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Animal Models of Colorectal Cancer

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Abstract

Colorectal cancer is a heterogeneous disease that afflicts a large number of people in the United States. The use of animal models has the potential to increase our understanding of carcinogenesis, tumor biology, and the impact of specific molecular events on colon biology. In addition, animal models with features of specific human colorectal cancers can be used to test strategies for cancer prevention and treatment. In this review we provide an overview of the mechanisms driving human cancer, we discuss the approaches one can take to model colon cancer in animals, and we describe a number of specific animal models that have been developed for the study of colon cancer. We believe that there are many valuable animal models to study various aspects of human colorectal cancer. However, opportunities for improving upon these models exist.

Keywords

sporadic; heritable; chemically-induced; genetically modified mice

I. Introduction

Human colorectal cancer is one of the most common malignancies in humans, particularly in the Western hemisphere (1,2). As such, there is an urgent need for research to improve our ability to diagnose, prevent, and treat this disease.

While human biopsy specimens, tumor xenotransplants, and *in vitro* cell culture models have been invaluable tools for developing and testing hypotheses regarding colorectal cancer, these approaches are limited in several critical ways. First, established tumors and colon cancer cell lines have a mutational complexity that limits clear understanding of the impact of individual mutations. Second, cancer cell lines can grow in the absence of normal microenvironments (e.g. stromal cell signals and matrix interactions) that are critical to normal colon tissue homeostasis. Third, human tumors develop over long periods and are generally identified after the third decade of life. Thus, biological events regulating cancer initiation and promoting tumor growth are difficult, if not impossible to study due to uncontrolled genetic and environmental diversity. Finally, ethical concerns limit the types of interventions (or non-interventions) that can be applied to patient populations.

For these reasons, controlled *in vivo* studies in animal models have been viewed as critical tools necessary to study the molecular mechanisms of colorectal carcinogenesis, to test potential preventative and therapeutic strategies, and to translate hypotheses derived from

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cell models into the complex physiology of the colon. This review will provide readers with a comprehensive review of the strategies one can use to model different aspects of human colorectal cancer and it will describe examples of models currently being used by the scientific community.

I. A. An Overview of our Current Understanding of Mechanisms Causing Human Colorectal Cancer

To effectively use animals to gain insight into the etiology of human colorectal cancer, or to test the potential of therapeutics, one must have a firm understanding of the human disease. In this section we will provide a brief overview of the molecular etiology and pathologic features of colorectal cancer in humans. This section will identify key concepts and molecular targets relevant to the challenge of modeling human colorectal cancer in animal models. For a comprehensive review of the molecular genetics of colorectal cancer, readers should consult the recent review by Fearon (3).

The vast majority of malignant colorectal cancers arise out of benign adenomatous polyps over a course of several decades, so that the peak incidence of colorectal carcinoma occurs between the ages of 60–80 years. A small percentage of human colorectal cancers are associated with defined familial syndromes, for which the genetic etiology has been determined (4). These diseases have revealed several mechanisms controlling the initiation and progression of sporadic colorectal cancer of the ascending colon and descending colon (Table 1).

The “Vogelstein model” (5) was a conceptual breakthrough in our understanding of the molecular etiology of colorectal cancer. It described the sequential accumulation of mutations in four genes that correlated with histological features that develop during colorectal cancer progression, i.e. inactivating mutations in the *APC* gene, *KRAS* gene activating mutations, inactivating mutations in chromosome 18 which later was identified as including the *DCC* gene (“deleted in colon cancer”) and SMAD family members, and inactivating mutations of the *TP53* gene (5). Others have since conducted a detailed analysis of the genome sequence in many human colorectal tumors and have described a “landscape” of mutations that characterize the disease (6,7). This analysis shows that the average human colorectal cancer contains approximately 76 somatic mutations and 1–2 chromosomal amplifications or deletions. An important functional distinction must be made among these mutations – some mutations are “drivers” that directly regulate carcinogenesis while others are “passenger” mutations that occur as a consequence of the driver mutations and which may have no functional significance in carcinogenesis. Of the somatic mutations observed in a typical tumor, 5–12 are “driver” mutations that modulate approximately 12–20 core pathways (7). The implication of the driver/passenger mutation model is that preventative and therapeutic approaches should be targeted to “driver” mutations or pathways influenced by “driver” mutations. Mutations commonly found in colorectal cancer are listed in Table 2 and described further below.

There is additional significance to the concept that mutations within core pathways drive colorectal carcinogenesis. The morphology of colorectal cancer is directly influenced by the molecular pathways that are disrupted. This relationship between molecular etiology and morphology may account for the differences in cancers that develop in the ascending (proximal or right) colon compared to the descending (distal or left) colon. Others have described the molecular origins and clinical behavior of cancer in the ascending and descending colon (4). The following section summarizes these differences.

I. A.1. Descending Colon/Rectal Cancers—Approximately 55% of colorectal cancers occur in the descending colon and rectum. These tumors are typically initiated through the

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mechanism of chromosomal instability (CIN) (4). The classic example for this mechanism is the rare human colorectal cancer susceptibility syndrome, Familial Adenomatous Polyposis (FAP). FAP accounts for less than 1% of colorectal cancers and is caused by germline mutations of the tumor suppressor gene *APC*. Consistent with Knudson's two hit hypothesis for tumor suppressor genes (8), loss of function of the remaining wild-type *APC* allele is required for tumor initiation. Being born with the "first hit", FAP patients have an accelerated development of colorectal cancer and develop hundreds of intestinal polyps at a relatively young age. Based on the role of *APC* mutations in FAP, researchers subsequently determined that spontaneous mutations in the *APC* gene also play a critical role in initiation of sporadic cancer of the descending colon and rectum (4,9).

APC is a 2843 amino acid protein that is part of the canonical WNT signaling pathway where it helps form a destruction complex that regulates the cytoplasmic level of β-catenin (10,11). Upon normal activation of WNT signaling by ligand, β-catenin is released from the destruction complex (containing glycogen synthase kinase 3β, Axin, and Casein Kinase) and accumulates in the cytoplasm and nucleus. In the nucleus, β-catenin is a co-activator for the TCF/LEF family of transcription factors. β-catenin target genes are numerous and diverse, but the protein products of these genes predominantly stimulate proliferation and delay differentiation. APC can also be localized in the nucleus where it inhibits β catenin binding to the TCF/LEF transcriptional complex (12) and mediates β catenin export to cytoplasm (13). When WNT signaling is activated out of this context, e.g. due to mutations that disrupt APC function, it promotes neoplastic transformation (14). In addition, APC is involved in several biologic functions within the cell that are independent of the canonical WNT pathway. The importance of these other roles of APC in colorectal cancer is uncertain, but has been reviewed elsewhere (15,16).

Over 90% of germline *APC* mutations in FAP patients (17) and somatic *APC* mutations in colorectal tumors (18) occur in a cluster between codons 1286-1513 that result in truncation of the APC protein. The ability of APC to regulate β-catenin stability is dependent upon three functional motifs in the central portion of the APC protein and mutation-induced truncations eliminate this regulation. Two of the functional motifs confer the ability of APC to bind β-catenin: three 15-amino-acid repeats between amino acids 1014-1210 (19) and seven 20-amino-acid repeats that occur between amino acids 1034-2130 (10). The third functional motif in APC is a series of three SAMP (serine-alanine-methionine-proline) repeats between amino acids 1034-2130 that control binding of APC to the destruction complex through Axin (20). Loss of APC's ability to bind either β-catenin or Axin prevents the normal regulation of β-catenin degradation and increases transcription of genes whose protein products drive cell proliferation.

Neoplastic transformation resulting from *APC* gene mutations requires inactivation or loss of both *APC* alleles. Even in FAP patients, the occurrence of the "second hit" in this process is random (8) - but the *behavior* of "second hit" *APC* mutations is non-random (21). There appears to be an optimal, or "just right", level of WNT signaling that is imparted by the truncated APC fragment resulting from somatic mutation or loss of heterozygosity (22). This selective advantage is imparted by the retention of one or two of the 20 amino acid repeats that bind β-catenin (22,23). The "just right" hypothesis is not an absolute requirement for neoplastic transformation in the colon, as many colorectal tumors do not follow this pattern of *APC* mutation (24). However, the pattern of *APC* mutation may affect the pathogenesis of colorectal cancer. This hypothesis is consistent with the phenotypic variability among mouse models that harbor truncating *Apc* mutations of different lengths (see below). In the context of selecting an appropriate experimental model, it is important that the researcher consider the potential influence of the length of the mutated Apc protein on the biologic effect of interest.

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Progression of pedunculated adenomatous polyps that typically result from *APC* gene mutations, to invasive and metastatic colorectal adenocarcinomas, is driven by accumulation of mutations in at least three additional pathways: activating mutations of *KRAS*, deletions that disrupt *SMAD* genes and TGFβ-induced differentiation, and inactivation of *TP53* (5). *KRAS* is a member of the Ras family of small-G proteins that facilitate downstream signaling from growth factor receptors to kinase cascades, including the mitogen activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K) pathways. KRAS mutations are rare in the early stages of colorectal cancer, but greater than 40% of colorectal cancers have acquired *KRAS* mutations by the time they grow beyond 1 cm in diameter (25). In addition, approximately 15–20% of colorectal cancers have somatic mutations in the *PI3KCA* gene that encodes class I PI3Ks (6). These mutations activate PI3K activity and elevate levels of its second messenger, phosphatidylinositol-3,4,5-triphosphate (PIP_3), an activator of Akt kinase (26). Proteins phosphorylated by activated Akt stimulate progression through cell cycle, cell growth, and cell survival. The PIP_3/Akt pathway is held in check by the phosphatase PTEN, which lowers PIP_3 levels by converting it to PIP_2. In this role, PTEN is a tumor suppressor whose function is lost in 15–20% of colorectal cancers (27).

