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Genetic and Chemical Models of Colorectal Cancer in Mice

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Abstract

Colorectal cancer (CRC) is a significant health concern because of its associated mortality. Most CRCs exhibit dysregulation of the Wnt signaling pathway, caused by mutational inactivation of the adenomatous polyposis coli tumor suppressor gene (*APC*) or mutational activation of β-catenin. Disease progression is accompanied by additional mutations in the *KRAS* oncogene and *p53* tumor suppressor gene. Other CRCs are microsatellite unstable because of mutational inactivation or epigenetic silencing of key molecules responsible for DNA mismatch repair. This review focuses on several common mouse models of CRC, highlighting the consequences of germline mutation of the aforementioned tumor suppressor genes or proto-oncogenes. This article also discusses chemical carcinogens that adversely affect the intestinal tissues with formation of colorectal neoplasia in mice. These mouse models have significantly contributed to the understanding of the mechanisms responsible for CRC pathogenesis and also may serve as potential vehicles for therapeutic intervention.

Keywords

Colorectal Cancer; Genetic factors; Wnt pathway; Mismatch repair; Chemical carcinogenesis; Mouse models

Introduction

Colorectal cancer (CRC) is one of the leading causes of cancer-related death in the United States and around the world. Each year, approximately 150,000 Americans are diagnosed with CRC, and approximately 50,000 die from it. Numerous factors, including genetic, epigenetic, and environmental causes, act independently or in coalition and cumulate in the formation of CRC. Animal models increasingly have been valuable in elucidating the pathogenic mechanisms of CRC. Mouse models are especially important because of their relatively low maintenance cost, short gestation period, and ease of genetic manipulation and because of the extensive information available on their genetic background. In this article, we discuss

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representative genetic and chemical mouse models of intestinal tumorigenesis that are closely relevant to the human disease.

Dysregulation of the Wnt Signaling Pathway in Colorectal Cancer and Related Mouse Models

The Wnt signaling pathway is critical for the maintenance of intestinal epithelial homeostasis [1••]. Wnts are secreted proteins that regulate proliferation and differentiation of recipient cells by a canonical or noncanonical mechanism. The canonical mechanism, which has been investigated extensively, commences with the binding of Wnt to the Frizzled (Fz) receptors on the plasma membrane [1••]. The critical mediator for Wnt signaling is β-catenin, the stability of which is regulated by a destruction complex containing the tumor suppressors axin and adenomatous polyposis coli (APC) as well as several isoforms of casein kinase (CK) and glycogen synthase kinase 3 (GSK3) [1••]. In the absence of Wnt, CK1 and GSK3 sequentially phosphorylate β-catenin, leading to its degradation by a ubiquitin-dependent mechanism [1••]. Upon receptor activation by Wnt ligands, the intrinsic kinase activity of the destruction complex is inhibited, with the consequent accumulation of stable, nonphosphorylated β-catenin. The stabilized β-catenin then translocates into the nucleus, where it interacts with T-cell factor and elicits a transcriptional cascade to activate expression of target genes, many of which drive cellular proliferation [1••].

Among the various Wnt pathway components, APC is the molecule most clinically relevant to CRC. The gene encoding APC is located on chromosome 5q21, which is linked to the autosomal dominant disorder familial adenomatous polyposis (FAP). Patients with FAP are predisposed to formation of adenomas in their intestinal tract and are at risk for CRC at an early age [2]. Subsequently, it was found that germline mutation of the *APC* gene causes FAP. Moreover, more than 80% of sporadic CRCs contain mutation in *APC* [3]. These findings directly link the Wnt signaling pathway to CRC pathogenesis.

