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Review Article

Mouse models for colorectal cancer

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Abstract: Colorectal cancer (CRC) is the third leading cause of cancer-related death in the United States, with the number of affected people increasing. There are many risk factors that increase CRC risk, including family or personal history of CRC, smoking, consumption of red meat, obesity, and alcohol consumption. Conversely, increased screening, maintaining healthy body weight, not smoking, and limiting intake of red meat are all associated with reduced CRC morbidity and mortality. Mouse models of CRC were first used in 1928 and have played an important role in understanding CRC biology and treatment and have long been instrumental in clarifying the pathobiology of CRC formation and inhibition. This review focuses on advancements in modeling CRC in mice.

Keywords: Colorectal cancer, human, mouse models, tumorigenesis, Apc, FAP, HNPCC, stem cells

Introduction

Colorectal cancer (CRC) is the third most common cancer worldwide, and the number one cause of nonsmoking cancer-related deaths in the world [1]. In the U.S., annual reported cases of CRC is approximately 142,000 and mortality 50,000 [2]. Clinically and histologically, colorectal cancer can be graded as 1 of 4 stages, with the highest grade and mortality associated with mainly liver or widespread metastasis [3]. CRC begins with specific molecular alterations in Wnt-β-catenin pathway. Additional loss of function or mutations in k-ras, DCC, DPC4 or JV18-1 or p53 contributes to CRC development [4, 5]. Further, combinations of alterations in other pathways including the mitogen-activated protein kinase (MAPK) pathway, phosphatidylinositol 3-kinase (PI3K) pathway, nuclear factor-kappa B (NF-κB) pathway, and activator protein 1 (AP-1) pathway are additional contributors to stepwise CRC development [6-9]. As intestinal tumors develop, they quickly outgrow the local blood supply and must recruit new capillary blood vessels in order to sustain adequate blood supply for continued growth [9-11].

Numerous mouse models of CRC have been developed, providing insights into pathogenesis mechanisms, tools for discovery, validation of novel therapeutic targets, and a predictive platform in which to test new chemoprevention strategies. There are several excellent reviews in the literature on this subject, so in this review we provide an overview and update some of the latest genetic, chemical, and bacterial CRC studies employing animal models.

Genetic models of early events

Mouse model for FAP

Colorectal cancers begin with intestinal epithelial cells that lose the function of the Apc pathway (gatekeeper function), part of the Wnt signaling pathway [12]. Upon Wnt binding to the Frizzled receptor and receptor activation, Apc forms a complex in the cytoplasm that results in ß-catenin phosphorylation by glycogen synthase kinase-3 (GSK-3). β-catenin phosphorylation results in its proteolytic degradation [13]. However, loss of Apc function results in nuclear accumulation of β-catenin, where, in cooperation with the transcription factor Tcf-4, it modulates expression of a variety of Tcf-4 responsive target genes. Loss of Apc function has been shown to act through Tcf-4 to upregulate, *c-Myc*, *Cdk4*, and *cyclin D1* proto-oncogene expression [14-16]. Therefore, *Apc* mutation affects the G_1

