contributing to this dramatic reduction in tumor multiplicity is *Mom1* (Gould *et al.* 1996). This locus acts as a semidominant modifier; one copy of the resistance allele ($Mom1^R$) reduces tumor multiplicity by a factor of two, whereas two copies reduce tumor multiplicity by a factor of four (Gould *et al.* 1996). Several other genetic modifiers and environmental factors affecting tumorigenesis in mouse models of FAP have been identified, using a wide variety of experimental approaches. The relevance of these genes and factors to human colorectal cancer continues to be examined.

The molecular basis of the Li–Fraumeni syndrome (LFS) is also beginning to be understood. A breakthrough occurred in early 1990 when Malkin and colleagues demonstrated that afflicted individuals carry a mutation in the *p53* tumor suppressor gene (Malkin *et al.* 1990). LFS is characterized by the development at an unusually early age of breast carcinoma, soft-tissue sarcoma, osteosarcoma, leukemia, brain tumors, and adrenocortical carcinoma (Li and Fraumeni 1969). This diversity in the tumor spectrum probably reflects the involvement of *p53* in numerous cellular processes, including cell cycle arrest, senescence, and apoptosis (reviewed in Harris and Levine 2005). For one specific example, p53 protein stimulates the transcription of *p21*, which encodes a protein that inhibits a complex of Cyclin E and Cdk2 and thereby blocks the cell cycle at G_1. Mutations in *p53* are among the most frequent genetic alterations found in sporadic forms of human cancer (Nigro *et al.* 1989). Changes in p53 activity may be a useful prognosticator of clinical outcome for patients with colorectal cancer (reviewed in Soussi and Beroud 2001).

Mice have been generated that either express a dominant-negative mutant allele of *p53* or lack p53 activity (Donehower *et al.* 1992; Jacks *et al.* 1994; De Vries *et al.* 2002). In particular, Donehower and his colleagues have generated a *p53* knockout allele ($p53^{BCM}$) in which intron 4 and exon 5 are replaced with a selectable marker cassette, and Jacks and his colleagues have generated a *p53* knockout allele ($p53^{tm1Tyj}$) in which exons 2–6 are replaced with a selectable marker cassette. Mice that are homozygous for either of these knockout alleles are viable but develop tumors that are similar to those commonly seen in LFS patients (Donehower *et al.* 1992; Jacks *et al.* 1994; De Vries *et al.* 2002). These mouse models have been treated with carcinogens and bred to other mice each predisposed to a particular cancer to understand more fully the role of p53 in suppressing tumorigenesis in particular tissues (Kemp *et al.* 1993; Clarke *et al.* 1995; Nacht *et al.* 1996; Xu *et al.* 1998; Reilly *et al.* 2000; Yamamoto *et al.* 2000; Halberg *et al.* 2000; Shirai *et al.* 2002; Sakai *et al.* 2003; Hirata *et al.* 2005).

This study extends earlier studies by exploring the phenotype of mice carrying *Apc* mutations as a function of several genetic variables: the *Apc* allele, the *p53* allele and its target *p21*, and the genetic background—C57BL/6J (B6) *vs.* 129S6/SvEvTac (129) *vs.* (B6 × 129)F_1 (F_1).

MATERIALS AND METHODS

Mice: All mice were bred, housed, and investigated in the McArdle Laboratory for Cancer Research, under approved guidelines set forth by the Institutional Animal Care and Use Committee of the American Association for the Assessment and Accreditation of Laboratory Animal Care at the University of Wisconsin. We bred several C57BL/6J (B6) congenic strains to test different combinations of two mutant alleles of *Apc* with two knockout alleles of *p53*. B6 mice carrying the *1638N* allele of *Apc* were obtained through the Mouse Models of Human Cancer Consortium and crossed to our B6 stock carrying $p53^{BCM}$ (Donehower *et al.* 1992; Yang *et al.* 1997). The resulting progeny were intercrossed to generate $Apc^{1638N/+}$

$p53^{+/+}$, $Apc^{1638N/+}$ $p53^{BCM/BCM}$, and $Apc^{+/+}$ $p53^{BCM/BCM}$ mice on the B6 genetic background. In addition, B6 mice carrying $p53^{tm1TyjJ}$ were obtained from The Jackson Laboratory in Bar Harbor, Maine (Jacks *et al.* 1994). Females were crossed to B6 $Apc^{Min/+}$ males from our laboratory stock (Moser *et al.* 1990). The resulting *p53*-heterozygous progeny were intercrossed to generate $Apc^{Min/+}$ $p53^{+/+}$, $Apc^{Min/+}$ $p53^{tm1TyjJ/+}$, $Apc^{Min/+}$ $p53^{tm1TyjJ/tm1TyjJ}$, and $Apc^{+/+}$ $p53^{tm1TyjJ/tm1TyjJ}$ mice on the B6 genetic background. To test whether the effect of *p53* on tumorigenesis in $Apc^{Min/+}$ mice was also affected by genetic background, the Apc^{Min} and $p53^{BCM}$ alleles present in our B6 stock were moved onto the129S6/SvEvTac (129) genetic background by backcrossing for 10 generations. Populations of $Apc^{Min/+}$ $p53^{+/+}$, $Apc^{Min/+}$ $p53^{+/BCM}$, $Apc^{Min/+}$ $p53^{BCM/BCM}$, and $Apc^{+/+}$ $p53^{BCM/BCM}$ mice on the 129 genetic background were generated by intercrossing *p53*-heterozygous mice from the N_{10} through N_{14} generations. In parallel, we generated test mice of all three *p53* genotypes on the (B6 × 129)F_1 hybrid background by crossing B6 $Apc^{+/+}$ $Mom1^{R/R}$ $p53^{+/BCM}$ females to 129 $Apc^{Min/+}$ $Mom1^{S/S}$ $p53^{+/BCM}$ males.

To assess the role of *p21*, (129 × B6)F_2 mice carrying a knockout allele of *p21* ($p21^{tm1Tyj}$) were obtained from The Jackson Laboratory. Females were bred to congenic 129 $Apc^{Min/+}$ mice from our laboratory stock. The resulting *p21*-heterozygous progeny were intercrossed to generate $Apc^{Min/+}$ $p21^{+/+}$, $Apc^{Min/+}$ $p21^{tm1Tyj/+}$, $Apc^{Min/+}$ $p21^{tm1Tyj/tm1Tyj}$, and $Apc^{+/+}$ $p21^{tm1Tyj/tm1Tyj}$ mice. Since these mice had a heterogeneous genetic background composed of 129 and B6 alleles, we moved the $p21^{tm1Tyj}$ allele onto the 129 genetic background by backcrossing for 10 generations. This congenic *p21*-deficient line was then bred to the congenic 129 $Apc^{Min/+}$ stock described above. The resulting *p21*-heterozygous progeny were intercrossed to generate $Apc^{Min/+}$ $p21^{+/+}$, $Apc^{Min/+}$ $p21^{+/tm1Tyj}$, $Apc^{Min/+}$ $p21^{tm1Tyj/tm1Tyj}$, and $Apc^{+/+}$ $p21^{tm1Tyj/tm1Tyj}$ mice. The test mice in this study were genotyped at *Apc*, *Mom1*, *p21*, and *p53* by analyzing DNA prepared from toe clips using PCR assays as described previously (Jacks *et al.* 1994; Halberg *et al.* 2000; Yang *et al.* 2001).

