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## Review

# Mouse models of colorectal cancer

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## Abstract

Colorectal cancer is one of the most common malignancies in the world. Many mouse models have been developed to evaluate features of colorectal cancer in humans. These can be grouped into genetically-engineered, chemically-induced, and inoculated models. However, none recapitulates all of the characteristics of human colorectal cancer. It is critical to use a specific mouse model to address a particular research question. Here, we review commonly used mouse models for human colorectal cancer.

**Key words** Colorectal cancer, mouse model, tumorigenesis, metastasis

Colorectal cancer is the third most common malignancy in the world. In the United States, colorectal cancer is the fourth most commonly diagnosed cancer and the second leading cause of cancer-related death<sup>[1]</sup>. The development of colorectal cancer is a process sequentially acquiring a number of genetic changes in normal epithelium, which enables precancerous cells to develop into an adenomatous polyp and progress into an invasive tumor (Figure 1)<sup>[2]</sup>. Inactivation of the adenomatous polyposis coli (*APC*) gene and activation of the *K-Ras* proto-oncogene are two early events of colorectal tumorigenesis<sup>[3,4]</sup>. Germline mutation of the *APC* gene causes the familial adenomatous polyposis (FAP) syndrome. However, lesions exhibiting *K-ras* mutation without *APC* alteration result mostly in non-dysplastic lesions with limited potential to progress to carcinoma<sup>[5]</sup>. The next step in progression from adenoma to carcinoma is the loss of heterozygosity of chromosome 18q, which contains candidate tumor suppressor genes including *SMAD2*, *SMAD4* and *DCC*<sup>[6]</sup>. Mutation of *p53* on chromosome 17q appears to be a late-stage event<sup>[2]</sup>. Some genetic changes do not affect the cell biology of the tumor but instead result in loss of genomic stability. As evidenced in patients with hereditary non-polyposis colorectal cancer (HNPCC),

loss of DNA mismatch repair (MMR) genes leads to microsatellite instability (MSI) and early-onset colorectal tumors<sup>[7,8]</sup>. In addition to MSI, colorectal cancer can develop chromosomal instability (CIN), which also occurs relatively early in tumor evolution<sup>[8-12]</sup>.

The laboratory mouse is one of the best model systems in biomedical research because of the availability of genetic/genomic information on individual murine lines and techniques to construct transgenic and knockout mice. Many mouse models for colorectal cancer have been generated and can be grouped as genetically-engineered, chemically-induced, and inoculated models. As none of these models recapitulate the process of colorectal cancer development in its entirety, it is important to use a specific model to address a particular scientific question. Here, we review commonly used mouse models for human colorectal cancer (Table 1).

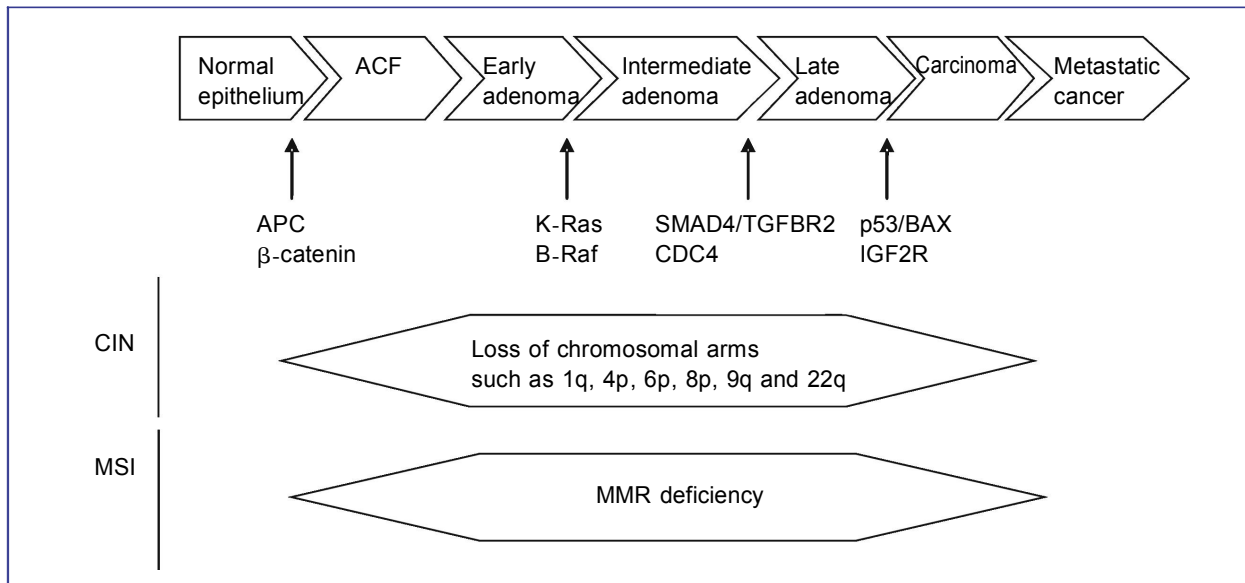
## Mouse Models for Familial Adenomatous Polyposis (FAP)

FAP is a hereditary disease with high penetrance that causes numerous polyps throughout the colon and rectum. Human *APC* gene is commonly deleted in many kindreds with FAP<sup>[13,14]</sup>. *APC* functions as a tumor suppressor to down-regulate the canonical WNT signaling pathway by binding to and promoting the degradation of  $\beta$ -catenin protein<sup>[15]</sup>. Loss of *APC* impairs  $\beta$ -catenin degradation<sup>[15,16]</sup>. The accumulated  $\beta$ -catenin moves to the nucleus, where it activates TCF/LEF transcription factors that transactivate Wnt-targeted genes<sup>[17]</sup>. Activation of the canonical WNT pathway is a key event in colorectal tumorigenesis<sup>[18]</sup>.