Disruption of TGFβ signaling is estimated to occur in at least 40% of colorectal cancers as they progress (28). TGFβ signals through cell surface receptors leading to phosphorylation of SMAD2 and SMAD3. When phosphorylated, the SMAD2/3 complex enters the nucleus where they join SMAD4 to regulate transcription of genes that promote cellular differentiation or apoptosis to exert a suppressive effect on tumor progression (28). Deletions within chromosome 18q, where the *SMAD2* and *SMAD4* genes reside, are observed in approximately 70% of colorectal carcinomas (25). Additionally, mutations of the TGFβ type II receptor occur in 30% of colorectal carcinomas (29) and mutations in the *SMAD3* gene on chromosome 15 are found in another 5% of colorectal cancers (7). The *DCC* gene was originally identified as the putative tumor suppressor gene responsible for the effects of deleting chromosome 18q (30). DCC was once viewed as a marker for the loss of TGFβ function but it has since been characterized as an inducer of apoptosis whose function is reduced by binding to its ligand netrin-1 (31). Experimental evidence from knockout mice suggests that DCC may play a role in limiting tumor progression (32).

Mutations in the *TP53* gene encoding the multifunctional p53 protein are found in more than 75% of colorectal carcinomas (25). The complex role of p53 in tumorigenesis has been reviewed extensively (33). The p53 protein is a central transcriptional regulator responsible for protecting genomic integrity, halting the cell cycle, and facilitating DNA repair. In the presence of catastrophic DNA damage, p53 initiates apoptosis. In addition, numerous other cellular functions have been attributed to p53 target genes, including responses to reactive oxygen species, angiogenesis, and autophagy.

I. A.2. Ascending Colon Cancers—For the 20% of colon cancers that originate in the ascending colon, cancer initiation is usually associated with microsatellite instability (MSI). Thus, cancers of the ascending colon are distinct from those that develop through the classic Vogelstein "adenoma to carcinoma" model (4). MSI results from genetic or epigenetic inactivation of DNA mismatch repair (MMR) mechanisms. The loss of MMR allows for the rapid accumulation of changes in the number of short tandem repeat sequences (microsatellites) within the genome. Failure of MMR also increases the potential for transformative mutations to occur in "driver" genes of colorectal cancer.

Germline mutations in one of two major mismatch repair genes, *MSH2* and *MLH1*, are important for tumor initiation in approximately 90% of cases of hereditary Lynch Syndrome, also known as Hereditary Non-Polyposis Colorectal Cancer (HNPCC). This disease accounts for 5–8% of all colon cancers (34). The remaining 10% of HNPCC cases involve

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alterations of other MMR genes, such as *MSH6*, *PMS2*, and *MLH3* (34). In addition to colon cancer, Lynch Syndrome is accompanied by early-onset cancers in several other epithelial tissues, including the stomach, ovary, and endometrium. A high percentage of HNPCC colon cancers also exhibit increased WNT signaling (35). However, in these tumors *APC* mutations are rare while β-catenin gene (*CTNNB1*) mutations are common (36).

Sporadic cancers of the ascending colon are also strongly associated with a failure of MMR (37). In contrast to Lynch Syndrome, sporadic colon cancer MSI commonly results from hypermethylation of MMR gene promoters, particularly *MLH1* (38). Another distinguishing feature underlying the molecular pathogenesis of sporadic, ascending colon cancers is a high frequency of mutations in the *BRAF* gene, whose gene product regulates MAPK signaling (39). Sporadic MSI colon cancers frequently exhibit activated WNT signaling resulting from hyper-methylation of the *APC* promoter or mutation of other components of the canonical WNT pathway, rather than from truncating mutations of the *APC* gene (40). Finally, MSI cancers deviate from the Vogelstein model in that *KRAS* and *TP53* mutations are uncommon (41). However, inactivation of the type II TGFβ receptor (42) and the pro-apoptotic gene *BAX* (43) are frequently found in MSI cancers.

In addition to these important differences in molecular pathogenesis, there are important morphologic differences between tumors exhibiting MSI in the ascending colon and those exhibiting CIN in the descending colon. Descending colorectal adenomas typically present as pedunculated tumors composed of neoplastic epithelial cells forming well-differentiated tubular glands. MSI-associated tumors, by contrast, frequently exhibit an exophytic or sessile gross phenotype. Microscopically, MSI-associated tumors are characterized by excessive mucin production, poor epithelial differentiation, and lymphocytic infiltration (37). A unique morphologic variant of hyperplastic polyp, exhibiting a serrated glandular morphology, is believed to represent a precursor lesion to MSI cancers. Lesions of this phenotype harbor *BRAF* mutations, and down-regulation of $p21^{(WAF1/CIP1)}$ that may lead to a hypermethylator phenotype that silences MMR genes (44,45).

II. Animal Models of Human Colorectal Cancer

While it is seems obvious, the goal of modeling human colorectal cancer in animals is to recapitulate the molecular etiology, pathology, and clinical progression of the disease. As a result, the known diversity of human colorectal cancer makes it impossible for a single animal model to adequately represent all forms of the disease. None-the-less, we believe that three characteristics are important to maintain the translational potential of the studies conducted in the animal models. First, the cancer that develops in the animal model should be limited to the large intestine so that researchers can study the development of the disease without the confounding effects of disease in other tissues. Second, the histologic and molecular features of colorectal lesions should be similar to those observed in human tissue. Finally, the models should capture the complex cellular interactions that are relevant to human colon cancer. For example, while xenografts of human tumor into nude mice are often cited as highly relevant to the study of human cancer, these mice are immune-compromised and this eliminates the impact of this critical system on the tumors.

Potential animal models for colorectal cancer fit into three broad categories: spontaneous intestinal cancers in various animal species, chemically or environmentally induced cancers in rodents, and cancers induced by genetic manipulation of mice.

II. A. Spontaneous Colorectal Cancer in Animals

II. A.1. Non-Rodent Species—The dog has become an attractive model for comparative oncology research, as is highlighted by the National Cancer Institute (NCI) Comparative

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Oncology Program (https://ccrod.cancer.gov/confluence/display/CCRCOPWeb/Home). There are many similarities between canine colorectal cancer and the human disease. Canine intestinal cancer occurs more commonly in the large intestine than in the small intestine (46). Like humans, pedunculated adenomas are more prevalent in the distal colon/rectum, whereas tumors in the middle or proximal colon are more likely to exhibit a sessile, annular phenotype that cause luminal stricture (46). Immunohistochemical evaluation of canine colorectal adenomas revealed cytoplasmic and nuclear accumulation of β-catenin, suggesting that dysregulation of the WNT signaling pathway is also an important driver of colorectal carcinogenesis in the dog (47). Canine colorectal adenomas demonstrate a tendency to progress to malignancy (48), but unlike human colorectal tumors this malignant behavior is not accompanied by acquisition of *Tp53* mutations (49,47). Despite all of the similarities between colon tumors in dogs and humans, the utility of dogs for colorectal cancer research is severely limited by the low prevalence of the disease in the pet dog population (less than 1%) (50,46).

The incidence of feline gastrointestinal adenocarcinoma is also less than 1% and over 70% of these tumors occur in the small intestine (51). The low incidence and disparity of tumor location from the human condition make feline gastrointestinal adenocarcinoma a poor model for colorectal cancer research.

The only other domestic veterinary species with a significant incidence of intestinal cancer is sheep. In New Zealand, intestinal adenocarcinomas were found in 1.6% of normal adult sheep (52). Sheep are an attractive model for human colorectal cancer because their adenocarcinomas share many histologic features with the human lesion and mimic many aspects of the metastatic behavior of the human disease (53). However, in contrast to the human disease, 100% of the intestinal adenocarcinomas of sheep are found in the small intestine. An obvious weakness of sheep as a model of human intestinal disease is the unique physiology of the ruminant forestomachs. The potential influence of this anatomic variant on intestinal carcinogenesis is unclear.

Among non-human primates, the cotton-top tamarin (*Saguinus oedipus*) is predisposed to idiopathic ulcerative colitis and a high percentage of animals with colitis develop colorectal adenocarcinomas (54). In some colonies, the incidence of colorectal adenocarcinomas at death can be as high as 39% (55). The adenocarcinomas, arose within the cotton-top tamarin, exhibit mucinous or signet-ring morphology and rarely originate from adenomatous polyps. In addition, they metastasize early and aggressively to regional lymph nodes (54). The average age of death due to colonic adenocarcinoma ranges from 5–7 years (55). Efforts to identify a heritable or familial cause for this syndrome have failed (55) and there are no reports detailing the molecular nature of these adenocarcinomas. The cause of the syndrome is unknown, but there is evidence that environmental stress (56) and luminal bacteria (57) may play a role in its pathogenesis. The similarities to the human disease and spontaneous nature of these adenocarcinomas are features beneficial in modeling therapeutic and preventative interventions. However, the long latency of carcinogenesis, costs, and ethical concerns of using non-human primates for biomedical research are barriers to wide-spread use of this model.

II. A.2. Rodents—Spontaneous gastrointestinal neoplasia is rare in rodents. In a 1969 survey of three early sublines of C57BL mice (designated C57BL green, C57BL blue, and C57BL Icrf), 9.5% of aged mice had neoplastic or hyperplastic lesions in the gastrointestinal tract (58). However, the majority of epithelial glandular adenomas or adenocarcinomas were found in the small intestine and only two epithelial glandular adenomas were found in the colon. A more contemporary study reported that the incidence of intestinal tumors in C57Bl/

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6J mice fed a common semi-purified diet (AIN-76A, (59)) was 1% in the large intestine and 4% in the small intestine (60).

