Chapter 2
Mouse Models of Intestinal Cancer

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Introduction

Intestinal cancers are a category of heterogeneous tumors that occur sporadically or through inherited susceptibility, each characterized by genetic alterations affecting a number of molecular pathways. As a result of this complexity, numerous genetically engineered mice (GEM) have been generated to model different genetic, morphologic, or clinical features of intestinal cancer. Mouse models of intestinal cancer can be broadly divided into six groups based on the underlying signaling pathway disrupted or by the means with which tumors were induced: Wnt-related GEM; GEM associated with alterations in TGF-beta (β) signaling; mismatch repair-deficient GEM; immune-deficient mice; carcinogen-treated mice; and others that do not neatly fit into the aforementioned categories. Although differences have been noted in lesions arising in these broadly grouped genetic and other models, some characteristics are shared. Adenomas are the most common lesion in mouse models of intestinal cancer. Unlike humans, lesions can be present throughout the intestinal tract, with no predilection for the colon. Invasion and metastasis occur rarely. This chapter will summarize the findings from most of the available mouse models of intestinal cancer.

GEM and the Wnt Signaling Pathway

Min/+ and Related Mice

The *APC* gene was initially identified by positional cloning as the disease gene for familial adenomatous polyposis coli (FAP) and was subsequently found to

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J.D. Potter and N.M Lindor (eds.), *Genetics of Colorectal Cancer*,
DOI: 10.1007/978-0-387-09568-4_3,

be mutated in the majority of sporadic colorectal tumors (Groden et al. 1991; Kinzler et al. 1991). As a result of these initial findings, research has focused on understanding the cellular pathways in which APC participates and how deregulation of these pathways can lead to tumorigenesis. Although APC has many roles in the cell, regulation of the protein β-catenin is the primary function. APC is part of a protein complex that phosphorylates β-catenin, marking it for ubiquitination and proteolytic degradation. In the absence of APC or in the presence of a Wnt signal, β-catenin is stabilized and shuttled to the nucleus where it can transcriptionally alter the expression of downstream Wnt target genes. Therefore, when *APC* is disabled by mutations, the Wnt signaling pathway is constitutively activated, allowing for uncontrolled growth and tumor progression. Many *APC* mutants have been identified in persons with FAP, in whom genotype/phenotype correlations are well known (reviewed by Nieuwenhuis and Vasen 2007). *APC* mutations in the first or last third of the gene are associated with an attenuated polyposis, characterized by late onset and a small number of adenomas. Conversely, mutations in the central region of the gene correlate with a severe phenotype with thousands of adenomas developing at a young age. To investigate these observations more closely and to gain insight into the mechanism of disease onset, several mutant *Apc* mouse models have been created.

Perhaps the most widely used GEM model of gastrointestinal (GI) tumorigenesis is the $Apc^{Min/+}$ mouse. Thus, it has become the basis of comparison for other GI cancer mouse models (Moser et al. 1990). The Apc^{Min} (multiple intestinal neoplasia) allele carries an ethylnitrosourea (ENU)-induced nonsense mutation at codon 850 (Su et al. 1992), which leads to embryonic lethality in homozygote animals. Most studies use the heterozygote mice, $Apc^{Min/+}$, which typically live 4 months (Moser et al. 1990). Mice carrying the Apc^{Min} allele on the *C57BL/6* background develop an average of 24 polyps per mouse in the small intestine and five per mouse in the colon by 4 months of age. Most polyps are adenomas, with none progressing to invasive adenocarcinoma and, as expected for adenomas, tumors in the $Apc^{Min/+}$ have been found not to metastasize.

Given the advances in gene-knockout technologies, several other *Apc* mutant mice have been created. The importance of their study stems from the knowledge that several mutations have been detected within the *APC* gene in human tumor samples and in persons with FAP, which may underlie variations in disease progression among patients; the results from these subsequent mouse models indicate that, indeed, not all *Apc* mutations are equivalent. The precise location and the type of mutation within *Apc* dictate the degree of tumor susceptibility, which is probably the result of the multifunctional nature of Apc and its contribution to various cellular pathways.

$Apc^{716/+}$ mice harbor a truncating mutation at codon 716 and, like $Apc^{Min/+}$ mice, develop polyps mainly in the small intestine (Oshima et al. 1995); they develop an average of 300 polyps as early as 3 weeks of age (Oshima et al. 1995), and typically have a reduced lifespan compared to $Apc^{Min/+}$ mice, even on the same *C57BL/6* background. $Apc^{1309/+}$ mice have a truncating mutation at codon 1309 (Quesada et al. 1998). These mice typically develop an average of 34 adenomas by 14 weeks of age, a slightly higher incidence of polyp formation than the $Apc^{Min/+}$ mouse, and

a lower incidence than the *Apc*$^{716/+}$ mouse. Again, these polyps are predominantly found in the small intestine. This GEM is particularly interesting because codon 1309 is the most frequently mutated residue in persons with FAP with severe polyposis (Nagase and Nakamura 1993). A 5-base-pair deletion results in truncation of APC three codons downstream from the mutation.