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**Figure 1.** The development of colorectal cancer is a process sequentially acquiring a number of genetic changes in normal epithelium, which enables precancerous cells to develop into an adenomatous polyp and progress into an invasive tumor. Inactivation of the adenomatous polyposis coli (*APC*) gene and activation of  $\beta$ -catenin are early events of colorectal tumorigenesis. Activation of the *K-Ras* and *B-Raf* proto-oncogenes promotes tumorigenesis. The next step in progression from adenoma to carcinoma is the loss functions of candidate tumor suppressor genes including *SMAD4/TGFBR2* and *CDC4*. Mutation of *p53/BAX* and *IGF2R* appears to be a late-stage event. Some genetic changes do not affect the cell biology of the tumor but instead result in loss of genomic stability. For example, colorectal cancer can develop chromosomal instability (CIN), which also occurs relatively early in tumor evolution. Loss of DNA mismatch repair (MMR) genes leads to microsatellite instability (MSI) and early-onset colorectal tumors. ACF, aberrant crypt foci.

**Table 1. Mouse models for human colorectal cancer**

Human disease	Mouse model	Advantages and disadvantages
FAP	<i>Apc</i> mutants or $\beta$ -catenin transgenic mice	Mimic <i>APC</i> mutation in human. However, most tumors located in the small intestine. Tumors are not metastatic.
HNPCC	<i>Msh2</i> <sup>-/-</sup> , <i>Msh6</i> <sup>-/-</sup> , and <i>Mlh1</i> <sup>-/-</sup> mice	Mimic MMR deficiency in human. However, MMR-deficient mice develop tumors in other organs. The colonic tumors are not metastatic.
Inflammation-related colorectal cancer	DSS-induced mouse models <i>IL10</i> <sup>-/-</sup> , <i>IL2</i> <sup>-/-</sup> , T-cell receptor <sup>-/-</sup> / <i>p53</i> <sup>-/-</sup> or <i>TGF-1</i> <sup>-/-</sup> / <i>Rag-2</i> <sup>-/-</sup> <i>Muc2</i> <sup>-/-</sup>	Easy and reproducible. Tumor incidence is low. AOM/DSS combination produces more tumors at earlier time point. Tumor incidence is low. Requires the involvement of enteric microflora.
Sporadic colorectal cancer	Carcinogen-induced mouse model Cre adenovirus-mediated <i>Apc</i> inactivation	Easy and reproducible. DMH/AOM/MAM have relatively high colorectal tumor incidence. IQ, PhIP, DMAB, MNNG or MNU target multiple organs and exhibit a low tumor incidence. The tumors are not metastatic. Require surgical procedures. Results are reproducible. Develop metastasis in ~20% of animals.
Metastatic colorectal cancer	Orthotopic inoculation model Intrasplenic inoculation model Intraportal inoculation model Intrahepatic inoculation model	Mimics colon tumor invasion, vascular spread, and metastasis to distal organ. Metastasis rates depend on cell lines and rodent strains. Reproducible and mimics vascular spread of colorectal cancer. Metastasis rates depend on cell lines and rodent strains. Mimics vascular spread of colorectal cancer metastasis and theoretically limits tumor growth predominantly to the liver. Metastasis rates depend on cell lines and rodent strains. Model is reproducible but does not mimic the generally accepted hypothesis of hematogenous spread of colorectal cancer.

FAP, familial adenomatous polyposis; HNPCC, hereditary nonpolyposis colorectal cancer; MMR, mismatch repair; DSS, dextran sulfate sodium; MAM, methylazoxymethanol; DMH, 1,2-dimethylhydrazine; AOM, azoxymethane; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine; IQ, 2-amino-33-methylimidazo[4,5-f]quinoline; DMAB, 3,2'-dimethyl-4-aminobiphenyl; MNU, methylnitrosourea; MNNG, N-methyl-N'-nitro-N-nitrosoguanidine.