II.B. Exogenous Promoters of Colorectal Cancer

II. B.1. Western Diet-Induced Rodent Neoplasia—Epidemiologic evidence points to diet as a strong modulator of colorectal cancer risk in humans (61). Several rodent studies have been conducted to examine the influence of a diet designed to model a typical "Western diet" on the incidence of colorectal cancer. Several modifications of this diet have been used, but the common features are increased concentrations of fat (20% vs. 5% in the AIN76A diet) with decreased levels of calcium (0.05% vs. 0.5% in the AIN76A diet) and vitamin D (100 IU/kg diet vs. 1000 IU/kg in the AIN76A diet) (62). Feeding the Western diet for as little as 12 weeks induces hyperplasia of colonic crypts in rats and mice (62). This effect can be prevented by increasing dietary calcium levels to 0.7% (63). In addition, when C57Bl/6J mice were fed the Western diet for two years, they developed dysplastic crypts and small (< 2mm) polypoid lesions predominantly within the distal colon. At the end of two years, approximately 70% of the mice fed the Western diet exhibited microscopic evidence of nuclear atypia in colonic epithelia (believed to be a precursor to dysplasia) and 40% of the mice had dysplastic crypts (64). In another study using a Western diet modified to have lower fiber, folate, methionine, and choline content (65), intestinal tumors developed in 25% of C57Bl/6J mice fed the diet for 2 years and the lesions demonstrated evidence of invasive adenocarcinoma (60). However, while feeding diets with elevated calcium (0.7%) and vitamin D (2300 IU/kg diet) content prevented colonic tumors, repletion of methyl-donor nutrients and fiber did not (60). Interestingly, almost all of these tumors developed in the small intestine, cecum, or most proximal extent of the colon (65,60). Feeding the Western diet to mice also induced a transcript profile in normal-appearing colonic mucosa that was similar to the pattern seen upon loss of an *Apc* allele in the *Apc$^{1638N/+}$* mouse (66). In addition, feeding the Western diet increased expression of transcripts for the Paneth cell and WNT signaling in the small intestinal villi and colon (67). These two observations suggest that the Western diet reprograms the intestinal mucosa to be "at risk" for colorectal cancer.

The Western diet model of spontaneous colorectal cancer is attractive because it appears to capture much of the complexity that underlies spontaneous colorectal carcinogenesis in humans. However, there are several weaknesses to the model. First, an analysis of the molecular mutations responsible for tumor formation after feeding the Western diet has not been reported so it is not clear whether carcinogenesis follows the Vogelstein model or another route. In addition, the dietary level of calcium used (1/10th of the level found in AIN76A diets) is clearly deficient based on studies published with bone and calcium metabolism as endpoints (68,69,70,71). As a result, the "Western diet" may change physiology in ways that do not reflect the etiology of human colorectal cancer.

II.B.2. Chemical Induced Models of Colorectal Cancer—A large number of chemicals are known to have mutagenic potential and many cancer studies have used this characteristic to controllably induce cancer (72). Below we will discuss the most commonly used chemical inducers of colorectal cancer in rodents.

II.B.2.a. DMH and AOM: The compound 1,2-dimethylhydrazine (DMH) and its metabolite, azoxymethane (AOM), are the two most commonly used carcinogens to induce and promote colorectal cancer in rats and mice (73). DMH and AOM are alkylating agents that are typically injected intraperitoneally or subcutaneously over several weeks to induce development of tumors in the distal colon. The majority of these tumors harbor mutations in the β-catenin gene (*Ctnnb1*), which is similar to HNPCC (36). These mutations affect the N-

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terminal amino acids of the β-catenin gene product, making the protein resistant to regulatory degradation, stabilizing β-catenin, and increasing WNT signaling to drive tumorigenesis (74). In addition, tumor incidence and multiplicity can be altered by both genetic background and by diet (73). This makes the models useful for the study of gene-gene and gene-environment interactions that influence the pathogenesis of colorectal cancer. However, there is little evidence that a large proportion of human sporadic colorectal cancer results from exposure to alkylating agents so some have questioned the translational potential of data generated with the model. Perse and Cerar (75) recently conducted a critical review of the morphologic and molecular changes related to the induction of colon cancer in rats by DMH and AOM that interested readers should consult for more details.

II.B.2.b PhIP: PhIP (2-amino-1-methyl-6-phenylimidozo [4,5-b] pyridine) is a heterocyclic amine produced during the cooking of meat and fish (76) that is a colon cancer-causing mutagen in rats (77). In mice PhIP induces formation of colonic aberrant crypt foci (78) but not colon tumors (79). However, combining PhIP with either DSS treatment or treating Apc^{Min} mice with PhIP can enhance tumorigenesis (80,81). Epidemiologic evidence links PhIP from cooked meat to increased colorectal cancer risk (82,83) and so the data obtained from the study of PhIP in rodents is highly relevant to human cancer. At typical PhIP doses (100–400 ppm), approximately 50% of male rats develop aberrant crypt foci or colonic adenomas within 1–2 years. In PhIP-induced tumors, mutations in the *Cnntb1*and *Apc* genes are common while *Kras* and *Tp53* mutations are rare (84,85). Colon cancers develop typically in the middle to distal regions of the colon and exhibit a polypoid, tubular adenoma morphology. Invasion and metastasis are rare. Similar to what others have shown in mice fed a Western diet (67), PhIP induces colonic adenomas in rats with a gene expression profile that includes markers of Paneth cell differentiation (86). PhIP-induced cancer is modulated by genetic background; BUF rats are highly responsive, F344 and Brown-Norway rats are moderately sensitive, and ACI are relatively resistant to PhIP-induced formation of aberrant crypt foci (87). For more information about this model, the reader should refer to the review by Nakagama *et. al.*(77)

II.B.2.c. N-methyl-N-nitro-N-nitrosoguanidine and N-methyl-N-nitrosourea: N-methyl-N-nitro-N-nitrosoguanidine (MNNG) and N-methyl-N-nitrosourea (abbreviated as both MNU and NMU) are direct-acting carcinogens that have been administered to mice and rats to induce neoplasia in a variety of organs (88). When given orally, cancer develops in the stomach, small intestine, large intestine, kidney, skin, lung, and thymus (89,90). Injection of MNU also induces prostate (91) and breast cancer (92). Unfortunately, the high incidence of extra-colonic neoplasia induced by these nitroso compounds is a confounding variable in this model. When administered via the rectum, MNU reproducibly causes a high incidence of colon cancer, but still induces thymic lymphoma and pulmonary cancers that can cause mortality (93). This local route of administration of MNU has been shown to cause DNA adduct formation and aberrant crypt foci (94). Intrarectal administration of 5 weekly MNNG doses to rats induced formation of colonic adenoma and carcinoma (1–2 per in rat) and was modified by the level of dietary fat (95,96). When compared to DMH, 3 week or 15 week courses of MNNG induced tumors with similar histopathologic features (97). MNU-induced colon cancer has been used to test a host of other potential preventive interventions against colorectal cancer (e.g. 1alpha-hydroxy-24-ethylcholechalciferol vitamin D analogue (98), dietary restriction (99), dietary fatty acids (100), ursodeoxycholic acid (101)). The complete molecular profile of mutations induced by MNU and MNNG is unknown, but 15- 30% of rat colonic tumors induced by MNU or MNNG have been found to contain *Kras* mutations (102,97). Endo *et. al.* (97) also found *Apc* mutations in 6% colon tumors induced by DMH or MNNG in rats. Interestingly, in aberrant crypt foci induced by MNNG in rats the

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MUC5AC gene product, gastric M1 mucin, is expressed. This is also observed in human colonic aberrant crypt foci (103).

II. C. Mutagen-Induced Germline Mutation Models

II. C. 1. The *Apc^Min^* Mouse—The workhorse for preclinical colorectal cancer research over the past 30 years has been the *Apc^Min^* mouse. This mouse was identified in 1990 from an ethylnitrosurea (ENU) mutagenesis screen in C57Bl/6J mice. The phenotype of the first of these mutant mice was severe, sometimes fatal, regenerative anemia that was attributed to multiple intestinal neoplasms or "Min". The Min mutation is autosomal dominant and homozygosity for the mutant allele is embryonic lethal. Heterozygous *Apc^Min^* mice became anemic by 60 days of age and often died by 120 days of age (104). Tumors occurred in the small and large intestine, but greater than 10-fold more lesions were found in the small intestine. The genetic basis for the intestinal phenotype is a T-to-A transversion at nucleotide 2549 of the mouse *Apc* gene that truncates the Apc protein at amino acid 850 (105). Similar to FAP, loss of heterozygosity of the remaining wild type *Apc* allele was required for adenoma formation (106). Because of its molecular and pathologic similarity to human FAP, *Apc^Min^* mice have been used extensively to study the development, treatment, and prevention of colorectal cancers that contain somatic *APC* mutations (107).

Although many researchers use the *Apc^Min^* model to study carcinogenesis or test treatment and prevention strategies, the *Apc^Min^* mouse has also been useful for identifying genetic modifiers of colorectal cancer risk, which are collectively referred to as Modifiers of Min (Mom) (108). Using classical genetic mapping strategies and congenic mouse lines researchers have identified 13 genetic modifiers of the *Apc^Min^* phenotype (108,108). These modifiers provide the underlying mechanistic basis for the diversity of the Min phenotype across inbred mouse lines (109). For example, C57BL/6J mice heterozygous for the *Apc^Min^* mutation have a large number of polyps and this causes early mortality due to intestinal bleeding (104) while hybrid F1 offspring from C57BL/6J; *Apc^Min/+^* mice crossed to either AKR/J, MA/MyJ, or *Mus musculus castaneus* mice have a significant decrease in polyp number (110). Information about "modifiers of Min" may help explain familial associations of cancer risk that account for 25% of human colorectal cancer cases (111). Because of the profound impact that genetic background has on the phenotype of the *Apc^Min^* mouse, it is critical for researchers to know and accurately report this information in their publications, especially if they are crossing their mice to other transgenic lines.