Tumor counts: Mice were killed between 90 and 100 days of age. The number of desmoid fibromas was scored on the abdominal wall and the intestinal tract was removed, opened longitudinally, and laid out as described previously (Moser *et al.* 1990). Samples were fixed in 10% buffered formalin for 16 hr and then placed in 70% ethanol for long-term storage. The number of intestinal tumors was scored under a dissecting microscope. All scoring was performed by a single observer (R. B. Halberg) blind to the genotype of the mice. Differences in tumor multiplicities between groups of mice were tested for statistical significance, using the two-sided Wilcoxon rank sum test. In addition, the pancreas and testes were removed from mice chosen at random. The tissue was fixed in 10% buffered formalin for 16 hr and then placed in 70% ethanol for long-term storage.

Histological analysis: Fixed desmoid fibromas and pancreatic, testicular, and intestinal tumors were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Sections were analyzed by two pathologists, H. C. Pitot and R. Sullivan, blind to the genotype of the mice.

***Apc*^{1638N} pyrosequencing:** Pyrosequencing primers were designed for the single-nucleotide polymorphism rs29821669 that was confirmed to be polymorphic between B6 and the ancestral 129/S6 allele of *Apc* that was targeted and gave rise to the 1638N transgenic line. The final PCR mix concentrations were as follows: 1× GoTaq clear buffer, 1.2 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 μM of each primer (forward, F; reverse, R), 0.6 unit of GoTaq Flexi (Promega, Madison, WI), 8 μl of DNA, and ddH_2O to 50 μl. The PCR cycling profile was 94° for 3 min, followed by 50 cycles of 94° for 15 sec, 57° for 1 min 30 sec, and 72° for 2 min, with a final elongation step of 72° for 10 min. Pyrosequencing was performed according to the manufacturer's protocols using Pyro Gold Reagents on a PSQ96MA machine and PSQ 96MA v2.1 software (Biotage, Uppsala, Sweden). A total of 40 μl of PCR reaction were used per well and only sequence reads with single-base peak heights of >120 units were included. Primer sequences were as follows: forward biotin, CCTTCCATTCTTCCGTCAAA; reverse, GGGGC TTCAATGTTCTCAAA; and pyrosequencing primer, TGTC TTGGTTTTGTACTG.

RESULTS

The *p53* effect is independent of the specific *Apc* and *p53* alleles: The ability of p53 to modulate tumorigenesis in mice carrying a mutant allele of *Apc* is evident with different mutant alleles of *Apc* and of *p53*. First, we tested whether a lack of p53 activity affected the development of intestinal tumors and desmoid fibromas in B6 mice carrying the *1638N* allele of *Apc*. B6 $Apc^{1638N/+}$ mice were crossed to B6 mice carrying the $p53^{BCM}$ knockout allele and the progeny were intercrossed to generate $Apc^{1638N/+}$ mice carrying $p53^{+/+}$ and $p53^{BCM/BCM}$. B6 $Apc^{1638N/+}$ $p53^{BCM/BCM}$ mice developed on average 15 intestinal tumors and 28 desmoid fibromas, compared to B6 $Apc^{1638N/+}$ $p53^{+/+}$ with an average of 1 intestinal tumor and 1 desmoid fibroma (Figure 1, A and B; $P = 2.2 \times 10^{-5}$ and $P = 2.4 \times 10^{-5}$, respectively). Previous reports have shown that a lack of *p53* affects intestinal neoplasia: Smits *et al.* (1998), using $Apc^{1638N/+}$ on a hybrid genetic background, and Halberg *et al.* (2000), using $Apc^{Min/+}$ on the inbred B6 background. The majority of tumors (8/10) from B6 $Apc^{1638N/+}$ mice maintain the wild-type allele of *Apc* during tumorigenesis in the intestine as determined by assessing the allelic ratio of a 129/B6 single-nucleotide polymorphism linked to the *Apc* gene by pyrosequencing. This observation is consistent with an earlier study (Haigis *et al.* 2004).

Next, we tested whether the effect of *p53* on tumorigenesis depended on a specific knockout allele of this gene. B6 mice carrying the $p53^{tm1TyjJ}$ knockout allele were bred to B6 $Apc^{Min/+}$ mice and the progeny were intercrossed to generate $Apc^{Min/+}$ mice carrying $p53^{+/+}$, $p53^{tm1TyjJ/+}$, and $p53^{tm1TyjJ/tm1TyjJ}$. B6 $Apc^{Min/+}$ $p53^{tm1TyjJ/tm1TyjJ}$ mice developed significantly more intestinal tumors and desmoid fibromas than B6 $Apc^{Min/+}$ $p53^{+/+}$ controls (Figure 1, C and D; $P = 0.003$ and $P = 7.8 \times 10^{-4}$, respectively). Interestingly, a heterozygous effect of *p53* deficiency was also observed. B6 $Apc^{Min/+}$ $p53^{tm1TyjJ/+}$ mice developed significantly more intestinal tumors than B6 $Apc^{Min/+}$ $p53^{+/+}$ controls (72 ± 49 *vs.* 53 ± 35, $P = 0.01$). These observations are consistent with results of our previous study utilizing Apc^{Min} and $p53^{BCM}$ (Halberg *et al.* 2000).

In summary (see Figure 1 and Table 1), the effect of *p53* deficiency on *Apc*-dependent intestinal and desmoid neoplasia is evident on the congenic B6 background for