The first murine *Apc* gene mutation, named *Apc<sup>Mn</sup>* (multiple intestinal neoplasia), was identified in a colony of mice following random mutagenesis<sup>[19]</sup>. This mutation is a truncating mutation at codon 850 of the *Apc* gene<sup>[20]</sup>. Homozygote *Apc<sup>Mn</sup>* mice are embryonic lethal, whereas heterozygote *Apc<sup>Mn</sup>* mice on a C57BL/6 background typically develop ~30 polyps, the majority of which occur in the small intestine<sup>[19,21]</sup>. Using homologous recombination in embryonic stem cells, several additional *Apc* mutants have been constructed: *Apc<sup>Δ716</sup>*, which contains a truncating mutation at codon 716<sup>[22]</sup>; *Apc<sup>1638N</sup>*, which contains a neomycin insertion in exon 15 that produces a truncating mutation at codon 1638; *Apc<sup>1638T</sup>*, which contains a hygromycin insertion in exon 15 resulting in a truncating mutation at codon 1638<sup>[23]</sup>; *Apc<sup>Δ14</sup>*, which contains a frameshift at codon 580<sup>[24]</sup>; *Apc<sup>1309</sup>*, which contains a frameshift at codon 1309<sup>[25]</sup>; *Apc<sup>Δ580</sup>*, which contains a frameshift at codon 580 and a truncation at codon 605<sup>[26]</sup>; and *Apc<sup>Δ474</sup>*, which contains a frameshit at codon 474<sup>[27]</sup>. The polyp adenomas of these *Apc*-mutant mice are histologically indistinguishable from each other, but the number of polyps developed is very different, even in the same C57BL/6J background. The *Apc<sup>Δ716</sup>* mouse develops ~300 polyps, *Apc<sup>1638N</sup>* mouse develops ~3, *Apc<sup>Δ14</sup>* mouse develops ~65, *Apc<sup>1309</sup>* mouse develops ~35, *Apc<sup>Δ580</sup>* mouse develops ~120, and *Apc<sup>Δ474</sup>* mouse develops ~30<sup>[16]</sup>. Similar to *Apc<sup>Mn</sup>* mice, these mutant mice primarily develop polyps in the small intestine. Comparing to *Apc<sup>Mn</sup>* mice, despite the significant increase in polyps in the small intestine, there is no increase in polyps in the colon in mice with some *Apc* mutants, such as *Apc<sup>Δ716</sup>*. In addition to early onset of gastrointestinal tumors, *Apc<sup>Mn</sup>* mice show progressive loss of immature and mature thymocytes from ~80 days of age, with complete regression of the thymus by 120 days<sup>[28]</sup>. Also, splenic natural killer cells, immature B cells, and B progenitor cells are depleted in the bone marrow. *Apc<sup>Mn</sup>* mice have perturbations in ammonia metabolism in the liver<sup>[29]</sup>. Circulating interferon-6 (IL-6) increases 10-fold, causing severe cachexia as exemplified by loss of muscle weight and fat tissues<sup>[30]</sup>. Due to these complications, most *Apc*-mutant mice die young (4 to 5 months).

Additional mouse models have been developed to test genes affecting tumorigenesis in mice with *Apc* mutations. *K-Ras* mutations are very common in colorectal cancers. Constitutively active mutant *K-ras* (*K-ras<sup>G12D</sup>*) promotes colorectal tumor development in *Apc*-heterozygous mice. Adenocarcinomas expressing *K-Ras<sup>G12D</sup>* invariably exhibit uniform high-grade dysplasia throughout the gut, but this does not occur in mice expressing wild-type *K-ras*<sup>[31]</sup>. Mice that are homozygous for the *Apc* conditional knockout (CKO) allele and heterozygous for a latent activated allele of *K-Ras* (*K-Ras<sup>tm45j/+</sup>*) develop advanced tumors and eventually

form liver metastasis<sup>[32]</sup>. COX-2 is expressed in early stage of polyp formation<sup>[33]</sup>. Knockout of *COX-2* gene (*Ptgs2*) in *Apc<sup>Δ716</sup>* mice dramatically reduces the number and size of polyps in these compound mutant mice<sup>[33,34]</sup>. Mutation in the *Cdx2* gene in *Apc<sup>Δ716</sup>* mice results in most polyps occurring in the colon<sup>[35]</sup>. *Cdx2*-mutant mice exhibit an increased frequency of loss of heterozygosity (LOH) of the *Apc* gene due to chromosomal instability, which may result from activation of the mTOR pathway and acceleration of the G<sub>1</sub> to S phase transition in the cell cycle. Introduction of a *Bub<sup>R1+/-</sup>* mutation into *Apc<sup>Mn</sup>* mice causes a 10-fold increase in the number of colonic tumors compared to *Apc<sup>Mn</sup>* mice<sup>[36]</sup>. Both *Cdx2*- and *BubR1*-mutant mice exhibit a higher rate of genomic instability<sup>[35,36]</sup>, making them good models for prevention and treatment of colon cancers with chromosomal instability. Introduction of *Smad4* mutation into *Apc<sup>Δ716</sup>* polyposis mice results in locally invasive malignant adenocarcinomas without metastasis<sup>[37]</sup>. The histopathologic manifestations are similar to human right-sided colon cancer, which is associated with mutations in the type II receptor of transforming growth factor (TGF)-β. Consistently, homozygous disruption of the type II receptor of the TGF-β gene (*Tgfb2*) in *Apc<sup>1638N</sup>* mice causes malignant transformation of the intestinal adenomas induced by *Apc* mutation<sup>[38]</sup>. Inactivation of cyclin-dependent kinase inhibitors (CDKI) *p21* and *p27* significantly enhances intestinal tumorigenesis in the *Apc<sup>1638N</sup>* mice<sup>[39,40]</sup>. These *Apc<sup>1638N</sup>* compound mouse models highlight the importance of *p21*, but not *p27*, in colon cancer prevention by non-steroidal anti-inflammatory drugs (NSAID)<sup>[39,40]</sup>. *Apc<sup>Mn</sup>* mice with heterozygous disruption of *Pten* develop invasive carcinomas that are large, making this a good animal model for studying persistent activation of the phosphatidylinositol 3-kinase/Akt pathway in colorectal cancer<sup>[41]</sup>. Recently, mutation of *Prox1* in *Apc<sup>Mn</sup>* mice has been reported to promote progression of colon adenoma to cancer<sup>[42]</sup>, suggesting that this model could be useful to study tumor progression.

