GASTROENTEROLOGY 2003;124:762–777

# SPECIAL REPORTS AND REVIEWS

## Pathology of Mouse Models of Intestinal Cancer: Consensus Report and Recommendations

GREGORY P. BOIVIN,* KAY WASHINGTON,† KAN YANG,§ JERROLD M. WARD,‖ THERESA P. PRETLOW,¶ ROBERT RUSSELL,# DAVID G. BESSELSEN,** VIRGINIA L. GODFREY,†† TOM DOETSCHMAN,§§ WILLIAM F. DOVE,‖‖ HENRY C. PITOT,‖‖ RICHARD B. HALBERG,‖‖ STEVEN H. ITZKOWITZ,## JOANNA GRODEN,§§ and ROBERT J. COFFEY¶¶

*Department of Pathology and Laboratory Medicine, University of Cincinnati and Cincinnati Veterans Affairs Medical Center, Cincinnati, Ohio; †Department of Pathology, Vanderbilt University Medical Center, Nashville, Tennessee; §Strang Cancer Research Laboratory at Rockefeller University, New York, New York; ‖National Cancer Institute, Frederick, Maryland; ¶Institute of Pathology, Case Western Reserve University, Cleveland, Ohio; #Lombardi Cancer Center, Georgetown University, Washington, DC; **University Animal Care, University of Arizona, Tucson, Arizona; ††Division of Laboratory Animal Medicine, University of North Carolina, Chapel Hill, North Carolina; §§Department of Molecular Genetics, Biochemistry, and Microbiology, University of Cincinnati College of Medicine, Cincinnati, Ohio; ‖‖McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, Wisconsin; ¶¶Medicine and Cell and Developmental Biology, Vanderbilt University Medical Center and Nashville Veterans Association, Nashville, Tennessee; and ##Division of Gastroenterology, Mount Sinai School of Medicine, New York, New York

High incidence, availability of premalignant lesions, and dominant inheritance of cancer predisposition in up to 15% of cases make colorectal cancer (CRC) one of the more intensively studied human malignancies. Identification of the genetic bases for familial adenomatous polyposis coli and hereditary nonpolyposis CRC has led to the development of mutant and genetically engineered mouse (GEM) models of intestinal neoplasia, such as the *Apc*$^{Min/+}$ mouse and the DNA mismatch repair (MMR) GEM models. In 1999, the National Cancer Institute funded the Mouse Models of Human Cancers Consortium to develop mouse models of human cancer. A gastrointestinal subgroup was formed to generate and characterize mouse models that recapitulate many of the features of human CRC, including germline and somatic cell genetics, histopathology, tumor distribution, and natural progression of the disease.

A panel of 7 pathologists and 4 basic scientists convened at the Jackson Laboratories in Bar Harbor, Maine, to examine examples of mouse models with intestinal neoplasia as part of a Mouse Models of Human Cancers Consortium–sponsored symposium "Mouse Models of Intestinal Neoplasia" and Jackson Laboratories–sponsored workshop "Techniques for Modeling Human Intestinal Cancer in Mice." The goals of the meeting were to describe the morphology of intestinal neoplasia in mouse models, develop standardized nomenclature for these lesions, develop recommendations for histologic handling of intestinal tissues from mouse models, and compare the morphology of intestinal lesions from mice with human colorectal neoplasia. The panel reviewed 83 H&E-stained slides from intestinal tumors taken from 17 different models of colorectal neoplasia, representing many of the commonly available and studied models of murine intestinal cancer. The following is a synopsis of that meeting and a summary of the histopathologic evaluation of more than 30 mouse models of intestinal cancer.

### Mouse Pathology Nomenclature

The committee came to a consensus on recommended nomenclature for intestinal neoplasia in mouse models (Table 1). The nomenclature parallels that used for humans. For a review of the pathology and genetics of human colorectal neoplasia, see the World Health Organization publication of the classification of tumors of the digestive system.[1]

Two major areas of general discussion will be summarized. First, guidelines for nomenclature were developed for microscopic and macroscopic lesions, with the introduction of the term "gastrointestinal intraepithelial neoplasia" (GIN) to represent putative preinvasive neoplastic lesions not grossly visible (Figure 1). GIN is synonymous with atypical hyperplasia, atypia, microadenoma, carci-

*Abbreviations used in this paper:* ACF, aberrant crypt foci; AOM, azoxymethane; CRC, colorectal cancer; GEM, genetically engineered mouse; GIN, gastrointestinal intraepithelial neoplasia; IL, interleukin; MMR, mismatch repair; TGF, transforming growth factor.


0016-5085/03/$30.00
doi:10.1053/gast.2003.50094

**Table 1.** Nomenclature for Histologic Assessment of Intestinal Tumors in the Rodent

| | |
|---|---|
| Hyperplasia | Gross thickening of the mucosa; some growths may be pedunculated; mitosis are always located in the lower two thirds of the mucosa; nuclei lack significant atypia, are basally located, ovoid to round, usually uniformly dark, with occasional visible nucleoli; crypts take on a star-shaped appearance in tangential sections; herniation (pseudoinvasion) of epithelium through the muscularis mucosae may occur. Hyperplasia can be further categorized as diffuse, focal, multifocal, and associated with inflammation (type and severity noted) and also can be graded as to severity. |
| ACF | Microscopically in whole-mount colonic tissue that usually is stained with methylene blue; one or more crypts larger than most crypts in the field, have a thickened layer of epithelial cells that stain more intensely with methylene blue, often have a slit-shaped luminal opening, have an increased pericryptal space, and are elevated from the focal plane of the microscope (ACF are not a histologic diagnosis) |
| GIN | Histologically apparent areas of dysplasia that are not visible grossly (< 0.5–1.0 mm); in human and veterinary pathology, these lesions may be referred to as microadenoma, microcarcinoma, carcinoma in situ, and focal areas of dysplasia; lesions may involve single or multiple glands; is compatible with dysplastic ACF that previously have been identified in the same unembeded tissue |
| Adenoma | Benign, circumscribed neoplasm composed of tubular and/or villous structures lined by dysplastic epithelium; herniation (pseudoinvasion) through the muscularis mucosae may occur; categorized by the following criteria:<br>(1) Macroscopic growth pattern; sessile, broad-based, or pedunculated (polypoid); attached by a stalk<br>(2) Histologic type: tubular, at least 75% of adenoma composed of branching tubules in lamina propria (usually pedunculated); villous, at least 75% of adenoma composed of leaf- or finger-like processes of lamina propria covered by epithelium (usually sessile; also known as papillary); tubulovillous, adenoma composed of 25%–75% of both tubular and villous structures (usually pedunculated; also known as papillotubular)<br>(3) Grade of dysplasia: based on the most severely dysplastic area of each tumor<br>(A) Low grade: branching or elongation of crypts with some reduction of interglandular stroma; low N/C ratio; cell nuclei elongated, crowded, appear stratified, with regular nuclear membranes, fine chromatin, and inconspicuous nucleoli; nuclei maintain polarity with respect to the basement membrane; mucus secretion usually present<br>(B) High grade: exhibits both architectural and cytologic changes; marked reduction of interglandular stroma with complex irregularity of glands with cribriform (sieve-like) structures and back-to-back glands; high N/C ratio; cell nuclei large, ovoid to round, with loss of polarity with respect to the basement membrane; cytologic atypia is more pronounced with marked, irregular nuclear membranes, with aberrant chromatin pattern not basally located (cleared, vesicular, clumped, or densely hyperchromatic chromatin); large, conspicuous nucleoli; mucus secretion usually absent; numerous mitoses with abnormal mitotic figures |
| Herniation | Glands have penetrated through the muscularis mucosae (see Table 3). |
| Adenocarcinoma | Malignant neoplasia of glandular epithelium composed of tubular and/or villous structures penetrating through the muscularis mucosa; categorized by the following criteria:<br>(1) Grade of differentiation: well differentiated, moderately differentiated, or poorly differentiated<br>(2) Histologic type: tubular/tubulovillous/villous adenocarcinoma; mucinous adenocarcinoma (>50% of tumor composed of extracellular mucin, signet-ring cells can be present); signet-ring cell adenocarcinoma (>50% of tumor composed of signet-ring cells); undifferentiated carcinoma (no glandular structure to differentiate; can be uniform or pleomorphic) |