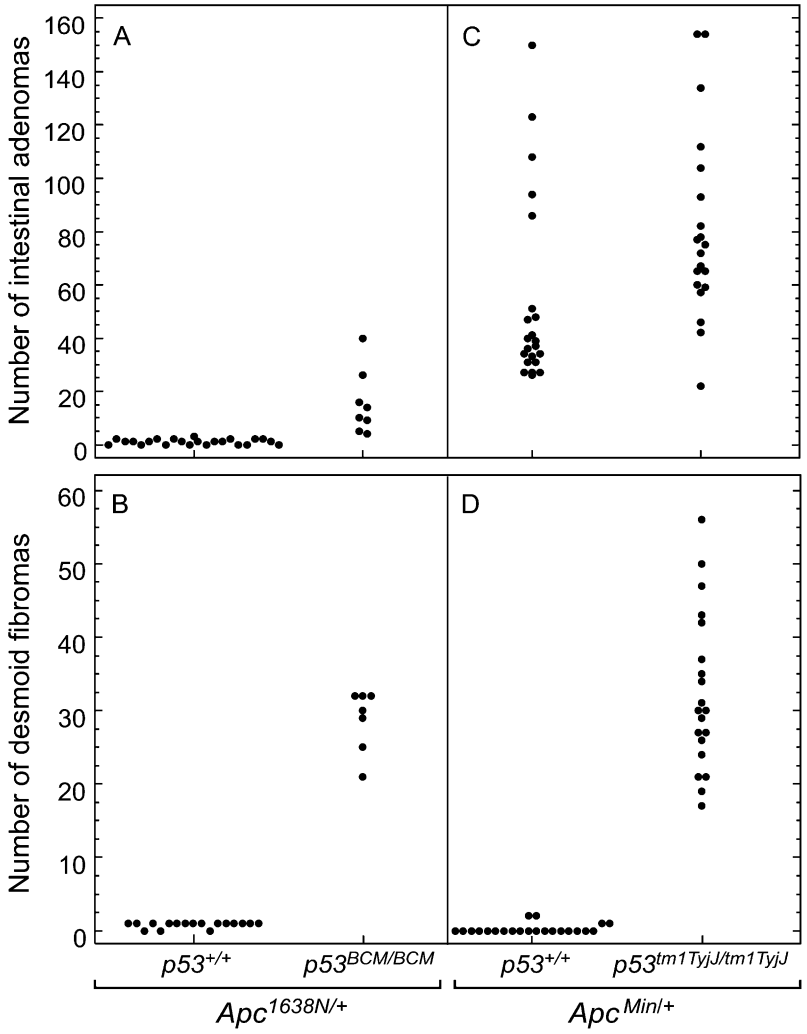

FIGURE 1.—The p53 effect on tumorigenesis in Apc mutants was observed with different *Apc* and *p53* alleles. Crosses between B6 congenic strains were performed to test the interaction between Apc^{1638N} and $p53^{BCM}$ (A and B: $P = 2.2 \times 10^{-5}$ and $P = 2.4 \times 10^{-5}$, respectively) or to test the interaction between Apc^{Min} and $p53^{tm1Tyjl}$ (C and D: $P = 0.003$ and $P = 7.8 \times 10^{-4}$, respectively). Progeny homozygous for the mutant *p53* allele were sacrificed at 100 days of age. The total numbers of intestinal tumors (A and C) and desmoid fibromas (B and D) were scored for each mouse.

each pairwise combination involving heterozygosity for either of two mutant alleles of *Apc* and homozygosity for either of two knockout alleles of *p53*. Notably, we also consistently detected a subtle but significant change in the multiplicity of intestinal tumors in $Apc^{Min/+}$ caused by heterozygosity for *p53* deficiency. The heterozygous effect of *p53* deficiency was observed on the F_1 background, consistent with the previous report of HALBERG *et al.* (2000) on the B6 genetic background: $Apc^{Min/+}$ $p53^{+/BCM}$ mice developed more intestinal tumors than controls but fewer than $Apc^{Min/+}$ $p53^{BCM/BCM}$ mice (Table 1; $P = 0.001$ and 0.03, respectively).

Suppression of tumorigenesis in $Apc^{Min/+}$ mice by *p53* is affected by polymorphic modifiers: Does the 129 genetic background differ from B6 by carrying polymorphic modifier alleles that affect the role of *Apc* and *p53* in intestinal or extracolonic neoplasia? A 129 congenic strain was bred, carrying Apc^{Min} and $p53^{BCM}$. 129 $Apc^{Min/+}$ $p53^{BCM/BCM}$ mice developed 46 ± 29 intestinal tumors, whereas 129 $Apc^{Min/+}$ $p53^{+/+}$ controls developed 50 ± 50 (Table 1; $P = 0.58$). The statistical power to detect an effect of *p53* deficiency on the multiplicity of intestinal tumors in this experiment was 0.8, based on the number of mice in each group and the variance in tumor counts among controls. Thus, the 129 genetic background carries at least one modifier allele that supplants the role of *p53* in suppressing tumorigenesis in the intestine. The 129 modifier system is formally recessive to B6 since the enhancing effect of homozygosity for *p53* deficiency is evident both on B6 and on the F_1 hybrid genetic background. $(B6 \times 129)F_1$ $Apc^{Min/+}$ $p53^{BCM/BCM}$ mice developed significantly more intestinal tumors than controls (Table 1; $P = 0.0002$).

Modifiers polymorphic between 129 and B6 also affect tumorigenesis in the pancreas. 129 $Apc^{Min/+}$ $p53^{BCM/BCM}$ mice and $(B6 \times 129)F_1$ $Apc^{Min/+}$ $p53^{BCM/BCM}$ hybrids develop large pancreatic tumors that are evident during necropsy (Table 1); multiple adenomas and adenocarcinomas, primarily derived from acinar cells, were present throughout the pancreas. Apc activity appeared to be reduced or lost in both types of pancreatic lesions since β-catenin was present throughout the nucleus (Figure 2). By contrast, B6 $Apc^{Min/+}$ $p53^{tm1Tyj/tm1Tyj}$ mice developed only microscopic adenomas in the

TABLE 1

Effect of *p53* and genetic background on the development of neoplasms in $Apc^{Min/+}$ mice

	Intestinal tumor count[a], mean ± SD (no. of mice)			Extracolonic tumors: Desmoid fibroma count, mean ± SD (no. of mice)			Extracolonic tumors: Pancreatic tumors, tumor bearing/total[b]		
Genotype	B6 $Mom1^{R/R}$[c]	129 $Mom1^{S/S}$	F_1 $Mom1^{R/S}$	B6 $Mom1^{R/R}$	129 $Mom1^{S/S}$	F_1 $Mom1^{R/S}$	B6 $Mom1^{S/S}$	129 $Mom1^{S/S}$	F_1 $Mom1^{R/S}$
$Apc^{Min/+}$ $p53^{+/+}$	27 ± 13 (13)	50 ± 50 (36)	62 ± 30 (27)	0 (8)	1 ± 2 (29)	3 ± 6 (19)	0/6	0/8	1/14
$Apc^{Min/+}$ $p53^{+/BCM}$	40 ± 19 (28)	47 ± 26 (31)	84 ± 36 (77)	1 ± 2 (20)	2 ± 4 (24)	6 ± 5 (67)	0/13	0/2	0/49
$Apc^{Min/+}$ $p53^{BCM/BCM}$	75 ± 25 (22)	46 ± 29 (10)	103 ± 36 (20)	33 ± 8 (22)	22 ± 4 (10)	28 ± 9 (17)	0/8[c]	9/9	8/9
$Apc^{+/+}$ $p53^{BCM/BCM}$	ND	0 (5)	0 (11)	0 (14)	0 (5)	0 (11)	0/6	0/3	0/11

[a] Total tract.

