Review

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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

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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 germline *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 germline *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 $Apc^{Min/+}$ and Apc^{1638N} 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).

$Apc^{1638N/+}$ 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, $Apc^{Min/+}$ 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 $Apc^{Min/+}$ and $Apc^{1322T/+}$ 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 ($Apc^{Min/+}$, $Apc^{\Delta 716/+}$, $Apc^{1322T/+}$, and $Apc^{\Delta e1\text{-}15/+}$; refs. 10, 34, 35). Wnt signaling is also elevated in the few polyps that develop in $Apc^{NeoR/+}$ and $Apc^{NeoF/+}$ mice (36, 37). $Apc^{mNLS/mNLS}$ mice have elevated Wnt signaling in intestinal epithelial cells (38, 39). $Apc^{1572T/1572T}$ embryonic stem cells also have elevated Wnt signaling (38, 39). Neither $Apc^{mNLS/mNLS}$ nor $Apc^{1572T/+}$ mice develop intestinal polyps (38, 39).

The "just right" hypothesis is supported by reports of increased polyp multiplicity in $Apc^{1322T/+}$ and $Apc^{\Delta e1\text{-}15/+}$ mice relative to $Apc^{Min/+}$ mice (34, 35). Compared with Apc^{Min}, Apc^{1322T} protein retains one 20-a.a. repeat that can bind to β-catenin and decrease Wnt signaling (34, 35). The $Apc^{\Delta e1\text{-}15}$ allele results in complete deletion of *Apc* and polyps in $Apc^{\Delta e1\text{-}15/+}$ mice also display less Wnt signaling than polyps in $Apc^{Min/+}$ mice (34). However, the "just right" hypothesis does not readily explain why $Apc^{\Delta 716/+}$ mice show higher activation of Wnt signaling and more polyps than $Apc^{Min/+}$ 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 of 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 $Apc^{\Delta 716/+}$ or $Apc^{Min/+}$ 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 $Apc^{1322T/+}$ mice relative to those from $Apc^{Min/+}$, despite lower

Table 1. Summary of rodent models with germline *Apc* mutations before MCR

Model (ref.)	*Apc* mutation	Intestinal phenotype	Polyp distribution	Extraintestinal phenotype
$Apc^{\Delta e1\text{-}15/+}$ (34)	Complete deletion of entire *Apc* gene	– ~160 polyps/male, ~190/female – Benign adenoma – Polyps show similar histopathology to those in $Apc^{Min/+}$ mice	– Similar distribution as in $Apc^{Min/+}$ mice	– Anemia
$Apc^{\Delta 242/+}$ (55)	*β-geo* gene trap cassette inserted between exons 7 and 8 leads to stop after codon 242	– 177 polyps – Benign adenoma – Polyps show similar histopathology to those in $Apc^{Min/+}$ mice	– Similar distribution as in $Apc^{Min/+}$ mice	– NR
$Apc^{\Delta 474/+}$ (86)	Insert of duplicated exons 7–10 leads to frameshift and stop after codon 474	– 122 polyps – Benign adenoma	– Mainly small intestine (SI) – Some in colon and stomach	– Mammary tumors in 18.5% females at 3–5 months (adenoacanthoma)
$Apc^{\Delta 580/+}$ (75)	Exon 14 deletion leads to frameshift and stop after codon 580	– 120 polyps – Adenomas	– Mainly SI	– Anemia
$Apc^{\Delta 14/+}$ (86)	Exon 14 deletion leads to frameshift and stop after codon 580	– 36 polyps – Benign adenoma to invasive carcinoma – More polyps in germ-free environment – Rectal prolapse (61%)	– SI – More colonic tumors than $Apc^{Min/+}$ mice	– Mammary tumors (9%) – Anemia
$Apc^{580D/+}$ (93)	Exon 14 deletion leads to frameshift and stop after codon 580	– Intestinal polyposis	– NR	– NR
$Apc^{\Delta 15/+}$ (76)	Deletion of the last exon (exon 15) including 3′ untranslated region	– 185 polyps – Adenoma – Few adenocarcinoma – Normal crypt maturation gradient lost	– Mostly SI – 77% in ileum	– Cutaneous cysts – Desmoid tumors – Anemia
$Apc^{\Delta 716/+}$ (10, 40)	Inserted Neo^R and diphtheria toxin α-subunit genes in exon 15 leads to stop after codon 716	– 58–256 polyps – Benign adenomas	– Mainly SI	– Anemia
$Apc^{Min/+}$ (19, 67, 79)	Generated by ENU screen Nonsense mutation after codon 850	– 20–100 polyps – Benign adenomas – Malignant transformation in old mice in some genetic backgrounds	– 60% in distal 1/3 of the SI – Few in colon – Very few in stomach	– Mammary tumors; 5% old females – Anemia – Splenomegaly – Abnormal hematopoiesis – Degeneration of ovarian follicles – Underdeveloped seminiferous tubules – Abnormal serum lipid profile
PIRC rat (9, 94)	Nonsense mutation after codon 1137	– 36 polyps and 178 microadenoma (<0.5 mm), males – 11 polyps and 35 microadenomas, females – Adenoma – Adenocarcinoma in older mice	– Tumors are in both SI and colon	– Benign epidermoid cysts – Jaw osteoma in old females

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.