N/C, nuclear/cytoplasmic.

noma in situ, and dysplasia. The term GIN was chosen to parallel similar classifications of neoplasia in other organ systems, such as MIN to describe mammary intraepithelial neoplasia, CIN to describe cervical intraepithelial neoplasia, and PIN to describe prostate intraepithelial neoplasia. Like other systems, GIN lesions are classified as low grade and high grade based on architecture and degree of cytologic atypia. Low-grade GIN is characterized by simple glandular architecture; crypts resemble those of tubular adenomas in humans. The neoplastic crypt cells are elongated and crowded, with hyperchromatic nuclei, but maintain polarity with respect to the basement membrane. High-grade GIN (high-grade dysplasia, carcinoma in situ, microcarcinoma) is characterized by more pronounced nuclear atypia, with loss of epithelial cell nuclear polarity. The neoplastic glands may show architectural complexities such as a cribriform appearance. However, the diagnosis of high-grade GIN may be made on cytologic grounds alone if markedly aberrant nuclei are present. These are microscopic lesions only and are distinguished from adenomas because they are not seen grossly and are not polypoid. Early morphologic changes in human colorectal neoplasia that may correspond to GIN are dysplastic aberrant crypt foci (ACF), putative precursors to CRC first described in colons of mice and rats treated with a carcinogen.[2] The finding of monoclonality in human ACF with only atypia or mild dysplasia makes ACF the earliest identified neoplastic lesion in the colon.[3] ACF occur at a higher frequency in the grossly normal colonic mucosa of humans with colon cancer than those without and at the highest frequency in patients with familial adenomatous polyposis.[4] Hyperplastic polyps, small sessile lesions characterized histologically by an expansion of the crypt replicative zone and a maturation of epithelial cells in the upper portion of the crypt, are commonly found in the

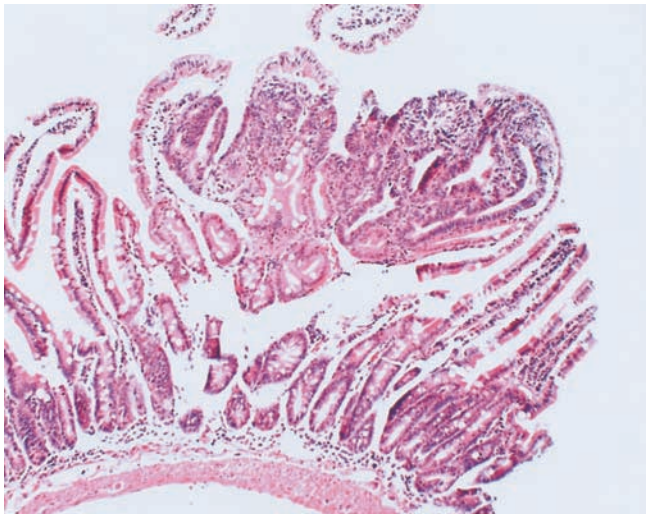

**Figure 1.** GIN from an AKR *Apc*$^{Min/+}$ mouse.

distal colon of humans but are not a feature of mouse models of CRC.

For grossly visible lesions, the term "adenoma" is recommended for preinvasive pedunculated, sessile, or flat plaque-like lesions. As for human colorectal adenomas, the qualifier "low grade" is not used in conjunction with "adenoma," because the diagnosis of adenoma implies at least low-grade dysplasia. More advanced adenomas with architectural complexity, increased cytologic atypia, and loss of tumor cell polarity are called adenomas with high-grade dysplasia.

Adenocarcinomas are malignant neoplasms of glandular epithelium composed of tubular and/or villous structures penetrating through the muscularis mucosa. They can be subclassified based on several different criteria, including degree of differentiation (well differentiated, moderately differentiated, or poorly differentiated) and