II. C. 2. The F344-*Pirc* Rat—An ENU mutagenesis approach also led to the development of the *Pirc* (polyposis in the rat colon) rat (112). The rat FAP model harbors a mutation at nucleotide 3409 of the *Apc* gene that results in truncation of the Apc protein at codon 1137. On a F344 background, heterozygous *Pirc* rats developed adenomas throughout the intestine, with 100% having at least one colonic tumor. There are several differences between the F344-*Pirc* rat and *Apc^Min^* mouse. Whereas *Apc^Min^* mice develop tumors with a small intestine-to-colon ratio of 40:1, the *Pirc* rat develops adenomas at a ratio approaching 1:1. As in the mouse, the adenomas of the *Pirc* rat mirror the morphology of human adenomas, including progression to invasive adenocarcinoma. The cancer incidence in *Pirc* rats is increased in males, while a gender effect has not been reported in the *Apc^Min^* mouse. The increased size of the rat over the mouse offers advantages for sample collection and use of advanced imaging techniques for longitudinal study.

II. D. Genetically Modified Mice

Genetically modified mice offer the potential to precisely recapitulate specific molecular etiologies relevant to human disease. When considering the features that would make an optimal mouse model of sporadic colon cancer, several features are desirable. First, a

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researcher should be able to increase or reduce the expression of genes that are hypothesized, or known, to influence human colorectal cancer. Chemically-induced cancer may lead to mutations in relevant human cancer genes, but they do not have the ability to target them, per se. Second, a researcher should be able to control the timing of the cancer-inducing molecular event. Like familial human cancers, traditional transgenic and knockout mice have germline modifications and express that modification embryonically. In contrast, most human cancers develop in adults (even if initiating events occur earlier in life). Later onset of controlled genetic modifications in animal models, rather than during the hormonal milieu of adolescent growth, could improve our ability to translate animal data to humans. Controlling the timing of induction is an advantage of chemically-induced cancers, but advances in inducible transgenic mouse models has made this feasible for genetically modified mice as well. Finally, the molecular event should be limited to the colon and/or rectum. Most genetically modified mouse models were generated to have a single gene defect in all cells in the body. While we've learned a lot about cancer from these models, they are often confounded by the existence of precancerous or cancerous lesions in other tissues. In this section we will describe the novel approaches one can use to make genetically modified mice that control the type, timing, or location of genetic alterations and we will then examine how these models have been used to disrupt expression of specific genes in the context of colon cancer.

II. D. 1 Tissue-specific and Inducible Genetic Modifications

II. D.1.a. Site-Specific Recombinases Provide Flexibility for Silent Modifications that can be Regulated: The use of DNA site-specific recombinases has been reviewed extensively by others (113,114). However, the recombination of genomic DNA by bacterial recombinases is an important and versatile tool used to generate conditional genetic modulation in mouse models, so the topic deserves a brief review. The most common system for controlled genomic recombination is the use of the bacteriophage enzyme Cre recombinase (Cre) to delete sequences between two LoxP (Locus of X-over of P1) DNA sequences by recombination (114). Use of this approach requires two genetically modified mice: a transgenic mouse expressing Cre and a mouse created by homologous recombination in ES cells to introduce LoxP sites flanking a biologically important DNA sequence into a gene. By using a gene promoter whose expression is specific to a cell or tissue type, Cre transgenic mice can be made that confer tissue-specific gene modifications (this will be discussed in greater detail below). In addition, a modified version of the Cre protein has been designed that allows tamoxifen-inducible control of Cre activity (115). In this system, the Cre coding region is fused to a modified estrogen receptor (ER) ligand binding domain with high affinity only for the estrogen analog tamoxifen (ER^{T2}). As such, when the Cre-ER^{T2} fusion protein is expressed in cells, it is restricted to the cytoplasm. When tamoxifen is administered and binds to the Cre-ER^{T2} protein, a nuclear localization signal is revealed and the Cre is translocated to the nucleus where it can delete floxed alleles in the genome.

LoxP sites can be used in two ways. When deletion of gene function is desired, LoxP sites are introduced to flank an exon, that when deleted, prevents the production of a functional protein. This is the way that researchers have created mice with *Apc* truncation mutations limited to the intestine (Table 4 and described in greater detail below). When over-expression of a natural or mutated protein is desired, a gene is designed where the LoxP sites flank an artificial STOP codon that precedes the coding sequence for a natural or mutated gene sequence. In this case, Cre mediated deletion of the STOP codon permits the protein to be translated. This was the strategy used to make mice expressing a constitutively active Kras protein (116) (Table 4).

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II.D.1.b. Methods to Make Genetic Modifications Intestine-Specific: Sporadic colorectal cancer in humans is not accompanied by primary tumors in other organs. In contrast, gene knockouts and transgenes driven by general promoters are expressed in every cell type and frequently cause tumors outside the large intestine. One advantage of the genome era is that we are identifying genes with tissue specific expression patterns. As a result, this information is being used to improve the tissue specificity of gene expression in genetically modified mice. Several intestine specific promoters have been developed and are proving useful for the study of intestinal cancers (Table 3). We will discuss these models below.

One of the first intestine-specific promoters to be developed was a 12.4 kb fragment of the *Villin* gene that contains promoter elements and enhancer regions necessary for intestine specific gene expression (117). This promoter drives expression of transgenes to the epithelial cells of the intestine along the entire crypt-villus axis and is expressed from the duodenum to the colon. A small amount of extra-intestinal expression is seen in the proximal tubules of the kidney, but no other tissues are transgene positive. Expression of a *Villin*-LacZ transgene is apparent at embryonic day 9 within the intestine. While this pattern of expression is earlier than desired, the restricted pattern of expression means that the use of the *Villin* 12.4 kb promoter is not likely to be embryonic lethal. There are two modifications of the *Villin* construct that make it even more useful: a *Villin*-Cre version that permits intestine specific deletion of floxed alleles and an inducible Cre-ER^{T2} version (118).

While the *Villin* promoter limits transgene expression to the epithelial cells of the intestine – and this was a large improvement from models that influence every cell in the body - the fundamental problem with the *Villin* promoter transgenic mouse is that it does not restrict intestinal expression to the epithelial cells of the colon. As a result, a number of groups have attempted to create models with a more restricted intestinal expression pattern. Saam and Gordon (119) created a mouse with Cre expression driven by a modified rat fatty acid binding protein gene (*Fabl*)(120). Intestinal expression was detected as early as embryonic day 13.5 in the epithelial cells of the jejunum, ileum, cecum, and colon but extra-intestinal expression was seen in the renal calyces, pelvis, ureter, and bladder (119). The predominant cytokeratin expressed in the epithelial cells of the intestine is cytokeratin 19 (CK19) (121). A 6.3 kb fragment of the *CK19* promoter has been used to drive expression of a Cre-ER^{T2} transgene that permits tamoxifen-inducible expression throughout the intestinal epithelium, but this gene is also expressed in the pancreatic ducts, hepatic ducts, and stomach (122). Lgr5 has been identified as a marker of the intestinal stem cell (123). Barker *et al.* (123) used homologous recombination of the *Lgr5* gene locus to create a gene that marks expression of Lgr5 (with an EGFP cDNA) and permits tamoxifen-inducible Cre-mediated deletion of floxed alleles from the intestinal stem cells (Lgr5-EGFP-IRES-Cre-ER^{T2} mice). However, *Lgr5* is expressed in the stem cells throughout the gastrointestinal tract, as well as in a number of other tissues (e.g. hair follicle) (124) so while this mouse may be useful to study early carcinogenic transformation *in vivo*, it may also lead to confounding phenotypes in other tissues that make it more difficult to study colon tumor formation or potential treatments in these mice.

Another model developed used a 9.5 kb fragment of the *CDX2* gene promoter to drive Cre-expression *(CDX2P9.5-NLS*-Cre*)*. In adults, the expression of this transgene is limited to the ileum, cecum, and colon. Unfortunately, during embryonic development the *CDX2P9.5-NLS* promoter is transiently expressed in multiple tissues of the distal half of the mouse, including the intestine, the muscle and bones of the rear legs, kidney, spleen and skin (125). This could lead to embryonic lethality or additional cancers outside the colon. In fact, embryonic lethality occurs when this mouse is used to remove a floxed STOP codon from a transgene encoding a constitutively active Kras protein (unpublished data from the Fleet lab). A variant of the *CDX2P9.5-NLS* promoter construct was used to construct the

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CDX2P9.5-G22-Cre mouse. The G22 addition is comprised of 22 guanine repeats in the transgene coding region prior to the normal translation start site of Cre. This addition is out of phase with the normal Cre start site, meaning that a somatic frameshift mutation must occur before Cre can be transcribed and translated from the *CDX2P9.5-G22* promoter and making embryonic expression unlikely. Spontaneous single-base deletions in the G22 repeat put the Cre coding sequence in phase, allows translation of a functional Cre enzyme, and this permits deletion of floxed alleles. β-galactosidase expression from a *CDX2P9.5*-G22-LacZ transgene was limited to the ileum and proximal colon (126).

Our lab recently developed a transgenic mouse that restricts gastrointestinal Cre expression to the cecum and colon (127). In this mouse, Cre expression is driven by a modified mouse *Carbonic anhydrase-1* promoter/enhancer. Transgene expression is mosaic, affecting approximately 10% of large intestinal epithelial cells. In the proximal colon, expression is mainly in the surface epithelium while distal colon transgene expression occurs from the crypt base to the luminal surface in groups of 3–6 crypts (unpublished data from our lab). The transgene drives expression of LacZ as early as 14.5 days post-coitum and a small amount of staining is also seen in the liver. When we crossed CAC mice to mice with a floxed *Apc* allele, 20% of the mice developed at least one colonic tumor by 20 weeks of age and no tumors developed in the small intestine or liver (127).