Apc$^{1638/+}$ mice carry an allele with a mutation at codon 1638 resulting in truncation of Apc; these mice also develop polyps mainly in the small intestine (Fodde et al. 1994; Oshima et al. 1995). However, *Apc*$^{1638/+}$ mice form only 3–5 tumors by 3.5 months of age and typically live 1 year (Yang et al. 1997). A modification to the *Apc*^{1638} allele design was engineered to produce a stable Apc protein (Smits et al. 1999). This allele, *Apc*^{1638T}, still encodes some of the β-catenin-binding motifs but lacks the C-terminal portion of Apc necessary for its interaction with other proteins important for growth control. On a mixed background, B6/129Ola, homozygosity for the *Apc*^{1638T} mutation does not result in embryonic lethality but leads to a number of phenotypic abnormalities in adult animals, including growth retardation and nipple-associated cysts. The same mutation on a B6 background leads to reduced postnatal survival. Heterozygous *Apc*^{1638T} mice are normal.

Given the predilection for intestinal tumors to form in the mouse models, conditional *Apc* mutant mice have been developed to investigate the initiation stage of intestinal adenoma formation (Shibata et al. 1997). *Apc*$^{580/+}$ mutant mice, on a mixed *129/BL6* background, carry an allele with *loxP* (or flox) sites flanking exon 14. Colonic introduction of a recombinant adenovirus expressing Cre recombinase, driven by the *SR-alpha* promoter into *Apc*$^{580/+}$ mice, induces a frameshift mutation at codon 580. Over 80% of homozygous animals (20 of 24 animals) have an average of 6.7 colonic adenomas 4 weeks after infection. No tumors were detected in either heterozygous or wild-type animals. Five of six homozygous mutants allowed to live after adenoviral infection survived over 1 year. Analysis of these animals showed invasion into the submucosal layer by tumor cells and hence progression to adenocarcinoma. Recent studies have used colon-specific promoters to drive Cre expression and generate colon tumors in the mouse, rather than the small intestinal distributions seen in the more established models.

Hypomorphic *Apc* mice were created in a study by Li et al. whereby the expression level of Apc was reduced to 10–20% of the wild-type *Apc* (Li et al. 2005). Polyp formation was reduced compared to the *Apc*$^{716/+}$ mice. These results argue that there is a threshold level (15% of wild type) of Apc expression that is required for proper growth control.

More recent studies have focused on conditionally inactivating *Apc* in order to understand the precise mechanism by which Wnt activation leads to polyps. Two mouse models have been generated, both making use of the *loxP* system. *Apc* is modified by an inducible *Cyp1A-Cre* transgene (Sansom et al. 2003) in one model, whereas the other uses a tamoxifen-regulated intestinal-specific *Villin-CreER* transgene (Andreu et al. 2005). Both studies reported that inactivation of *Apc* led to the rapid translocation of β-catenin to the nucleus and subsequent changes in the appearance of enterocytes and intestinal crypts. Following *Apc* loss, many of the epithelial cells along the crypt-villus axis enter S-phase. These studies

establish that a single event, loss of *Apc*, is enough to promote early phenotypic changes in the crypt.

β-Catenin Transgenic Mice

β-Catenin is a multifunctional protein component of the Wnt signal transduction pathway (Sheng et al. 1998). It is also a mediator of cell adhesion through its interaction with cadherins. It is known that β-catenin rapidly translocates to the nucleus upon loss of APC, resulting in transcriptional alteration of downstream target genes involved with proliferation, apoptosis, and cell-cycle regulation. Therefore, overexpressed β-catenin is considered oncogenic, resulting from either a nonfunctional *APC* gene or a gain of function mutation within β-catenin. The finding of mutations in the β-catenin gene (*CTNNB1*) in human colon cancer cell lines, with no detectable mutations in *APC*, has led to the hypothesis that β-catenin acts as an oncogene in the development of intestinal neoplasia (Iwao et al. 1998; Morin et al. 1997; Sparks et al. 1998). Several groups have investigated the role of activated β-catenin using in vivo mouse models.

Wong et al. designed a transgenic mouse expressing a human β-catenin N-terminal truncation mutant (N89β-catenin) in the intestine driven by the fatty acid-binding protein gene (*Fabp1*) promoter (Wong et al. 1998). The absence of GSK-3β phosphorylation sites, normally targeting degradation of β-catenin, was associated with a longer half-life than wild-type β-catenin in cell culture studies (Aberle et al. 1997; Cadigan and Nusse 1997; Miller and Moon 1996; Munemitsu et al. 1996; Yost et al. 1996). The deletion of these amino acids did not affect the ability of β-catenin to interact with E-cadherin, α-catenin, or Tcf (Wong et al. 1998). Although there were some changes in the architecture of the villi and an increase in the rate of cell division within undifferentiated cells in the crypts of Liberkühn, no dysplasia was detected in the transgenic mice.

Romagnolo et al. generated a similar β-catenin transgenic mouse, but had dramatically different results (Romagnolo et al. 1999). This transgenic mouse expressed activated β-catenin in the epithelial cells of the intestine using a transgene with an N-terminal truncation, N131β-catenin, lacking both the GSK-3β phosphorylation site, important for protein stabilization, and the α-catenin-binding site, necessary for adhesive properties of β-catenin (Barth et al. 1997; Hulsken et al. 1994). A calbindin-D9K promoter and its regulatory sequences, active in differentiated epithelial cells of the villi and the kidney (Colnot et al. 1998; Romagnolo et al. 1996), and the enhancer of the adolase B gene were used to drive expression. Overexpression of N131β-catenin resulted in small intestine adenomas by 3–4 weeks of age. The intestines were characterized by multifocal dysplastic lesions and a 3- to 4-fold higher number of apoptotic cells than in nontransgenic mice. Further analysis of these animals was inhibited by mortality from polycystic kidney disease.