The *Apc^{Min}* Mouse Model

In accordance with the recognition of APC as an important mediator of tumorigenesis in FAP, a mutant Apc mouse model was developed previously [4]. Like FAP patients, the mutant mice, appropriately named *Min mice* for “multiple intestinal neoplasia,” are predisposed to spontaneous intestinal adenomas and carcinomas in an autosomal dominant fashion with full penetrance [4]. Shortly after the identification of the Min mice, the causative mutation was identified as a nonsense mutation resulting in a truncated mouse Apc protein at amino acid (aa) position 850 [5]. The mice, now commonly referred to as *Apc$^{Min/+}$* or *Apc^{Min}* mice, develop an average of 30 adenomas per mouse throughout the intestinal tract, with most adenomas located in the small intestine [4]. The similarity between *Apc^{Min}* mice and FAP patients with regard to the nature of mutation of the *APC* gene and the intestinal phenotype made the *Apc^{Min}* mouse an excellent model to study intestinal tumorigenesis [5]. Homozygous *Apc^{Min}* mutation leads to embryonic lethality 6.5 days post coitus (dpc) [6]. The adenomas formed in the heterozygous *Apc$^{Min/+}$* mice display loss of the wild-type *Apc* allele [7], a condition similar to the loss of heterozygosity (LOH) of the *APC* gene in the tumors of FAP patients [8], confirming the role of APC as a tumor suppressor.

Additional somatic changes also modify the Min mouse phenotype. For example, crosses of Min mice, which were propagated on a C57BL/6J background, to other inbred mouse strains showed a decrease in adenoma formation and an increase in longevity in the resulting progeny [9]. One of the major markers that modify the *Apc* mutation in Min mice is located on chromosome 4 and is labeled the modifier of Min 1 (*Mom1*) [10]. *Mom1* controls 50% of the genetic variations in tumor number from the Min mouse [10]. The *Mom1* locus was dissected

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further to involve the secreted phospholipase A_2 gene (*Pla2g2a*) and *D4Mit64* [11]. Subsequently, several other modifiers of the Min phenotype were described, especially *Mom2, Mom3, Mom6,* and *Mom7* [12••].

Other *Apc* Mouse Models

Several other models that involve inactivation of the *Apc* gene have been developed and characterized [12••]. The main variations in these models from the Apc^{Min} mouse model lie in the number and location of tumors. Among the well-documented strains of genetically engineered *Apc* mice are the $Apc^{\Delta 716/+}$ and the $Apc^{1638N/+}$ mice.

The $Apc^{\Delta 716/+}$ mouse was developed using homologous recombination in embryonic stem cells containing a truncation of the *Apc* gene at aa 716 [13]. The homozygous mutants were embryonic lethal before day 8 of gestation. The study reported an average of about 300 small intestinal tumors per mouse at 16 weeks of age [13], significantly higher than the number in the $Apc^{Min/+}$ mouse. All the adenomas displayed LOH of the wild-type *Apc* allele but showed expression of the truncated allele. Therefore, truncation of *Apc* at aa 716 conferred a far greater susceptibility of the C57BL/6J mouse strain to develop adenomas than did truncation at aa 850 (ie, the $Apc^{Min/+}$ mouse model).

Similarly, a chain termination mutation in the 15th exon of the mouse *Apc* gene resulted in $Apc^{1638N/+}$ mice that developed adenomas throughout the intestinal tract, with an average of fewer than 10 adenomas per mouse [14]. These mice also showed a greater propensity to develop adenomas and carcinomas in the small intestine than in the colon and survived longer than the $Apc^{Min/+}$ or the $Apc^{\Delta 716/+}$ mice [14]. However, similar to the other two genotypes, homozygous mutants displayed embryonic lethality post implantation.

Among several other *Apc* mouse models is $Apc^{\Delta 14/+}$ [15], in which exon 14 of the *Apc* gene was deleted. These mice showed a shift in tumors to the distal colon and rectum including prolapse. The Apc^{1322T} mouse model was created through targeted deletion and selection. These mice showed increased polyposis and dysplastic adenomas with an earlier onset compared with the $Apc^{Min/+}$ mice [16]. Similar to the Apc^{1322T} mice, Apc^{1572T} mice also showed intermediate levels of β-catenin nuclear accumulation compared with the $Apc^{Min/+}$ mice [17]. However, these mice did not display intestinal tumorigenesis but rather developed mammary tumors with pulmonary metastases. These results indicate the rather fickle relationship between Wnt/β-catenin activation and the development of intestinal tumors.