Table 2. Summary of rodent models with germline *Apc* mutations within or after MCR

Model (ref.)	*Apc* mutation	Intestinal phenotype	Polyp distribution	Extraintestinal phenotype
$Apc^{1309/+}$ (70, 95, 96)	Neo^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 $Apc^{Min/+}$; only 1/3 distal	– Centrilobular cholestasis in liver – Microvesicular fatty liver – Abnormal serum lipid profile
$Apc^{1322T/+}$ (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 $Apc^{Min/+}$ mice	– Most in SI – Few in colon and stomach – SI polyps more proximal than $Apc^{Min/+}$ (<20% in distal 1/3 of SI)	– Anemia – Splenomegaly
$Apc^{1572T/+}$ (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
$Apc^{1638T/1638T}$ (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
$Apc^{1638N/+}$ (71)	Neo^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^R, neomycin resistance gene; SI, small intestine.

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

Model (ref.)	*Apc* mutation	Intestinal phenotype	Polyp distribution	Extraintestinal phenotype
$Apc^{mNLS/mNLS}$ (39)	Inactivating mutations in the 2 nuclear localization signals	– Increased cellular proliferation in intestinal epithelial cells – Few spontaneous intestinal polyps – Enhanced polyposis in $Apc^{mNLS/Min}$ mice	N/A	NR
$Apc^{\Delta SAMP}$ (65)	Deletion of codons between 1322 and 2006	– Similar to $Apc^{1322T/+}$	Similar to $Apc^{1322T/+}$	Similar to $Apc^{1322T/+}$
Apc^{NeoR} and Apc^{NeoF} (36, 37)	Neo^R gene in intron 13 in reverse (Apc^{NeoR}) and forward (Apc^{NeoF}) direction. Reduced Apc level to 10% and 20%, respectively	– 0.2 polyps in Apc^{NeoR} – 1 polyp in Apc^{NeoF} – Dysplastic adenomas	SI	$Apc^{NeoR/NeoR}$ embryos show severe developmental abnormalities and die *in utero*
$Apc^{\Delta 716/+/+}$ (98)	Mutant *Apc* allele truncated after codon 716 inserted as transgene in mouse with 2 wild-type *Apc* alleles	– None	N/A	Abdominal hamartoma in one mouse
$Apc^{\Delta 716/\Delta 716/+}$ (98)	Mutant *Apc* truncated after codon 716 inserted as transgene in $Apc^{\Delta 716/+}$	– Similar to $Apc^{\Delta 716/+}$	Similar to $Apc^{\Delta 716/+}$	Similar to $Apc^{\Delta 716/+}$

NOTE: *Apc* mouse models reported in this table are on C57Bl/6 background, but with different backcross isogenicity from N2 to > N20. All models are mouse models.
Abbreviations: NR, not reported; N/A, not applicable; Neo^R, neomycin resistance gene.

Wnt signaling in polyps from the former model relative to those from $Apc^{Min/+}$ 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 $Apc^{Min/+}$ mice, $Apc^{1322T/+}$ mice have a higher proportion of Paneth cells and cells that express stem cell markers (Lgr5, Bmi1, Msi1, and CD44), 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 $Apc^{Min/+}$ 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 modifier loci have been found to affect intestinal polyposis in $Apc^{Min/+}$ 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 $Apc^{Min/+}$ 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 $Apc^{Min/+}$ and $Apc^{\Delta 242/+}$ 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 $Apc^{Min/+}$ mice (57), $Apc^{\Delta 14/+}$ mice raised in pathogen-free conditions showed significant increases in intestinal polyp number (58).