A third β-catenin GEM was generated in which exon 3 could be deleted by inducible homologous recombination using *loxP* sites (Harada et al. 1999). The loss

of exon 3 does not alter the frame of the RNA. In this model, nearly 3,000 adenomas develop by 3 weeks of age, primarily in the duodenum and jejunum and with little involvement of the ileum, cecum, or colon. *Fabp1* regulatory regions were used to express Cre, resulting in a mutant β-catenin driven by its own enhancer and promoter. Differences in promoters, transgene copy numbers or locations, mouse strains, and or different types of dominant mutations may explain the dramatic differences in these three mouse models. Each, however, underscores the importance of the Wnt signaling pathway in mouse GI tumorigenesis.

Genes that Modify the Wnt Pathway

Cyclo-oxygenases (Cox) 1 and 2 are the key enzymes in prostanoid production and are the targets of nonsteroidal anti-inflammatory drugs (NSAIDs) and aspirin (Vane 1971, 1994). Both Cox-1 and Cox-2 enzymes convert arachidonic acid to prostaglandin G2 and then to prostaglandin H2 (DeWitt and Smith 1988; Hemler and Lands 1976; Miyamoto et al. 1976). Cox-1 is constitutively expressed in several mammalian tissues, whereas the distribution of Cox-2 expression is restricted to inflammatory cells such as monocytes and macrophages upon stimulation by cytokines, mitogens, serum, and endotoxins (Lee et al. 1992; Maier et al. 1990; O'Banion et al. 1992; O'Neill and Ford-Hutchinson 1993) Cyclo-oxygenase-2 (Cox-2) is expressed at early stages of adenoma formation, suggesting its importance as a therapeutic target. Cox-1 seems to work with Cox-2 in adenoma development by producing prostaglandin E2 (PGE_2) and stimulating angiogenesis (Takeda et al. 2003). Introduction of a *Cox-2* deletion onto the $Apc^{Min/+}$ background dramatically decreases tumor number (Oshima et al. 1996). The combination of *Cox-2* deletion with the $Apc^{716/+}$ mutation also leads to a dramatic decrease in the number and size of tumors. Not surprisingly, introduction of a *Cox-1* mutation to the $Apc^{Min/+}$ mouse reduces the number and size of tumors to about 80% of the reduction seen in *Cox-2;Apc* mutant mice (Chulada et al. 2000). As might be predicted, treatment of $Apc^{Min/+}$ mice with PGE_2 increases the number and size of intestinal adenomas (Wang et al. 2004). Clinical trials are ongoing to investigate Cox-2 inhibitors in FAP (Higuchi et al. 2003; Steinbach et al. 2000). See also Chap. 5.

To probe the arachidonic acid cascade for its contribution to intestinal tumorigenesis, several other compound mice were developed. Cytosolic phospholipase A_2 ($cPLA_2$) is one of the key enzymes responsible for cleavage of arachidonic acid, a substrate of Cox, from membrane phospholipids. Knockout of $cPla_2$ in $Apc^{716/+}$ mice reduces tumor number (Takaku et al. 2000). Additional studies have investigated the role of the G-protein coupled receptor Ep_2, which binds PGE_2, in tumor formation in $Apc^{Min/+}$ mice. Double heterozygotes displayed a marked reduction of tumor number (Sonoshita et al. 2001). PGE_2 indirectly transactivates peroxisome-proliferator activity receptor delta (PPARδ) through PI3K/Akt signaling. Deletion of *PPARδ* in $Apc^{Min/+}$ mice treated with PGE_2 negated the increase of intestinal adenomas found with treatment of PGE_2 alone (Wang et al. 2004).

Methylation contributes to the silencing of many genes which, in turn, leads to deleterious phenotypic changes depending on which genes have been affected. DNA methyltransferase 1 (DNMT1) is one of the enzymes responsible for methylating cytidine residues within genes. Mutations in the *Dnmt1* gene, in combination with an enzyme inhibitor, reduced the tumor number in $Apc^{Min/+}$ mice from one hundred to two or less (Laird et al. 1995). Mutation in the *Mbd2* gene, encoding a methyl-CpG-binding repressor, also reduced tumor numbers in $Apc^{Min/+}$ mice (Laird et al. 1995; Sansom et al. 2003). These results suggest a role for methylation in the development of intestinal polyposis.

Other modifier genes include the reqQ-like DNA helicase gene, *BLM*, which, when mutated, is responsible for the development of Bloom syndrome. When *Blm* heterozygous mice were bred to $Apc^{Min/+}$ mice, an increase in adenomas was observed as well as a change in the degree of tumor dysplasia (Goss et al. 2002; Luo et al. 2000). Mutation of the gene encoding the matrix metalloproteinases matrilysin (Mmp7), implicated in cancer invasion and metastasis, also reduces tumor number in $Apc^{Min/+}$ mice (Wilson et al. 1997).

