Understanding Phenotypic Variation in Rodent Models with Germline Apc Mutations

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Abstract

Adenomatous polyposis coli (APC) is best known for its crucial role in colorectal cancer suppression. Rodent models with various Apc mutations have enabled experimental validation of different Apc functions in tumors and normal tissues. Since the development of the first mouse model with a germline Apc mutation in the early 1990s, 20 other Apc mouse and rat models have been generated. This article compares and contrasts currently available Apc rodent models with particular emphasis on providing potential explanations for their reported variation in three areas: (i) intestinal polyp multiplicity, (ii) intestinal polyp distribution, and (iii) extraintestinal phenotypes. Cancer Res; 73(8); 2389–99. ©2013 AACR.

Introduction

Tumor suppressor adenomatous polyposis coli (APC) is critical for maintaining cellular homeostasis in the intestine (1, 2). APC is a large (2,843 amino acids), multidomain protein that has been implicated in many cellular functions including cellular proliferation, differentiation, cytoskeleton regulation, migration, and apoptosis (3). Mechanistically, APC is best known for its ability to antagonize Wnt signaling by targeting the oncoprotein β-catenin for proteasomal degradation (4).

Acquiring a somatic APC mutation is an early, if not initiating, event in the great majority of colorectal tumors (5). Inheriting a germline APC mutation results in the development of hundreds to thousands of colonic polyps, a condition termed familial adenomatous polyposis (FAP). These precancerous polyps are thought to initiate following a somatic mutation in the wild-type APC allele (6, 7). To avoid the progression of these polyps into invasive carcinoma, prophylactic colon removal is recommended for FAP patients (8). There are no reports of humans with germline mutation of both APC alleles, consistent with early developmental lethality associated with complete loss of APC function (9–11). Germline and somatic APC mutations typically result in premature APC protein truncation and group between codons 1250 and 1464, a region termed the "mutation cluster region" (MCR; ref. 12).

A meta-analysis of genotype–phenotype correlation in patients with FAP showed that germline mutations in the MCR result in the most severe intestinal polyposis phenotype, with up to 5,000 polyps (13). Mutations on either side of the MCR are associated with an intermediate intestinal polyposis phenotype, whereas mutations that result in a truncation in APC after amino acid (a.a.) 1595 or before a.a. 157 are associated with an attenuated phenotype (AFAP), characterized by development of only a few polyps (13). Complete deletion of APC has been reported only rarely and results in an intermediate phenotype (14, 15).

More than two thirds of patients with FAP also have extracolonic manifestations (13). Chronic hypertrophy of retinal pigment epithelium (CHRPE) is the most frequent phenotype, associated with APC truncation between a.a. 311 and 1446. Desmoid tumors, on the other hand, are associated with APC truncations 3′ to the MCR, after a.a. 1400. Duodenal and gastric tumors have been associated with APC mutations in 2 different regions, downstream of codon 1395 and between codons 564 and 1465 (13). It is important to note that these genotype–phenotype correlations are not rigid or complete, suggesting roles for other genetic and environmental factors in tumor development (13, 16).

For the past two decades, rodent models have been valuable for analysis of APC functions in intestinal homeostasis and tumor suppression (17, 18). APC is well conserved between human and rodent, with 92% similarity at the amino acid level (9, 19). Furthermore, some rodent models with germline Apc mutations that result in Apc protein truncation develop intestinal polyposis similar to that seen in patients with FAP (18). A brief summary of all published rodent models with germline Apc mutations appears in Tables 1 to 3, with a schematic provided in Figure 1.

Characterization of the many available Apc mouse and rat models has aided in discovery of various pathways important in colon carcinogenesis. Apc rodent models were also useful for elucidating the effect of various environmental and genetic factors on intestinal tumorigenesis and for testing potential chemoprevention and therapeutic agents. The many positive contributions of Apc mouse models have been reviewed previously (20, 21). As with most experimental systems, studies of...
the Apc models have also led to unanswered questions, particularly regarding phenotypic variation among the different models. Here, we review some of these variations, provide potential explanations, and pose challenges for future investigation.