Each of the promoters described so far target transgene expression to all epithelial cell types within the intestine. However, there are two promoters that drive expression specifically to cells of the secretory lineage. Gum *et al.* used either the *Intestinal trefoil factor (ITF)* gene promoter (128) or the *Mucin-2* gene promoter (129) to drive expression of the SV40 T antigen in the intestine of mice. Earlier, Itoh *et al.* (130) reported that the −6,353 to +24 *ITF* gene promoter caused goblet cell specific expression of a β-galactosidase reporter gene in transgenic mice. Gum *et al.* (128) found that the *ITF* promoter-SV40 T antigen transgene limited transgene expression to the proximal small intestine and colon. It also induced formation of tumors in the proximal colon that were similar to human small cell carcinoma of the colon and which originated from cells of the enteroendocrine lineage. Gum *et al.* (129) had previously shown that the *Mucin-2* promoter drove SV40 T antigen transgene expression in the small intestine (proximal≫distal), spleen, and lymph nodes, and that this caused goblet cell death by increased apoptosis.

II.D.1.c. Inducible Models: A limitation of both traditional transgenic mice and transgenic mice made with intestine specific promoters is that they do not replicate the post-natal acquisition of somatic mutations that cause the vast majority of human sporadic colorectal cancers. To address this challenge, several systems have been used to control the temporal expression of Cre in transgenic mice. The most popular method for inducible transgene expression is the Cre-ER^{T2} system we described briefly above. This Cre-ER^{T2} fusion protein has been expressed under the control of the *Villin* promoter (131,118) and the *Lgr5* promoter (123). While Cre-ER^{T2} has proven very effective at inducing precisely timed deletion of floxed alleles in the intestine when linked to these promoters, the liability of this approach is that it requires injection of a potent estrogenic compound (tamoxifen). Another system for tissue-specific inducible transgene activation is the reverse tetracycline activator system (132,133). This approach requires development of two or three transgenic mice. The first transgenic mouse uses a general or tissue-specific promoter to drive expression of the reverse tetracycline-regulated transactivator (rtTA). The rtTA is a tetracycline-responsive transcription factor that binds to the bacterial tetracycline (*tet*) operator sequence. When crossed to a second mouse that has a transgene that uses the *tet* operator sequence combined with a minimal human cytomegalovirus promoter (*tet*O-P_{hCMV}-), the promoter will drive expression of a transgene in the tissues specifically expressing rtTA. If a Cre transgene is being driven, then a third mouse with a gene containing a floxed allele is needed. So far,

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only one system relevant to intestinal biology has used this approach. Saam and Gordon (119) generated a triple transgenic mouse where floxed *Apc* alleles were recombined by Cre expression from a *tet*O-P_{hCMV}-Cre transgene that was induced by rtTA expressed from the intestinal fatty acid binding protein promoter (*Fabpl*$^{4xat\text{-}132}$). A variation on this system can also be used to limit Cre expression only to when tetracycline antibiotic administration is stopped (134), but there are no reports of this particular induction system being used for the study of colon cancer.

There are two inducible systems that are made tissue-specific by non-transgenic methods. *AhCre* mice use the rat *cytochrome P4501A1* promoter to drive Cre expression through an aryl hydrocarbon receptor (AhRc)-dependent mechanism. By treating mice with the AhRc ligand, β-napthoflavone, Cre is induced and can recombine floxed alleles (135). Injection of β-napthoflavone induced Cre expression in the liver, gallbladder, pancreas, esophagus, stomach, and small intestine (including the crypt stem cell compartment) (135). However, when β-napthoflavone is delivered orally, Cre expression is limited to the transit amplifying compartment and differentiated cells of the intestine (136). This model was used to delete floxed *Ctnnb1* (β-catenin) (135) or *Apc* (137) alleles in mouse intestine. An important limitation of the β-napthoflavone- *AhCre* model is that Cre induction is very weak in the colon (135).

A unique approach to restrict the temporal-spatial activity of Cre was developed by Hung *et. al.* (138). This group introduced an adenovirus vector expressing Cre through the rectum to infect colonic epithelial cells. While this approach provides very precise control over when and where Cre will be expressed, its implementation is limited by the surgical procedure required for efficient adenoviral delivery. The technique requires anesthesia of the mice, applying surgical clamps to the distal colon, creating abrasive injury to the colonic mucosa, followed by 30 minutes for adenoviral infection before removing the clamps and closing the abdomen. Without abrasive injury to stimulate cell proliferation, the colonic epithelial cells are less receptive to adenovirus infection and this approach will not lead to deletion of the floxed alleles in self-renewing cells (139).

II. E. Phenotypes of Mice with Specific Gene Deletions

In this section we will describe the mouse lines that have been created to model human cancer. Fearon recently reviewed the molecular genetics of colorectal cancer and listed 22 genes with recurrent somatic mutations driving the disease (3). In addition, Poulogiannis *et al.* (140) recently identified 39 genes that are involved in mismatch repair (MMR)-deficient colorectal cancers. Mouse models have not been developed to study the impact of each of these mutations in the context of the colon. However, we will describe the molecular and histological features for a number of mice whose mutations reflect changes in human cancer and we will identify the strengths or weaknesses of these models.

III. E. 1. Apc Mutant Mice—Several researchers have used homologous recombination in embryonic stem cells to generate mice with germline mutations in the *Apc* gene (Table 5). These *Apc* mutations are present in all cells and affect all cells in the body to some extent. As such, these mice are a model of human FAP. The goal of making these models was to overcome limitations of the *Apc*^{Min} mouse, e.g. either to shift the distribution of tumors away from the small intestine and towards the colon, to create models whose truncated Apc proteins are more like those found in humans, or to test the role of specific regions in the Apc protein. Given the differences between the small intestine and colon in terms of cell biology (e.g. existence of Paneth cells in small intestine), embryonic origin (e.g. foregut for duodenum, midgut for distal small intestine and proximal colon, hindgut for distal colon), and luminal environment (e.g. low pH and aseptic in upper small intestine, high pH with a

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diverse microflora in colon), targeting Apc mutations to the colon may be critical for studies of colon carcinogenesis and colon cancer prevention.

Oshima *et. al.* (141) generated a mouse expressing an *Apc* allele mutated to truncate translation at nucleotide 2150 and making an Apc protein 716 amino acids long, the $Apc^{\Delta 716}$ mouse. These mice develop more intestinal polyps than the Apc^{Min} mouse, but the majority of polyps still develop in the small intestine. Microadenomas from $Apc^{\Delta 716}$ exhibit loss of heterozygosity and invasive or metastatic adenocarcinomas do not develop. As a result, these mice were not a significant improvement upon the Apc^{Min} mouse. A mouse created by Saisi *et. al.* (142) introduced duplication of exons 7–10 and caused a frameshift of the *Apc* gene that truncated the Apc product at 474 amino acids ($Apc^{\Delta 474}$ mice). The $Apc^{\Delta 474}$ intestinal tumor phenotype was virtually identical to that of the Apc^{Min}, i.e. small intestinal adenomas and rare colon tumors. These mice also developed mammary adenocarcinoma between 3 and 5 months of age (142). The Apc^{1322T} mouse was designed to express a truncated 1322 amino acid Apc protein that retains one of the 20 amino acid repeats that bind β-catenin (143). This models a truncation common in human sporadic colon cancer and so is useful for testing the “just right” hypothesis proposed for the development of *APC*-mediated tumors in humans (22) (i.e. that cells which retain one 20 amino acid repeat express an optimal level of WNT signaling that favors neoplastic transformation and progression). Apc^{1322T} mice develop intestinal adenomas at a faster rate than Apc^{Min} and they have a more proximal distribution of polyps within the small intestine. Relative to Apc^{Min} mice, Apc^{1322T} mice exhibited less nuclear β-catenin within intestinal epithelial cells, a greater frequency of Paneth cell differentiation, and increased crypt fission within adenomas and morphologically normal crypts (143).

Fodde *et. al.* (144) targeted a mutation at nucleotide 4913 of the *Apc* gene that truncates the Apc protein at codon 1638. These Apc^{1638N} mice developed small and large intestinal adenomas similar to the Apc^{Min} mouse. The authors found few invasive adenocarcinomas in the small intestine and one case of metastasis to the liver (144). Interestingly, the truncated 1638 amino acid protein was not detected in tissues, suggesting that the truncated protein was unstable. By reversing the transcriptional orientation of their selection cassette, the Apc^{1638T} mouse was generated and this animal stably expressed the truncated 1638 amino acid Apc protein. In contrast to the Apc^{1638N} (and most other Apc truncation mutants) the Apc^{1638T} allele was not homozygous lethal to embryos, nor did this mouse develop intestinal adenomas (145). As such, this model is useful for studying the functions of the C-terminal aspect of the Apc protein, but is not very useful for studying intestinal carcinogenesis. Lewis *et al.* (146) recently made a mouse with deletion of the central portion of the *Apc* gene, but retained the C-terminus region by engineering an in-frame deletion of codons 1322–2006 to create the $Apc^{\Delta SAMP}$ mouse. The tumor phenotype of the $Apc^{\Delta SAMP}$ mouse was indistinguishable from that of the Apc^{1322T} mouse (146), confirming the observation that the C-terminus of Apc has no significance in intestinal tumorigenesis. This group subsequently created a mouse with truncation of the *Apc* gene at codon 1572 (Apc^{1572T}) that deleted the only SAMP repeat that was remaining in Apc^{1638T} mice (147). This mouse had a moderate level of WNT/β-catenin activation (more than WT or Apc^{1638T} but less than other truncation mutants and Apc^{Min} mice). No intestinal cancer was observed in these mice but multifocal tumors developed in the mammary gland. This demonstrates that the cancer risk conferred by some *Apc* gene mutations may be tissue specific. The Apc^{1309} mouse harbors a mutation identical to the most common form of FAP in humans and behaves similar to the Apc^{Min} mouse (148,149). However, this mouse is not available in the United States and a detailed description of the mouse has not been reported in the English-language literature.