GEM and the TGFβ Signaling Pathway

TGFβ1$^{-/-}$ *and Related Mice*

The transforming growth factor β (TGFβ) pathway plays an important role in both human and murine colon cancer. TGFβ controls cell growth, regulates epithelial cell differentiation and cell matrix interaction, and protects the epithelium from genetic damage caused by inflammatory cells (Brandes et al. 1991; Kulkarni et al. 1993; Roberts et al. 1992; Shull et al. 1992; Wahl et al. 1987). The multifunctional nature of the TGFβ family suggests several mechanisms by which defects in TGFβ signaling can lead to initiation, promotion, or progression of cancer. This hypothesis is supported by evidence from tumor-derived human colon cancer cell lines which are frequently resistant to the growth-inhibitory effects of TGFβ1 (Manning et al. 1991; Mulder et al. 1988). Mutations have been detected in *TGFβR2* in both sporadic and inherited colon cancers (Markowitz et al. 1995; Parsons et al. 1995). Additionally, inactivating mutations in *SMAD2* and *SMAD4*, two members of the family of intracellular proteins responsible for transducing signals from the activated TGFβ receptors, are present in many human colon cancers (Eppert et al. 1996; Takagi et al. 1996; Thiagalingam et al. 1996).

Inactivation of Tgfβ1 in mice results in autoimmune disease and death before 1 month of age. In order to study the role of Tgfβ1 in the development and progression of GI cancer, the *Tgfβ1*$^{-/-}$ mouse strain was crossed onto the immunodeficient *Rag2*$^{-/-}$ (Engle et al. 1999). Tgfβ1 deficiency (+/− or −/−) on the *Rag2*$^{-/-}$ background leads to cecal and colonic neoplasms (Engle et al. 1999). A marked increase in tumor incidence and severity was observed in the *Tgfβ1*$^{-/-}$ mice: adenomas are

detectable at 2 months of age and carcinomas are detectable at 3–6 months with 100% penetrance. The carcinomas show no mutations of *Apc*, *Ras*, or *Ctnnb1*, which suggests that the tumor-suppressive function of Tgfβ is independent of other known signaling pathways disrupted in intestinal cancers. Notably, many of the tumors have a mucinous histopathology.

Smad$^{-/-}$ Mice

Signaling by Tgfβ family ligands is mediated by the Smad family of intracellular proteins (Graff et al. 1996). The Smad proteins are the core of the Tgfβ pathway through their translation of cellular signals into responses. There are eight Smad proteins encoded by the human and mouse genomes, five of which act as substrates for the Tgfβ family of receptors (Massague 1998). Smads 1, 2, 3, 5, and 8 are commonly referred to as receptor-regulated Smads (RSmads). Smad4, also called Co-Smad, serves as a common partner for all Smads. Smads 6 and 7 are inhibitory and serve as decoys by interfering with Smad-receptor and Smad-Smad interactions. Smads undergo a continuous nuclear-cytoplasmic shuttling cycle. Phosphorylation leads to nuclear accumulation by destabilizing the RSmad interaction with cytoplasmic anchors and increases their affinity for nuclear factors (Shi and Massague 2003; Xu and Massague 2004). This then allows Smads to transcriptionally regulate Tgfβ downstream targets. Dephosphorylation has the opposite effect, sequestering Smads to the cytoplasm (Inman et al. 2002). Because Tgfβ signaling affects cell division, differentiation, migration, adhesion, organization, and death, and because Smads are the translators of these signals, Smad deregulation could have many deleterious cellular affects. Therefore, several Smad GEM models have been generated, some of which have developed intestinal tract tumors.

Smad2 is 91% homologous to Smad3; however, it differs biologically. Unlike Smad3 and 4, Smad2 does not bind directly to DNA and has a unique thirty amino acid region absent from other Smad proteins (Dennler et al. 1998; Jonk et al. 1998; Kim et al. 1997; Labbe et al. 1998; Yingling et al. 1997; Zawel et al. 1998). Pertinent to human GI tumors, *SMAD2* is the only RSMAD for which mutations have been associated with colorectal cancer (Eppert et al. 1996). To investigate whether Smad2 can act as a tumor suppressor, knockout mice were generated. Homozygous deletion of *Smad2* results in embryonic lethality at day 8.5 (Heyer et al. 1999; Nomura and Li 1998; Waldrip et al. 1998; Weinstein et al. 1998). Heterozygous mice (*Smad2*$^{+/-}$) had no abnormalities when aged to 1.5 years. Hamamoto et al. generated double heterozygous mice that carried *Apc* and *Smad2* null alleles (Hamamoto et al. 2002). Inactivation of *Smad2* in heterozygous *Apc* mutant mice did not change the total number of intestinal tumors but decreased the time to death from intestinal obstruction due to extremely large tumors. Additionally, these mice developed multiple invasive cancers not observed in *Apc* heterozygotes. These results suggest that deletion of *Smad2* alone does not initiate tumor formation, but accelerates progression of tumors initiated by loss of *Apc*.

Unlike other Tgfβ-family null mice, *Smad3* null mice are viable and reasonably healthy. They develop intestinal adenomas that sometimes progress to adenocarcinoma (Zhu et al. 1998). The *Smad3* mutant allele was generated by homologous recombination and established in both *129/Sv* and *129/Sv C57BL/6* mixed background mice. Most tumors are mucinous. Metastatic spread (uncommon in mouse models) was detected in a small number of animals. There was great variability in the time-course of disease, but tumors were smaller and less aggressive in mixed background mice. These in vivo studies have defined a new role for Smad3 as a tumor suppressor protein in the intestine. *Smad3* mutant mice display many of the histopathological stages observed in human colon cancer progression; to date, no *SMAD3* mutations have been detected in human colorectal cancers.