Variation in Intestinal Polyp Multiplicity

As shown in Table 1 to 3, the average number of polyps varies greatly between different mouse models with germ-line Apc mutations. In addition, the number of polyps also varies in the same Apc mouse model maintained in different laboratories (17). These variations in intestinal polyp number in different models likely stem from the nature of the Apc mutations as well as environmental and genetic factors (17, 18). We propose that the number of intestinal tumors that develop in different Apc models and in the same model analyzed by different laboratories is influenced by one or more of the following factors.

Different rates and mechanisms of wild-type Apc allele loss (e.g., LOH, mutation of wild-type Apc, gene silencing)

In both patients with FAP and rodent models with germ-line Apc mutations, loss or inactivation of the wild-type APC/Apc allele is required for polyp formation (22, 23). The mechanism by which the second wild-type Apc allele is lost appears to depend on the Apc mouse model (24). Because this second Apc "hit" is essential for polyp initiation (10, 22, 25), the rate at which the second "hit" occurs will directly affect the number of intestinal polyps. Increasing the expected rate of these second "hits" through introduction of genomic instability, X-ray exposure, or injection with a mutagen significantly increases the number of polyps in ApcMin/+ and Apc1638N mice (26–30). It has been suggested that certain Apc mutations might lead to chromosomal instability, which could affect the rate of wild-type Apc loss (31).

Apc1638N/+ mice develop relatively few intestinal polyps and the second Apc "hit" is usually inactivation of the wild-type Apc allele, predicted to be a rare event (24). On the other hand, ApcMin/+ mice, in which the wild-type Apc allele is lost by means of a more frequent LOH event, develop considerably more polyps (24). Loss of the wild-type Apc allele in both ApcMin/+ and Apc1322T/+ mice, however, is reported to occur via LOH, yet these two mouse models have widely different polyp numbers (32). Although the rate and underlying mechanism of wild-type Apc allele loss might contribute to intestinal polyp numbers in Apc mouse models, it is unlikely that these are sole defining parameters.

Different rates of polyp growth due to differences in Wnt signaling

Polyps must reach a certain size to be detectable. If two polyps are initiated at the same time, a more rapidly growing polyp should be detectable earlier than a slower growing polyp. The most recognized function of Apc is to antagonize the Wnt signaling pathway through inhibition of the activity of β-catenin as a transcription cofactor (4). As Wnt signaling can drive cellular proliferation, we might expect that different Apc mutations would lead to different levels of Wnt signal activation and different corresponding changes in cellular proliferation. In patients with FAP, mutations in the MCR are associated with the most severe intestinal phenotypes, whereas mutations outside the MCR lead to reduced polyp multiplicity (13). Notably, Apc mutations 5’ and 3’ to the MCR result in higher and lower activation of Wnt signaling, respectively (33). This observation has led to the proposal that submaximal upregulation of Wnt signaling promotes more polyp growth than higher or lower elevation of Wnt signaling, the "just right" hypothesis (34, 35).

Wnt signaling has been assessed in many Apc mouse models. Some models have high polyp multiplicity and show elevated Wnt signaling in these polyps (ApcMin/+; Apc716/+; Apc1322T/+; and Apc716D15A; refs. 10, 34, 35). Wnt signaling is also elevated in the few polyps that develop in Apc1322T+ and ApcNsdR/+ mice (36, 37). ApcMinSLS/mNLS mice have elevated Wnt signaling in intestinal epithelial cells (38, 39). Apc1322T/1572T embryonic stem cells also have elevated Wnt signaling (38, 39). Neither ApcMinSLS/mNLS nor Apc1322T/+ mice develop intestinal polyps (38, 39).