[b] Mice were scored as tumor bearing if a tumor was detected during necropsy at 90–100 days of age. Microscopic adenomas in the pancreas of some B6 $Apc^{Min/+}$ $p53^{tm1Tyj/tm1Tyj}$ mice were detected only when the pancreas was sectioned and stained with hematoxylin and eosin.

[c] Mom1 is a semidominant modifier of intestinal tumorigenesis that acts independently of p53 (Halberg *et al.* 2000). Testicular tumors were also observed in mice homozygous for $p53^{BCM/BCM}$ and heterozygous or homozygous for the 129 background, but not depending on *Apc* deficiency.

pancreas (Figure 2). Thus, pancreatic neoplasms arise when both *Apc* and *p53* are deficient and a dominant 129 susceptibility modifier system is present. This observation extends the report by Clarke *et al.* (1995).

The polymorphic modifier(s) affecting the development of tumors in the intestine and pancreas of B6 and 129 mice do not affect the development of desmoid fibromas on the abdominal body wall. 129 $Apc^{Min/+}$ $p53^{BCM/BCM}$ mice developed significantly more desmoid fibromas than 129 $Apc^{Min/+}$ $p53^{+/+}$ controls (Table 1; $P = 5.5 \times 10^{-8}$). A similar result was observed in (B6 × 129)F_1 hybrids (Table 1; $P = 1.4 \times 10^{-6}$). Thus, the activity of polymorphic modifiers can be tissue specific. Note that we demonstrated in our earlier study that the activity of the modifier locus *Mom1* is also tissue specific (Halberg *et al.* 2000).

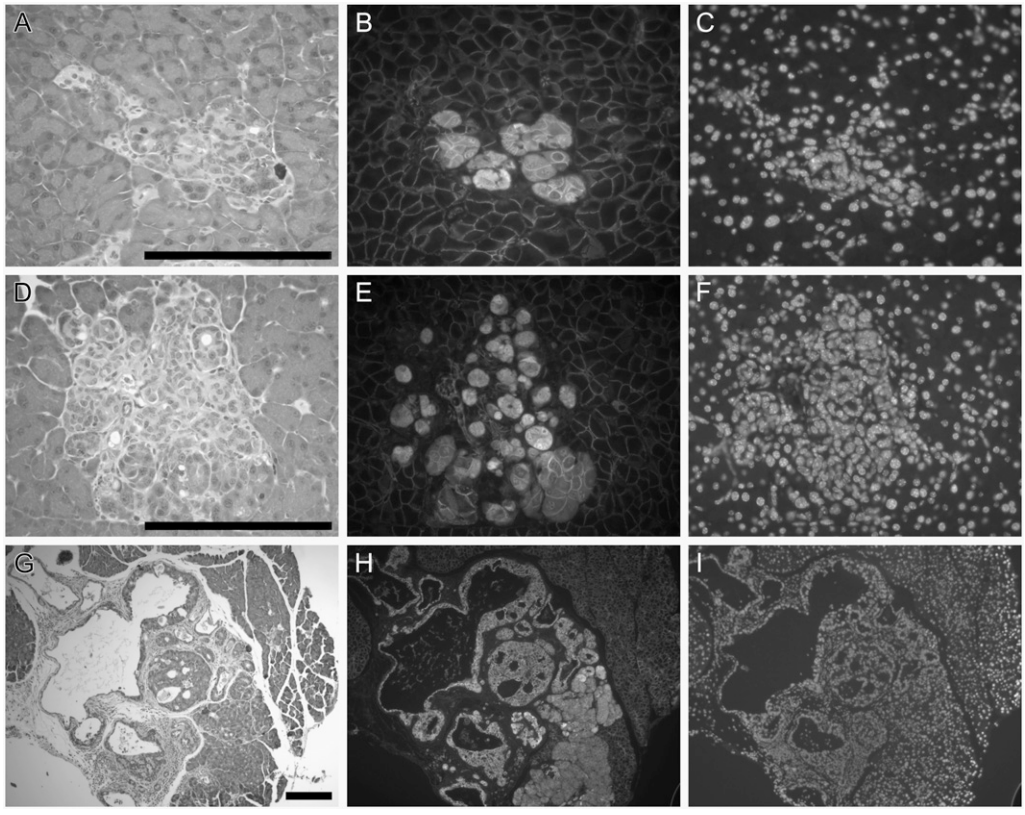

Figure 2.—β-Catenin is detectible in the nucleus of neoplastic cells within the pancreas. The pancreas was removed from a B6 $Apc^{Min/+}$ $p53^{tm1Tyj/tm1Tyj}$ mouse (A–C) or a 129 $Apc^{Min/+}$ $p53^{BCM/BCM}$ mouse (D–F and G–I) at 100 days of age, embedded in paraffin, and sectioned. Every 10th slide was stained with hematoxylin and eosin (A, D, and G). Adenomas (A and D) and adenocarcinomas (G) were apparent. The levels of β-catenin antigens (B, E, and H) within cells were determined by immunofluorescence microscopy. Nuclei were counterstained with DAPI (C, F, and I). Bars, 200 μm.

TABLE 2

Effect of *p21* and genetic background on the development of neoplasms in $Apc^{Min/+}$ mice

Genotype	Intestinal tumor count,[a] mean ± SD (no. of mice)		Desmoid fibroma count, mean ± SD (no. of mice)		Pancreatic tumors, tumor bearing/total[b]	
	Mixed background[c]	129	Mixed background[c]	129	Mixed background[c]	129
$Apc^{Min/+}$ $p21^{+/+}$	59 ± 30 (7)	54 ± 34 (11)	0 ± 0 (4)	2 ± 3 (10)	0/7	0/11
$Apc^{Min/+}$ $p21^{+/-}$	120 ± 76 (20)	97 ± 51 (26)	2 ± 3 (9)	3 ± 3 (25)	0/20	0/26
$Apc^{Min/+}$ $p21^{-/-}$	178 ± 136 (16)	101 ± 39 (12)	1 ± 2 (9)	4 ± 4 (12)	0/16	0/12
$Apc^{+/+}$ $p21^{-/-}$	0 ± 0 (10)	0 ± 0 (8)	0 ± 0 (7)	0 ± 0 (8)	0/10	0/8

[a] Total tract.
[b] Mice were scored as tumor bearing if a tumor was detected during necropsy at 90–100 days of age.
[c] Heterozygous $p21^{+/-}$ progeny of the cross (B6 × 129)F_2 $p21^{+/-}$ × 129 $Apc^{Min/+}$ were intercrossed to generate these mice.