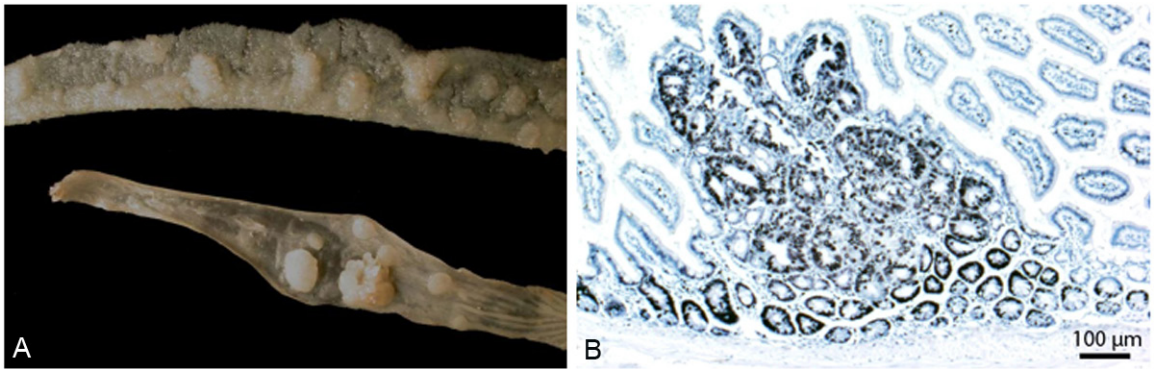

Figure 1. Gross and microscopic images of the intestinal polyps. A: Multiple raised polyps are present within the small (top) and large (bottom) intestine. B: Ki67 staining of the microscopic section of the polyp showing prolifertive nuclear staining.

to S transition of the cell cycle, causing cell growth dysregulation in intestinal epithelial cells, with resultant formation of intestinal polyps (**Figure 1**).

Patients with familial adenomatous polyposis (FAP) carry a germline mutation in one *APC* allele. They develop hundreds to thousands polyps within the large intestine and they are at high risk for developing CRC [17, 18]. Mouse models have been useful for modeling FAP; the adenomas that arise in *Apc* mutant mice are similar to development in FAP patients, in that they are at least in part nonimmunogenic and arise in immunocompetent mice. On the other hand, *Apc* mutant mice develop large numbers of adenomas in their small intestine and fewer in the large intestine and rarely progress to invasive adenocarcinoma (perhaps due to a short lifespan), whereas FAP patients develop low numbers of adenomas in their small intestine and large numbers of adenomas in their large intestine which progress to invasive adenocarcinoma [19-21]. Homozygous knockout of the *Apc* gene in mice is embryonic lethal, but heterozygous mutant Apc mice ($Apc^{-/+}$) develop between 3 and 300 intestinal adenomas/polyps in the intestine, with the overall number depending on the location of the truncating mutation, and other modifiers [22]. The first *Apc* mutant mouse model, multiple intestinal neoplasia (Min), was developed by Moser et al, with several other subsequent models showing multiple adenomas within the small and large intestine [19, 22-27]. McCart et al reviewed these models and the application of the model in drug testing [28]. Studies have shown that NSAID's inhibit adenoma formation in the *Apc* mutant mice [29-31]. This is in agreement with epidemiological studies suggesting that NSAID's decrease colorectal cancer occurrence in humans [32, 33]. While the *Apc* mutant mouse model is currently the best available model to study prevention strategies targeting early events in CRC development, one disadvantage of *Apc* mutant mice as a CRC model is that progression to malignant cancer and metastases occur late in the course of disease, so it is infrequently observed [19, 20]. Robanus-Maandag and colleagues developed a new *Apc* mutant mouse with tumors developing mainly in the large intestine, similar to human FAP patients. This model, Fabpl*Cre*; $Apc^{15lox/+}$, had an extended lifespan and developed a significant number of adenomas and adenocarcinomas in the large intestine, which should be useful to study the genetic alterations associated with the adenoma-carcinoma sequence in the mouse [34].

Mouse model for HNPCC

Hereditary Non-polyposis Colorectal Cancer (HNPCC), also known as Lynch Syndrome (LS), is the most common of the inherited CRC syndromes, and accounts for 3-5% of CRC cases [35, 36]. HNPCC is caused by mutations in one of the DNA mismatch repair (MMR) genes MSH2, MLHJ, PMSJ, and PMS2. The disease is inherited in an autosomal dominant pattern and the mutations are associated with development of cancers [37, 38]. There is also a limited, but increased incidence of hematological malignancies in patients with HNPCC [39]. Similarly, mouse models carrying disruptions of MMR genes develop lymphoma in addition to

intestinal neoplasia [40-42]. A novel conditional knockout mouse was developed, in which the Msh2 is knocked down in villin-expressing tissues, mainly the small and large intestine, but normal MMR activity is preserved in the rest of the body. This model has similarities to HNPCC, as the mice do not develop lymphoma; however, they do develop intestinal adenomas and adenocarcinomas [43]. Lastly, mice homozygous for the Mlh1 gene are predisposed to developing tumors of the gastrointestinal tract. Introduction of the Apc gene (gatekeeper) in MMR homozygous mice enhanced Apc mediated intestinal tumorigenesis [44].