SMAD4 was initially cloned as a tumor suppressor that is mutated in more than 50% of human pancreatic cancers (Hahn et al. 1996). *SMAD4* is also mutated in more than 30% of human sporadic colon cancers; germline mutations are associated with familial juvenile polyposis (Friedl et al. 1999; Nagatake et al. 1996). *Smad4* null mice die at embryonic day 6.5; therefore, *Smad4*$^{+/-}$ mice are often used for tumorigenesis studies (Sirard et al. 1998; Yang et al. 1998). Polyps can be detected in the fundus and antrum of the stomach of *Smad4*$^{+/-}$ mice; polyps found in the antrum can develop into adenocarcinoma with aging (Xu et al. 2000). Polyps can also be found in the duodenum and cecum, albeit at a lower frequency. From these studies, it seems reasonable to infer that Smad4 is particularly important for tumor suppression in the stomach. *Smad4*$^{+/-}$ mice have also been bred with *Apc*$^{+/-}$ mice; double heterozygotes develop intestinal adenocarcinomas that lack wild-type alleles at both loci (Takaku et al. 1998).

GEM and DNA Mismatch Repair

Individuals with Lynch Syndrome (see Chap. 6) carry heterozygous germline mutations in one of six DNA mismatch repair (MMR) genes. Tumors that arise have typically lost the wild-type copy of the gene through somatic events and are characterized by microsatellite instability (MSI). The mammalian MMR system detects and repairs base substitution or small nucleotide insertion/deletion mutations, sends apoptotic signals in response to DNA damage, and suppresses incorrect homologous recombination events. In eukaryotes, initiation of the repair process requires three different MutS yeast homologs: MSH2, MSH3, and MSH6. MSH2 and MSH6 form a heterodimeric complex that initiates base-base mispairing as well as single base insertion/deletion mispairs. The MSH2-MSH3 heterodimeric complex repairs larger insertion/deletion mispairs of 2–4 bases. Both complexes require interaction with eukaryotic MutL homologs to activate subsequent repair events. The four MutL homologs are: MLH1, PMS1, PMS2, and MLH3. Three heterodimeric complexes form: MLH1–PMS2 to provide the primary function for mitotic MMR, MLH1–PMS1, and MLH1–MLH3. The MLH1–PMS2 complex also interacts with the two MutS complexes. The majority of Lynch Syndrome

mutations occur in three MMR genes, *MLH1*, *MSH2*, or *MSH6*, although in rare cases mutation in other MMR genes have been identified. Mouse lines carrying mutations all of the *MutS* and *MutL* genes have been generated, some of which have resulted in phenotypes similar to Lynch Syndrome.

Deletion of *Msh2*, *Msh6*, or *Mlh1* results in intestinal tumors, although there is great variation of the phenotypes. Given that Msh2 participates in two "MutS" complexes, it is not surprising that the *Msh2*$^{-/-}$ mice have a severe phenotype (de Wind et al. 1995; Reitmair et al. 1995). Fifty percent of *Msh2*$^{-/-}$ mice die by 6 months and all animals by 1 year. Mice develop adenomas of the small intestine and, after 6 months, adenocarcinoma (Reitmair et al. 1996b). *Msh3*$^{-/-}$ mice develop tumors very late in life, with an overall tumor spectrum somewhat similar to wild-type animals. This mild phenotype may be the result of only moderate repair defects being caused by deletion of *Msh3*, or by compensation by intact Msh2 and Msh6. These data are reminiscent of the absence of detectable *MSH3* mutations in Lynch Syndrome families. *Msh6*$^{-/-}$ mice develop a similar tumor spectrum of intestinal adenomas and adenocarcinomas as the *Msh2*$^{-/-}$ mice but with a delayed onset and subsequent increased survival (up to 16 months of age) (Edelmann et al. 1997). This delayed onset of tumor formation is attributed to the impairment of the repair of base-base mismatches, but retention of the 2- to 4-base-pair insertion/deletion repair. Also, as a result of this retention of 2- to 4-base-pair insertion/deletion repair, the MSI phenotype in tumors is absent (de Wind et al. 1999; Edelmann et al. 2000). Given the redundancy in function between MMR genes, compound-knockout mice have also been generated. Inactivation of both *Msh3* and *Msh6* in mice is associated with adenocarcinoma of the small intestine and decreased survival compared to the single-gene-inactivation controls. These phenotypes are more similar to *Msh2*$^{-/-}$ mice.

Mutation of *Mlh1* results in a severe phenotype and a markedly reduced lifespan (6 months) similar to *Msh2*$^{-/-}$ mice (Baker et al. 1996; Edelmann et al. 1996, 1999; Prolla et al. 1998). Intestinal adenocarcinoma, skin tumors, and T-cell lymphomas have also been detected. As a result of the complete ablation of repair mechanisms in *Mlh1*$^{-/-}$ mice, MSI is a characteristic of their tumors.

Because the lifespan of many homozygous MMR mice is markedly shortened by aggressive lymphomas, studies of spontaneous intestinal tumors are more complicated. To circumvent this, intestinal tumorigenesis can be accelerated by breeding homozygous mutant MMR mice to carry an *Apc* mutation. *Msh3*, *Msh6*, *Mlh1*, *Pms2*, and *Msh3/Msh6* deficient mice have all been bred with mutant *Apc* mice (Baker et al. 1998; Edelmann et al. 1999; Kuraguchi et al. 2001; Reitmair et al. 1996a; Wei et al. 2002). In each case, there is a significant increase in tumor number and a consequent decreased lifespan compared to controls.