The p53 effect is partially mediated by p21: Yang and her colleagues reported that $Apc^{1638N/+}$ mice homozygous for a knockout allele of *p21* with a mixed genetic background of B6 and 129 on a high-fat "Western diet" developed on average 7.5 ± 4.9 tumors in the proximal small intestine, significantly more than the average of 4.3 ± 1.9 developed by controls homozygous for the wild-type allele of the negative regulator *p21* (Yang *et al.* 2001). One of the targets activated by the p53 transcription factor is *p21*. Thus, the suppressive effect of p53 function on tumorigenesis in $Apc^{Min/+}$ mice might be mediated by p21 function. To test this possibility, (129 × B6)F_2 mice carrying a knockout allele of *p21* ($p21^{tm1Tyj}$) were bred to congenic 129 $Apc^{Min/+}$ mice from our laboratory stock. The resulting heterozygous progeny were intercrossed to generate $Apc^{Min/+}$ mice carrying $p21^{+/+}$, $p21^{tm1Tyj/+}$, and $p21^{tm1Tyj/tm1Tyj}$. The multiplicity of intestinal tumors in $Apc^{Min/+}$ $p21^{tm1TyjJ/tm1TyjJ}$ mice ranged from 35 to 426 with an average of 178 ± 136 whereas the multiplicity of intestinal tumors in $Apc^{Min/+}$ $p21^{+/+}$ mice ranged from 29 to 107 with an average of 59 ± 30 (Table 2). Here again, the *p21* deficiency displayed a heterozygous effect on intestinal tumors, but not on desmoid fibromas or pancreatic tumors.

The *p21* knockout allele was then bred onto the 129 genetic background to determine whether our initial analysis of the role of *p21* is confirmed under congenic conditions on the background that obscures the *p53* deficiency on *Apc*-dependent intestinal neoplasia. The multiplicity of intestinal tumors in 129 $Apc^{Min/+}$ $p21^{tm1TyjJ/tm1TyjJ}$ and $Apc^{Min/+}$ $p21^{tm1TyjJ/+}$ mice was again significantly higher than that in 129 $Apc^{Min/+}$ $p21^{+/+}$ mice (Table 2). Thus, the dominant 129 modifier system that supplants the *p53* effect on intestinal tumorigenesis in $Apc^{Min/+}$ mice does not supplant the *p21* effect. Here too, a lack of p21 activity did not lead to the development of desmoid fibromas or pancreatic tumors in 129 $Apc^{Min/+}$ mice (Table 2). Taken together, these results indicate that *p21* mediates the *p53* effect only on intestinal tumorigenesis, and in that case only in part.

DISCUSSION

FAP patients carrying the same *Apc* mutation often have different clinical presentations, varying in both multiplicity of colonic tumors and incidence of extracolonic lesions. Perhaps these two modes of variation reflect polymorphic genetic modifiers. In the Min mouse model, such modifiers have been associated with the alterations in tumor multiplicity (*Mom1* on chromosome 4 and *Mom2*, *-3*, and *-7* on chromosome 18—linked to *Apc*). In this study, we have investigated whether the distinct tissues in which $Apc^{Min/+}$ mice develop neoplasms involve a contribution by the negative regulators *p53* and *p21*. Further, we have used controlled variation in the genetic background to ascertain whether polymorphic genetic modifiers act in a tissue-specific manner to influence further the qualitative *Apc*-dependent neoplastic phenotype.

The difference in intestinal tumor multiplicity between two *Apc* alleles, the nonsense allele *Min* and the insertion allele *1638N*, can be explained either by a dominant-negative activity of the *Min* allele or, as for *Mom2* (Baran *et al.* 2007), by a position effect in which the *1638N* insertion affects expression of a neighboring cell-vital gene (Haigis *et al.* 2004). In contrast to the vast preponderance of loss-of-heterozygosity (LOH) seen in Min tumors on the B6 background (Luongo *et al.* 1994; Haigis *et al.* 2002; Haigis and Dove 2003), the majority of tumors in B6 $Apc^{1638N/+}$ mice do not show LOH. That these tumors can be cleanly dissected away from normal tissue is shown by finding that a minority of tumors from B6 $Apc^{1638N/+}$ mice do show LOH. Instead, as inferred by the regional distribution of these tumors (Haigis *et al.* 2004) and as demonstrated by Kucherlapati *et al.* (2007), the major pathway for tumorigenesis in $Apc^{1638N/+}$ mice involves intragenic mutations in the wild-type *Apc* allele. Thus, the nature of the predisposing *Apc* allele can influence the pathway whereby the function of the wild-type allele is lost. The scenarios described here (LOH *vs.* intragenic *Apc* mutation) are not the only

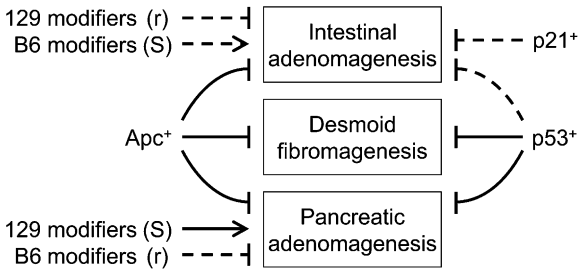

FIGURE 3.—The development of three distinct neoplasms in the laboratory mouse is affected by *Apc*, *p21*, *p53*, and modifiers that are polymorphic between 129 and B6. The tumor suppressor genes and polymorphic modifiers can have a partial (dashed line) or complete (solid line) effect. Whether modifiers act in a recessive fashion to resist (r) or a dominant fashion to enhance susceptibility (S) was ascertained by analyzing two parental strains and their F_1 hybrid.

factors to consider in understanding allelic specificity of the intestinal phenotype. Dominant negative alleles, haploinsufficiency, alternative splicing, and variable levels of Wnt signaling each have been raised as possible contributing factors to allele-specific effects on intestinal multiplicity (SOLOMON *et al.* 1987; THLIVERIS *et al.* 1994; SAMOWITZ *et al.* 1995; ROWAN *et al.* 2000; ALBUQUERQUE *et al.* 2002; SIEBER *et al.* 2002).

Inspection of Table 1 and Figure 3 shows several distinct patterns of interaction between the two tumor suppressor genes *Apc* and *p53*. First, for intestinal tumorigenesis, the dependence on *Apc* deficiency is complete within the range of variables tested in this study. However, genetic background affects the influence of *p53* deficiency. Second, for desmoid fibromas, either *Apc* or *p53* function can suppress development but no significant effects of genetic background have been observed. Finally, for acinar pancreatic neoplasms, either *Apc* or *p53* function can suppress the pathway, but here there is a strong effect of genetic background. The instances of desmoid fibromagenesis and acinar pancreatic adenocarcinomagenesis, in which either *Apc* or *p53* activity is sufficient to suppress a neoplastic pathway, provide formal examples of functional redundancy, as elaborated in the notion of robustness (HALBERG *et al.* 2000; HARTWELL 2004).

One interpretation of the data in Table 2 is that *p21* alone can mediate a portion of the *p53* effect on intestinal tumorigenesis. But note that *p21* does not regulate desmoid fibromagenesis or pancreatic neoplasia. An interesting possibility is that *p21* function is not important for specific neoplastic pathways in the intestine—the microsatellite instability (MSI) and CpG island methylator (CIMP) pathways (see OGINO *et al.* 2006). By extrapolation of this hypothesis, desmoid fibromagenesis and acinar pancreatic neoplasia would share features with the MSI and CIMP intestinal pathways and not with the LOH pathway. Evaluation of the silencing pathway in these several *Apc*-dependent neoplastic processes may shed light on this possibility.