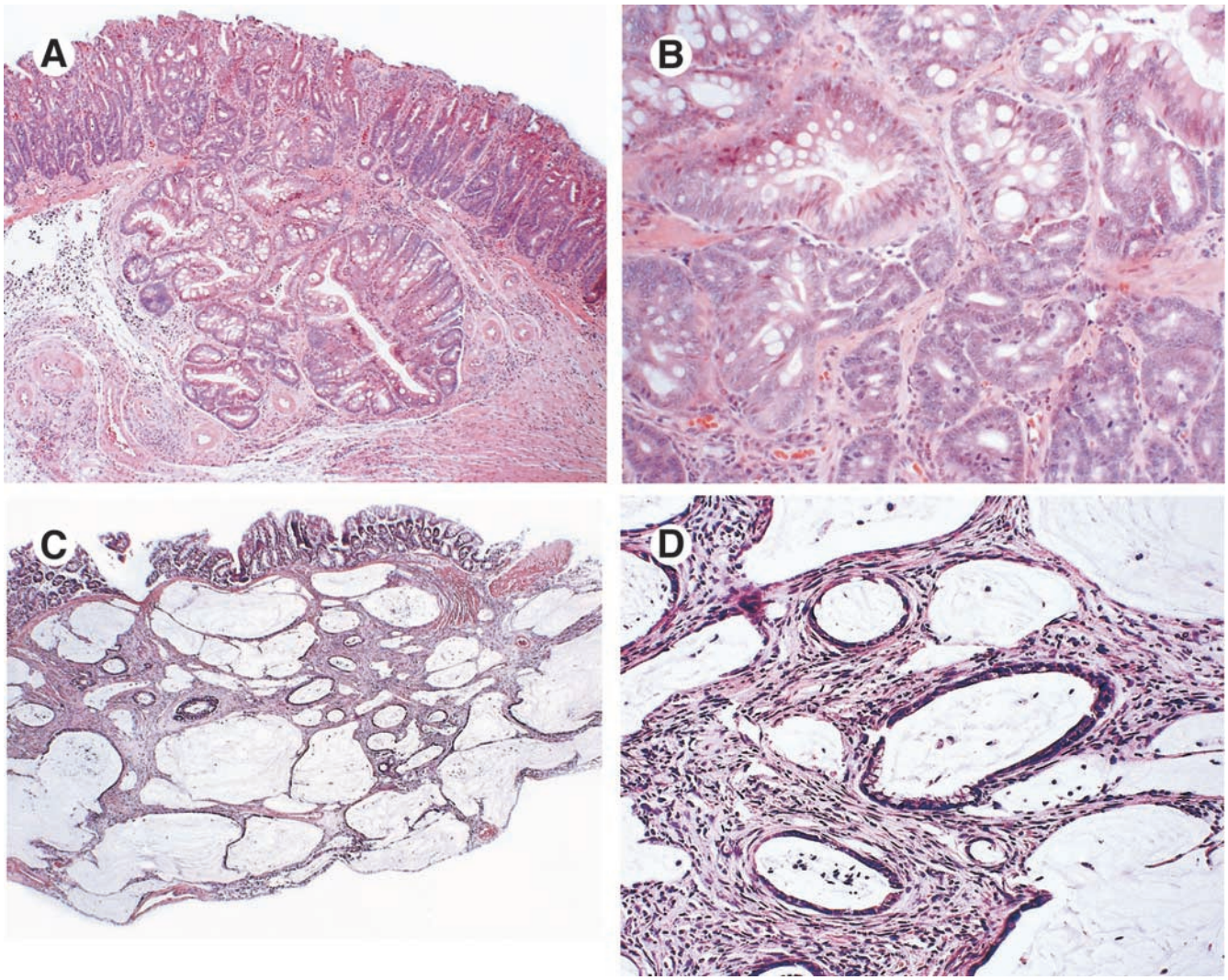


**Figure 2.** (*A*) Herniation of the intestinal mucosa of an *Msh3*$^{-/-}$ mouse. (*B*) Note the multiple cellular types in the invading cells and absence of a desmoplastic response in the herniated intestinal lesion. (*C*) Carcinoma in a *Tgfb1*$^{-/-}$ *Rag2*$^{-/-}$ mouse. (*D*) There is severe desmoplasia and loss of mucosa in the carcinoma.

histologic type. Histologic types described in mice include tubular, tubulovillous, villous, mucinous (>50% of tumor composed of extracellular mucin), signet-ring cell (>50% of tumor composed of signet-ring cells), and undifferentiated carcinoma (minimal gland formation; can be uniform or pleomorphic).

Secondly, guidelines for differentiation of invasive cancer and herniation of the gut epithelium were developed. Mucosal herniation (Figure 2), a common finding in the murine intestinal tract, can be independent of cancer development. It is likely due to the relative thinness of the muscularis mucosae. In the mouse, this muscular layer is only a few cells thick; therefore, the mucosa is able to penetrate the muscle with relative ease. Two mechanisms of traversing the muscularis are suggested: (1) herniation of the epithelium through weak points where vessels and lymphatics traverse the muscle, and (2) increased pressure from the mucosal lesion (polyp) pushing the basal crypt cells through the muscularis mucosa. Either of these mechanisms of epithelial displacement may be enhanced in inflammatory conditions in which there is even greater potential for disruption of the muscularis mucosa.

Similarly, determination of invasion in human CRC is occasionally a diagnostic problem when pseudoinvasion, or noninvasive displacement of the epithelium, arises. In pedunculated adenomas, torsion and trauma to the lesion may result in displacement of adenomatous epithelium into the head of the polyp. A thin layer of lamina propria is associated with the displaced epithelium, and hemosiderin is present in the stroma as evidence of trauma. The displaced epithelium has the same degree of dysplasia as the nondisplaced epithelium of the lesion. The lack of a desmoplastic response helps distinguish pseudoinvasion from invasive carcinoma. Displacement of nonneoplastic epithelium into deeper layers of the bowel wall also occurs in colitis cystica profunda, which is associated with inflammatory bowel disease.

As in mouse models, mucosal prolapse in humans may cause changes in the mucosa that mimic invasive adenocarcinoma. Solitary rectal ulcer syndrome, in which the rectal mucosa prolapses, is characterized by fibromuscular obliteration of the lamina propria with distortion and displacement of crypts and may on occasion be mistaken for neoplasia. Distinguishing the smooth muscle proliferation from the desmoplastic response to the tumor and recognizing that the displaced mucosa is not atypical or neoplastic are keys to distinguishing mucosal prolapse from infiltrating carcinoma in humans.

In an attempt to provide a simple classification system to differentiate carcinoma from mucosal herniation in the mouse, several characteristics of the penetrating epithelium were evaluated (Table 2). The most important characteristics in this evaluation were high-grade dysplasia, desmoplasia, loss of mucosal lining in invading glands, and presence of irregular, sharp, or angulated glands. To classify a lesion as a carcinoma, most of these features should be present in the invading epithelium. Herniated epithelium is usually characterized by cystically dilated glands, absence of dysplasia, no pleomorphism, absence of desmoplasia, and no loss of acinar architecture. Caution is needed when evaluating inflamed areas because of severe fibrosis, as well as frequent disruption and herniation of glandular tissue that accompanies many chronic inflammatory bowel disease models. As with all questionable diagnoses, consultation between pathologists may help in differentiating herniated and invasive lesions.

**Table 2.** Features That Distinguish Invasive Carcinoma From Herniation

1. Invasive cells are different from overlying mucosal component with atypia exceeding low-grade dysplasia
2. Presence of desmoplasia that is not associated with a prominent inflammatory cell infiltrate
3. Presence of irregular, sharp, or angulated glands in invasive component
4. Invading crypts spread laterally deep to the surface mucosal component
5. Cell loss from invading mucosa
6. More than 2 invading glands
7. Absence of basement membrane around invading glands
8. Evidence of progression to invasive cancer in other mice of the same genotype

## Morphology of Intestinal Neoplasia in GEM and Comparison With Human Lesions

Mouse models of intestinal neoplasia may be broadly divided into 5 groups: *Apc* and related models with mutations in Wnt signaling, MMR GEM, GEM with alterations in transforming growth factor (TGF) β signaling, immune-deficient mice with colitis, and carcinogen-treated mice. The following descriptions are a compilation of meeting findings as well as published reports on murine models of intestinal cancer. Original terminology from the published reports has not been changed to reflect the proposed nomenclature. Summary tables list the primary features of the different models (Table 3) and relationship to human disease (Table 4). Terminology similar to that used for human lesions is used for descriptive purposes and does not imply similarities in clinical behavior of morphologically similar lesions in mice and humans, because disease progression may vary significantly for the 2 species.