The "just right" hypothesis is supported by reports of increased polyp multiplicity in Apc1322T/+ and Apc1322T/1572T mice relative to ApcMin/+ mice (34, 35). Compared with ApcMin, Apc1322T protein retains one 20-a.a. repeat that can bind to β-catenin and decrease Wnt signaling (34, 35). The Apc1322T/+ allele results in complete deletion of Apc and polyps in ApcMin/+ mice also display less Wnt signaling than polyps in ApcMin/+ mice (34). However, the "just right" hypothesis does not readily explain why Apc716D15/+ mice show higher activation of Wnt signaling and more polyps than ApcMin/+ mice (40). In addition, several groups have reported that although loss of both Apc alleles is required to activate Wnt signaling (as assessed by nuclear translocation of β-catenin), this Apc loss is not sufficient for full Wnt signal activation (11, 41, 42). To establish the extent to which Wnt signaling and polyp growth contribute to phenotypic variation, Wnt signaling activities and proliferation rates must be directly compared in different Apc mouse models.

Different abilities to evade growth-inhibitory effects

Another explanation for variation in polyp number among different Apc mouse models is negative selection of particular Apc genotypes. This negative selection could contribute to the "just right" hypothesis. Support for negative selection contributing to polyp phenotypes is provided by the observation that addition of Cdx2 or BubR1 mutations to Apc716D15/+ or ApcMin/+ mice, respectively, results in reduced polyp multiplicity and increased apoptotic indices in the small intestines, despite the increased proliferation index in these cells (43, 44). Similarly, induction of a conditional Apc mutation in hematopoietic stem cells results in upregulation of Wnt signaling and increased stem cell proliferation with increased apoptosis and eventual exhaustion of the stem cell population (45). If this phenotype holds true for intestinal tissues, the "just right" hypothesis might explain the increased stem cell number in polyps from Apc1322T/+ mice relative to those from ApcMin/+ , despite lower
Table 1. Summary of rodent models with germline Apc mutations before MCR

<table>
<thead>
<tr>
<th>Model (ref.)</th>
<th>Apc mutation</th>
<th>Intestinal phenotype</th>
<th>Polyp distribution</th>
<th>Extraintestinal phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apc\textsuperscript{\texttextsuperscript{S înt-15\texttextsuperscript{þ}}/\texttextsuperscript{þ}}\textsuperscript{(34)}</td>
<td>Complete deletion of entire Apc gene</td>
<td>\textsim160 polyps/male, \textsim190/female - Benign adenoma - Polyps show similar histopathology to those in Apc\textsuperscript{\texttextsuperscript{Min\texttextsuperscript{þ}}} mice</td>
<td>Similar distribution as in Apc\textsuperscript{\texttextsuperscript{Min\texttextsuperscript{þ}}} mice</td>
<td>Anemia</td>
</tr>
<tr>
<td>Apc\textsuperscript{\texttextsuperscript{5242\texttextsuperscript{þ}}/\texttextsuperscript{þ}}\textsuperscript{(55)}</td>
<td>\beta geo gene trap cassette inserted between exons 7 and 8 leads to stop after codon 242</td>
<td>177 polyps - Benign adenoma - Polyps show similar histopathology to those in Apc\textsuperscript{\texttextsuperscript{Min\texttextsuperscript{þ}}} mice</td>
<td>Similar distribution as in Apc\textsuperscript{\texttextsuperscript{Min\texttextsuperscript{þ}}} mice</td>
<td>NR</td>
</tr>
<tr>
<td>Apc\textsuperscript{\texttextsuperscript{14\texttextsuperscript{þ}}/\texttextsuperscript{þ}}\textsuperscript{(75)}</td>
<td>Exon 14 deletion leads to frameshift and stop after codon 580</td>
<td>120 polyps - Adenomas</td>
<td>Mainly SI</td>
<td>Anemia</td>
</tr>
<tr>
<td>Apc\textsuperscript{\texttextsuperscript{716\texttextsuperscript{þ}}/\texttextsuperscript{þ}}\textsuperscript{(10, 40)}</td>
<td>Inserted Neo\textsuperscript{5\texttextsuperscript{þ}} and diphtheria toxin \alpha\texttextsuperscript{-subunit genes in exon 15 leads to stop after codon 716</td>
<td>58–256 polyps - Benign adenomas</td>
<td>Mainly SI</td>
<td>Anemia</td>
</tr>
<tr>
<td>Apc\textsuperscript{\texttextsuperscript{Min\texttextsuperscript{þ}}/\texttextsuperscript{þ}}\textsuperscript{(19, 67, 79)}</td>
<td>Generated by ENU screen Nonsense mutation after codon 850</td>
<td>20–100 polyps - Benign adenomas - Malignant transformation in old mice in some genetic backgrounds</td>
<td>60% in distal 1/3 of the SI - Few in colon - Very few in stomach</td>
<td>Mammary tumors; 5% old females - Anemia - Splenomegaly - Abnormal hematopoiesis - Degeneration of ovarian follicles - Underdeveloped seminiferous tubules - Abnormal serum lipid profile - Benign epidermoid cysts - Jaw osteoma in old females</td>
</tr>
<tr>
<td>PIRC rat\textsuperscript{(9, 94)}</td>
<td>Nonsense mutation after codon 1137</td>
<td>36 polyps and 178 microadenoma (&lt;0.5 mm), males - 11 polyps and 35 microadenomas, females - Adenoma - Adenocarcinoma in older mice</td>
<td>Tumors are in both SI and colon</td>
<td></td>
</tr>
</tbody>
</table>