Alternatively, p21 might act independently of p53 to affect intestinal tumorigenesis in Apc mutants. CHEN *et al.* (2004) demonstrated that clusterin-mediated apoptosis is independent of *p53* but dependent on *p21*. Clusterin expression and apoptosis were induced in HCT116 cells and HCT116 cells lacking p53 activity following treatment with 5-fluorouracil. By contrast, clusterin was not induced and apoptosis was inhibited in HCT116 cells lacking p21 activity. Action of p21 independent of p53 could explain why the dominant 129 modifier system supplants the *p53* effect on intestinal tumorigenesis in $Apc^{Min/+}$ mice but not the *p21* effect.

We are interested in the consistent observation of a subtle but significant change in multiplicity of intestinal tumors in $Apc^{Min/+}$ observed in both homozygous and heterozygous *p53* deficiency. These effects can be detected only by analyzing a cohort of mice with a homogenous genetic background, where the random segregation of polymorphic modifiers has been eliminated. These observations point to an important basic issue in the genetics of "tumor suppressor" genes: Do they commonly have a heterozygous mutant phenotype, even if finally the emergent tumor is homozygous for the mutant allele or doubly heterozygous for two mutant alleles?

Normal regulation in the intestine and pancreas of *p53*-deficient $Apc^{Min/+}$ mice is partially restored by modifiers polymorphic between 129 and B6. $Apc^{Min/+}$ $p53^{-/-}$ mice develop significantly more intestinal tumors than $Apc^{Min/+}$ $p53^{+/+}$ controls on B6 and (B6 × 129)F_1, but not on the 129 genetic background (Table 1 and Figure 3). Thus, homozygosity for a recessive 129 allele or alleles blunts the enhancement of intestinal adenomagenesis observed in *p53*-deficient backgrounds. An analogous situation exists in the pancreas. 129 and (B6 × 129)F_1 $Apc^{Min/+}$ $p53^{-/-}$ mice develop massive adenocarcinomas in the pancreas, whereas B6 $Apc^{Min/+}$ $p53^{-/-}$ mice develop only microscopic adenomas (Table 1 and Figure 3). In this case, homozygosity for a recessive B6 allele attenuates the enhancement of acinar pancreatic tumorigenesis when both *Apc* and *p53* are mutated. These polymorphic differences between B6 and 129 apparently cannot be explained by differences in the coding region of either *p21* or *p53*. The 129S6/SvEvTac strain has not been resequenced, but the 129S1 and 129X1 strains do not show any coding-region differences from B6 in either *p21* or *p53* (dbSNP database build 129, http://www.ncbi.nlm.nih.gov/SNP/).

Recessive resistance may reflect a polymorphism that reduces or eliminates the activity of a tumor-promoting factor. For example, a single-nucleotide polymorphism in the promoter of the human ornithine decarboxylase (*ODC*) gene that reduces the level of ODC expression also reduces the risk of adenoma recurrence in patients using aspirin for chemoprevention of colonic neoplasia (ERDMAN *et al.* 1999). Consistent with this observation, treatment of $Apc^{Min/+}$ mice with dimethylfluoroorni-

thine, a suicide inhibitor of ODC activity, reduces tumor multiplicity in the small intestine by a factor of two (Martinez *et al.* 2003). High-resolution mapping and positional cloning are required to identify the proposed modifiers polymorphic between B6 and 129 affecting tumorigenesis in $Apc^{Min/+}$ $p53^{-/-}$ mice. These modifiers may be novel targets for chemotherapeutics because some human pancreatic tumors lack Apc and p53 activity (Abraham *et al.* 2001, 2002; Dong *et al.* 2005). Thus, polymorphic modifiers can profoundly affect tumorigenesis in a specific tissue pattern under certain genetic conditions that predispose mice to cancer, emphasizing further the importance of characterizing mutant alleles on a series of homogenous genetic backgrounds—for example, as reported here, on two distinct inbred backgrounds and the F_1 between them.

We thank Harlene Edwards, Jen Triemstra, and Jane Weeks for highly capable technical assistance and Jeff Bacher, Norman Drinkwater, Paul Lambert, and Alexandra Shedlovsky for critical review of this manuscript. This is publication no. 3640 from the Laboratory of Genetics. This work was supported by grants R37 CA63677 and U01 CA-58085 (The Mouse Models of Human Cancer Consortium) from the National Institutes of Health.

LITERATURE CITED

Abraham, S. C., T. T. Wu, D. S. Klimstra, L. S. Finn, J. H. Lee *et al.*, 2001 Distinctive molecular genetic alterations in sporadic and familial adenomatous polyposis-associated pancreatoblastomas: frequent alterations in the APC/beta-catenin pathway and chromosome 11p. Am. J. Pathol. **159:** 1619–1627.

Abraham, S. C., T. T. Wu, R. H. Hruban, J. H. Lee, C. J. Yeo *et al.*, 2002 Genetic and immunohistochemical analysis of pancreatic acinar cell carcinoma: frequent allelic loss on chromosome 11p and alterations in the APC/beta-catenin pathway. Am. J. Pathol. **160:** 953–962.

Albuquerque, C., C. Breukel, L. R. van der Luijt, P. Fidalgo, P. Lage *et al.*, 2002 The 'just-right' signaling model: APC somatic mutations are selected based on a specific level of activation of the beta-catenin signaling cascade. Hum. Mol. Genet. **11:** 1549–1560.

Baran, A. A., K. A. Silverman, J. Zeskand, R. Koratkar, A. Palmer *et al.*, 2007 The modifier of Min 2 (Mom2) locus: embryonic lethality of a mutation in the Atp5a1 gene suggests a novel mechanism of polyp suppression. Genome Res. **17:** 566–576.

Bertario, L., A. Russo, P. Sala, M. Eboli, M. Giarola *et al.*, 2001 Genotype and phenotype factors as determinants of desmoid tumors in patients with familial adenomatous polyposis. Int. J. Cancer **95:** 102–107.

Bertario, L., A. Russo, P. Sala, L. Varesco, M. Giarola *et al.*, 2003 Multiple approach to the exploration of genotype-phenotype correlations in familial adenomatous polyposis. J. Clin. Oncol. **21:** 1698–1707.

Chen, T., J. Turner, S. McCarthy, M. Scaltriti, S. Bettuzzi *et al.*, 2004 Clusterin-mediated apoptosis is regulated by adenomatous polyposis coli and is p21 dependent but p53 independent. Cancer Res. **64:** 7412–7419.

Clarke, A. R., M. C. Cummings and D. J. Harrison, 1995 Interaction between murine germline mutations in p53 and APC predisposes to pancreatic neoplasia but not to increased intestinal malignancy. Oncogene **11:** 1913–1920.