More recent studies of the role of MMR genes in intestinal tumor formation have shifted to knock-in allele designs, to analyze individual Lynch Syndrome mutations. Often these are missense mutations, which have quite different outcomes than gene deletions. The first of the knock-in MMR mice, *Msh2*^{GA}, carries a mutation at codon 674 (glycine to alanine) in the *Msh2* coding region (Lin et al. 2004). This mutation affects a conserved ATPase domain of Msh2 that is crucial for initiation of repair by MutS homologs (Alani et al. 1997; Drotschmann et al. 1999;

Wu and Marinus 1994). Analysis of cells from *Msh2*$^{GA/GA}$ mice showed that, although apoptotic responses were comparable to wild-type cells, ATP-mediated mismatch release was impaired, similar to *Msh2*$^{-/-}$ cells. This repair defect results in cancer predisposition in vivo that is similar to *Msh2*$^{-/-}$ mice: all *Msh2*$^{GA/GA}$ mice succumb to lymphoid or intestinal tumors by 1 year. The delayed onset of cancer in *Msh*$^{GA/GA}$ mice compared to *Msh2*$^{-/-}$ mice indicates that the remaining functional apoptotic response can stall the onset of tumorigenesis.

Another knock-in mouse model carries a mutation at codon 1217 (threonine to aspartate) in the *Msh6* gene (Yang et al. 2004). The *Msh6*^{TD} mutation impairs ATP-binding or its processing steps in the repair process (Hess et al. 2002). Studies from mutant cell extracts found that the DNA damage response and mismatch-binding capacity was not impaired; however, cells were deficient in ATP-induced mismatch release. *Msh6*$^{TD/TD}$ cell extracts were deficient in repair of both base substitutions and dinucleotide insertion/deletion loops, in contrast to *Msh6*$^{-/-}$ cell extracts that were not. *Msh6*$^{TD/TD}$ mice had a cancer phenotype similar to *Msh6*$^{-/-}$ mice, although they were characterized by a delayed tumor onset.

Two additional genes involved with DNA MMR, Flap endonuclease 1 (Fen1) and exonuclease 1 (Exo1) have been studied to determine their potential contribution in GI tumors. Fen1 was found to promote tumor progression when combined with *Apc*$^{1638N/+}$ (Kucherlapati et al. 2002). *Exo1* in combination with *Apc*$^{1638N/+}$ showed a moderate increase in tumor incidence and multiplicity when compared to *Apc*$^{1638N/+}$ siblings (Kucherlapati et al. 2007). These mice have decreased median survival, which is due to infections resulting from an impaired immune response. Triple mutant mice *Apc*$^{1638N/+}$ *Exo1Fen1* mice survive longer and display invasive GI tumors with MSI.

Immune-Deficient GEM

Inflammatory bowel disease (IBD) in humans has been divided into two major forms, ulcerative colitis (UC) and Crohn's disease (Podolsky 1991). Although the underlying mechanisms of IBD development are not fully understood, it certainly involves an immune response to intestinal bacterial and subsequent inflammation. IBD very markedly increases the risk of GI cancer above that of the general population (Eaden et al. 2001; Itzkowitz 1997). The risk of colitis-associated colon cancer (CACC) among patients is related to the severity of colitis. Although the pathogenesis of CACC remains unclear, it is characterized by an increased rate of epithelial proliferation associated with repetitive cycles of inflammation, tissue damage, and regeneration. Various immune-deficient mouse models have been generated to model IBD and are commonly characterized by inflammation of the large bowel with proliferative lesions that occasionally progress to adenocarcinoma. Many of these models, when rederived in a germ-free (bacteria-free and virus-free) environment, have a less severe phenotype than those maintained under normal conditions, suggesting roles for both pathogens and inflammatory responses in tumor susceptibility.

Cytokine-Deficient Mice

Il-2 was initially believed critical for the proliferation of T-cells in vitro; however, in vivo studies indicate that this is not the case (Hatakeyama et al. 1989). More recent studies point to a newly defined role for Il-2 in the development and homeostasis of regulatory T-cells (Burchill et al. 2007). *Il-2*$^{-/-}$ mice develops symptoms of UC (Sadlack et al. 1993). Half of the mice die within 9 weeks from severe anemia while the rest die within 6 months due to wasting. None of these mice develop GI cancer. When *Il2*$^{-/-}$ mice are crossed with *β2-microglobulin* null mice, 32% develop colonic adenocarcinoma between 6 and 12 months of age (Simpson et al. 1995; Sohn et al. 2001). The late onset of adenocarcinoma suggests that prolonged chronic inflammation may be required for tumorigenesis. All tumors from these compound mice carry mutations in *Apc*; more than half carry *p53* mutations. *Il10*$^{-/-}$ mice develop symptoms characteristic of Crohn's disease; 60% of mice develop colonic adenocarcinoma (Berg et al. 1996; Kuhn et al. 1993). These adenocarcinomas are not associated with mutations in genes typical of GI cancer, such as *p53*, *Apc*, *Msh2*, or *K-ras*. Gαi2-knockout mice develop inflammation limited to the colon; 31% develop neoplasms throughout the colon anywhere from 15 to 36 weeks (Rudolph et al. 1995). A recent study by Edwards et al. (2008) found that the Gi2-α$^{-/-}$ colonic epithelium is hyperproliferative even before the onset of colitis and resistant to induction of apoptosis. They concluded from their study that Gi2α is a direct negative regulator of colonic epithelium. Seventy-five percent of these mice die by 28 weeks, preventing long-term studies. Recent work by Ko et al. (2008) investigated the effect of deletion of *IL-4Rα* gene on AOM-induced aberrant crypt foci number and size in Balb/c mice. IL-4Rα-dependent signaling was found to have a protective, anti-neoplastic role during the post-initiation phase of AOM-induced colorectal carcinogenesis in Balb/c mice. Deletion of the *IL-4Rα* gene led to high serum levels of IL-4. Additionally IL-13, which can signal through the IL-4Rα receptor normally, instead signals via the IL-13Rα2 receptor leading to induction of TGFβ, which has pro-tumororigenic activity at early stages of intestinal tumorigenesis.