## Hereditary Non-polyposis Colorectal Cancer (HNPCC) Models

HNPCC (Lynch syndrome) is one of the most prevalent malignancies in the western world and accounts for about 5% of all colorectal cancers. Patients with HNPCC develop early-onset tumors in the colon and rectum, and a subset of patients also develop tumors in the stomach, small intestine, ovaries, and endometrium<sup>[43]</sup>. Patients with HNPCC carry a mutant allele of the DNA MMR genes, such as *MLH1*, *MSH2*, and *MSH6*<sup>[44-46]</sup>. Upon mutation of the wild-type allele by somatic events, the cells become MMR-deficient, and their genomic DNA

displays increased rates of replication errors at short repeat sequences, which is termed microsatellite instability (MSI)<sup>[47-49]</sup>.

Heterozygous *Msh2*, *Msh6*, and *Mlh1* knockout mice do not develop early-onset tumors, but homozygous *Msh2*, *Msh6*, and *Mlh1* knockout mice are cancer-prone, developing tumors in multiple organs including the gastrointestinal tract. These homozygous knockout mice die prematurely due to aggressive lymphomas, which are very similar to patients with biallelic mismatch repair mutations<sup>[50,51]</sup>. These phenotypes in humans and mice suggest that the basic mechanisms of DNA repair and tumor suppression are conserved.

*Msh2*<sup>-/-</sup> murine cells are unable to repair single-base mismatches and 1- to 4-base insertion/deletion loops (IDLs). The loss of MMR in these mice causes a severe reduction in survival and a strong cancer predisposition phenotype<sup>[52,53]</sup>. Most *Msh2*<sup>-/-</sup> mice die from T-cell lymphomas by 6 to 8 months, and those that survive often develop small intestinal adenomas and invasive adenocarcinomas. Similar to tumors in patients with HNPCC, the tumors in *Msh2*<sup>-/-</sup> mice have high MSI<sup>[53]</sup>. To avoid early death caused by tumorigenesis in other organs, conditional *Msh2* knockout mice, in which intestine-specific gene inactivation is permitted, have been generated using either *Villin-Cre* or *Cdx2-NLS-Cre*. These mice develop tumors that highly mimic tumors developed by patients with Lynch syndrome, which make the mice useful preclinical models<sup>[54]</sup>.

*Msh6*-deficient mice survive longer (up to 18 months) and develop tumors at an older age than *Msh2*-deficient mice<sup>[55]</sup>. *Msh6*-deficient cells exhibit dysfunctional repair of base substitution mutations and single-base IDLs. Because *Msh6*<sup>-/-</sup> mice predominantly have base substitution mutations rather than frame shift mutations, tumors in these mice do not display the MSI phenotype that is a characteristic of HNPCC. In parallel, individuals with germ-line *MSH6* mutations frequently have atypical HNPCC characterized by cancer onset at more than 60 years old and a variable MSI phenotype<sup>[56]</sup>. Moreover, *Msh6*<sup>-/-</sup> mice also develop endometrial cancers, which is consistent with a significant number of patients with *MSH6* mutations<sup>[57]</sup>.

*Mlh1*<sup>-/-</sup> mice exhibit complete MMR deficiency, have a shortened life span (up to 12 months) and a strong cancer predisposition, similar to *Msh2*-deficient mice. The tumor spectrum of *Mlh1*<sup>-/-</sup> mice includes T-cell lymphomas, intestinal adenomas and adenocarcinomas, and skin tumors, which are high MSI<sup>[58-60]</sup>.

A significant number of MSI-positive human colorectal cancers carry somatic mutations in the *APC* gene, indicating that loss of APC function is critical for tumor initiation and/or progression in MMR-deficient tumors. Mice that have homozygous mutations of *Msh2*, *Msh6*, *Mlh1*, or *Pms2* and heterozygous germ-line

mutations of *Apc* develop tumors almost exclusively in the intestinal tract<sup>[61-64]</sup>. Specifically, in *Apc*-mutant mice, functional loss of *Msh2* or *Mlh1* dramatically increases intestinal tumors<sup>[61,62]</sup>, while functional loss of *Msh6* or *Pms2* moderately increases intestinal tumors<sup>[63,64]</sup>. Functional loss of *Msh3*, on the other hand, does not increase the tumor load<sup>[64]</sup>. The incidence of tumors correlates with the severity of the MMR defects in MMR knockout mice.