NOTE: Apc mouse models reported in this table are on C57BL/6 background, but with different backcross isogenicity from N2 to > N20. Apc rat models reported in the table are on F344 background. Apc models are mouse models unless otherwise noted. Abbreviations: ENU, ethyl nitrosourea; SI, small intestine; NR, not reported.
| Model (ref.)   | Apc mutation | Intestinal phenotype | Polyp distribution | Extraintestinal phenotype |
|...............|--------------|----------------------|--------------------|--------------------------|
| Apc\textsuperscript{1309/}\textsuperscript{+} (70, 95, 96) | Neo\textsuperscript{R} gene inserted leads to truncation after codon 1309 | 33–37 polyps on average – Benign adenoma | – Mainly SI – Few stomach and colon – SI polyps more proximal than Apc\textsuperscript{Min+/+}, only 1/3 distal | – Centrilobular cholestasis in liver – Microvesicular fatty liver – Abnormal serum lipid profile – Anemia – Splenomegaly |
| Apc\textsuperscript{1322T/}\textsuperscript{+} (32, 35) | Deletion after codon 1322 | 200 polyps – Benign adenomas with severe dysplasia in large polyps – Polyps have less Wnt signaling but more stem cells relative to those from Apc\textsuperscript{Min+/+} mice | – Most in SI – Few in colon and stomach – SI polyps more proximal than Apc\textsuperscript{Min+/+} (<20% in distal 1/3 of SI) | |
| Apc\textsuperscript{1572T/}\textsuperscript{+} (38) | PGK-Hygromycin cassette inserted in sense orientation leads to stop at codon 1572 | None | N/A | Mammary-invasive adenocarcinoma in 100% of females and 30% of males |
| Apc\textsuperscript{1638T/1638T} (69, 97) | PGK-Hygromycin cassette inserted in sense orientation leads to stop at codon 1638 | None | N/A | Viable homozygous mutant – Postnatal growth retardation – Cutaneous cysts in nipples – Absent preputial glands – Aberrant response of thyroid gland to thyroid-stimulating hormone |
| Apc\textsuperscript{1638N/}\textsuperscript{+} (71) | Neo\textsuperscript{R} gene inserted in antisense orientation leads to stop after codon 1638 | <10 polyps – Benign adenoma and adenocarcinoma – Aberrant crypt foci – Liver metastasis in one mouse | – SI, colon, and stomach – Uniformly distributed along SI | Desmoid tumors – Cutaneous cysts |
| KAD rat (68) | Nonsense mutation in Apc codon 2523 | No spontaneous intestinal tumors – Homozygous mutant rats have increased incidence and multiplicity of colonic tumors when treated with AOM-DSS relative to treated wild-type rats | Colon (AOM-DSS-induced) | Homozygous mutant animals are viable |