Mucin-Deficient Mice

Mucins are highly glycosylated proteins that are the major component of the mucus that lubricates and protects underlying intestinal epithelium (Gendler and Spicer 1995). Alterations of mucin expression and glycosylation have been detected in human colon cancer, but their role in tumorigenesis is not well understood (Kim et al. 1996). MUC2 is the most abundant secreted gastrointestinal apomucin (Kim and Gum 1995; van Klinken et al. 1999). *Muc2*-deficient GEM were generated by replacing exons 2–4 of *Muc2* with a *PGK-neo* cassette (Velcich et al. 2002). The resultant *Muc2*$^{-/-}$ mice were characterized by the absence of recognizable goblet cells throughout the intestine. By 12 months, 65% of *Muc2*$^{-/-}$ mice

had developed adenomas with an average of >1.5 tumors per mouse. Adenomas occurred in the small and large intestine, as well as the rectum. In older mice, adenomas spontaneously progressed to adenocarcinoma. The formation of rectal tumors distinguishes the *Muc2*$^{-/-}$ mouse from many of the other mice presented here and may reflect the disorganized inflammatory processes occurring in response to the loss of normal mucins. To understand the impact of the MUC2 and APC interaction on tumorigenesis, Yang et al. (2008) crossed *Muc2*$^{-/-}$ mice with both the *Apc*$^{1638N/+}$ and *Apc*$^{Min/+}$ mice respectively. They found that introduction of *Muc2* into *Apc*$^{1638N/+}$ and *Apc*$^{Min/+}$ greatly increased transformation induced by the *Apc* mutation and significantly shifted tumor development toward the colon as a function of *Muc2* gene dosage.

MUC1 is an epithelial cell glycoprotein overexpressed and hypoglycosylated in the majority of human adenocarcinomas; its expression is also increased in IBD (Vlad et al. 2004; Campbell et al. 2001; Rhodes 1996). *Il10*$^{-/-}$ mice display some of the characteristics of human IBD; however, this mouse model lacks Muc1 expression. To explore the importance of MUC1 in IBD, Beatty et al. (2007) introduced the human MUC1 molecule into the *Il10*$^{-/-}$ mouse model. These mice develop IBD, but the disease is characterized by an earlier age of onset, greater inflammation, and higher number of colon cancers than *Il10*$^{-/-}$ controls.

Carcinogen-Induced Models of Intestinal Tumorigenesis

Intestinal tumors can be induced in rodents by a number of carcinogens including *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (Schoental and Bensted 1969), *N*-ethyl-*N*′-nitro-*N*-nitrosoguanidine, 1,2-dimethylhydrazine (Colussi et al. 2001), 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline (Fujita et al. 1999), and *N*-methyl-*N*-nitrosourea (Qin et al. 2000). Azoxymethane (AOM), a metabolite of 1,2-dimethylhydrazine (DMH), is the most widely used compound and offers a number of advantages over the parent compound including enhanced potency and chemical stability. In AOM-treated rodents, most intestinal tumors arise in the colon and form grossly visible exophytic polypoid or plaque-like growths. The microscopic appearance of low-grade lesions in these models is similar to human colonic adenomas. There is also evidence that AOM-treated mice may be a useful model for studying metastatic colorectal cancer (Ochiai et al. 2001). Studies of AOM-treated mice have identified some of the molecular abnormalities associated with these tumors and suggest that in many ways they are indistinguishable from tumors initiated by activation of Wnt signaling (Perantoni and Rice 1999; Takahashi et al. 2000; Kaiser et al. 2007). The dramatic differences in tumor number and penetrance associated with AOM-treatment in different mouse strains also highlight the ability of the mouse to model the complexities of genetic background and possibly environment (e.g., intestinal bacteria) and their effects on tumor susceptibility and eventual response to therapy in the human.