## Chemically Induced Colorectal Cancer Models

Carcinogen induced colorectal cancer in mice is rapid and reproducible, and recapitulates the adenoma-to-adenocarcinoma sequence that occurs in humans. The availability of genetically-engineered or specific inbred mice add further value to these models. Commonly used carcinogens include the following: 1) methylazoxymethanol (MAM), 1,2-dimethylhydrazine (DMH), and azoxymethane (AOM); 2) heterocyclic amines (HCAs), such as 2-amino-1-methyl-6-phenylimidazo [4,5-b]pyridine (PhIP) and 2-amino-3,4-dimethylimidazo [4,5-f] quinoline (IQ); 3) aromatic amines, such as 3,2'-dimethyl-4-aminobiphenyl (DMAB); and 4) alkylnitrosamide compounds, such as methylnitrosourea (MNU) and N-methyl-N'-nitro-N-nitrosoguanidine (MNNG).

MAM was first identified in Cycad flour and found to be carcinogenic in both humans and rats<sup>[65,66]</sup>. DMH and AOM (DMH metabolite) are MAM precursors, which require metabolic activation to form DNA-reactive products<sup>[67-69]</sup>. The reactive metabolite, MAM, readily yields a methyl diazonium ion that can alkylate macromolecules in the liver and colon<sup>[67,70,71]</sup>, including guanine (by adding methyl groups at either the O6 or N7 position to form O6-methyl-deoxyguanosine or N7-methyl-deoxyguanosine). Repetitive treatment with DMH produces colon tumors in rodents that exhibit many of the pathologic features associated with the human disease<sup>[72-74]</sup>; thus, it represents a reliable, reproducible experimental system for studying sporadic (non-familial) colorectal cancer. AOM is advantageous over DMH because of its stronger potency and greater stability during administration<sup>[75]</sup>. As in human populations, inbred murine strains differ in their sensitivity to colon carcinogens. SWR/J and A/J mice are sensitive to AOM and develop up to 20 tumors in the distal colon, whereas AKR/J mice are resistant and rarely develop tumors<sup>[76,77]</sup>. Also, FVB/N and Balb/c murine strains that are commonly used for constructing gene knockout and transgenic animals, develop approximately four tumors and one tumor per mouse, respectively, upon treatment with AOM<sup>[76,77]</sup>. The 129SvJ, C57Bl/6J, AKR/J, and DBA/2J mice are relatively resistant to AOM and DMH<sup>[77]</sup>. In contrast, DMH induces colon cancer with moderate

success in Balb/cHeA and SWR/J mice and with high success in A/J, P/J, STS/A, and ICR/Ha mice<sup>[78-82]</sup>. Interestingly, the morphology of AOM-induced dysplasia appears nearly identical between these mouse strains even though they exhibit a difference in their sensitivity to colon cancer development<sup>[83]</sup>. The reason for this strain-specific difference in carcinogen sensitivity is not clear, but data suggest involvement of subsequent carcinogenic steps such as tumor promotion rather than the result of differences in carcinogen metabolism<sup>[84]</sup>.

HCAs, including IQ and PhIP, are mutagens usually formed upon broiling fish and meat<sup>[85-87]</sup>. The precursors of IQ-type HCAs are creatinine, amino acids, and sugars in fish and meat<sup>[87]</sup>. IQ requires metabolic activation by liver microsomes for conversion to its ultimate carcinogen, and subsequently form high levels of DNA adducts in a number of organs<sup>[88-90]</sup>. PhIP (4 weekly intraperitoneal injection at 50 mg/kg body weight) increases the number of small tumors and cystic crypts in the small/large intestines in *Apc*<sup>Mn</sup> mice, but only induces a few aberrant crypt foci (ACF) in the large intestines in wild-type mice<sup>[91]</sup>. Both IQ and PhIP induce intestinal tumors in *Apc*<sup>Mn</sup> mice associated with inactivation of the wild-type *Apc* allele, either by causing LOH or truncation mutations<sup>[92]</sup>. Rats who receive either IQ or PhIP have a high frequency of colon, mammary gland, and prostate cancers<sup>[93-96]</sup>. However, the incidence of colon tumor is low, ranging from 5% to 28% when these agents are administered in the diet for up to 52 weeks<sup>[97]</sup>. Short-term intermittent feeding of 400 mg/kg PhIP in combination with a high-fat diet results in accelerated tumor formation<sup>[98]</sup>. Thus, the HCA-induced colon cancer model is useful to investigate the chemopreventive activity of potential agents against colon carcinogenesis.

DMAB, an aromatic amine, was first reported to be a colonic carcinogen in rats in 1952<sup>[98]</sup>. Interestingly, injections of DMAB to male F344 rats (weekly subcutaneous injection at 50 mg/kg body weight) induced multiple colon tumors in 27% and 75% of rats fed either a low-fat or high-fat diet, respectively<sup>[99,100]</sup>. DMAB induced both adenomas and adenocarcinomas, with a multiplicity of 1.2 to 2.7 tumors per tumor-bearing rat. DMAB is less potent in rodent models than DMH or AOM. It is less organ-specific and induces neoplasms in mammary and salivary glands, ear ducts, skin, forestomach, and urinary bladder<sup>[73,101,102]</sup>.