Cormier, R. T., and W. F. Dove, 2000 Dnmt1N/+ reduces the net growth rate and multiplicity of intestinal adenomas in C57BL/6-multiple intestinal neoplasia (Min)/+ mice independently of p53 but demonstrates strong synergy with the modifier of Min 1(AKR) resistance allele. Cancer Res. **60:** 3965–3970.

Croner, R. S., W. M. Brueckl, B. Reingruber, W. Hohenberger and K. Guenther, 2005 Age and manifestation related symptoms in familial adenomatous polyposis. BMC Cancer **5:** 24.

de Vries, A., E. R. Flores, B. Miranda, H. M. Hsieh, C. T. van Oostrom *et al.*, 2002 Targeted point mutations of p53 lead to dominant-negative inhibition of wild-type p53 function. Proc. Natl. Acad. Sci. USA **99:** 2948–2953.

Donehower, L. A., M. Harvey, B. L. Slagle, M. J. McArthur, C. A. Montgomery, Jr. *et al.*, 1992 Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. Nature **356:** 215–221.

Dong, M., G. Ma, W. Tu, K. J. Guo, Y. L. Tian *et al.*, 2005 Clinicopathological significance of p53 and mdm2 protein expression in human pancreatic cancer. World J. Gastroenterol. **11:** 2162–2165.

Enomoto, M., M. Konishi, T. Iwama, J. Utsunomiya, K. I. Sugihara *et al.*, 2000 The relationship between frequencies of extracolonic manifestations and the position of APC germline mutation in patients with familial adenomatous polyposis. Jpn. J. Clin. Oncol. **30:** 82–88.

Erdman, S. H., N. A. Ignatenko, M. B. Powell, K. A. Blohm-Mangone, H. Holubec *et al.*, 1999 APC-dependent changes in expression of genes influencing polyamine metabolism, and consequences for gastrointestinal carcinogenesis, in the Min mouse. Carcinogenesis **20:** 1709–1713.

Fearon, E. R., 1997 Human cancer syndromes: clues to the origin and nature of cancer. Science **278:** 1043–1050.

Garber, J. E., and K. Offit, 2005 Hereditary cancer predisposition syndromes. J. Clin. Oncol. **23:** 276–292.

Giardiello, F. M., G. J. Offerhaus, D. H. Lee, A. J. Krush, A. C. Tersmette *et al.*, 1993 Increased risk of thyroid and pancreatic carcinoma in familial adenomatous polyposis. Gut **34:** 1394–1396.

Gould, K. A., W. F. Dietrich, N. Borenstein, E. S. Lander and W. F. Dove, 1996 Mom1 is a semi-dominant modifier of intestinal adenoma size and multiplicity in Min/+ mice. Genetics **144:** 1769–1776.

Groden, J., A. Thliveris, W. Samowitz, M. Carlson, L. Gelbert *et al.*, 1991 Identification and characterization of the familial adenomatous polyposis coli gene. Cell **66:** 589–600.

Gurbuz, A. K., F. M. Giardiello, G. M. Petersen, A. J. Krush, G. J. Offerhaus *et al.*, 1994 Desmoid tumours in familial adenomatous polyposis. Gut **35:** 377–381.

Haigis, K. M., and W. F. Dove, 2003 A Robertsonian translocation suppresses a somatic recombination pathway to loss of heterozygosity. Nat. Genet. **33:** 33–39.

Haigis, K. M., J. G. Caya, M. Reichelderfer and W. F. Dove, 2002 Intestinal adenomas can develop with a stable karyotype and stable microsatellites. Proc. Natl. Acad. Sci. USA **99:** 8927–8931.

Haigis, K. M., P. D. Hoff, A. White, A. R. Shoemaker, R. B. Halberg *et al.*, 2004 Tumor regionality in the mouse intestine reflects the mechanism of loss of Apc function. Proc. Natl. Acad. Sci. USA **101:** 9769–9773.

Halberg, R. B., D. S. Katzung, P. D. Hoff, A. R. Moser, C. E. Cole *et al.*, 2000 Tumorigenesis in the multiple intestinal neoplasia mouse: redundancy of negative regulators and specificity of modifiers. Proc. Natl. Acad. Sci. USA **97:** 3461–3466.

Harris, S. L., and A. J. Levine, 2005 The p53 pathway: positive and negative feedback loops. Oncogene **24:** 2899–2908.

Hartwell, L., 2004 Genetics. Robust interactions. Science **303:** 774–775.

Hirata, A., T. Tsukamoto, M. Yamamoto, H. Sakai, T. Yanai *et al.*, 2005 Organ-specific susceptibility of p53 knockout mice to N-bis(2-hydroxypropyl)nitrosamine carcinogenesis. Cancer Lett. **238:** 271–283.

Jacks, T., L. Remington, B. O. Williams, E. M. Schmitt, S. Halachmi *et al.*, 1994 Tumor spectrum analysis in p53-mutant mice. Curr. Biol. **4:** 1–7.

Kemp, C. J., L. A. Donehower, A. Bradley and A. Balmain, 1993 Reduction of p53 gene dosage does not increase initiation or promotion but enhances malignant progression of chemically induced skin tumors. Cell **74:** 813–822.

Kinzler, K. W., M. C. Nilbert, L.-K. Su, B. Vogelstein, T. M. Bryan *et al.*, 1991 Identification of FAP locus genes from chromosome 5q21. Science **253:** 661–665.

Kucherlapati, M., A. Nguyen, M. Kuraguchi, K. Yang, K. Fan *et al.*, 2007 Tumor progression in Apc(1638N) mice with Exo1 and Fen1 deficiencies. Oncogene **26:** 6297–6306.

Kuraguchi, M., X. P. Wang, R. T. Bronson, R. Rothenberg, N. Y. Ohene-Baah *et al.*, 2006 Adenomatous polyposis coli (APC) is required for normal development of skin and thymus. PLoS Genet. **2:** 1362–1374.

Li, F. P., and J. F. Fraumeni, Jr., 1969 Soft-tissue sarcomas, breast cancer, and other neoplasms. A familial syndrome? Ann. Intern. Med. **71:** 747–752.

Luongo, C., A. R. Moser, S. Gledhill and W. F. Dove, 1994 Loss of Apc^{+} in intestinal adenomas from Min mice. Cancer Res. **54:** 5947–5952.

Malkin, D., F. P. Li, L. C. Strong, J. F. Fraumeni, Jr., C. E. Nelson *et al.*, 1990 Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. Science **250:** 1233–1238.

Martinez, M. E., T. G. O'Brien, K. E. Fultz, N. Babbar, H. Yerushalmi *et al.*, 2003 Pronounced reduction in adenoma recurrence associated with aspirin use and a polymorphism in the ornithine decarboxylase gene. Proc. Natl. Acad. Sci. USA **100:** 7859–7864.