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Apc$^{\Delta 14}$ mice were created by homologous recombination to remove exon 14 in the mouse *Apc* gene (150). Removal of exon 14 causes a frameshift in the *Apc* coding region and results in a truncated protein containing 580 amino acids of the Apc protein. They have polyps and microadenomas throughout the gastrointestinal tract but a greater percentage of their polyps are in the distal colon and they exhibit earlier mortality than *Apc*^{Min} mice. In addition, *Apc*$^{\Delta 14}$ mice raised under specific pathogen free conditions have fewer tumors, demonstrating strong environmental effects on incidence (150). The increased colonic exposure and environmental modification are attractive features of this model.

Two laboratories have independently generated mice with a floxed exon 14 of the *Apc* gene. Shibata *et. al.* (139) called their model the *Apc*^{580S} mouse and Kuraguchi *et. al.* (151) called theirs the *Apc*^{CKO} mouse. Upon recombination of the LoxP sites, the mice were designated as *Apc*^{580D} or *Apc*$^{\Delta 580}$, respectively. A recent review by Phelps *et. al.* refers to the existance of an *Apc*$^{fl/fl}$ mouse in which the entire *Apc* allele is floxed (16); this model is actually the *Apc*^{580S} mouse. Adenovirus-Cre mediated recombination of LoxP sites in mice homozygous for the *Apc*^{580S} allele leads to formation of colonic polyps within 4 weeks (139). When β-napthoflavone was used to induce Cre expression from an AhRc driven *CYP1A*-Cre transgene, *Apc*$^{580D/580D}$ mice experienced severe morbidity within 5 days and so tumor formation could not be studied. However, there was rapid nuclear localization of β-catenin, an increased number of proliferating cells, loss of discrete crypt structure, and loss of differentiated phenotypes (e.g. reduced expression of absorptive enterocyte markers and decreased goblet cells) (152).

Using the *CDX2P9.5-NLS* or *CDX2P9.5-G22* promoter to drive Cre expression and recombine the *Apc*^{580S} allele shifts polyp development distally, but some tumors still develop in the ileum (125,126). *CDX2P9.5-NLS;Cre;Apc*$^{580D/580D}$ embryos were not viable but *CDX2P9.5-NLS;Cre;Apc*$^{580D/+}$ mice developed three ileal tumors and five colonic tumors per mouse by 200 days of age (125). In contrast to *CDX2P9.5-NLS;Cre;Apc*$^{580D/+}$ mice, the *CDX2P9.5-G22;Cre;Apc*$^{580D/+}$ mice failed to develop grossly apparent intestinal adenomas (126). Cre expression is delayed in *CDX2P9.5-G22;Cre;Apc*$^{580D/580D}$ mice so they survive to develop fatal intestinal tumor burdens by four weeks of age.

Apc^{CKO} mice were not originally used to study intestinal cancer (151) but they are the mutant Apc line available in the National Cancer Institute Mutant Mouse Repository. When we crossed these mice to the CAC mouse (with large-intestine specific Cre expression driven by a modified *Carbonic anhydrase-1* promoter) to generate CAC,*APC*$^{\Delta 580/+}$ mice, 20% of mice developed adenocarcinoma in the distal colon by 20 weeks of age (127). The incidence of adenoma formation was significantly increased by a 5–7 day course of 2% DSS given at 10 weeks of age. Thus, these mice can be used to study the interaction between inflammation and *Apc* gene mutations. In addition, since Cre expression is limited to the large intestine in embryos and adults, CAC, *Apc*$^{\Delta 580/\Delta 580}$ mice are viable and all have extensive colonic hyperproliferation in the distal colon at weaning that develops into adenocarcinoma by 6 weeks of age. Although there is a small amount of Cre expressed from the CAC transgene in the liver, no lesions form in that organ in either CAC,*Apc*$^{\Delta 580/+}$ or CAC,*Apc*$^{\Delta 580/\Delta 580}$ mice. The restricted embryonic expression of Cre in this mouse may make it useful for assessing the impact of combining gene mutations.

Robanus-Maandag *et. al.* (153) created a mouse with LoxP sites surrounding exon 15, the *Apc*$^{\Delta 15}$ mouse. Cre-mediated deletion of exon 15 results in a truncated 650 amino acid Apc protein that, like the *Apc*^{1638N} mouse, is detected only at low levels (5% of the wild type Apc level). However, unlike *Apc*^{1638N} mice, *Apc*$^{\Delta 15}$ mice exhibit a severe *Apc*^{Min}-like phenotype characterized by a high intestinal tumor burden with 95.5% of lesions occurring

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in the small intestine. In addition, intestinal obstruction usually caused death by 2.5–4.5 months of age, making these mice a poor choice for colorectal cancer studies.

Exon 15 also contains two nuclear localization signals (NLS) necessary to allow for APC movement into the nucleus; when localized in the nucleus APC can also impair β catenin mediated gene transcription by promoting β catenin nuclear export (13). Zeineldin et al. (154) recently created a mouse whose Apc gene has mutated NLS signals ($Apc^{mNLS/mNLS}$). Compared to mice with wild-type Apc alleles, $Apc^{mNLS/mNLS}$ mice have elevated Wnt target gene expression and an increased crypt cell proliferation index. When crossed to $Apc^{Min/+}$ mice, the resulting $Apc^{Min/mNLS}$ mice have increased 50% more polyps in the jejunum and ileum but no increase in the low number of colon polyps normally found in $Apc^{Min/+}$ mice.

II. E. 2. DNA Mismatch Repair Genes—DNA MMR systems were first identified in bacteria and the bacterial *MutL*, *MutS*, and *MutH* genes are well conserved in mammals (155). Mice heterozygous or homozygous for a null MutL homologue 1 (*Mlh1*) gene exhibit defects in DNA MMR that result in microsatellite instability (156). The $Mlh1^{-/-}$ mice develop cancer in both lymphoid tissue and in the intestine and they die as a result of these tumors by 9 months of age. Approximately 70% of MutS homologue 2 (*Msh2*) knockout mice developed intestinal tumors after 6 months of age, but these mice succumb primarily to lymphomas (157). Tumors from $Msh2^{-/-}$ mice acquire *Apc* gene mutations (157) and when either *Mlh1* or *Msh2* knockout mice are crossed to mice heterozygous for a mutated *Apc* allele, intestinal tumorigenesis is markedly increased (158,156). However, small intestinal tumors are more common than colon tumors in both the *Mlh1* and *Msh2* knockout models. Combining *Msh2* null mice with mice carrying an inducible *Kras* mutant allele increased the number of adenomas per mouse 5-fold, but there were still 3 times more tumors in the jejunum compared to the colon (159). Recently, Kucherlapati *et al.* (160) made a *Msh2* mutant mouse with LoxP sites surrounding exon 12 ($Msh2^{LoxP}$ mice) and used *EIIa*-Cre mice for embryonic recombination or *Villin*-Cre mice to recombine the floxed alleles in intestine. While *EIIa*-Cre;$Msh2^{LoxP/LoxP}$ mice had both lymphoma and small intestinal tumors, the *Villin*-Cre;$Msh2^{LoxP/LoxP}$ mice had only small intestinal tumors (100% incidence, 1.6 tumors per mouse).

Although the focus of DNA MMR gene mutations has been on *MLH1* and *MSH2*, other MMR genes are also relevant. MLH1 heterodimerizes with MSH3 or PMS2 to have activity and *PMS1* and *PMS2* genes can be mutated in HNPCC (161), while MSH6 forms a dimer with MSH2 (162). Unlike *Msh1* and *Msh2* knockout mice, neither *Pms1* nor *Pms2* null mice develop intestinal tumors (163). However, 50% of *Msh3* knockout mice develop both adenomas and adenocarcinomas in stomach, small intestine, colon, and rectum (163). By crossing *Msh3* knockout mice to $Apc^{1638N/+}$ mice, there was an increase in frameshift mutations in the wild type *Apc* allele that caused higher incidence and more tumors per mouse compared to the $Apc^{1638N/+}$ mice (164). On their own, *Msh6* knockout mice develop many tumors, but few of these are in the intestine (<10%) (162).

II. E. 3. β-catenin (*Ctnnb1*)—β-catenin is the downstream target of the tumor suppressor Apc. The complex that includes Apc facilitates phosphorylation of serine and threonine residues of the N-terminus of β-catenin by glycogen synthase kinase 3β (GSK3β). All of these serine/threonine residues (S33, S37, T41, and S45) are transcribed from exon 3 of the β-cateinin gene, *Ctnnb1*. Harada *et. al.* (165) inserted LoxP sites to flank exon 3 in the *Ctnnb1* gene to make $Catnb^{lox(ex3)/+}$ mice which were then crossed to keratin19 promoter-Cre (*K19*-Cre) or intestinal fatty acid binding protein promoter-Cre (*Fabp*-Cre) transgenic mice. Upon recombination, the mice produce a form of β-catenin that cannot be phosphorylated and is therefore stabilized and localized in the nucleus leading to

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uncontrolled β-catenin function. Although recombination of the floxed allele was seen in all intestinal segments of the *K19*-Cre; $Catnb^{lox(ex3)/+}$ mouse, 3000 polyps formed in the small intestine (with the highest densities in the duodenum and proximal jejunum) and only microadenomas were found in the colon. In contrast, 200–700 polyps were seen in the small intestine of mice crossed to the *Fabp*-Cre line. Histologically, the tumors that developed were similar to those seen in $Apc^{\Delta 716}$ mice (165).