**Table 3.** Mouse Models of Intestinal Cancer

| Name of model | Background strain | Predominant location | Predominant neoplasm | Average no. of tumors/mouse | Age when analyzed (*mo*) | Other lesions | Metastasis | Reference |
|---|---|---|---|---|---|---|---|---|
| WNT pathway | | | | | | | | |
| *Apc*$^{Min/+}$ | C57BL/6 | Small intestine | Adenoma | 30 | 4 | Carcinoma | No | Moser et al.[7] |
| *Apc*$^{1638N/+}$ | C57BL/6 | Small intestine | Adenoma/ carcinoma | 4 | 12 | Desmoid tumors | Yes (1 reported) | Fodde et al.[13]; Smits et al.[15] |
| *Apc*$^{\Delta 716/+}$ | C57BL/6 | Small intestine | Adenoma | 300 | 4 | | No | Oshima et al.[11] |
| *Apc*$^{1309/+}$ | NR | Small intestine | Adenoma | 34 | 3 | | No | Quesada et al.[18] |
| ΔN131 β-catenin | C57BL/6 × DBA | Small intestine | Adenoma | 1 | 1 | Polycystic renal disease | No | Romagnolo et al.[20] |
| Activated β-catenin | C57BL/6 × NR | Small intestine | Adenoma | 3000 | 1 | | No | Harada et al.[22] |
| MMR genes | | | | | | | | |
| *Msh3*$^{-/-}$ | C57BL/6 × 129/ Sv × SJL/J | Small intestine | Adenoma/ carcinoma | <1 | 24 | Lymphomas, skin tumors | No | Edelmann et al.[29] |
| *Msh3*$^{-/-}$ *Apc*$^{1638/+}$ | C57BL/6 | Small/large intestine | Adenoma/ carcinoma | 5 | 9 | Skin tumor | No | Kuraguchi et al.[91] |
| *Msh6*$^{-/-}$ | C57BL/6 × 129/ SvJ × SJL/J | Small intestine | Adenoma/ carcinoma | <1 | 11 | Lymphomas, skin tumors | No | Edelmann et al.[30] |
| *Msh6*$^{-/-}$ *Apc*$^{1638/+}$ | C57BL/6 | Small/large intestine | Adenoma/ carcinoma | 26 | 5 | Skin tumor | No | Kuraguchi et al.[91] |
| *Msh3*$^{-/-}$ *Msh6*$^{-/-}$ | 129/Ola × FVB/ N | Small intestine | Adenoma/ carcinoma | 1 | 6 | Skin tumor | No | Edelmann et al.[29] |
| *Msh3*$^{-/-}$ *Msh6*$^{-/-}$ *Apc*$^{1638/+}$ | C57BL/6 | Small/large intestine | Adenoma/ carcinoma | 40 | <3 | | No | Kuraguchi et al.[91] |
| *Mlh1*$^{-/-}$ | C57BL/6 × 129/ Ola | Small intestine | Adenoma/ carcinoma | 2 | 6 | Lymphomas, skin tumors | No | Edelmann et al.[48] |
| *Mlh1*$^{-/-}$ *Apc*$^{1638/+}$ | C57BL/6 | Small intestine | Adenoma/ carcinoma | 2 | 6 | Skin tumor | No | Edelmann et al.[48] |
| *Mlh1*$^{-/-}$ *Apc*$^{1638/+}$ | C57BL/6 × 129/ Ola | Small intestine | Adenoma/ carcinoma | 45 | 3 | | No | Edelmann et al.[48] |
| *Mlh1*$^{-/-}$ *Apc*$^{Min/+}$ | C57BL/6 | Small intestine | Adenoma | 139 | 2 | | No | Shoemaker et al.[9] |
| *Msh2*$^{-/-}$ | C57BL/6 × 129/ Ola | Small intestine | Adenoma/ carcinoma | <1 | 6 | Lymphoma | No | Reitmair et al.[28] |
| TGF-β models | | | | | | | | |
| *Rag2*$^{-/-}$ *Tgfβ1*$^{-/-}$ | 129S6 × CF1 | Cecum/colon | Mucinous carcinoma | 2 | 2–6 | | No | Engle et al.[40] |
| *Rag2*$^{-/-}$ *Tgfβ1*$^{+/-}$ | 129S6 × CF1 | Cecum/colon | Adenoma | 2 | 2–6 | | No | Engle et al.[40] |
| *Smad4*$^{+/-}$ | C57BL/6 | Stomach/ duodenum | Hamartomatous polyp | 2 | 24 | | No | Taketo et al.[44] |
| *Smad4*$^{+/-}$ *Apc*$^{\Delta 716/+}$ | C57BL/6 | Small intestine | Carcinoma | 300 | 4 | Signet-ring cell carcinoma | | Taketo et al.[46] |
| *Smad3*$^{-/-}$ | 129/Sv | Colon | Mucinous carcinoma | NR | 6 | Rectal prolapse | Yes | Zhu et al.[42] |
| *Smad3*$^{-/-}$ | 129 × C57BL/6 | Cecum/colon | Mucinous carcinoma | NR | NR | Rectal prolapse | NR | Zhu et al.[42] |
| Immunodeficient | | | | | | | | |
| *IL-10*$^{-/-}$ | C57BL/6 × 129 | Colon/rectum | Colitis/carcinoma | NR | 2–6 | | No | Berg et al.[56] |
| IL-2 β2-microglobulin$^{-/-}$ | C57BL/6 × 129 | Rectum/colon | Carcinoma | NR | 6–12 | | No | Shah et al.[57] |
| *Tcrα*$^{-/-}$ | C57BL/6 | Rectum/colon | Colitis | NR | 3–12 | | | Mizoguchi et al.[49] |
| $G\alpha_{i2}^{-/-}$ | 129Sv × C57BL/ 6J | Colon | Colitis/carcinoma | 1 | 3–9 | Altered thymocyte maturation | No | Rudolph et al.[52] |
| Carcinogen-treated | | | | | | | | |
| AOM | A/J | Colon | Adenoma/ carcinoma | 36 | 6 | | NR | Papanikolaou et al.[92] |
| AOM | SWR/J | Colon | Adenoma/ carcinoma | 16 | 6 | | NR | Papanikolaou et al.[92] |
| AOM | AKR/J | Colon | Adenoma/ carcinoma | <1 | 6 | | NR | Papanikolaou et al.[92] |
| MNU | NR | Small intestine | Adenoma/ carcinoma | 1.5 | 3–12 | | NR | Qin et al.[27] |
| MNG | C3H | Colon/small intestine | Adenoma/ carcinoma | <1 | 11–20 | Stomach SCC | NR | Schoental et al.[74] |
| Other models | | | | | | | | |
| *Cdx2*$^{+/-}$ | 129/Sv × C57BL/6 | Colon | Gastric and intestinal heterotopia | 10 | 3 | | No | Tamai et al.[62] |
| *Muc2*$^{-/-}$ | C57BL/6 × 129/ SvOla | Small/ large intestine | Adenoma/ carcinoma | 1 | 6–12 | | No | Velcich et al.[67] |
| PI(3)kinase$\gamma^{-/-}$ | C57BL/6 | Rectum/colon | Carcinoma | 1+ | 3–6 | | Peritoneal | Sasaki et al.[90] |
| N-cadherin$^{-/-}$ | C57BL/6 × 129/ Sv | Small intestine | Colitis/adenoma | NR | 2–19 | | No | Hermiston et al.[63] |

NR, not reported; MNU, *N*-methyl-*N*-nitrosourea; MNG, *N*-methyl-*N*-nitroso-*N*′nitroguanidine; SCC, squamous cell carcinoma.

**Table 4.** Histopathologic Classification of Human Intestinal Tumors and Representative Mouse Models

| Human colorectal tumor | Features of human tumors | Equivalent mouse model |
|---|---|---|
| Adenocarcinoma | Most common histologic type occurring in sporadic form and with inherited predisposition in familial adenomatous polyposis and hereditary nonpolyposis CRC | *Mlh1*$^{-/-}$ *Apc*$^{1638N/+}$ *Msh6*$^{-/-}$ *Apc*$^{1638N/+}$ *Msh3*$^{-/-}$ *Apc*$^{1638N/+}$ |
| Mucinous carcinoma | Mucinous component >50%; may exhibit high levels of microsatellite instability | *Tgfb1*$^{-/-}$ *Rag2*$^{-/-}$ *Smad3*$^{-/-}$ |
| Signet-ring cell carcinoma | >50% signet-ring cells | *Smad4*$^{+/-}$ *Apc*$^{\Delta 716/+}$ |
| Adenosquamous carcinoma | Rare; pure squamous cell carcinoma is very rare | Not reported |
| Small cell undifferentiated carcinoma | Neuroendocrine differentiation; highly aggressive | Not reported |
| Undifferentiated carcinoma | At least 70% solid growth pattern | Not reported |
| Medullary carcinoma | Solid or trabecular growth pattern; little mucin production; tumor-infiltrating lymphocytes; no evidence of neuroendocrine differentiation; high levels of microsatellite instability | Not reported |