MNNG and MNU are direct alkylating agents that do not require metabolic activation. Intrarectal instillation of MNU or MNNG in rodents induces colorectal tumors<sup>[103-105]</sup> that are predominantly sessile or polypoid lesions. For example, intrarectal administration of MNNG (1 to 3 mg/week) for 20 weeks induced colon tumors at the injection site in all male F344 rats (57% adenomas, 43% adenocarcinomas)<sup>[104,105]</sup>. Most of the resultant

adenocarcinomas were well differentiated with infiltration into the submucosa, but a minority was poorly differentiated and showed mucinous cancer cells infiltrating into the submucosa. As no biochemical activation is required for MNU or MNNG action, these carcinogens are ideal for studying the modifying effects of xenobiotics in colorectal tumorigenesis without involving carcinogen metabolism<sup>[106]</sup>.

Taken together, all of these carcinogens cause mutations and deletions in a spectrum of genes that are known to be involved in human colon cancer tumorigenesis. *K-Ras* activating mutations (G to A) were found in 66% of DMH-induced colon carcinomas<sup>[107]</sup> and 33% of MNU-generated carcinomas<sup>[108]</sup>. *p53* mutations are frequent in MNU-induced rat colon tumors. In contrast, *K-Ras* but not *Apc* and *p53* mutations are frequently observed in AOM-induced lesions<sup>[109-111]</sup>. *Apc* mutations are frequent<sup>[112,113]</sup> but *K-Ras* mutations are rare in PhIP-induced colon tumors<sup>[114]</sup>. In carcinogen-induced rodent colon tumors not having *Apc* mutations, *β-catenin* mutations can occur<sup>[115-117]</sup>, indicating the importance of the Wnt/*Apc*/*β-catenin* signaling pathway in chemically-induced rodent colon carcinogenesis.

## Inflammation-Related Colorectal Cancer Models

Inflammation plays important roles in the development of colorectal cancer; the risk increases with the extent and duration of inflammation. Colorectal cancer is one of the most serious complications associated with long-standing inflammatory bowel disease (IBD). Several mouse models for inflammation-related colorectal cancer have been developed, including dextran sodium sulphate (DSS)-induced colitis models and genetically engineered models.

### DSS-induced colitis model

The most commonly used colitis mouse model uses DSS<sup>[118]</sup>. Colorectal cancer development following DSS-induced inflammation suggests that chronic inflammation in IBD plays a critical role in epithelial malignant neoplasia of the colon and rectum<sup>[119]</sup>. This model is DSS dose-dependent and typically requires a relatively long exposure period and low repeated cycles of DSS administration<sup>[120]</sup>. The incidence and/or multiplicity of DSS-induced colorectal tumors are relatively low<sup>[121]</sup>. To promote tumorigenesis, mice are often treated with AOM and DSS and then develop tumors after a relatively short-term exposure<sup>[122]</sup>. Different strains of mice exhibit distinct susceptibilities to AOM/DSS-induced colon carcinogenesis<sup>[120]</sup>. For example, nearly 100% Balb/c mice developed colonic

adenocarcinoma, with a multiplicity of  $7.7 \pm 4.3$ ; in contrast, only 50% of C57BL/6N mice formed tumors, with a multiplicity of  $1.0 \pm 1.2$ ; and even less robustly, both C3H/HeN and DBA/2N mice developed only a few colonic adenomas<sup>[120]</sup>. In addition, DSS-treated *Apc*<sup>Mn</sup> mice also develop more colon tumor than vehicle control-treated ones<sup>[123]</sup>.

In the AOM/DSS model, dysplastic lesions are initially formed in the colonic mucosa. Both dysplasia and neoplasia stain positive for  $\beta$ -catenin, COX-2, and inducible nitric oxide synthase but not *p53*. Mutations of the  $\beta$ -catenin gene occur in 80% to 100% of AOM-induced or DMH/DSS-induced colonic adenocarcinomas<sup>[123,124]</sup>. Mice developing colonic adenocarcinomas often have mutations of the  $\beta$ -catenin gene at codons 32 to 34 if the mice receive AOM/DSS<sup>[123]</sup> or at codons 33, 37, and 41 if the mice receive AOM<sup>[117]</sup>. Collectively, these results suggest that mutations at codons 33 and 34 might be caused by AOM exposure, whereas mutations at codon 32 may result from DSS exposure<sup>[117,125]</sup>. Gene expression of Wnt inhibitory factor 1 (*Wif1*), plasminogen activator (*Plat*), myelocytomatosis oncogene (*Myc*), and phospholipase A2 group IIA (platelets and synovial fluid) (*Plscr2*) are up-regulated, and the inflammation-related gene, peroxisome proliferator-activated receptor- $\gamma$  (*Ppar $\gamma$* ), PPAR-binding protein (*Pparbp*), and *Tgfb3* are down-regulated at 5 to 10 weeks of AOM/DSS exposure<sup>[126]</sup>. Similar to the AOM/DSS model, mice receiving the colon carcinogens (PhIP and DMH) followed by DSS treatment have a higher incidence of tumors<sup>[124,127]</sup>.