Tissue-Specific Mutant *Apc* Mouse Models

The $Apc^{fl/fl}$ mice were developed by targeted mutagenesis of the *Apc* gene in which LoxP sites were introduced to flank exon 14 [18]. Upon expression of Cre recombinase, exon 14 of the *Apc* gene was deleted, causing a frameshift mutation at codon 580 (Apc^{580S}). The homozygous $Apc^{580S/580S}$ mice were phenotypically normal in the absence of Cre recombinase expression. However, when the homozygotes were anally infected with Cre-expressing adenovirus, multiple rectal adenomas were formed at 3 months of age [18]. Genomic analyses of adenomas also revealed the homozygous deletion of the Apc^{580S} locus (Apc^{580D}).

Recently, several systems were developed to induce intestine-specific expression of Cre recombinase. Among the more commonly used mouse models are the Fabpl-Cre, Villin-Cre, and AhCre transgenic models. The Fabpl-Cre model has a promoter element comprising nucleotides −596 to +21 of the rat liver fatty acid binding protein gene (*Fabpl*), with four additional tandem repeats of nucleotides −172 to −133 added at nucleotide −132, preceding the Cre gene [19]. This mouse shows vigorous Cre recombinase expression in the small intestinal and colonic epithelial cells, beginning from embryonic day 13.5. Cre expression also

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can be detected in the ureter and bladder epithelial cells. The same group also generated an inducible version of the Fabpl-Cre mouse [19]. The Villin-Cre model, established by two different groups as an intestine-specific Cre expression system, is well characterized. A 9-kb mouse villin regulatory region was used to drive Cre recombinase expression in intestinal epithelial cells [20]. The expression of the transgene also was found in the epithelial cells of the proximal tubules of kidney cells. The Cre expression from the villin promoter was turned on in the intestinal epithelial cells from embryonic 12.5 dpc. Similarly, another group developed a 12.4-kb villin promoter-driven Cre mouse in which Cre also is specifically expressed in intestinal epithelial cells [21]. Recently, an inducible Villin-Cre expression system was developed that places tamoxifen-driven Cre recombinase expression under the control of the mouse villin promoter (vil-Cre-ERT2) [22].

The AhCre transgenic model was created as an inducible system in which Cre expression is controlled by a cytochrome P-450 promoter element inducible with β-naphthoflavone [23]. Expression of Cre recombinase from AhCre mice was detected in the intestine, liver, pancreas, gallbladder, and stomach. The AhCre mouse model was used to conditionally delete the *Apc* gene by crossing it with the $Apc^{fl/fl}$ mice [24]. The $Cre^{+}Apc^{fl/fl}$ mice showed loss of crypt–villus architecture, replaced by a distinct crypt-like phenotype and altered differentiation patterns. There also was induction of the Wnt signaling pathway with nuclear localization of β-catenin. However, the mice became visibly morbid within 5 days of β-naphthoflavone administration and had to be sacrificed [24]. These mice revealed a severe reaction in the intestinal epithelial cells to homozygous deletion of the *Apc* gene, confirming the importance of APC as a tumor suppressor.

A recent study exploited the $Apc^{\Delta 14/+}$ mouse [15] by crossing it with the Fabpl-Cre mouse to generate *Fabpl-Cre*; $Apc^{2lox14/+}$ mice [25•]. These mice had pedunculated, focal adenocarcinomas in the colon that were heterogeneous, showing both low- and high-grade tumorigenic regions. Lastly, a recent review extensively described the *Apc* mouse models and their implications [12••]. Several compound mouse models involving *Apc* mutants also have been reported [26••].

Mutant β-Catenin Mouse Models

Other mouse models of intestinal tumorigenesis due to modifications of the Wnt signaling pathway involve activation of β-catenin. One model expressed stable, activated, amino terminal-truncated β-catenin protein that had lost its GSK3β phosphorylation sites from the calbindin D9K (CaBP9K) promoter linked to the enhancer of the aldolase B gene [27]. These mice showed multifocal dysplastic lesions similar to those observed in FAP patients. The mice also developed severe polycystic kidney disease due to activated β-catenin [27]. Another group generated mice containing exogenous LoxP sequences flanking exon 3 of the endogenous β-catenin gene. These mice, when crossed with either Fabpl-Cre or cytokeratin 19-Cre mice, developed multiple intestinal tumors and decreased survival compared with their normal counterparts [28]. Thus, modifications in the Wnt signaling pathway that lead to increased nuclear accumulation of β-catenin, either through β-catenin activation or *Apc* inactivation, severely affect the normal homeostasis of the intestinal epithelia. Accumulated mutational events in these affected epithelial cells promote increased proliferation and tumorigenesis.