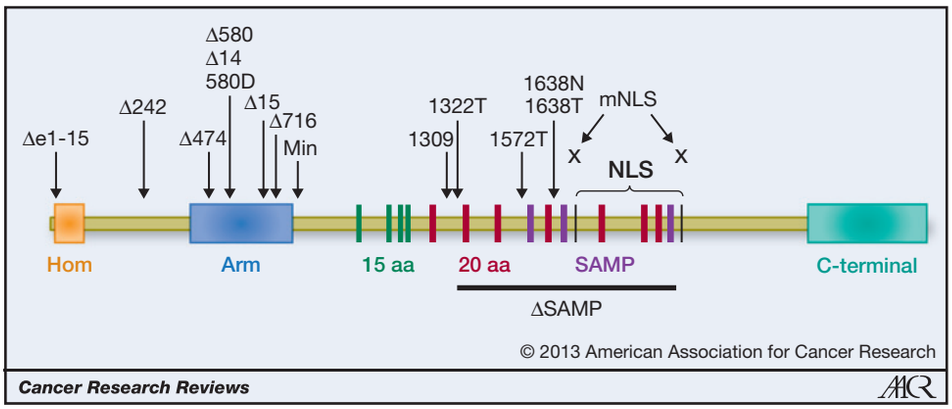

Figure 1. Sites of *Apc* mutations in different Apc mouse models relative to Apc domains. Domains of Apc are indicated as follows: Hom, homodimerization; Arm, Armadillo repeats; serine–alanine–methionine–proline (SAMP), axin binding; NLS, nuclear localization signals. C-terminal includes microtubule-, EB1-, and PDZ-binding domains. The MCR is between codons 1250 and 1464.

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 polyp multiplicity in our $Apc^{Min/+}$ mouse colony. We found that the polyp burden per mouse significantly increased from 45.9 ± 4.5 in 10 $Apc^{Min/+}$ mice on Lab diet 5001 (Purina) to 81 ± 9.3 in 25 age-matched $Apc^{Min/+}$ 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 Apc region to Apc^{1322T} (as in $Apc^{\Delta SAMP}$ 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*–induced DNA excision. The $Apc^{Min/+}$ mouse, PIRC rat, and KAD rat were generated by chemical mutagenesis which resulted in a single basepair change in the *Apc* gene (9, 67, 68). Many other models, such as Apc^{1309}, Apc^{1638N}, and Apc^{1638T}, were generated through insertion of an antibiotic resistance gene into the *Apc* gene, thus introducing a nonsense mutation (69–71). In Apc^{neoF} and Apc^{neoR} alleles, the antibiotic resistance gene disrupts an enhancer sequence in intron 13 (36, 37). Other mouse models with *Apc* truncation including $Apc^{1322T/+}$ and $Apc^{\Delta e1-15}$ were generated using Cre-*lox*–mediated deletion of specific Apc regions. The later technology allowed removal of most exogenous DNA sequences originating from the targeting vector including the antibiotic resistance gene. The Apc^{mNLS} model contains mutations "knocked into" the *Apc* gene, with the antibiotic resistance gene subsequently removed by Cre-*lox*–mediated deletion (39).

The $Apc^{1638N/+}$ and $Apc^{1638T/+}$ models, which differ only by orientation of the inserted neomycin resistance gene, provide clear evidence for the contribution of extraneous DNA to phenotypic variation (69). $Apc^{1638N/+}$ mice express so little truncated Apc protein that they might be considered virtually null (69, 72); yet the described phenotype of $Apc^{1638N/+}$ mice is not similar to that of the $Apc^{\Delta e1-15}$ model, which has a complete deletion of the *Apc* gene (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 $Apc^{1322T/+}$ and $Apc^{1309/+}$ mice, which differs by only 13 amino acids, stems from the different technology used in their generation: Cre-*lox*–mediated deletion in $Apc^{1322T/+}$ versus insertion of an antibiotic resistance cassette in $Apc^{1309/+}$. However, other genetic and environmental factors may contribute to the variation between these two mouse models as well (32, 70). A final illustration of the challenges in generation of Apc mouse models is the $Apc^{\Delta 474/+}$ 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 $Apc^{\Delta 716}$, $Apc^{Min/+}$, Apc^{1322T}, and Apc^{1638T} mice, this is not universally the case (32, 69, 74). In Apc^{580D}, $Apc^{\Delta 14}$, $Apc^{\Delta 474}$, and $Apc^{\Delta 242}$ models, the truncating mutation occurs before the final exon

(15), and thus there is the possibility of a nonsense-mediated RNA decay. Truncated Apc was not detected in intestinal polyps from $Apc^{\Delta 580/+}$ mice or embryonic stem cells from $Apc^{\Delta 15/+}$ mice (75, 76), which suggests that these alleles might also be virtually null. A related consideration is the effect of the introduced mutation (and possibly the antibiotic selection cassette) on Apc folding. Although most of Apc is thought to be natively unfolded (77), the effects of mutations on inherently folded domains of Apc, and the consequences of potential folding defects in relation to phenotype, are not understood.