### Genetically engineered IBD mouse models

The immune system plays an important role in the etiology of colonic inflammation. Disruption of the immune response to antigenic stimuli is strongly associated with IBD and IBD-related colorectal cancer. IL-2- and IL-10-defective mice develop IBD similar to humans. The level of inflammation in IL-10-defective mice correlates with the incidence of colorectal adenocarcinomas<sup>[128]</sup>. The combination of IL-2 deficiency with 2-microglobulin deficiency in mice causes colonic inflammation, and some of these mice develop adenocarcinoma of the proximal colon<sup>[129,130]</sup>. Notably, IL-2- and IL-10-defective mice, as well as either T-cell receptor/*p53*- or TGF-1/*Rag-2*-deficient mice, do not develop chronic inflammation or intestinal tumors when maintained under germ-free conditions, suggesting that the enteric microflora plays an important role in the development of IBD and IBD-associated cancers in these mice<sup>[131-134]</sup>.

Activation of nuclear factor- $\kappa$ B (NF- $\kappa$ B) and correlated pro-inflammatory cytokines and adhesion molecules are required for inflammation-related intestinal

neoplasia<sup>[135,136]</sup>. Studies with *Nod2* (*Card15*) knockout mice indicate *Nod2* is a positive regulator of NF- $\kappa$ B and IL-1 $\beta$  secretion and increases susceptibility to inflammation induced by bacteria in the intestine<sup>[137,138]</sup>. Blocking NF- $\kappa$ B signaling reduces colonic inflammation and carcinogenesis in the AOM/DSS model<sup>[135,137-140]</sup>. Chronic colitis appears to require activation of the Toll-like receptor-4 (TLR4). Genetically, Tlr4-deficient mice have decreased levels of Cox-2 and Pge2 expression, as well as activation of the epidermal growth factor receptor signaling pathway. These mice are also more resistant than wild-type mice to the development of colitis<sup>[139]</sup>. Inhibition of TLR4 or tumor necrosis factor- $\alpha$  signaling could be useful in treating IBD-associated dysplastic colonic tissue.

Another genetically engineered colorectal mouse model is the *Muc2*<sup>-</sup> mouse. *Muc2* encodes the major gastrointestinal mucin, which forms an insoluble mucous barrier that protects the intestinal lumen<sup>[141-143]</sup>. Targeted inactivation of *Muc2* causes tumor formation throughout the intestinal tract, including the colon and rectum<sup>[141]</sup>. *Muc2*<sup>-</sup> mice have a chronic intestinal inflammation associated with up-regulation of Cox-2 and cytokines, increased cell migration and proliferation, and decreased apoptosis. However, unlike other mouse colon cancer models, such as *Apc*<sup>Mn</sup> and *Apc*<sup>1638+</sup> mice<sup>[141,144]</sup>, the  $\beta$ -catenin pathway is not involved in tumorigenesis<sup>[141,145,146]</sup>. Inactivation of *p21* in *Muc2*<sup>-</sup> mice significantly increases tumorigenesis, possibly through down-regulating *p27* and up-regulating *c-Myc*<sup>[40]</sup>. *Muc2*<sup>-</sup> mice possessing an *Apc* mutation have significantly increased colorectal carcinogenesis, perhaps through an interaction between  $\beta$ -catenin and chronic inflammation<sup>[146]</sup>.

### Metastatic Colorectal Cancer Models

Almost a third of patients with colorectal cancer have metastatic disease at the time of diagnosis. Moreover, half of the patients who are diagnosed with and undergo resection for early-stage disease subsequently develop metastasis. Metastatic colorectal cancer is difficult to treat and, ultimately, nearly all patients die of their disease<sup>[147]</sup>. Mice that are genetically-engineered or carcinogen-induced to develop colon cancer rarely develop metastasis. In contrast, models using transplant colorectal cancers can exhibit high rates of metastasis and recapitulate some of the desired characteristics.

Orthotopic implantation is defined as the inoculation of tumor cells/tissue in the intestine, for example, cecum or rectum<sup>[148]</sup>. This method mimics colon tumor invasion, vascular spread, and metastasis to distal organs. The metastasis rates depend on cell type, implantation site, and rodent strain. For example, colon cancer MCA-38

and RCN-9 cells produced liver metastasis in 40% to 65% of mice 8 weeks after intramural injection into the cecal wall of C57BL/6J mice<sup>[149,150]</sup>. Liver metastases have also occurred by inoculating human colon cancer tissues orthotopically in nude mice<sup>[151,152]</sup>. The histological features of the transplanted tumors may help determine the success of liver metastasis formation<sup>[152]</sup>. However, neither mouse colon carcinoma CT-26 cells (in Balb/c), MCA-38 cells (in C57BL/6J), nor DHD/K12-PROb cells (in BD-IX rats) developed liver metastasis when injected into the wall of the rectum or cecum<sup>[153,154]</sup>. Orthotopic implantation of colon cancer cells into mice has also resulted in metastasis to lymph nodes and other sites. Three human colon cancer cell lines (HCT-116, SW-620, and DLD-1) orthotopically injected into the cecal wall of nude mice showed varying degrees of mesenteric and retroperitoneal lymphatic invasion, hematogenous dissemination to the liver and lungs, as well as peritoneal carcinomatosis (29% to 100%)<sup>[155]</sup>. Another series of experiments placing human colon cancer in the cecal wall of immunodeficient mice showed that lymph node metastases occurred most frequently using HCC2998 and HT29 cells. SW620 cells gave rise to multiple small (2 to 3 mm) metastatic hepatic nodules, and CaCo2, WiDr, and Co205 cells had very low rates of metastasis<sup>[156]</sup>.