Modifiers that affect early events

Identification of mouse tumor susceptibility factors are an important strategy in finding second site modifier alleles that influence intestinal tumor development. The modifier of Min (*Mom1*) was identified in 1993 by introducing 35cM of distal AKR chromosome 4 from into C57BL/6 mouse. The results were that *Mom1* is a semi-dominant modifier of intestinal adenoma size in Min mice [45]. The *Mom2* locus, which is on chromosome 18, was introduced into $Apc^{-/+}$ mice leading to greater reduction in adenoma multiplicity in both the small and large intestines than the *Mom1* locus of $Apc^{+/-}$ mice [46]. Later, six recombinant lines presenting with limited intraline variation in adenoma multiplicity were established through selective breeding for homozygosity for distal chromosome 18 markers [47]. *Mom5* was reported in 2009 to determine the impact of estrogen receptor β (ERβ) signaling on intestinal carcinogenesis in $Apc^{-/+}$ mice. The results show 50% reduction in in adenoma formation [27]. Kwong *et al.* found that *Mom7* on chromosome 18 regulates the loss of heterozygosity of distal elements and could be another pathway useful in chemoprevention [48]. The identification of *Mom12* and *Mom13* loci on chromosome 6 highlights the effects of residual donor DNA on tumorigenesis in $Apc^{-/+}$ mice. *Mom12,* is linked to the D6Mit33 marker and results in increased tumorigenesis compared to $Apc^{-/+}$ controls. *Mom13* increases intestinal tumor multiplicity in the absence of the *Mom12* [49].

Additional non-*Apc* gene considerations such as undefined genetic background effects and environmental factors can also act as modifiers. Genetic background affects adenoma multiplicity in $Apc^{-/+}$ mice [24, 45]. The average number of adenomas in a C57BL/6J background were around 29. However, the number of adenomas reduced to 6 when C57BL/6J was crossed to AKR mice [50]. Similarly, environmental factors such as chemical and bacterial agents have been shown to have implications for intestinal tumorigenesis. Exposure of MMR-deficient cells to mutagens and alkylating agents potentiate tumorigenesis and fail to induce apoptosis [51, 52]. Dietary factors also play a role in CRC formation and inhibition; obese mice (*ob/ob*) are more susceptible to chemical- induced colon cancer. Tumor cell lines grew more rapidly in obese mice compared to lean mice [53]. Bacteria appear to be another important cofactor in CRC formation. Exposure of *Apc* mutant mice to enteriotoxigenic bacterial fragiles (ETBF) leads to enhanced high tumor load [54]. Further, *Apc* mutant mice have a high number of tumors upon infection with *Citrobacter rodentium* [55]. *Smad3-/-* mice develop colorectal adenocarcinoma after being inoculated with either *Helicobacter bilis* or *Helicobacter hepaticus*. Taken together, specific second site genetic modifiers, environmental factors, and genetic background can mediate dramatic differences in the dynamics of tumorigenesis in models of CRC.

Other elements in tumor multiplicity include the location of the mutation within the *Apc* gene and this has been reviewed in detail by McCart et al [28]. Additional factors such as posttranslational modifications affect formation of intestinal tumorigenesis. Laird et al found that DNA hypomethlyation suppresses intestinal neoplasia in Apc-/+ mice [56]. Similarly, hypermethylation of the APC promoter 1A has been described in sporadic CRC in humans with associated partial reduction in transcript levels [57].