Other GEM Models of Intestinal Cancer

$Rb^{MI/MI}$ Mice

In addition to mouse models engineered to perturb known pathways in the development of GI cancer, interesting findings have emerged from mouse models targeting pathways not associated with GI cancer. One of these is the *$Rb^{MI/MI}$* mouse, which carries a knock-in mutation that eliminates the C-terminal caspase-cleavage site of the retinoblastoma (Rb) protein, a known regulator of cell proliferation and cell death (Chau et al. 2002). Apoptosis was attenuated in the intestine of the *$Rb^{MI/MI}$* mice following endotoxic shock; embryo-derived fibroblasts were resistant to apoptosis induced by the type I receptor for tumor necrosis factor (TNFRI) (Chau et al. 2002). These results suggested that caspase cleavage of Rb is required for TNFRI-induced cell death and that the antiapoptotic function of the *$Rb^{MI/MI}$* allele might promote tumor formation when tumor suppression function is altered. Borges et al. (2005) explored this hypothesis by combining the *$Rb^{MI/MI}$* allele with a *p53*-null background. Introduction of *$Rb^{MI/MI}$* statistically significantly increased the incidence of colonic adenomas as well as lymphoma. Colonic tumors are a rare phenotype in *p53*-null mice (Donehower et al. 1995; Jacks et al. 1994); 26% of *$Rb^{MI/MI}$;$p53^{-/-}$* mice developed colonic tumors versus 3% of *$p53^{-/-}$* mice (Borges et al. 2005). In recent studies by Kucherlapati et al. (2008), mice were generated with an *Apc(1638N)* allele, *Rb(tm2brn)* floxed alleles, and a villin-cre transgene (RBVCA) to examine the role of Rb1 in GI tumors. RBVCA mice were found to have reduced median survival due to increased tumor incidence and multiplicity in the cecum and proximal colon. These results indicate that Rb1 may influence the location of the tumor within the GI tract, and that both cecal and duodenal tumors initiate through inactivation of Apc.

PI(3)K-Deficient Mice

Phosphoinositide-3-OH kinases (PI(3)Ks) constitute a family of evolutionarily conserved lipid kinases that regulate numerous fundamental cellular responses, including proliferation, transformation, differentiation, and protection from apoptosis (Leevers et al. 1999; Toker and Cantley 1997). Homozygous gene-targeted deletion of the *p110γ* catalytic subunit of PI(3)K leads to the development of invasive colorectal adenocarcinomas in mice (Sasaki et al. 2000). Epithelial tumors were detected in the colon and represented all stages of histopathology, including tubular and villous adenomas and invasive adenocarcinoma. The large carcinomas demonstrated transmural, local invasion, and metastasis into the peritoneal cavity. No tumors were found in the small intestine, stomach, or other tissues.

Cdx2$^{-/-}$ Mice

Cdx2, one of the mouse homologs of the *Drosophila melanogaster* protein, caudal (Mlodzik and Gehring 1987), is a key transcription factor for intestinal development and differentiation (Beck et al. 1995; Lorentz et al. 1997; Traber and Silberg 1996). Homozygous knockout of the *Cdx2* gene in mice results in embryonic lethality (Chawengsaksophak et al. 1997; Tamai et al. 1999). Ninety percent of *Cdx2*$^{+/-}$ mice develop multiple (up to ten) intestinal adenomas by 3 months of age; these adenomas primarily occur in the proximal colon. To test whether reduced expression of Cdx2 may be responsible for colon tumor progression, the *Cdx2*-knockout allele was introduced into the *Apc*$^{716/+}$ background to generate double heterozygote mice, *Apc*$^{716/+}$;*Cdx2*$^{+/-}$ (Aoki et al. 2003). These mice develop colonic adenomas that are characterized by loss of heterozygosity (LOH) at the *Apc* locus. *Apc*$^{716/+}$; *Cdx2*$^{+/-}$ mice rarely survive more than 30 weeks, preventing the study of malignant progression.

Dominant Negative N-Cadherin Mice

Cadherins are transmembrane glycoproteins that mediate homophilic adhesive interactions between cells (Kemler 1993; Ranscht 1994). Their conserved cytoplasmic domains interact directly with β-catenin or plakoglobin and are essential for linkage to the actin cytoskeleton and for productive cell–cell adhesion (Hinck et al. 1994; Nathke et al. 1994). Control of cell adhesion is important during embryogenesis, and perturbations of cell adhesion are associated with tumor invasion and metastasis. To understand the role of cadherins in intestinal tumorigenesis, Hermiston and Gordon (1995) generated a transgenic mouse line on the *129SV/B6* background that expresses dominant negative N-cadherin in the crypt-villus epithelium of the small intestine using a *Fabp* promoter. By 3 months of age, the mice developed features of Crohn's disease; by 6 months, adenomas; this suggested relationships among the structural integrity of the intestinal epithelium, inflammatory responses, and, ultimately, tumor initiation.

Conclusions

This chapter highlights many of the mouse models currently in use that allow us to learn about the initiation and progression of intestinal cancers. It is important to highlight some considerations concerning mouse models while thinking about such studies. Species, strain, and sex of the mice may affect experimental outcomes. The same gene mutated in two mouse strains may lead to dramatically different phenotypes, with great variation in expressivity and penetrance. Male mice are more susceptible to gastric and hepatic cancers; therefore, studies without male

mice may under-represent these tumors (Rogers and Fox 2004). Additionally, the environment in which mice are bred and housed can affect experimental outcomes. Microbial populations most certainly differ between facilities and perhaps even across rooms and cages and, as described earlier, can affect inflammatory responses and subsequent gastrointestinal disease. Dietary differences also affect tumor susceptibility. However, despite the variables affecting outcome in these long-term in vivo experiments, the ability to simulate the complex germline and somatic alterations that occur in intestinal tumor formation is very powerful. The effects of aging and environmental exposures can also be queried in these complex in vivo systems in order to model human cancer.

Acknowledgments The authors are supported by NIH awards CA-009338 (EMP), CA-98013 (JG), and CA-63517 (JG). For further information or ongoing updates, the NCI website (http://emice.nci.nih.gov/mouse_models/organ_models/gastro_models) on mouse models of cancer in an outstanding resource.

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