Intrasplenic injection of tumor cells mimics the vascular spread of colorectal cancer. This procedure is relatively easy and consistently induces liver metastases. Mortality caused by either splenic injection or local tumor growth can be controlled by splenectomy following injection<sup>[157]</sup>. Paradoxically, in one series of experiments, liver metastasis occurred more frequently when moderate to well differentiated cells (CX-1, HT-29, CCL188, and CCL235) but not poorly differentiated cells (MIP-101, Clone A, CCL222, and CCL231) were intrasplenically injected<sup>[158]</sup>. In another series of intrasplenic injection-induced liver metastasis experiments, many of the human colon cancer cells (metastatic capability: COLO320DM and HCT116 > HT-29, WiDr and LoVo > LS174T) spread to the liver, with a frequency ranging from 50% to 100% of mice. CaCo2, COLO201, LS123, SW48, and SW1417 cells showed no metastasis after  $1 \times 10^4$  cells were injected<sup>[159]</sup>. Rodent colorectal cancer cells also exhibit different metastatic capabilities. Mouse colon cancer MCA-38 cells and mucinous colon adenocarcinoma WB-2054-M4 cells efficiently developed liver metastases in C57BL/6J mice and Wistar/Furth  $\times$  Brown-Norwegian hybrid rats, respectively, when injected intrasplenically<sup>[160,161]</sup>. However, K12-TR cells failed to develop liver metastases after injection into the spleens of BD-IX rats, as well as nude rats and mice<sup>[162]</sup>.

Intraportal injection of tumor cells mimics the vascular spread of colorectal cancer metastasis and

theoretically limits tumor growth to the liver. Current data show that this procedure reproducibly results in liver metastases in almost all animals. Partial hepatic ischemia before injection of tumor cells further increases the number of hepatic metastases, likely due to up-regulation of expression of adhesion molecules induced during hepatic ischemia<sup>[163,164]</sup>. WB-2054-M cells, originally from a lung metastasis, yielded liver metastases in 50% of Wistar/Furth  $\times$  Brown-Norwegian hybrid rats<sup>[165]</sup>. Colon cancer CC531, LDLX40, DHDK12/TR, and LMCR cells developed liver metastasis in all Wistar, WAG/Rij, and BDIX rats when injected into the portal vein<sup>[166-172]</sup>.

Intrahepatic (subcapsular or intraparenchymal) implantation of tumor cells is a widely used method to create liver metastases. The model is reproducible and has acceptable complication rates. However, as this method does not mimic the generally accepted hypothesis of hematogenous spread of colorectal cancer, these tumors might not reflect the human situation, and, therefore, may behave aberrantly to therapeutic interventions. Despite this disadvantage, such induction of tumors has been used by many investigators to study local therapy of metastases. For example, CC531 as well as N-methyl-N-nitrosoguanidine (NNG) induced colon adenocarcinoma cells develop tumors in all animals in several weeks when injected into the liver of WAG/Rij or Wistar rats respectively<sup>[173-178]</sup>, and DHDK12/TR cells develop tumors in BD-IX rats in all animals 6 weeks after injection<sup>[179]</sup>. Intrahepatic implantation of tumor fragments of human colon cancer, derived from a liver metastasis of a patient, resulted in 100% liver metastases after only 10 days<sup>[180]</sup>.

## Conclusions and Future Direction

A variety of mouse models of human colorectal cancer have been developed, and each imitates, in part, human colon carcinogenesis. These models allow rapid and repeated interrogation of hypotheses, and each produces a method to test various therapeutic modalities that would not be possible in humans. Genetically-engineered mice models are useful for studying the importance of specific genomic alterations in the development and progression of colorectal cancer and their sensitivity to various therapies. The chemically-induced mouse models mimic human sporadic colorectal cancer and are often used to study dietary influences on carcinogenesis. The inoculated colorectal cancer models recapitulate some features of colorectal cancer metastasis and are useful models for anti-metastatic drug evaluation. Even though each mouse model recapitulates an aspect of human colorectal cancer, the power of these models to predict



clinical efficacy of treatments may be limited. Genetic and chemical mouse models may not reproduce the complexity of the human disease, and injection of human and murine colon cancer cell lines might be hampered because the long-term cultured cell line might have accumulated features that no longer reflect the characteristics of freshly isolated tumor cells. Also, differences in mouse size and physiology, as well as variations in colon cancer that develops in mice and humans may also lead to translational limitations. Nevertheless, each system has the exciting and robust capacity to model human colon cancer, facilitating a

better and more rapid understanding of its etiology and providing new opportunities for developing and rapidly testing novel therapies.

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