Spontaneous and chemically induced intestinal tumorigenesis models

As the spontaneous incidence of colorectal cancer in mice is low (1%-4%), many chemicals have been used to induce CRC. These carcinogens include dimethyhydrazine (DMH) or its metabolites, azoxymethane (AOM), dextran sulfate sodium (DSS), 2-amino-1-methyl-6-phenylimidazol (4,5-b) pyridine (PhIP), N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), MNU, 3,2'-dimethyl-4-aminobiphenyl (DMBA). The pro-

gression of these cancers depends on the duration and dosage of the chemical. Also, the background of the mice plays a significant role in the development of colorectal tumors [58-61]. AOM/DSS treatment in mice offers a powerful model in the initiation of aberrant crypt foci (early lesions) and is useful in evaluation of CRC chemopreventive strategies [58]. For example, it has been shown AOM/DSS treatment increases aberrant crypt foci in Nrf2 knockout mice (the transcription factor Nrf2 recognizes the antioxidant response element in the promoter of target genes) [62, 63]. MNNG, DMBA, and PHIP have been used more frequently in rat models to date. MNNG does not require biochemical activation and can be injected directly in the rectum; therefore, it is considered a topical agent and is not an ideal model for humans due to the route of administration [59]. DMBA tumorigenic activity is less potent in inducing colorectal tumors and requires multiple doses [60]. In mice, PHIP has been used most widely in *Apc*$^{-/+}$ mice and has been shown to increase the number and size of intestinal adenomas [61, 64-66].

Mouse models of invasion and metastasis

Many models have been developed to monitor the invasiveness and metastasis of the implanted or injected tumors. Nude mice that lack T cell function or SCID mice that lack both B and T cell function have been useful for developing orthotopic tumor implantation models. Grafts from either human (xenografts) or murine (syngeneic autografts or allografts) tumors can be implanted into recipient mice. The tumor cells or tumor tissue can be implanted or injected at primary or metastatic tumor sites in immunodeficinet mice. In addition, spleen and kidney capsule can be useful for tumor cell implantation. The advantage of the model is that the starting material is from a parallel relevant site representing human cancer directly, as opposed to standard subcutaneous xenografts. In addition, intravascular and intrasplenic injection mimic vascular or portal spread of CRC [67-70]. However, the disadvantage of the xenograft implantation is that the tumor development is not exactly the same as human CRC development due to species differences. In addition, there are differences between native intestine and the subcutaneous microenvironment. Immunocompetent mice can be used as models as well. Mouse cell lines that escape immune detection have been used in these mice. Cell lines that lack major histocompatibility complex are able to grow without rejection in immunologically incompatible recipient mice [11, 71]. These are useful in some cases where syngeneic cell lines are not available.

Orthotopic implantation has been used to produce a model more similar to human cancers than subcutaneous xenografts. In this model, the implant (colon cancer cell lines) is directly placed on the serosa of the intestine [11]. The advantage of orthotopic implantation is it's relevance and that the metastatic site can be monitored by imaging. The disadvantage is that the orthotopic implantation is challenging procedure and can be associated with inflammation of the implanted site if stringent surgical technique is not followed.

Recently, a new colonoscopy system was developed for implanting human colorectal cancer into the mouse colonic submucosa. This promising model is non-invasive, fast, and was not associated with significant inflammation [72]. Magnetic resonance imaging and other related imaging modalities can effectively monitor internal tumor growth and invasiveness *in vivo* [73]. Similarly, many *in vivo* studies have successfully used a luciferase construct to monitor tumor growth, invasiveness, and metastasis [74, 75].

Other models

In addition to the models mentioned above, Ramanathan et al found that a mutation in p53 gene increases progastrin-dependent colonic proliferation and subsequent formation of aberrant crypt foci [76]. Recently, a novel mouse model demonstrated the expression in the intestine of a dominant active form of the PI3K protein resulted in highly invasive mucinous adenocarcinomas [77]. p110, catalytic subunit of class Ib PI3-kinase, produces PIP3 in response to chemokines and other G protein-coupled receptor agonists [78]. Sasaki et al reported that *p110*$^{\gamma-/-}$ mice developed spontaneous malignant CRC [79].