Inactivation of the Mismatch Repair System in Colorectal Cancer and Related Mouse Models

Hereditary nonpolyposis colorectal cancer (HNPCC), also known as Lynch syndrome, accounts for about 3% of all CRC cases in the United States. It is an autosomal dominant disorder with high penetrance and early onset of colorectal cancer compared with the general

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population [29••]. HNPCC is caused by a germline mutation in one of the genes encoding proteins involved in DNA mismatch repair (MMR), with resultant microsatellite instability (MSI) [30]. Microsatellites are short di- and trinucleotide repeats that must be replicated faithfully. Slippage may occur when DNA polymerase skips sequences that need to be replicated. MMR proteins normally correct this defect. However, a deficient MMR system in the cell leads to MSI. The MMR system comprises members of the mutS and mutL protein families. Mutations in the gene encoding mutS member hMSH2, which recognizes the mismatched DNA region, or mutL member hMLH1, which excises the mismatched region, result in high MSI compared with mutations in the other mutS/mutL genes. Notably, mutations in *hMSH2* and *hMLH1* cause most HNPCC cases. It has been noted that about 15% of sporadic CRCs show the MSI phenotype; however, these mutations are somatic not germline [30].

The *mutS* Mouse Models

Several mouse models of MMR mutations have been described. The $Msh2^{-/-}$ mice did not have any developmental defects and were fertile; however, they developed multiple tumors in the colorectal, gastric, and endometrial regions, along with lymphoblastic lymphomas, and survived for only 2 to 5 months [31]. Unlike HNPCC patients, these mice did not have early onset of CRC, but they did show MMR defects in the lymphomas [31]. A recent study has shown that the tissue-specific Villin-Cre-mediated deletion of *Msh2* ($VCMsh2^{LoxP/LoxP}$) results in MSI specifically in the intestinal epithelium [32•]. A significantly higher percentage of the $VCMsh2^{LoxP/LoxP}$ mice showed a propensity to develop intestinal tumors compared with the $Msh2^{-/-}$ mice. Interestingly, it also was noted that the adenomas from $VCMsh2^{LoxP/LoxP}$ mice showed *Apc* gene inactivation [32•].

Defects in the *hMSH3* and *hMSH6* genes occur less frequently in HNPCC patients. MSH3-inactivating mutations resulted in defective insertion/deletion repair but intact base/base repair mechanisms. $Msh3^{-/-}$ mice did not show a predisposition to CRC and rarely developed tumors later in life [33]. Similar to the $Msh3^{-/-}$ mice, the $Msh6^{-/-}$ mice also showed defects in insertion/deletion repair [34]. They developed a spectrum of tumors, mainly of the gastrointestinal tract, and lymphomas. The tumors, however, did not show any signs of MSI [34]. Mice with double homozygous deletions ($Msh3^{-/-}/Msh6^{-/-}$) had an increased predisposition to intestinal tumors, decreased survival, and loss of MMR activity [35]. Other mutS gene deletions, *Msh4* and *Msh5*, failed to show any predisposition to cancers or decreased survival but did show reduction in fertility of the progeny [36••].

The *mutL* Mouse Models

hMLH1 is one of the genes implicated in high MSI in humans upon mutation. *Mlh1*-null ($Mlh1^{-/-}$) mice showed a wide spectrum of tumors, including lymphomas and intestinal tumors [37]. The $Mlh1^{-/-}$ tumors displayed increased MSI and decreased levels of *Apc* expression [38]. Like *Msh2*-null mice, the $Mlh1^{-/-}$ mice displayed decreased survival and increased susceptibility to cancers [37].