II. E. 4. Kras—Kras is a membrane associated GTPase that functions downstream of a variety of receptor tyrosine kinase growth factor receptors. Oncogenic missense mutations in codons 12, 13, or 61 dramatically decrease the GTPase activity of the protein and lead to constitutive activation. Approximately 40% of human colorectal cancers harbor somatic mutations of the *KRAS* gene, usually within codon 12 (3). Jackson *et. al.* (116) used homologous recombination to replace an endogenous *Kras* allele with an allele that had a floxed translational stop site (Lox-STOP-Lox) in front of a gene encoding the oncogenic $Kras^{G12D}$ allele (i.e. where the mutated codon 12 replaces an aspartate with a glycine, LSL-$Kras^{G12D}$ mice). In the presence of Cre activity, the STOP codon is removed and the constitutively active $Kras^{G12D}$ is expressed. Deletion of the STOP codon throughout the body using a CMV promoter-Cre transgene is embryonic lethal (166). This demonstrates the importance of limiting the distribution of mutant *Kras* during embryonic development when one wants to model human colorectal cancer.

Fabl-Cre; LSL-$Kras^{G12D/+}$ mice have diffuse colonic hyperplasia with extreme lengthening of the crypts and development of large goblet cells, however, no tumors developed (167). When combined with the $Apc^{\Delta 14}$ mouse to make *Fabl-Cre;LSL-*$Kras^{G12D/+}$;$Apc^{\Delta 14/+}$ mice, the tumors that developed were more advanced than those from the $Apc^{\Delta 14}$ mouse and showed uniform, high-grade hyperplasia, a complete lack of terminally differentiated cells, loss of cell polarity, and fused glands with serrated borders. *Villin-Cre; LSL-*$Kras^{G12D/+}$ (168) and *CDX2P9.5-G22;Cre;*$Kras^{G12D/+}$ (169) mice exhibited a similar phenotype of epithelial hyperplasia. In addition, others have shown that combining the $Kras^{G12D}$ allele with either the Apc^{Min} genotype or AOM chemical induction increased the number of colonic lesions (170). Similarly, although neither *Villin-Cre; LSL-*$Kras^{G12D/+}$ nor *Villin-Cre;*$TGFb2R^{loxp/loxp}$ mice form intestinal neoplasms, *Villin-Cre; LSL-*$Kras^{G12D/+}$;$TGFb2R^{loxp/loxp}$ triple transgenic mice form tumors that metastasize through a β-catenin-independent mechanism (171).

Luo *et al.* (159) have also created a Lox-STOP-Lox mouse for the *Kras* mutation that is thought to be the most aggressive *KRAS* mutant found in humans ($KRAS^{G12V}$). When an AhRc-regulated promoter was used to drive inducible Cre expression and recombination of the LSL-$Kras^{G12V}$ mice, only minor effects were observed in the intestine (170). However, this mutant did accelerate tumorigenesis in Apc^{Min} mice (170) as well as cancer caused by DMH treatment (172).

II. F. Other Models

Table 2 summarizes the genes whose mutations have been shown to contribute to colon cancer (3). We cannot describe all of the models that have been used for the study of colon cancer, but we will review several others that we think are worth consideration.

II. F. 1. p53, p21, p27—Although mutations in the *p53* gene are common in human colorectal cancers, intestinal cancers are rarely seen in heterozygous or homozygous *p53* null mice, where lymphomas and sarcomas are the most common malignancies (173). In a survery by Harvey *et. al.*(174), only 2% of $p53^{+/-}$ mice developed intestinal adenocarcinomas and $p53^{-/-}$ mice failed to develop intestinal tumors. *p53* null mice do

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develop increased numbers of intestinal tumors when treated with carcinogens, such as AOM (175,176,177) and DMH (178). In addition, *p53* null genotypes increase susceptibility to intestinal tumorigenesis driven by inflammation (179) or *Apc* mutation (180). When Mdm2, a negative regulator of p53, is conditionally deleted from intestinal epithelial cells in *Villin*-Cre;*Mdm2*$^{int\Delta}$ mice, epithelial cells paradoxically increased proliferation via WNT and EGFR signaling, apparently in an attempt to compensate for increased p53-mediated apoptosis (181). Other p53 target genes and negative regulators of the cell cycle, such as p21 and p27, have been studied in transgenic mice. Mice null for p21 (*p21*$^{-/-}$) do not form intestinal tumors on their own, but increased tumor susceptibility of *Apc* mutant mice, especially when fed a Western diet (182). In contrast, *p27*$^{-/-}$ mice developed small and large intestinal tumors in the absence of other cancer-related promoters (183), in addition to increasing susceptibility in mice with *Apc* mutation (184) or on a Western diet (183).

II. F. 2. PTEN—The negative regulator of PI3K signaling, PTEN, is a tumor suppressor involved in numerous cancers, including colorectal cancer (169). *Pten*$^{-/-}$ mice do not exhibit an increased incidence of intestinal neoplasia, but when crossed with *Apc*^{Min} mice, *Pten*$^{-/-}$ mice have larger, more invasive tumors (185). Marsh *et. al.* (186) later confirmed this observation by demonstrating that conditional deletion of one *Apc*^{580S} and one floxed *Pten* allele in intestinal epithelial cells led to more advanced adenocarcinomas than what was seen in mice with one deleted *Apc* allele.

II. F. 3. Mucin 2—Although it is not an oncogene or tumor suppressor gene, Mucin 2 is a major component of the mucus layer that lubricates and protects the intestinal epithelium from physical damage and from chemicals in the intestinal lumen (187). As a result, even though the *Muc2* gene is not mutated in human colon cancer, this deletion may accelerate the spontaneous development of intestinal cancer in mice by increasing the rate of mutations caused naturally by the contents of the lower bowel. Consistent with this, *Muc2* knockout mice have bacteria in direct contact with the intestinal epithelium and bacteria can be found deep within the crypt where their metabolic products could have negative effects on intestinal stem cells (188). Microarray analysis of *Muc2* knockout mice show that they have a transcript profile reflective of low level inflammation and increased oxidative stress (189). *Muc2* knockout mice have a higher rate of intestinal epithelial cell proliferation than normal mice and 68% of *Muc2* knockout mice develop intestinal cancer within 1 year (2:1 ratio of small intestine to colon) (189). Deletion of *Muc2* from *Apc*$^{1638N/+}$ and *Apc*$^{Min/+}$ mice increased the incidence of tumors, increased the number of tumors per mouse, and shifted tumor burden towards the large intestine (190), suggesting an increased rate of cellular transformation in the absence of the protection provided by Muc2.

II. F. 4 Fbxw7—FBXW7 is a component of the Skp1/Cullin/F-box protein E3 ubiquitin ligase complex and a tumor suppressor gene for human colorectal cancer (3). This ubiquitin ligase is involved in the ubiquitin-mediated degradation of proto-oncogenes, like cyclin E (191) and c-Myc (192). Babaei-Jadidi *et. al.* (193) deleted *Fbxw7* from the intestine of *Villin*-Cre;*Fbxw7*$^{flox/flox}$ mice and found that intestinal adenomas formed by 10 months of age. Tumorigenesis was accelerated when *Villin*-Cre;*Fbxw7*$^{flox/flox}$ mice were crossed to *Apc*^{Min} mice. In addition, the small intstine of *Villin*-Cre;*Fbxw7*$^{flox/flox}$ mice had a reduction in Mucin 2-positive goblet cells and Paneth cells, but increased Notch and c-Jun activation, increased nuclear levels of the proto-oncogene DEK, and increased proliferation of progenitor and transit amplifying cells.

II. F. 5. The Sleeping Beauty Model—A unique model has been used to screen for additional genes that may play a critical driver role in intestinal carcinogenesis. Starr *et. al.* (194) used a “Sleeping Beauty” system to drive genomic transposition in the gastrointestinal

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tract of triple transgenic *Villin*-Cre; LSL-*SB11 transposase; T2/Onc transposon* mice. This transgenic combination facilitates random transposon insertion into the genome, where by insertion into a tumor suppressor gene will lead to inactivation and tumor formation. Analysis of the most common insertion sites within intestinal tumors from these mice identified 77 candidate tumor suppressor genes, including 17 novel genes. This model may be useful for other studies wishing to explore tumor suppressor genes of colorectal cancer.

II.G. Models of Colorectal Cancer Metastasis

A general weakness of animal models of colorectal carcinogenesis is the rarity by which colorectal adenocarcinomas metastasize beyond local invasion of the colon wall. Aside from xenotransplantation of human colorectal tumors or cancer cell lines into mice, researchers studying colorectal cancer metastasis have very few *in vivo* tools at their disposal. One mouse model that seems to be predisposed to more aggressive and metastatic colorectal adenocarcinomas is the *Smad3* knockout mouse. Smad3 is a signaling molecule within the TGFβ pathway, and TGFβ is disrupted in a high percentage of human colorectal cancers (28). *Smad3* knockout mice on the 129/Sv background developed aggressive colorectal adenocarcinomas within 4–6 months and this was accompanied by frequent metastasis to regional lymph nodes (195). Tumors were present in 100% of the mice, they developed independent of *Apc* mutations, and they were found in both the proximal and distal colon. The importance of genetic background was highlighted by the observation that only 30% of 129/SV x C57Bl/6 mice developed colon tumors. In addition, cancer driven by *Smad3* gene deletion is dependent upon the presence of gut microflora; when *Smad3* knockout mice are grown in specific pathogen free housing they do not develop colon tumors (196).