Another interesting model with relevance for age-dependent carcinogenesis relates to telomere maintenance. With each cell division and with aging, telomeres shorten and display degenerative defects. Both CAST/EiJ and mTR

knockout mice can develop short telomeres and, in parallel, these mice have been shown to develop intestinal microadenomas [80]. In addition, a mouse model of obesity and colorectal cancer has been developed. Basically, (db/db) mouse-an animal model of type II diabeteswas bred to the *Apc*$^{-/+}$ mouse. The double mutant mice, *db/db- Apc*$^{-/+}$, developed larger numbers of adenomas when compared to *Apc*$^{-/+}$ mice [81]. Similarly, a mouse model of alcohol consumption revealed that alcohol-fed *Apc* mutant mice exhibited an increase in number and sizes of adenomas in the intestine. Alcohol intake lead to increases in the number of mast cells and subsequent invasion of tumor cells [82].

Recent advances

Rapid advances have been made in identifying and understanding stem cell pathways affecting intestinal cell differentiation and proliferative capacity. These, in turn, have provided insights into gastrointestinal stem cell dynamics and CRC. Increasing evidence shows that stem cells are involved in development of CRC and other cancers [83-85]. The mucosal layer of the intestine is composed of epithelial cells, with villi at the luminal surface and crypts at the base of the villi [86]. The intestinal stem cells are located near the base of each crypt [87, 88]. Each crypt contains about 30 stem cells with 4-6 lineage ancestors [89]. Stem cells, which develop and differentiate as they migrate from the crypt up to the villus, are the source of enterocytes, goblet cells, enteroendocrine, and paneth cells [90]. Similar to other cells, stem cells can go through apoptosis, which is believed to be a defensive mechanism against cancer development [91]. The signaling pathways which control stem cell propagation share some similarities to other non-stem cells. It is known that tumors arising from normal cells require many gene alterations [92]. Mutations in terminally differentiated cells, such as enterocytes, would in theory have little pathological significance for cancer in the intestine, since these cells are turned over constantly, in less than one week [90, 93]. In contrast, mutations in stem cells, long term residents of the mucosa, can pass alterations to their progeny through self-renewal [94]. Consequently, the accumulation of mutations results in an opportunity for cells to go through a multistep carcinogenesis process and the eventual development of malignant cancer. Epithelial tumors may arise from adult stem cells and early daughter cells, because they are the only cells in the gut that persist long enough to accumulate multiple mutations.

Identifying intestinal cancer stem cells is a new important strategy for the identification of novel cancer biomarkers and developing more effective therapeutic interventions. The main intestinal stem cell biomarkers have been recently reviewed in detail [95]. Barker, et al identified that Lgr5-expressing crypt base columnar cells resist apoptosis, undergo self-renewal, give rise to terminally-differentiated cells, and have all the criteria of putative intestinal stem cells [96]. Another putative intestinal stem cell marker, DCAMKL-1 (doublecortin and CaM kinase-like-1), is predominantly observed in a unique quiescent cell population in the lower third of the intestinal crypt [97]. Furthermore, *Prom1* (*CD133* in humans), has been identified in colorectal, hepatocellular, and pancreatic cancer as a cancer stem cell marker, and has been used as a marker to predict colon cancer recurrence in humans [98-100]. It was recently found that Prom1 is a marker for stem cells and early progenitors in mouse small intestine (**Figure 2**) [101]. Similarly, Prom1-positive cells mark intestinal stem cells that are susceptible to neoplastic information [102]. In addition, Bmi1-positive cells are located at the bottom of crypts and have features of a stem cell marker [103]. Mouse telomerase reverse transcriptase (mTert) marks slowly cycling intestinal stem cells [104]. In addition, a sensitive model was recently developed to obtain a quantitative comprehensive *in situ* description of the location of stem-cell markers at the single-transcript level. In this model, co-expression of *Lgr5*, *Bmi1*, *Dcamkl1,* and *mTert* genes were detected at the crypt base [105].