Another member of the mutL family involved in the human MMR complex is hPMS2. Male *Pms2* mutant mice were sterile and showed MSI abnormalities in the germline [39]. The *Pms2*-null mice also showed tumor susceptibility, but the phenotype was less severe than observed in the *Mlh1*-null mice [38]. Lastly, the *Mlh3*-null mice developed adenomas and carcinomas in the intestinal tract with increased MSI [40]. *Mlh3* and *Pms2* double-null mice showed increased tumor susceptibility and reduced survival, similar to the results observed in *Mlh1*-null mice [40].

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Other Genetic Mouse Models of Human Colorectal Cancer

One of the earliest known regulators of tumorigenesis that has been investigated extensively is the *p53* tumor suppressor gene. However, a host of mouse models that involved *p53* inactivation developed lymphomas and sarcomas [41] but not colonic tumors. The following sections summarize several other mouse models of CRC not involving *Apc* or *p53*.

The Transforming Growth Factor-β Pathway Mouse Models

Transforming growth factor-β (TGF-β) plays an important role in inhibiting proliferation of normal epithelial cells while promoting tumor progression. Nontransformed epithelial cells are inhibited by TGF-β because of upregulation of p21, which arrests cell cycle progression [42]. In contrast, many tumors display impaired TGF-β signaling as a result of inactivation of the pathway components, including TGF-β receptor II (TβRII), or activation of other mediators that can severely inhibit TGF-β signaling, such as RAS. Inactivating mutations in TβRII have been observed in about 25% of all human CRCs [42]. *Tgfbr2*-null mice did not show any signs of intestinal cancer, but they did show increased CRC and metastases when crossed with mice expressing oncogenic *KRAS* [43•].

Targeted homozygous deletion of the *Tgfb1* gene in mice resulted in death shortly after birth as a result of inflammatory cell infiltration into major organs and acute wasting [44]. However, elimination of the intestinal flora and subsequent breeding of the $Tgfb1^{-/-}$ mice in germ-free conditions alleviated inflammation and cancer formation in these mice [45]. Because immune cell infiltration was one of the leading causes of death in *Tgfb1*-null mice, crossing these mice with *Rag2*-null immune-deficient mice could rescue the *Tgfb1*-null mice [46]. The $Tgfb1^{-/-}$/$Rag2^{-/-}$ mice showed increased cancer in the cecum and other colonic regions of the intestine. These mice did not display any signs of MSI or *Apc* inactivation in the tumors [46].

The SMAD family of proteins are known mediators of TGF-β signaling. Smad2 and Smad3 proteins are activator proteins, whereas Smad6 and Smad7 are inhibitory proteins, with Smad4 functioning as an intermediary [42]. *Smad2* and *Smad4* knockout mice were embryonic lethal [47,48]. $Smad3^{-/-}$ mice, however, developed extensive colorectal adenocarcinomas at 4 to 6 months of age [49]. The tumors contained the whole spectrum of type and size, from microadenomas to deeply invasive carcinomas. *Apc* expression was normal in the tumors [49]. However, in a subsequent exon 8 knockout of the *Smad3* gene, no adenocarcinomas were observed in the intestine [50]. $Smad4^{+/-}$ mice were examined about 1 year after birth and were found to display gastric and duodenal polyps [51].

K-Ras Mouse Model

Another important player in human colorectal carcinogenesis is the *RAS* oncogene. Activating mutations in the *RAS* genes, particularly *KRAS*, are present in approximately 50% of CRCs [52]. The RAS signaling cascade is triggered by growth factors [53]. Homozygous deletions of the *K-Ras* gene resulted in embryonic lethality around 12.5 dpc [54]. Overexpression of an activated *K-Ras* gene ($K\text{-}Ras^{LA}$) in mice resulted in tumorigenesis, especially in the lungs, but also produced thymic lymphomas and skin papillomas [55]. These mice did not develop adenomas in the colon but did show aberrant crypt foci (ACF), one of the earliest lesions of dysplasia. Expression of activated K-Ras ($K\text{-}Ras^{V12G}$) under the control of the villin promoter led to intestine-specific induction of RAS signaling, as demonstrated by elevated pERK activity [56]. The mice developed multiple intestinal lesions varying from aberrant crypt foci to adenocarcinomas. These tumors retained normal expression from the *Apc* gene [56]. The floxed *K-Ras* mouse model also has been used to achieve intestine-specific expression using other promoter-Cre mice. Intestine-specific expression of activated K-Ras in Fabpl-Cre mice resulted in increased proliferation and dysplasia of the colonic epithelium [57]. One study used