### *Apc* and Related GEM

The *Apc*$^{Min/+}$ mouse was the first germline mutant mouse model of gastrointestinal tumorigenesis.[5] Thus, data on tumor incidence, severity, and location in other mouse models are often compared with the phenotype of this mouse. The *Apc*$^{Min}$ allele carries an ethylnitrosourea-induced nonsense mutation that leads to embryonic lethality in the homozygous state.[6] Studies are conducted in *Apc* heterozygous (*Min*/+) mice. Mice carrying the *Apc*$^{Min}$ allele on the C57BL/6 background develop, on average, 30 pedunculated and flat adenomas per mouse in the small intestine and 5 per mouse in the colon by 4 months of age. Most polyps are adenomas, with occasional progression to invasive adenocarcinoma[7,8]; tumors in *Apc*$^{Min/+}$ have not been observed to metastasize. Cystic crypts are observed in Min mice.[9] However, spontaneous colonic ACF are rare or absent in these mice.[10] The average mouse life span is 119 days.[5]

Two other mouse strains with targeted insertion mutations at other sites in the *Apc* gene were examined by the panel. *Apc*$^{\Delta 716/+}$ develop a similar distribution of polyps as the *Apc*$^{Min/+}$ mice (i.e., most lesions were in the small intestine with very few in the large intestine)[11] and also lack colonic ACF.[12] However, there is a 10-fold increase in polyps compared with *Apc*$^{Min/+}$ mice.[11] In the *Apc*$^{\Delta 716/+}$ mouse, as in the other *Apc* models, loss of heterozygosity or mutation of the remaining normal *Apc* allele is a required step in the formation of adenomas. The committee also examined the *Apc*$^{1638N/+}$ mutant mouse on the C57BL/6 background. These mice develop significantly fewer adenomas than either the *Apc*$^{\Delta 716/+}$ or *Apc*$^{Min/+}$ mice, with 1–6 polypoid hyperplastic lesions or intestinal tumors developing by 3–5 months of age.[13] In addition, these mice develop colonic ACF spontaneously.[14] The mice have an average life span of about 15 months. Invasive carcinomas are sometimes seen in the small intestine; liver metastasis has been observed infrequently in *Apc*$^{1638N/+}$ mice.[13] Similar to tumors occurring in *Apc*$^{Min/+}$ and *Apc*$^{\Delta 716/+}$ animals, tumors from *Apc*$^{1638N/+}$ animals show loss of the normal *Apc* allele and loss of heterozygosity for markers on the entire chromosome 18 but do not carry mutations in K-*ras* and *p53*.[15] One mechanism of chromosome-wide loss of heterozygosity in the *Apc*$^{Min/+}$ mouse is homologous somatic recombination.[16]

Two other *Apc* GEM have been described in the literature but were not examined by the panel. The *Apc*$^{1638T/+}$ mice do not develop intestinal tumors. Interestingly, mice homozygous for the *Apc*$^{1638T}$ allele survive to adulthood, although they are runted.[17] *Apc*$^{1309/+}$ mice develop an average of 34 adenomas by 14 weeks of age, a slightly higher incidence of polyp formation than in *Apc*$^{Min/+}$ mice. However, the distribution of polyps in the intestine has not been described.[18]

Although onset, severity, and location of tumors vary between mice carrying different *Apc* mutations, the histologic appearance of all tumors is similar (Figure 3). Tumors arising in the *Apc*$^{Min/+}$ mice and related models are pedunculated adenomas that protrude into the gut lumen and arise in mucosa without inflammation. Most of the polyps occur in the small bowel, with a smaller number in the colon. The microscopic appearance of the tumors is similar to colonic adenomas in humans, with the exception of differences in surface involvement. In human adenomas, dysplastic cells are found on the superficial mucosal surface; this is in contrast to *Apc*$^{Min/+}$, in which adenomas are covered by a surface layer of nondysplastic epithelium.

Early lesions in *Apc*$^{Min/+}$ mice are characterized by neoplastic crypts that are enlarged relative to normal crypts and lined by atypical epithelial cells with hyperchromatic, crowded, elongated nuclei and increased nucleus-to-cytoplasmic ratios. Goblet cell and Paneth cell differentiation can be identified in some of the cells of the

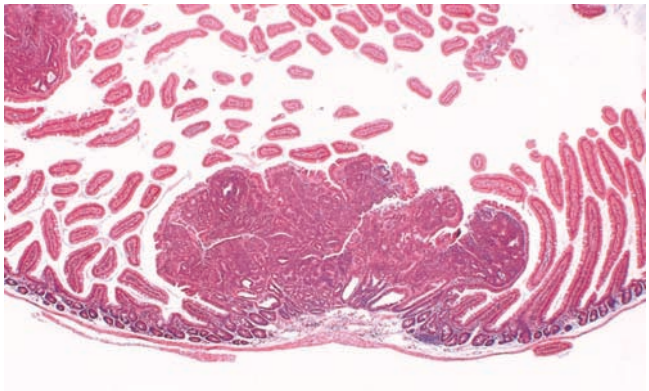

**Figure 3.** Papillary adenoma from an *Apc*$^{Min/+}$ mouse.

adenoma by routine light microscopy; neuroendocrine differentiation in some cells may be identified by immunohistochemical stains.[7] In more advanced lesions, the neoplastic glands have a more complex architecture characterized by small, densely packed back-to-back glands or the formation of large cribriform glands. Adenocarcinoma arising in these lesions is rare and is characterized by invasion of the submucosa or deeper layers of the bowel wall accompanied by a desmoplastic stromal response. The central portion of adenocarcinomas may be ulcerated. Increased numbers of apoptotic bodies and mitoses are commonly seen in both early and advanced lesions.

### β-Catenin Transgenic Mice

β-Catenin is a multifunctional protein that is a component of the WNT signal transduction pathway as well as cadherin-mediated cell adhesion complexes.[19] It associates with the APC tumor suppressor protein, which facilitates its proteasomal degradation. In intestinal tumors of both humans and *Apc*$^{Min/+}$ mice, β-catenin is transported from the cytoplasm to the nucleus, an indicator of activated WNT signaling.[19] Three groups have described transgenic overexpression of mutated β-catenin, which acts as a dominant activator of WNT signaling. ΔN131β-catenin overexpression by the calbindin promoter leads to development of adenoma in transgenic mice by 3–4 weeks of age, although further analysis was inhibited by mortality from polycystic kidney disease that also developed in these mice.[20] In contrast, Wong et al.[21] found no oncogenic effect of overexpression of a human $NH_2$ amino-terminal truncation mutant (N89 β-catenin) in the 129/Sv embryonic stem cell–derived component of the small intestine of adult C57BL/6-ROSA26 129/Sv chimeric mice. A third transgenic mouse overexpressing an activated β-catenin developed

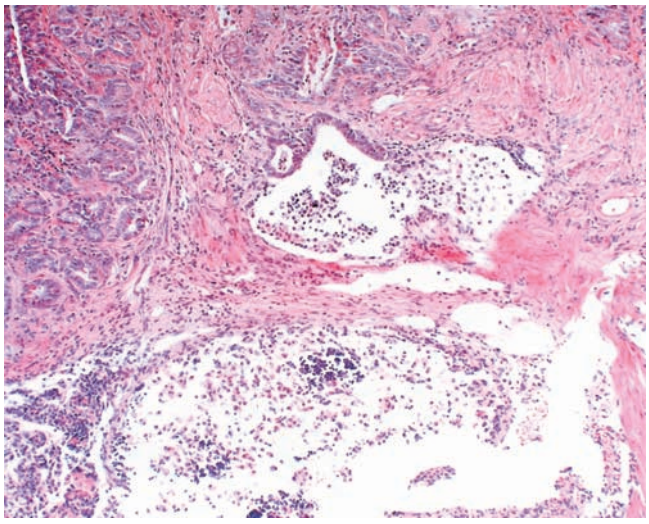

**Figure 4.** Adenocarcinoma with mucinous differentiation in the small intestine of an *Msh3*$^{-/-}$ *Apc*$^{1638/+}$ GEM. There is a strong desmoplastic reaction to the carcinoma.