**NOTE:** Apc mouse models reported in this table are on C57BL/6 background, but with different backcross isogenicity from N2 to N20. Apc rat models reported in the table are on F344 background. Apc models are mouse models unless otherwise noted. Abbreviations: AOM-DSS, azoxymethane-dextran sodium sulfate; N/A, not applicable; Neo\textsuperscript{R}, neomycin resistance gene; SI, small intestine.
emerge in what is considered a congenic strain (46). Several genes are present in different mouse strains and can even modi- 

cy between laboratories (20–100/mouse; refs. 17, 18). This inconsistency might result from variations in diet, emer- 
gence of genetic modifiers, and even from different methods of polyp detection. A genetic modifier is a genetic locus that modifies the effect produced by a nonallelic locus. Modifier genes are present in different mouse strains and can even emerge in what is considered a congenic strain (46). Several modifier loci have been found to affect intestinal polyposis in ApcMin/+ mice and are named modifier of min (Mom; reviewed in ref. 18). Some modifiers are single genes, others are thought to represent contiguous genes and some remain less well defined (47). The modifiers appear to function as recessive, dominant, or semidominant loci (17). The first identified modifier gene, Mom-1 (Pla2g2a), works in a cell nonautonomous manner, possibly by reducing inflammatory response in the gut (48–50). The Mom-2 (Atp5a1) allele is on the same chromosome as Apc (chromosome 18) and appears to inhibit loss of the wild-type Apc allele (48, 51). The mechanisms of action of other modifiers such as Mom-3, Mom-7, Mom-12, and Mom-13 are not understood (52–54).

Although identified in ApcMin/+ mice, Mom genes likely also affect phenotypes of other Apc mouse models. For instance, the C3H/HeJ mouse strain carries at least one Mom allele that is absent from the C57BL/6 strain Mom-1 (48). Both ApcMin/+ and ApcA242/+ mice show reduced polyp multiplicity in the first generation mixed C57BL/6; C3H/HeJ mice compared with C57BL/6 mice (55). At present, there appears to be no direct examination of the effect of specific modifiers of Min on different Apc mouse models.

Environmental factors, such as intestinal flora, might also contribute to phenotypic variation (56). While intestinal flora appear to increase the number of polyps in ApcMin/+ mice (57), ApcA14/+ mice raised in pathogen-free conditions showed significant increases in intestinal polyp number (58).

### Table 3. Summary of mouse models with other germline Apc mutations

<table>
<thead>
<tr>
<th>Model (ref.)</th>
<th>Apc mutation</th>
<th>Intestinal phenotype</th>
<th>Polyp distribution</th>
<th>Extraintestinal phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>ApcNeoR and ApcmNLS (39)</td>
<td>Inactivating mutations in the 2 nuclear localization signals</td>
<td>Increased cellular proliferation in intestinal epithelial cells</td>
<td>N/A</td>
<td>NR</td>
</tr>
<tr>
<td>ApcNeoF (36, 37)</td>
<td>Deletion of codons between 1322 and 2006</td>
<td>Similar to Apc1322T/−</td>
<td>Similar to Apc1322T/−</td>
<td>Similar to Apc1322T/−</td>
</tr>
<tr>
<td>Apc1322T/− (98)</td>
<td>Mutant Apc allele truncated after codon 716 inserted as transgene in mice with 2 wild-type Apc alleles</td>
<td>Dysplastic adenomas</td>
<td>SI</td>
<td>ApcNeoR/NeoR embryos show severe developmental abnormalities and die in utero</td>
</tr>
<tr>
<td>Apc1322T/− (98)</td>
<td>Mutant Apc truncated after codon 716 inserted as transgene in Apc−716/+</td>
<td>Reduced Apc level to 10% and 20%, respectively</td>
<td>None</td>
<td>Abdominal hamartoma in one mouse</td>
</tr>
<tr>
<td>Apc−716/+ (98)</td>
<td>Mutant Apc inserted as transgene in Apc−716/+</td>
<td>Similar to Apc1322T/−</td>
<td>Similar to Apc1322T/−</td>
<td>Similar to Apc1322T/−</td>
</tr>
</tbody>
</table>

NOTE: Apc mouse models reported in this table are on C57B/6 background, but with different backcross isogenicity from N2 to N20. All models are mouse models.