Hung *et al.* (138) used surgical introduction of a Cre expressing adenovirus to induce recombination of floxed alleles in the distal colon of $Apc^{CKO/+}$; LSL- $Kras^{G12D/+}$ mice. Over 90% of the mice developed tumors within 3 weeks (3.6 tumors per mouse). Carcinomas appeared in these mice within 20 weeks of the adenovirus injection and spontaneous gross liver metastases were observed by 24 weeks after the injection (138). As we described above in section II.E.4, others have combined *Apc* mutations with the activated $Kras^{G12D}$, but this is the only report to identify carcinoma and metastasis. The major difference between the Hung *et al.* study and these other reports is that the Hung group was able to restrict tumor formation to the distal colon and they studied the development of the phenotype for a much longer period. Similarly, although neither *Villin-Cre; LSL-*$Kras^{G12D/+}$ nor *Villin-Cre;*$TGFb2R^{loxp/loxp}$ mice form intestinal neoplasms, 15% of *Villin-Cre; LSL-*$Kras^{G12D/+}$*;*$TGFb2R^{loxp/loxp}$ triple transgenic mice developed lymph node and lung metastases by 22 weeks of age (171).

III. Conclusion

This review demonstrates that there are many animal models from which researchers can gain valuable insights into the biology of human colorectal cancer. However, no single animal model faithfully recapitulates all aspects of the human disease, so researchers must carefully select the model that is most appropriate for testing their specific hypothesis. Despite the progress made in the development of animal models of human colorectal cancer, there are several clear weaknesses with these models. First, all of the global gene deletion and chemically-induced models develop cancer outside the colon; in some models colon cancer is a minor phenotype. As a result, long-term studies of colorectal cancer development are limited due to high morbidity and mortality from these other phenotypes. Second, only some of the genes relevant to human colorectal cancer have been modified to make mice with floxed alleles. As a result, we are often left with models where the cancers develop early in life and their development may be confounded by the anabolic environment of growth. In addition, the lack of mice with floxed gene alleles limits the use of tools for both

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intestine-specific and inducible gene modifications in mice. Third, only a few of the animal models for colorectal cancer are relevant to natural initiating events that drive human colorectal cancer. This limits our ability to study the initial stages of colorectal carcinogenesis as well as primary cancer prevention in animal models. Finally, only a few mouse models have been developed to study metastatic events. Thus, we lack models for testing late-stage colorectal cancer. This is an untapped opportunity for mouse model development.

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Table 1

Summary of Major Differences Between Colorectal Cancer of the Ascending and Descending Colon.

Ascending Colon		**Descending Colon/Rectum**
Microsatellite Instability	**Predominant Initiation Mechanism**	Chromosomal Instability
Often arise de novo or out of hyperplastic serrated adenomas	**Precursor Lesion**	Predominantly arise out of benign adenomatous polyps
Exophytic or sessile growths with poor differentiation and excess mucin production	**Typical Morphology**	Well differentiated, pedunculated tubular adenomas
Lynch Syndrome	**Classic Human Familial Disease**	Familial Adenomatous Polyposis
β-catenin stabilizing mutations	**WNT Signaling**	*APC* inactivating mutations
Inactivating mutations common	**DNA Mismatch Repair Genes**	Mutations uncommon
BRAF mutations	**Growth Factor Receptor Pathways**	*KRAS* mutations
TGFβIIR mutations common	**TGFβ Signaling**	*SMAD* mutation or deletion
Activating mutations of *BAX*	**Apoptosis/Cell Survival**	*TP53* inactivating mutations

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Table 2

Recurrent Somatic Gene Mutations in Human Colorectal Cancer.

Pathways					
MMR	**Wnt**	**MAPK**	**TGF**	**p53**	**PTEN/Akt**
MLH1 MSH2 MLH3 MSH6	APC (>70%)[1] CTNNB1 (<5%) CMYC (5–10%) MYB (<5%) CCNE1 (5%) TCF7L2 (5%)* CDK8 (10–15%) FBXW7 (20%)	KRAS (40%) NRAS (<5%) EGFR (5–15%) HER2 (<5%) BRAF (5–10%)	SMAD2 (5–10%) SMAD3 (5%) SMAD4 (10–15%) TGF βIIR (10–15%)* ACVR2 (10%)*	P53 (>60%) BAX (5%) CDK8 (10–15%)	PTEN (10%)* PIK3CA (15–20%) HER2 (<5%)

[1] frequency seen in all sporadic colon cancers

* associated with MMR defects

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Table 3

Promoters and Methods for Inducible or Intestine-Specific Gene Expression.

Promoter	Small Intestine			Large Intestine			Extra Intestinal Expression	Transgene(s) Available	Inducible	Citation
	Dd	Je	Il	Ce	ACo	DCo				
Epithelial Cell Expression										
Villin	x	x	x	x	x	x	Stomach, kidney	Cre Cre-ER^{T2}	Yes*	(117)
CK-19	x	x	x	x	x	x	Pancreatic ducts, hepatic ducts, stomach	Cre-ER^{T2}	Yes*	(122)
Lgr5	x	x	x	x	x	x	Stem cells	Cre-ER^{T2}	Yes*	(123)
Cyp1A1	x	x	x	x	x	x	broadly	Cre	Yes†	(135)
Fabl		x	x	x	x	x	Renal calyces, pelvis, ureter, bladder	Cre tetO-PhCMV- Cre	Yes‡	(119,120)
CDX2P9.5			x	x	x	x	Embryo (kidney, spleen, hind limbs, skin)	Cre	No	(125)
CDX2P9.5-G22			x	x	x			Cre	No	(126)
Car1				x	x	x	Liver	Cre	No	(127)
Secretory Cell Expression										
ITF	xx	xx	x	x		x	stomach	SV40 T Ag	No	(128)
Muc2	xx	xx	x				Stomach, spleen, lymph node	SV40 T Ag	No	(129)

Dd= duodenum, Je = jejunum, Il=Ileum, Ce= Cecum, Co= Colon. Inducible by (*) Tamoxifen, (†) β-napthoflavone, or (‡) tetracycline.

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Table 4

Mouse Models with Floxed Alleles

Gene	Floxed Allele	Published Cre Promoters	Observed Tumor Phenotype	Citation
Apc	Exon 14	Numerous intestinal specific promoters and intrarectal adenovirus-Cre	Tubular adenoma formation in regions matching promoter expression	(139,151)
	Exon 15	Fabl	Severe Apc^{Min}-like phenotype	(153)
Ctnnb1	Exon 3	*CK-19* *Fabl*	Tubular adenoma formation consistent with promoter expression	(165)
Kras	LSL- $Kras^{G12D}$	*Fabl* *CDX2P9.5-G22* *Villin*	Epithelial hyperplasia, but no tumor formation unless combined with carcinogens or *Apc* mutations	(116)
	LSL-$Kras^{G12V}$	*AhCre*	Same as $Kras^{G12D}$	(159)
Msh2	Exon 12	*Villin*	Small intestinal tumors only	(181)
TGF βR2	Exon 2	*Villin*	No tumors unless combined with other relevant mutations	(197)
Fbxw7	Exon5	*Villin*	Small polyps and increased crypt fission	(193)

LSL= LoxP-STOP-LoxP.

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Table 5

Models with Germline *Apc* Mutation.

Apc Mutation	Apc Product Length (amino acids)	Estimated No. Tumors per Mouse		Homozygous Embryonic Lethal	Citation
		Small Intestine	Large Intestine		
Apc^{WT}	2843	0	0	No	
Apc^{Min}	850	30	3	Yes	(104)
F344-Pirc rat	1137	15	10	Yes	(112)
$Apc^{\Delta 716}$	716	300	3	Yes	(141)
$Apc^{\Delta ex14}$	580	40	4	Yes	(150)
$Apc^{\Delta 474}$	474	30	3	Yes	(142)
Apc^{1322T}	1322	200	3	Yes	(143)
Apc^{1638N}	0	3	0	Yes	(144)
Apc^{1638T}	1638	0	0	No	(145)
$Apc^{\Delta SAMP}$	1322 + (2006–2843)	200	3	Yes	(146)
$Apc^{\Delta 15}$	650	175	8	Yes	(153)
Apc^{1309}	1309	30	3	Yes	(148,149)
Apc^{mNLS}	Full length, mutant nuclear localization signals	0	0	No	(154)

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Table 6

Other Relevant Rodent Models

Model	Phenotype	Citation
Genetic Models		
Msh2 $^{-/-}$	L tumor, reduced MMR	(198)
Mlh1 $^{-/-}$	Non-colon 2X > C tumor, reduced MMR	(156)
Msh3–/–	ST, SI, C tumor, reduced MMR	(163)
Msh6–/–	< 10% intestinal tumors, reduced mismatch repair	(162)
Pms1 $^{-/-}$	None	(163)
Pms2 $^{-/-}$	None	(163)
p53 $^{-/-}$	No intestinal tumors alone but increased AOM-induced tumor in intestine	(174)
Muc2 $^{-/-}$	SI 2X > C tumor by 12 mos. of age	(189)
p27 $^{-/-}$	SI = C tumor, more c-myc, cyclin D1	(183)
p21 $^{-/-}$	None	(182)
Pten $^{-/-}$	No intestinal tumors	(199)
Smad3–/–	Metastatic C tumors, no Apc mutation	(195)
TGFβ1 $^{+/-}$	C tumor only with *Rag2* $^{-/-}$	(200)
Chemical-Induced		
AOM/DMH	C and other tissues, MSI, KRas, Cnntb1 mutations, no p53 mutation	(201)
PhIP	C (in rats), SI (in mice), P, M, L tumor; MSI, Apc and Cnntb1 mutations, no KRas or p53 mutations	(77)
MNU/MNNG	C, M, P tumors; KRas, p53 mutations	(201,202)

C = colon, L = lymphoid, M = mammary, MMR = mismatch repair, MSI = microsatellite instability, N/A = not available, P = prostate, SI = small intestine, ST= stomach, Tg = transgenic, ITF = intestinal trefoil factor