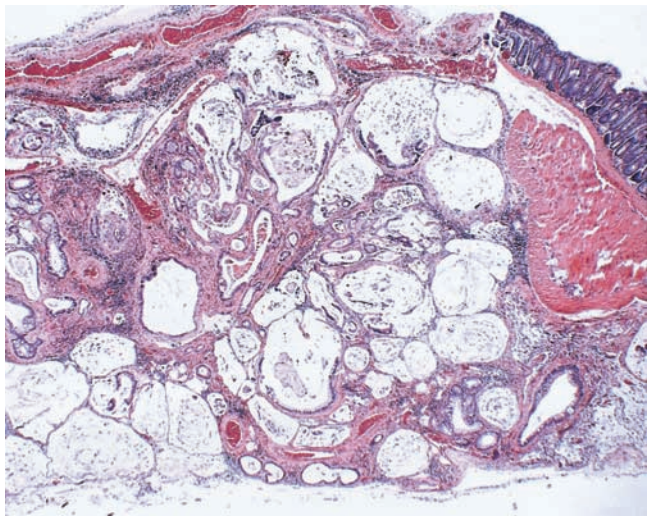

**Figure 5.** A *Tgfb1*$^{-/-}$ *Rag2*$^{-/-}$ mouse with cecal mucinous adenocarcinoma. Large mucin lakes are present in the muscular wall.

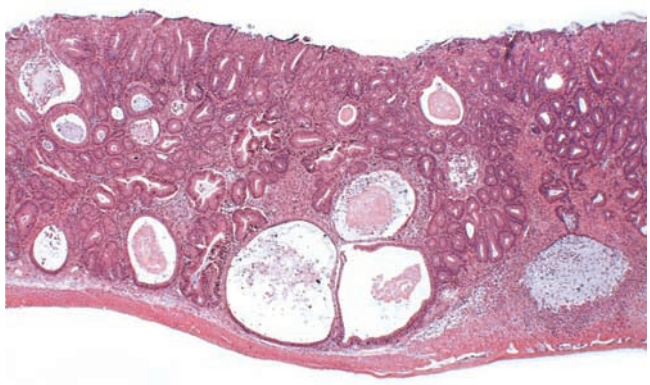

**Figure 6.** Severe hyperplasia and inflammation in the colon of an IL-10$^{-/-}$ mouse.

3000 tumors, primarily in the duodenum and jejunum with fewer in the ileum and little involvement of the cecum and colon. These tumors develop around 3 weeks of age and are similar histopathologically to those of $Apc^{\Delta 716/+}$ mice but occur in greater numbers.[22] Strain differences, transgene promoter sequences, and/or differences in β-catenin sequence may explain these differences in phenotype. These models underscore the importance of the WNT signaling pathway in mouse gastrointestinal tumorigenesis.

### MMR GEM

Mismatches in DNA result from DNA replication errors, genetic recombination, and chemical modification. Several human genes encode proteins that correct DNA mismatches; the most studied of these are *MSH2* and *MLH1*, which are homologues of bacterial DNA MMR genes *MutS* and *MutL,* respectively. Some human sporadic CRCs have defects in DNA MMR genes and are characterized by microsatellite instability. Microsatellite instability is used as a marker for homozygous loss of MMR genes (epigenetic silencing of alleles by promoter hypermethylation has been reported) and is characteristic of tumors that form in humans with hereditary nonpolyposis CRC caused by germline mutations of MMR genes. Certain histologic subtypes, such as medullary carcinoma, mucinous carcinoma, undifferentiated carcinoma, and signet-ring cell carcinoma, are overrepresented among tumors with high levels of microsatellite instability, defined as those tumors exhibiting microsatellite instability in more than 30%–40% of markers tested.[23–25]

Several MMR GEM have been developed. $Mlh1^{-/-}$, $Pms1^{-/-}$, and $Pms2^{-/-}$ mice were derived in mixed C57 black mixed and 129/Sv backgrounds; of these, only the $Mlh1^{-/-}$ mice developed intestinal tumors. Most of the lesions occur in the jejunum and ileum, with a few neoplasms developing in the colon. Most mice develop small numbers of neoplasms, including carcinomas, between 4 and 12 months of age. Similar to other MMR GEM, these mice develop lymphomas, skin tumors, and sarcomas.[26] Although spontaneous gastrointestinal tumors were not detected in the *Pms2* mutant mice, heterozygotes are more susceptible to *N*-methyl-*N*-nitrosourea–induced adenomas and carcinomas in the small intestine than wild-type mice (2.3 per mouse compared with 1.3 per mouse).[27] Double mutant $Apc^{Min/+}$ $Mlh1^{-/-}$ mice show an enhancement of adenoma multiplicity, often accompanied by de novo nonsense mutations in the $Apc^{+}$ allele rather than loss of heterozygosity. The *Mlh1* deficiency also enhances the formation of cystic crypts in $Apc^{Min/+}$ mice.[9]

$Msh2^{-/-}$ mice in a mixed background (C57BL/6 and 129/Ola) develop lymphomas that kill about 50% of mice between 2 and 6 months of age.[28] In mice that survive 6 months or longer, intestinal adenomas occur at a high frequency (15 of 22), with a high proportion of lesions in the duodenum and jejunum. Adenomas are typically plaque lesions, different from the pedunculated tumors seen in $Apc^{Min/+}$ mice and other MMR GEM. These intestinal neoplasms have a prominent inflammatory component, unlike many of the MMR models described.[28] In addition, 7 of 13 mice originally described developed spontaneous ACF in their colons. Some of these mice (7%) developed skin neoplasms, including sebaceous carcinomas that are also seen in humans in the setting of Muir–Torre syndrome.[28] The double mutant $Apc^{Min/+}$ $Msh2^{-/-}$ mice developed more adenomas at an earlier age than $Apc^{Min/+}$ mice in the small and large intestine and more ACF compared with either $Apc^{Min/+}$ or $Msh2^{-/-}$ mice. The adenomas in $Apc^{Min/+}$ $Msh2^{-/-}$ mice do not progress to carcinomas, perhaps because of the shorter life span of these mice compared with $Msh2^{-/-}$ mice.[10]

$Msh3^{-/-}$ mice on a mixed background of C57BL/6 (60%), 129/Sv (37.5%), and SJL/J (2.5%) strains have a life span similar to their control littermates and a low incidence of mice with tumors (10%), with roughly equal numbers of intestinal adenomas and carcinomas in each affected mouse.[29] Intestinal lesions in $Msh3^{-/-}$ mice include low-grade polypoid tumors classified as adenomas, with deep cystically dilated crypts lined by goblet cells. Rectal prolapse with mucosal hyperplasia, erosion of the surface mucosa with acute inflammation, expansion of the replicative zone, and displacement of epithelium in the submucosa also occurs in this model. In the $Apc^{1638N/+}$ $Msh3^{-/-}$ double mutant mice, adenomas with numerous apoptotic bodies are found. Adenocarcinomas in this model consist of infiltrative glands in a desmoplastic stroma (Figure 4). Rectal prolapse also occurs in these double mutants.