Matushansky, I., R. G. Maki and C. C. Cardo, 2008 A context dependent role for Wnt signaling in tumorigenesis and stem cells. Cell Cycle **7:** 720–724.

Moser, A. R., H. C. Pitot and W. F. Dove, 1990 A dominant mutation that predisposes to multiple intestinal neoplasia in the mouse. Science **247:** 322–324.

Moser, A. R., A. R. Shoemaker, C. S. Connelly, L. Clipson, K. A. Gould *et al.*, 1995 Homozygosity for the *Min* allele of *Apc* results in disruption of mouse development prior to gastrulation. Dev. Dyn. **203:** 422–433.

Nacht, M., A. Strasser, Y. R. Chan, A. W. Harris, M. Schlissel *et al.*, 1996 Mutations in the *p53* and *SCID* genes cooperate in tumorigenesis. Genes Dev. **10:** 2055–2066.

Nagy, R., K. Sweet and C. Eng, 2004 Highly penetrant hereditary cancer syndromes. Oncogene **23:** 6445–6470.

Nigro, J. M., S. J. Baker, A. C. Preisinger, J. M. Jessup, R. Hostetter *et al.*, 1989 Mutations in the p53 gene occur in diverse human tumour types. Nature **342:** 705–708.

Nishisho, I., Y. Nakamura, Y. Miyoshi, Y. Miki, H. Ando *et al.*, 1991 Mutation of chromosome 5q21 genes in FAP and colorectal cancer patients. Science **253:** 665–668.

Ogino, S., M. Brahmandam, T. Kawasaki, G. J. Kirkner, M. Loda *et al.*, 2006 Epigenetic profiling of synchronous colorectal neoplasias by quantitative DNA methylation analysis. Mod. Pathol. **19:** 1083–1090.

Oshima, H., M. Oshima, M. Kobayashi, M. Tsutsumi and M. M. Taketo, 1997 Morphological and molecular processes of polyp formation in $Apc^{\Delta 716}$ knockout mice. Cancer Res. **57:** 1644–1649.

Reilly, K. M., D. A. Loisel, R. T. Bronson, M. E. McLaughlin and T. Jacks, 2000 Nf1;Trp53 mutant mice develop glioblastoma with evidence of strain-specific effects. Nat. Genet. **26:** 109–113.

Rowan, A. J., H. Lamlum, M. Ilyas, J. Wheeler, J. Straub *et al.*, 2000 APC mutations in sporadic colorectal tumors: a mutational "hotspot" and interdependence of the "two hits." Proc. Natl. Acad. Sci. USA **97:** 3352–3357.

Sakai, H., T. Tsukamoto, M. Yamamoto, N. Shirai, T. Iidaka *et al.*, 2003 High susceptibility of nullizygous p53 knockout mice to colorectal tumor induction by 1,2-dimethylhydrazine. J. Cancer Res. Clin. Oncol. **129:** 335–340.

Samowitz, W. S., A. Thliveris, L. N. Spirio and R. White, 1995 Alternatively spliced adenomatous polyposis coli (APC) gene transcripts that delete exons mutated in attenuated APC. Cancer Res. **55:** 3732–3734.

Shibata, H., K. Toyama, H. Shioya, M. Ito, M. Hirota *et al.*, 1997 Rapid colorectal adenoma formation initiated by conditional targeting of the Apc gene. Science **278:** 120–123.

Shirai, N., T. Tsukamoto, M. Yamamoto, T. Iidaka, H. Sakai *et al.*, 2002 Elevated susceptibility of the p53 knockout mouse esophagus to methyl-N-amylnitrosamine carcinogenesis. Carcinogenesis **23:** 1541–1547.

Shoemaker, A. R., A. R. Moser, C. A. Midgley, L. Clipson, M. A. Newton *et al.*, 1998 A resistant genetic background leading to incomplete penetrance of intestinal neoplasia and reduced loss of heterozygosity in $Apc^{Min/+}$ mice. Proc. Natl. Acad. Sci. USA **95:** 10826–10831.

Sieber, O. M., H. Lamlum, M. D. Crabtree, A. J. Rowan, E. Barclay *et al.*, 2002 Whole-gene APC deletions cause classical familial adenomatous polyposis, but not attenuated polyposis or "multiple" colorectal adenomas. Proc. Natl. Acad. Sci. USA **99:** 2954–2958.

Smits, R., W. Van Oordt, A. Luz, C. Zurcher, S. Jagmohan-Changur *et al.*, 1998 *Apc*1638N: a mouse model for familial adenomatous polyposis-associated desmoid tumors and cutaneous cysts. Gastroenterology **114:** 275–283.

Smits, R., M. F. Kielman, C. Breukel, C. Zurcher, K. Neufeld *et al.*, 1999 Apc1638T: a mouse model delineating critical domains of the adenomatous polyposis coli protein involved in tumorigenesis and development. Genes Dev. **13:** 1309–1321.

Solomon, E., R. Voss, V. Hall, W. F. Bodmer, J. R. Jass *et al.*, 1987 Chromosome 5 allele loss in human colorectal carcinomas. Nature **328:** 616–619.

Soussi, T., and C. Beroud, 2001 Assessing TP53 status in human tumours to evaluate clinical outcome. Nat. Rev. Cancer **1:** 233–240.

Su, L. K., K. W. Kinzler, B. Vogelstein, A. C. Preisinger, A. R. Moser *et al.*, 1992 Multiple intestinal neoplasia caused by a mutation in the murine homolog of the APC gene. Science **256:** 668–670.

Thliveris, A., W. Samowitz, N. Matsunami, J. Groden and R. White, 1994 Demonstration of promoter activity and alternative splicing in the region 5′ to exon 1 of the *APC* gene. Cancer Res. **54:** 2991–2995.

Xu, Y., E. M. Yang, J. Brugarolas, T. Jacks and D. Baltimore, 1998 Involvement of p53 and p21 in cellular defects and tumorigenesis in Atm−/− mice. Mol. Cell. Biol. **18:** 4385–4390.

Yamamoto, M., T. Tsukamoto, H. Sakai, N. Shirai, H. Ohgaki *et al.*, 2000 p53 knockout mice (−/−) are more susceptible than (+/−) or (+/+) mice to N-methyl-N-nitrosourea stomach carcinogenesis. Carcinogenesis **21:** 1891–1897.

Yang, K., W. Edelmann, K. Fan, K. Lau, V. R. Kolli *et al.*, 1997 A mouse model of human familial adenomatous polyposis. J. Exp. Zool. **277:** 245–254.

Yang, W. C., J. Mathew, A. Velcich, W. Edelmann, R. Kucherlapati *et al.*, 2001 Targeted inactivation of the p21WAF1/cip1 gene enhances Apc-initiated tumor formation and the tumor-promoting activity of a Western-style high-risk diet by altering cell maturation in the intestinal mucosa. Cancer Res. **61:** 565–569.

Communicating editor: T. R. Magnuson