Variation in Polyp Distribution

Tumors in most Apc mouse models occur mainly in the small intestine, whereas germline mutations of *APC* in humans result in tumors predominantly in the large intestine (21, 78). The PIRC Apc rat model has tumors in both small and large intestines (9, 13, 79). A pig model with germline *Apc* mutations was recently reported to develop polyps in the colon (80). In addition to this interspecies variation, mouse models with different germline *Apc* mutations show different distributions of intestinal polyps. Analysis of $Apc^{Min/+}$ mice with different genetic backgrounds has led to the hypothesis that polyp distribution is somehow linked to the mechanism by which the wild-type *Apc* allele is lost (24). Haigis and colleagues showed that in a B6 background, $Apc^{Min/+}$ mice develop polyps mainly in the distal half of the small intestine, and loss of the wild-type *Apc* allele occurs by means of LOH. In an AKR background, $Apc^{Min/+}$ mice develop polyps predominantly at the ileocecal junction, and inactivation of the wild-type *Apc* allele is achieved through allelic silencing. In the B6 background, $Apc^{Min/+}$ mice with additional mutations that inactivate the mismatch repair gene *Mlh* develop polyps all over the small intestine, and loss of the wild-type *Apc* allele is achieved through a point mutation. $Apc^{1638N/+}$ mice develop polyps in a similar distribution and appear to retain the wild-type *Apc* allele (24).

Mechanistically, two models have been proposed to explain the connection between polyp distribution and loss of the wild-type *Apc* allele. In the first model, the molecular machinery in different intestinal regions determines the mechanism of the second *Apc* "hit" and hence the distribution of polyps. This model is supported by the finding that mice in which the wild-type *Apc* allele is inactivated by the same mechanism (e.g., $Apc^{Min/+}/Mlh^{-/-}$ and $Apc^{1638N/+}$) have similar polyp distributions (24). However, the finding that both $Apc^{1322T/+}$ and $Apc^{Min/+}$ mice lose the wild-type *Apc* allele through LOH, yet have different polyp distributions, does not support this model. A second model proposes that polyp growth is dictated by the *Apc* status but also by the particular environment of the different intestinal regions, independent of the mechanism of the second *Apc* mutation. Supporting this hypothesis, $Apc^{\Delta 716/+}$ mice with an additional mutation of *Cdx2* exhibit more colonic and fewer small intestinal polyps. Yet, loss of the wild-type *Apc* allele occurs via LOH regardless of *Cdx2* status (44). Similarly, a colonic shift of polyps has been described in $Apc^{Min/+}$ mice with an additional *BubR1* mutation, although the mechanism of loss of the wild-type *Apc* allele in these mice was not reported (43). Mutation of both *Cdx2* and *BubR1* increases chromosomal instability and changes the proliferation and apoptotic indices in intestines of $Apc^{\Delta 714/+}$ and $Apc^{Min/+}$ mice, respectively (43, 44). Further support for the second model comes from $Apc^{Min/+}$ mice in a 129/Sv background, 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 $Apc^{Min/+}$ 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 $Apc^{Min/+}$ and $Apc^{1322T/+}$ 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 $Apc^{Min\text{-}FCCC}$ mice, which were generated by mating C57Bl/6J $Apc^{Min/+}$ males with $Apc^{+/+}$ females from an independent colony of C57Bl/6 mice maintained at Fox Chase Cancer Center (Philadelphia, PA). $Apc^{Min\text{-}FCCC/+}$ mice develop more colon polyps than do $Apc^{Min/+}$ 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 $Apc^{\Delta 14/+}$ and $Apc^{580D/+}$ mice, which carry similar mutations (truncating the Apc protein at amino acid 580) but appear to have different polyp distributions. $Apc^{\Delta 14/+}$ mice develop more colonic polyps than do $Apc^{Min/+}$ mice. $Apc^{580D/+}$ mice develop a similar number of colonic polyps as $Apc^{Min/+}$ mice, although direct comparison of $Apc^{580D/+}$ and either $Apc^{\Delta 14/+}$ or $Apc^{Min/+}$ 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).

Apc rodent models also develop some of these extraintestinal manifestations; for example, $Apc^{1638N/+}$ mice develop desmoid tumors (72) and PIRC 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, adenoacanthoma 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 $Apc^{Min/+}$ and $Apc^{\Delta 474/+}$ models. Very few $Apc^{Min/+}$ mice develop mammary tumors, whereas $Apc^{\Delta 474/+}$ mice develop mammary tumors at a rate that is almost double that seen in $Apc^{Min/+}$ mice (73, 91). In contrast, there are no reports of mammary tumor development in Apc mouse models with the most severe intestinal polyposis ($Apc^{\Delta 714}$, Apc^{1322T}, and $Apc^{\Delta SAMP}$; refs. 32, 40, 65). Perhaps mice with severe polyposis die too early, before mammary tumors have a chance to develop. $Apc^{1572T/+}$ mice, which develop no intestinal polyps, have a fully penetrant mammary tumor phenotype in females. K14-cre-$Apc^{CKO/+}$ mice are a conditional model in which the $Apc^{\Delta 580}$ 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.

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Authors' Contributions

Conception and design: M. Zeineldin, K.L. Neufeld
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): M. Zeineldin
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): K.L. Neufeld, M. Zeineldin
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