$Msh6^{-/-}$ mice developed on the same mixed background as the $Msh3^{-/-}$ mice had a median survival of 11 months. Thirty-eight percent of $Msh6^{-/-}$ mice developed duodenal and jejunal tumors, with a high ratio of carcinomas to adenomas.[29] On the C57BL/6 background, *Msh6*-deficient mice develop intestinal adenomas with densely packed crypts and high-grade cellular dysplasia primarily in the duodenum and jejunum; these tumors had a median onset of 10 months.[30] Extraintestinal lesions, including lymphomas, and benign skin and hepatic neoplasms occur in a significant proportion of these mice.[30] On a different mixed background (129/OLA and

FVB/N), *Msh6*$^{-/-}$ mice rarely develop intestinal tumors (2 of 22 at an average of 52 weeks), the features of which were not further described.[31] This highlights the importance of genetic background in modifying the phenotype of intestinal tumor formation resulting from a predisposing mutation.

To assess the overlapping functions of the MMR proteins, mice null for both *Msh3* and *Msh6* were generated. Compared with *Msh6*$^{-/-}$ mice, the incidence of intestinal tumors in the double knockout mice doubled to 2 tumors per mouse and the frequency of mice with gastrointestinal tumors increased to 75%. The life span of the mice was markedly decreased (50% survival: *Msh3*$^{-/-}$, 22 months; *Msh6*$^{-/-}$, 11 months; *Msh3*$^{-/-}$ *Msh6*$^{-/-}$, 6–7 months), although the ratio of carcinomas to adenomas was unchanged.[29] Tumors in the *Msh6*$^{-/-}$ mice occur primarily in the small intestine with rare occurrence in the colon. These studies support a role for *Msh3* in preventing the development of neoplasia in an *Msh6*$^{-/-}$-deficient environment.

### GEM With Alterations in TGF-β Signaling Pathway

The role of TGF-β signaling in cancer is complex.[32] Normal epithelial cells are growth inhibited by TGF-β, whereas many cancers become resistant to TGF-β growth inhibition. TGF-β can stimulate tumor progression, suggesting that TGF-β can act bifunctionally in neoplasia. In addition, mutations in the human *TGFBR2* have been found in both sporadic and inherited colon cancers exhibiting increased microsatellite instability (i.e., patients with hereditary nonpolyposis CRC).[33–35] Inactivating mutations in *SMAD2* and *SMAD4*, 2 members of the family of intracellular proteins responsible for transducing signals from the activated TGF-β receptors, are also present in many human colon cancers.[36–38]

Targeted inactivation of *Tgfb1* results in autoimmune disease and death before 1 month of age.[39] To examine the role of TGF-β1 in the development and progression of intestinal cancer, this mouse strain was crossed onto an immunodeficient background. *Tgfb1*$^{-/-}$ *Rag2*$^{-/-}$ mice were developed in a mixed 129S6 (97%) and CF-1 (3%) background. All combinations of *Tgfb1* ($^{+/+}$, $^{+/-}$, and $^{-/-}$) with *Rag2*$^{-/-}$ can develop spontaneous cecal and colonic neoplasms.[40] A marked increase in tumor incidence and severity was observed in the *Tgfb1*$^{-/-}$ *Rag2*$^{-/-}$ mice; sessile adenomas were detectable by 2 months of age, carcinomas were observed by 3–6 months with 100% penetrance, and no carcinomas were detectable in 39 *Tgfb1*$^{+/+}$ *Rag2*$^{-/-}$ mice before 6 months of age and in only 1 of 5 mice at 6 months of age. More advanced lesions are present in the cecum compared with the colon. Carcinomas often included areas of mucin-filled cysts (Figure 5) and showed no evidence of *Apc* pathway inactivation by immunohistochemical analysis, suggesting that the tumor-suppressive effects of TGF-β1 are not mediated through an *Apc*-dependent pathway.[40] Importantly, *Tgfb1*$^{-/-}$ *Rag2*$^{-/-}$ mice derived in a germ-free environment did not develop neoplasms until recolonization with normal flora containing *Helicobacter hepaticus*.[41] The role of microflora in the development of these lesions is a critical area for future studies. Slides from *Tgfb1*$^{-/-}$ *Rag2*$^{-/-}$ mice and *Tgfb1*$^{-/-}$ *p21*$^{-/-}$ mice were examined by panel members, who observed invasive colonic crypts and pools of mucin extending through the wall of the intestine. In both of these GEM, adenocarcinoma arose in areas of mucosal hyperplasia and transmural inflammation.

The SMAD family of proteins are downstream effectors of the TGF-β signaling pathway. Two *Smad*-deficient mice, *Smad3*$^{-/-}$ mice and *Smad4*$^{+/-}$ mice, develop intestinal lesions. All of the *Smad3*$^{-/-}$ mice on the 129/Sv background had proximal and/or distal colonic tumors by 6 months of age.[42] Lesions in these mice included mucosal hyperplasia, sessile adenomas, adenocarcinomas, and metastatic carcinomas with metastasis to the liver and mesenteric lymph nodes. There were mucinous variants, and many of the carcinomas showed a marked desmoplastic reaction. Tumors were also characterized by a transmural inflammatory component.[42] Interestingly, another *Smad3*$^{-/-}$ GEM failed to develop intestinal tumors.[43] Strain, mutational differences, and intestinal flora may all contribute to the marked difference observed in these 2 laboratories. Unfortunately, the bacterial flora of the intestinal tract, specifically *Helicobacter* status, was not examined in either group of mice.

A range of histopathologic lesions was observed in *Smad3*$^{-/-}$ GEM examined by the panel. In some areas of the colon, slight mucosal hyperplasia with reactive change and a modest increase of chronic inflammatory cells were found in the lamina propria. In other cases, the colitis was more pronounced, with prominent mucosal hyperplasia, expansion of the crypt replicative zone, and a mixed acute and chronic inflammatory infiltrate in the lamina propria that extended into the submucosa. Polypoid colonic tumors with large cystic crypts filled with cellular debris and mucin pools extending through the bowel wall into perirectal tissue were also present. The cells lining the mucin pools showed minimal nuclear atypia. Similar to the tumors examined in the *Tgfb1*$^{-/-}$ *Rag2*$^{-/-}$ mice, there was no indication of *Apc* inactivation in the cancers. On a hybrid background of 129/Sv

and C57BL/6, mice developed histologically similar lesions; however, tumor onset was delayed and neoplasms were only seen in 30% of the mice.[42]

*Smad4*-deficient mice have been described in which homozygotes die during embryonic development.[44] The *Smad4*$^{+/-}$ mice develop polyps in the duodenum and stomach after 1 year of age. The duodenal polyps are sessile and consist of cystic deep glands with overlying hyperplastic mucosa. Most of the gastric polyps in this model appear hamartomatous. A mild inflammatory component is associated with the tumors, and there are no lesions in the distal intestines of the *Smad4* mice. The human counterpart of the *Smad4* GEM may be a subset of patients with juvenile polyposis, in whom germline mutations of *SMAD4* have been identified.[45]

### Combinations of *Smad4*, MMR-Deficient, and *Apc* GEM

Several mice with combinations of targeted mutations and mutant *Apc* have been developed. *Smad4*$^{+/-}$ *Apc*$^{\Delta 716/+}$ mice develop larger tumors in the small intestine and colon, although tumor number did not change in comparison with *Apc*$^{\Delta 716/+}$ mice. There is also a significant increase in progression of the lesions, including greater desmoplasia, invasion, and the presence of signet-ring cells, suggesting that mutation of *Smad4* plays a role in histologic progression of intestinal lesions.[46]

Unlike *Smad4*$^{+/-}$ *Apc*$^{\Delta 716/+}$ mice, there was no significant change in tumor progression when mice deficient in MMR genes were crossed with mice carrying *Apc* mutations, although most showed increased tumor multiplicity. For example, *Pms2*$^{-/-}$ *Apc*$^{Min/+}$ mice were characterized by a 3-fold increase in small intestinal adenomas and a 4-fold increase in colonic adenomas but no increased incidence of carcinoma.[47] Similar increases in tumor multiplicity were found in a study of *Msh2*$^{-/-}$ *Apc*$^{Min/+}$ mice.[10] *Mlh1*$^{-/-}$ *Apc*$^{Min/+}$ mice were characterized by a 3-fold increase in average tumor number but no difference in tumor size or progression.[9] Similarly, *Mlh1*$^{-/-}$ *Apc*$^{1638N/+}$ mice also have increased tumor numbers.[48] Evaluation of mice carrying combinations of different gene mutations should improve our understanding of the effects of gene interactions on tumor initiation and progression.