To understand the role of stem cells in intestinal cancer stem cell initiation, an inducible Lgr5-EGFP-IRES-creERT2 cassette was used to delete the *APC* gene in crypt base columnar stem cells. After tamoxifen administration, β-catenin accumulation was observed in isolated Lgr5-EGFP+ stem cells and these transformed cells quickly became associated with clusters of β-catenin-expressing progeny migrating up the crypt [106].

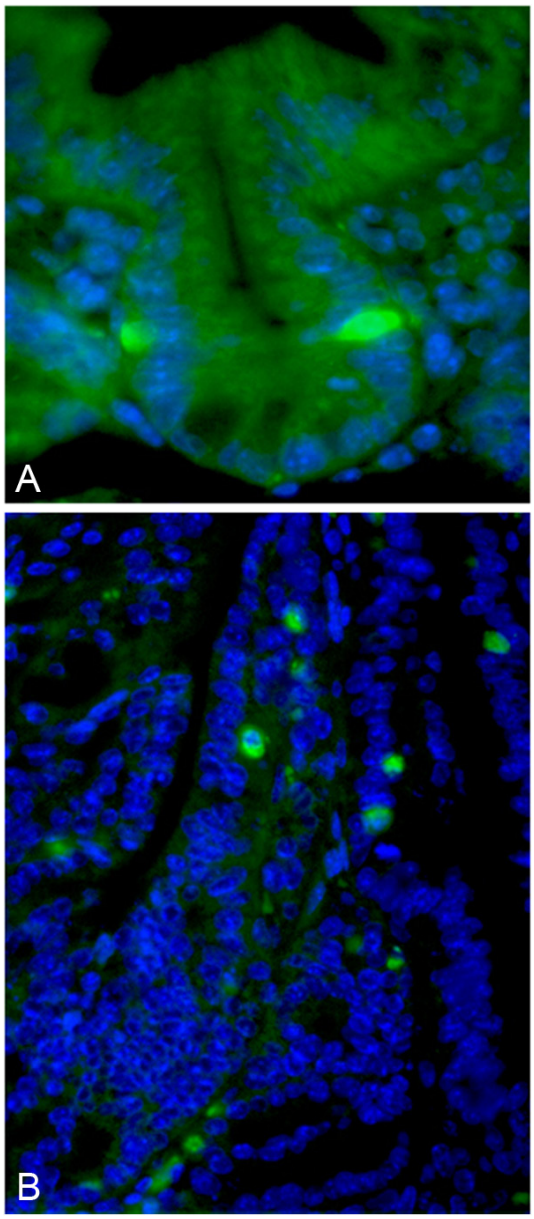

Figure 2. Prom1 immunofluroescsnese staining of normal crypt and polyp. A: Notice rare positive (green color) staining at the +4 crypt position. B: Notice expansion of the positive cells (green color) within the polyp.

Conclusion

In summary, genetically modified mouse models continue to play an important role in understanding the genome's role in formation, progression, and inhibition of CRC. These models also offer robust methods to study naturally occurring and synthetic compounds for the inhibition or treatment of CRC. The spontaneous and chemically induced models are often used to study effect on the treatment or prevention of CRC formation. The mouse models for invasion and metastasis are useful for understanding the pathogenesis of progression and metastasis of CRC. Other models have been used to address specific questions like how aging, or alcohol consumption, or diabetes affect the risk of developing CRC. Finally, the recent advances in identifying roles for intestinal stem cells in CRC provide new insights for understanding the formation and inhibition of CRC.

With the advances in genomic sequencing of human CRC, the functional analysis of identified genomic alterations is necessary to distinguish driver gene alterations from passenger alterations in CRC [107, 108]. Therefore developing mouse models and related methods to discover and validate candidate genomic CRC drivers that play an important role in human CRC is urgently needed for translation of CRC sequencing advances into new, safe and effective chemopreventives and treatments.

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Disclosure of conflict of interest

All the authors do not have any conflict of interest.

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