Abbreviations: NR, not reported; N/A, not applicable; NeoR, neomycin resistance gene.

Wnt signaling in polyps from the former model relative to those from Apc−716/+ mice.

**Distinctive effects on differentiation**

It is possible that the effect of Apc genotypes on enterocyte differentiation contributes to differences in intestinal polyp number. For instance, compared with ApcMin/+ mice, Apc1322T/− mice have a higher proportion of Paneth cells and cells that express stem cell markers (Lgr5, Bmi1, Msi1, and CD4,4), not only in adenomas but also in apparently normal intestinal epithelial cells (35). Cell fates that result from different Apc genotypes might alter tumor initiation or growth. Again, Wnt signaling is one of several factors proposed to affect differentiation.

**Contributions of genetic modifiers or environmental factors**

It is well established that genetic and environmental factors affect intestinal polyp multiplicity in Apc mouse models. Polyp multiplicity in ApcMin/+ mice varies greatly between laboratories (20–100/mouse; refs. 17, 18). This inconsistency might result from variations in diet, emergence of genetic modifiers, and even from different methods of polyp detection. A genetic modifier is a genetic locus that modifies the effect produced by a nonallelic locus. Modifier genes are present in different mouse strains and can even emerge in what is considered a congenic strain (46). Several
Diet is another major environmental factor that clearly impacts the mouse phenotype (59–61). Although typically defined, the concentration of various vitamins, fiber, and total fat varies greatly between laboratory mouse diets. In our own experience, switching the mouse diet had a dramatic effect on polypl multiplicity in our Apc<sup>Cmin</sup>-/- mouse colony. We found that the polypl burden per mouse significantly increased from 45.9 ± 4.5 in 10 Apc<sup>Cmin</sup>-/- mice on Lab diet 5001 (Purina) to 81 ± 9.3 in 25 age-matched Apc<sup>Cmin</sup>-/- mice on Harlan 2018 diet (P = 0.0006). Notably, the new diet (Harlan 2018) has a 24% increase in fat and decreased fiber, vitamin D, and folic acid by 42%, 67%, and 44%, respectively. Unfortunately, these interlaboratory variables such as diet confound direct comparison of the phenotypes of Apc mouse models studied in different laboratories.

**Differences in cellular migration and adhesion**

APC interaction with cytoskeletal components, including actin filaments and microtubules, is thought to affect cell adhesion and migration (62, 63). Decreased cellular adhesion and migration in cells with APC mutations is expected to contribute to tumor formation (64). APC interacts with cytoskeletal proteins through its C-terminal region, which is absent in Apc from most mouse models (Fig. 1). Adding the C-terminal domain of APC to Apc<sup>1322T</sup> (as in Apc<sup>Cmin</sup>-/- mice) did not change the phenotype (65). However, it is possible that cytoskeletal alterations affect later stages of tumor progression such as invasion and metastasis, which do not occur in most Apc mouse models (66). Currently, evidence supporting a direct role of the Apc C-terminus in intestinal phenotype variation among different Apc mouse models is lacking.