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the AhCre transgenic mouse to express floxed *K-Ras* gene in the intestinal epithelium [58•]. No major changes were noted in the crypt-villus architecture or the proliferative compartment. Also, the migration and differentiation of the intestinal epithelial cells remained intact [58•]. A subsequent study crossing Villin-Cre mice with floxed *K-Ras* mice generated another model of intestine-specific K-Ras activation [59]. These mice displayed an overall widespread disorganization of the colonic crypts similar to that observed with the Fabpl-Cre-mediated K-Ras expression [57].

The Toll-Like Receptor Signaling Mouse Models

Toll-like receptor (TLR) signaling, a key mediator of the innate immune system, protects the host from harmful microbes. The TLR family of pattern-recognition receptors includes various members that signal intracellularly through a common intermediate, myeloid differentiation factor 88 (MyD88) [60]. Mice with targeted disruption of *MyD88* did not display any overt intestinal phenotype but showed defects in T-cell proliferation and induction of cytokines in response to interleukin (IL)-1 [61]. However, $MyD88^{-/-}$ mice showed severe morbidity and mortality with severe colonic injury in response to dextran sodium sulfate (DSS) treatment [62].

TLR4 is one of the key receptors in the recognition of lipopolysaccharides from gram-negative bacteria [63]. TLR4 expression is low in normal intestinal epithelium but gets significantly induced in inflammatory bowel disease (IBD)- and colitis-associated tumors [64]. *Tlr4*-deficient mice did not show any overt phenotypic changes compared with the wild-type mice [65], and chemically induced carcinogenesis was considerably attenuated in the *Tlr4*-deficient mice [64].

Both pathogenic and commensal bacteria possess flagella, which are composed of flagellin that can be secreted into the intestinal lumen. Only pathogenic flagellin can translocate to the basolateral membrane of intestinal epithelial cells to interact with TLR5 and activate proinflammatory gene expression [66]. *Tlr5* knockout mice (TLR5KO) showed an increase in spontaneous colitis, and 10% to 12% of the mice developed rectal prolapse [67•]. The cecum and proximal colon displayed a high incidence of hyperplasia and focal crypt epithelial destruction. A potential reason for the spontaneous colitis may have been stimulation of TLR4 signaling. The *Tlr4*/*Tlr5* double-knockout mouse did not show any signs of inflammation or spontaneous colitis [67•].

Other Mouse Models of Colorectal Cancer

Immune-deficient models of intestinal inflammation have been well characterized. IL-10-deficient mice suffered from growth retardation and chronic enterocolitis, but when these mice were raised in specific pathogen-free microbial environments, the severity of the symptoms was attenuated [68]. However, when the mice were raised in similar pathogen-free conditions for a longer period (6 months), they developed colonic lesions and adenocarcinomas [69]. Similarly, IL-2-deficient mice also developed severe forms of colitis that were alleviated by raising the mice in germ-free conditions [70].

Cdx2, a caudal-related homeobox family member of transcription factors, is specifically expressed in the intestinal epithelium and plays a significant role in development. Homozygous deletion of *Cdx2* in mice resulted in embryonic lethality at 3.5 to 5.5 dpc. The heterozygous mutants survived but developed multiple adenomas, mainly in the proximal colon, within 3 months of age [71]. Recently, a study used the intestine-specific expression of *Cdx2* to generate a *CDX2P-NLS Cre* transgenic mouse. This mouse expresses the Cre recombinase from the *Cdx2* promoter [72•]. Upon crossing this mouse with an $Apc^{fl/fl}$ mouse, colon-specific

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adenocarcinomas and carcinomas developed in 15% to 20% of the progeny [24]. These tumors also had morphologic and molecular similarities to human colon cancer [72•].