**Differences in technologies used to generate the mouse model**

Apc rodent models have been generated using three different technologies: chemical mutagenesis screen, insertion of an antibiotic resistance gene, and Cre-lox-mediated deletion of specific Apc exons. These models differ by only 40 bp (69–72); yet the described phenotype of Apc<sup>1322T</sup>/- mice is not similar to that of the Apc<sup>^{ΔNLS}</sup> model, which has a complete deletion of the Apc exons (34, 72). The neomycin resistance gene clearly affects the phenotypes of these mice and if inserted in reverse orientation might affect not only Apc expression but also expression of genes upstream of Apc. It is possible that the 6-fold difference in intestinal polyp number between Apc<sup>1322T</sup>-/- and Apc<sup>1309/-</sup>-/- mice, which differs by only 13 amino acids, stems from the different technology used in their generation: Cre-lox–mediated deletion in Apc<sup>1322T</sup>-/- versus insertion of an antibiotic resistance cassette in Apc<sup>1309/-</sup>-/- mice. However, other genetic and environmental factors may contribute to the variation between these two models as well (32, 70). A final illustration of the challenges in generation of Apc mouse models is the Apc<sup>Δ474/-</sup> mice, which have a duplication of Apc exons 7 to 10. This feature complicates dissection of the contribution of exon duplication to the phenotype (73).

**Differences in expression of the mutant allele**

When analyzing the phenotypes of different Apc mouse models, another consideration is the level of expression of the mutant allele. Apc is a large multidomain protein. Truncations of Apc in most patients with FAP and rodent models leave N-terminal domains intact (Fig. 1). Although normal expression levels of truncated Apc protein have been verified in Apc<sup>Δ716</sup> mice, this is not universally the case (32, 69, 74). In Apc<sup>Δ580</sup>, Apc<sup>Δ14</sup>, and Apc<sup>Δ474</sup> models, the truncating mutation occurs before the final exon

![Figure 1. Sites of Apc mutations in different Apc mouse models relative to Apc domains.](Image of Figure 1)
different intestinal regions determines the mechanism of the Apc model is supported by the Apc distribution is somehow linked to the mechanism by which genetic backgrounds has led to the hypothesis that polyp variations (24). However, the first model comes from ApcMin/+ mice in a 129/Sv back-ground, where additional mutations that inactivate Smad3 result in increased colonic tumors; yet, in both cases, loss of the wild-type Apc allele is achieved through LOH (81). Finally, PPARγ agonists increase colonic but not small intestinal tumors in ApcMin/+ mice (82, 83). PPARγ is expressed in higher quantities in the colon and cecum relative to the small intestine, which might account for this differential effect (83).

An expansion of the "just right" hypothesis has been proposed to explain the variation in polyp distribution among patients with FAP and ApcMin/+ and Apc1322T/+ mice. The basal level of Wnt signaling is not the same in different intestinal regions. It was proposed that changes in Wnt signaling that result from specific Apc mutations cause optimal Wnt signaling for polyp growth only in certain intestinal regions. On the other hand, in other intestinal regions, these same Apc mutations will result in a higher or lower Wnt signaling level than what is optimal for tumor growth (84).

Perhaps some of these mechanisms can be clarified by studying ApcMin-FCCC mice, which were generated by mating C57Bl/6J ApcMin/+ males with ApcMin/+ females from an independent colony of C57Bl/6 mice maintained at Fox Chase Cancer Center (Philadelphia, PA). ApcMin-FCCC/+ mice develop more colonic polyps than do ApcMin/+ mice, but the molecular basis behind this polyp shift has not been determined (85). Further clarification of the underlying mechanisms that control polyp distribution might also be achieved through careful analysis of Apc1322T/+ and Apc580D/+ mice, which carry similar mutations (truncating the Apc protein at amino acid 580) but appear to have different polyp distributions. Apc1322T/+ mice develop more colonic polyps than do ApcMin/+ mice. Apc580D/+ mice develop a similar number of colonic polyps as ApcMin/+ mice, although direct comparison of Apc580D/+ and either Apc1322T/+ or ApcMin/+ mice has not been reported (75, 86).