Muc2 is the most abundant secreted apomucin in the gastrointestinal epithelium. Mucins play an important role in enveloping and protecting the luminal layer of the intestinal epithelium. Muc2 deficiency in mice caused gastrointestinal abnormalities leading to adenomas and invasive adenocarcinomas at approximately 1 year of age [73].

Chemical-Induced Mouse Models of Colon Cancer

Chemical induction of carcinogenesis was recapitulated extensively in a recent review article [74••]. An earlier report of colon cancer caused by a carcinogen involved feeding cycad meal and cycasin, which contain the chemical methylazoxymethanol glycoside (MAM), to rats [75]. 1,2-Dimethylhydrazine (DMH), a metabolic precursor to MAM, was used in later studies as a model for sporadic colon carcinogenesis. Subsequently, azoxymethane (AOM), a DMH metabolite, was the preferred choice of researchers to generate colon cancer [76]. Other chemical mutagens include heterocyclic amines, such as 2-amino-1-methyl-6-phenylimidazo [4,5-*b*]pyridine; aromatic amines, such as 3-2′-dimethyl-4-aminobiphenyl; and alkyl nitrosamides, such as methylnitrosourea and *N*-methyl-*N*’-nitro-*N*-nitrosoguanidine [74••].

Both DMH and AOM are metabolized in cells to produce MAM with high intracellular stability. This stability helps the distribution of MAM to the colon, which then produces a methyldiazonium ion that causes alkylation events leading to DNA damage [76]. Carcinogens in mice target a large number of genes for mutations. Both DMH- and AOM-induced tumors in mice show activating mutations in the *K-Ras* gene [77]; 66% of tumors derived from DMH treatment and 20% to 33% of the AOM-treated tumors developed *K-Ras* mutations [77,78].

Wnt pathway gene mutations also are very common in both chemical models of carcinogenesis. Mutations in the *Apc* gene have been observed with AOM treatment in rats. Loss of full-length Apc protein was observed in tumors derived from AOM-treated mice [79]. Several mutational events in the β-catenin gene were observed in mice treated with AOM [80]. Tumors showed mutations in the β-catenin gene specifically at regions phosphorylated by GSK3β. These tumors appropriately displayed increased nuclear accumulation of β-catenin [80].

CRC pathogenesis may involve the increased synthesis of prostaglandins through elevated expression of cyclooxygenase 2 (COX-2). COX-2 usually is not expressed in normal epithelium but shows an elevated expression pattern in response to growth factors and oncogenes [81]. Mice treated with AOM showed increased expression of COX-2 in the adenocarcinomas along with elevated levels of prostaglandin E_2 [82]. Finally, TGF-β and SMAD proteins also are affected by AOM treatment because of the reduction in expression of TGFβRII receptor. Although no major changes in TGF-β, SMAD3, or SMAD7 proteins were found in the tumors, they did show increased expression of c-Myc [83].

The AOM/DSS model is aimed at recapitulating IBD-associated colorectal carcinogenesis. DSS causes inflammation in the colon of mice upon short exposure, but prolonged exposure might result in CRC [84]. A two-stage treatment model of AOM followed by DSS is used widely to generate CRC in mice. In this model, AOM treatment was carried out in an initial single dose followed by 1-week treatment with 2% DSS in drinking water. Mice sacrificed after 20 weeks all had adenomas and adenocarcinomas with strong expression of COX-2, β-catenin, and inducible nitric oxide synthase [85].

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Conclusions

Numerous genetic and chemical mouse models of CRC have been generated over the years. Most mouse models of human CRC faithfully replicate the characteristics of their human counterparts. However, several genetic mouse models generate tumors predominantly in the small intestine, in contrast to human CRC, in which tumors are found in the colonic epithelium, with only rare occurrences in the small bowel. Carcinogen treatment of mice does generate colonic neoplasia, but these mice show specific gene expression patterns that may not represent the entire gamut of human CRC. Additional mouse models aimed at resolving the particular issue of region-specific tumor distribution are needed for the study of human CRC.

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•• Of major importance

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