Variation in Extraintestinal Phenotypes

Although best known for its role to suppress colorectal tumorigenesis, Apc mutations have been seen in other tumors including breast and liver carcinomas (4). In addition, both patients with FAP and rodent models with germline Apc mutations develop extraintestinal phenotypes (see Tables 1–3). As with the intestinal phenotype, the underlying mechanism for variation in extraintestinal phenotypes between patients with FAP and Apc rodent models as well as among different Apc rodent models is not completely understood. Patients with FAP have increased susceptibility to hepatic, pancreatic, thyroid, and brain tumors. They also develop desmoid tumors, dental anomalies, and congenital hypertrophy of retinal pigment epithelium. It is important to note that the penetrance of these extraintestinal phenotypes is variable in patients with FAP (16, 87). The basis behind this variation is not completely understood, although it seems to correlate with the Apc germline as well as the acquired somatic mutations. (16, 33).

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Apc rodent models also develop some of these extraintestinal manifestations; for example, Apc<sup>HtHt</sup>, mice develop desmoid tumors (72) and PIBR rats show mandibular osteoma (9). Other phenotypes described in patients with FAP have not been reported for Apc rodent models. The short life span of most Apc rodent models could prevent the full expression of some of these phenotypes. On the other hand, Apc rodent models manifest some other extraintestinal phenotypes that have not been described in patients with FAP (Tables 1–3). For example, many mouse models with germline Apc mutations develop mammary tumors. Although APC mutations and promoter methylation have been found in up to 70% of sporadic human breast cancers, patients with FAP do not appear at an increased risk for breast tumors (88–90). In addition, adenocanthoma is a common type of mammary tumor that develops in Apc mouse models but it has not been reported in humans (91). Other extraintestinal phenotypes described in Apc rodent models include splenomegaly, abnormal hematopoiesis, changes in the serum lipid profile, gonadal changes, cutaneous cysts, and thyroid abnormalities. Differences in physiology, life span, and genetic content between human, mouse, and rat could be underlying causes.

Among different Apc mouse models, some extraintestinal phenotypes, such as anemia and splenomegaly, seem to correlate with the severity of intestinal polyposis. In contrast, mammary gland tumors in Apc mouse models appear to correlate with the severity of polyposis in only a few cases, such as in the Apc<sup>Min</sup> and Apc<sup>CAT/CAT</sup> mouse models. Very few Apc<sup>Min</sup>/ mice develop mammary tumors, whereas Apc<sup>CAT/CAT</sup>/ mice develop mammary tumors at a rate that is almost double that seen in Apc<sup>Min</sup>/ mice (73, 91). In contrast, there are no reports of mammary tumor development in Apc mouse models with the most severe intestinal polyposis (Apc<sup>N143D</sup>, Apc<sup>1527Tt</sup> and Apc<sup>SSAMP</sup>, refs. 32, 40, 65). Perhaps mice with severe polyposis die too early, before mammary tumors have a chance to develop. Apc<sup>1527Tt</sup>/ mice, which develop no intestinal polyps, have a fully penetrant mammary tumor phenotype in females. K14-cre-ApcCKO/ mice are a conditional model in which the Apc<sup>5306</sup> allele is expressed only in ectoderm-derived tissues including the mammary gland (75, 92). Mammary tumors from these mice have mutations in the wild-type Apc allele that cluster around codon 1530 consistent with the requirement of an optimal level of Wnt signaling for mammary tumorigenesis (38). It is likely that some of the genetic and environmental factors previously described also account for the variability in extraintestinal phenotypes among different Apc rodent models.

Conclusions and Future Directions

APC research has benefitted greatly from different rodent models with germline Apc mutations. However, genotype–phenotype correlation of these different models is confounded by many genetic and environmental factors. Use of standardized genetic backgrounds and environmental conditions in different laboratories should enable reliable genotype–phenotype analysis of these animals. This standardization will also shed light on the role of different Apc mutations in tumorigenesis. When possible, a direct comparative analysis of different models in the same laboratory will illuminate the contribution of many factors described in this review to phenotypic variation in rodent models with germline Apc mutations.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Disclaimer

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Authors' Contributions

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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): M. Zeineldin

Analysis and interpretation of data (e.g., statistical analysis, biosstistics, computational analysis): K.L. Neufeld, M. Zeineldin

Writing, review, and/or revision of the manuscript: M. Zeineldin, K.L. Neufeld

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