### Immune-Deficient Mouse Models: Interleukin 10, Interleukin 2, G$\alpha_{i2}$, and *Tcr*$\alpha$

Several immune-deficient mouse models have been described in the literature and generally are characterized by inflammation of the large bowel with proliferative lesions that occasionally progress to adenocarcinomas.[49–52] Of particular importance in these models of inflammatory bowel disease is the absence of disease when the mice are rederived in a germ-free (bacteria- and virus-free) environment. In specific pathogen–free environments in which known pathogenic agents are not present (normal flora is still present in the gastrointestinal tract), interleukin (IL)-10– and IL-2–deficient mice have a reduced number and size of lesions.[50,53]

The specific bacteria associated with lesion development in GEM are *Helicobacter* species, although the precise role of these organisms is debated. *H. hepaticus* infection was not required for the development of colitis in IL-10$^{-/-}$ mice.[54,55] In a contrasting study, IL-10–deficient mice failed to develop colitis when cleared of *Helicobacter* infections with antibiotics; conversely, infection of pathogen-free IL-10$^{-/-}$ mice with *H. hepaticus* was associated with colitis.[50] It is possible that antibiotic treatment intended to eliminate *H. hepaticus* may also have eliminated the causative agent of colitis and that *H. hepaticus* may only have exacerbated an ongoing process. There is little doubt, however, that intestinal flora plays an important pathogenic role in the immune-deficient inflammatory bowel disease models.

Sixty percent of IL-10$^{-/-}$ mice (C57BL/6 × 129) develop colonic lesions by 6 months of age,[56] and 32% of IL-10$^{-/-}$ $\beta_2$-microglobulin$^{-/-}$ mice (C57BL/6 × 129) develop colonic adenocarcinomas between 6 and 12 months of age.[57] These data suggest that colitis is a significant precursor of adenocarcinoma in mice. Histopathologic interpretation is complicated by the frequent occurrence of rectal prolapse and mucosal herniation associated with inflammation. Alterations in β-catenin expression and *Apc* loss of heterozygosity were not observed in adenocarcinomas from IL-10$^{-/-}$ mice.[58]

Colitis-associated tumors in GEM are plaque-like lesions arising in hyperplastic colitic mucosa or in areas of rectal prolapse (Figure 6). The distribution and morphology of the colitis differs among the various models, but, in general, the affected colonic mucosa contains an increased mixed inflammatory infiltrate in the lamina propria. The colitic mucosa is thickened and hyperplastic compared with normal mucosa. Erosion and ulceration with prominent smooth muscle fibers in the lamina propria are seen in prolapsed mucosa. Mucosal herniation, or displacement of nonneoplastic epithelium into the submucosa and deeper layers of the bowel wall, is common in rectal prolapse and difficult to distinguish from carcinoma. Intraepithelial neoplasia arising in areas of hyperplasia is frequently seen in reactive mucosa.

In humans, adenocarcinomas arising in the setting of chronic inflammatory conditions are most often associated with ulcerative colitis but are occasionally seen in

Crohn's disease and schistosomiasis. The incidence of CRC in individuals with ulcerative colitis varies with duration and extent of disease but is estimated at 15%–20% after 30 years of disease.[59] Such CRCs develop in areas of inflammation and in flat or plaque-like areas of mucosal dysplasia, similar to the mouse. Many do not have an exophytic component and are inconspicuous on gross examination. Most tumors arising in ulcerative colitis are typical CRCs on microscopic examination, although mucinous and signet-ring cell carcinomas and other rare tumor subtypes are overrepresented.[59] Ulcerative colitis in humans differs morphologically from colitis in GEM, with human colitis exhibiting greater mucosal architectural distortion, loss of crypts, branching and budding crypts, and crypt atrophy. Diffuse nonpolypoid mucosal hyperplasia is seen in immunodeficient GEM but is not a feature of human ulcerative colitis.

### Other GEM With an Intestinal Phenotype

***Cdx2*$^{-/-}$ mice.** Cdx2 is an intestine-specific transcription factor that is a member of the caudal-related homeobox gene family.[60,61] *Cdx2*-deficient mice develop colonic lesions very early in life, with reprogramming of mucosal differentiation leading to gastric and intestinal heterotopia. These mice have villiform structure formation in the cecum and proximal colon with some polyp formation in the colon. Many of the lesions are lined by smooth muscle and show loss of goblet cell development. The duplication of the mucosa begins at embryonic day 11.5 as an outpocketing of the gut epithelium opposite the cecal bud. Connection of the outpocketing to the colonic lumen is retained up to 3 months of age.[62]

**N-cadherin transgenic mice.** Mice overexpressing a dominant negative N-cadherin along the entire crypt-villus axis develop inflammation localized to the jejunum and portions of the duodenum and ileum and occasionally form adenomas. Inflammation is characterized by an infiltration of the lamina propria with lymphocytes, immunoglobulin G– and immunoglobulin A–secreting plasma cells, and histiocytes. Inflammatory lesions are present by 6 weeks of age; by 3 months, the inflammation becomes transmural. Neutrophilic infiltration, Paneth cell hyperplasia, perturbed crypt-villus architecture, and ulceration are evident in the earlier lesions.[63]

***Lkb1*$^{+/-}$ mice.** Peutz–Jeghers syndrome is a hereditary disorder characterized by gastrointestinal hamartomatous polyposis associated with mucocutaneous pigmentation. Germline mutations of the gene encoding *LKB1* (*STK11*), a serine/threonine kinase, are identified in most patients with Peutz–Jeghers syndrome. To investigate the role of LKB1 in the Peutz–Jeghers syndrome phenotypes, a germline mutation in the mouse *Lkb1* gene was introduced by homologous recombination in mouse embryonic stem cells by 3 groups.[64–66] All 3 groups reported development of hamartomatous polyps in the gastrointestinal tract at approximately 20 weeks of age. Polyps were found from the stomach to the colon with some variation among groups, but in no instance did the polyps progress to carcinoma. Whereas 2 of the groups found that the polyps retained the wild-type allele, Bardeesy et al.[66] found loss of heterozygosity of the wild-type allele in 3 of 12 polyps.

**Muc-2$^{-/-}$ mice.** Muc-2 is the most abundantly secreted apomucin in the intestinal tract. Muc-2$^{-/-}$ knockout mice were examined for development of colon cancer at 6 and 12 months of age.[67] The incidence of neoplasms increased from 16% to 68% with increasing dysplasia of the tumors with age. Both adenomas and invasive carcinomas were described in the small intestine with a few in the large intestine, including the rectum. In addition, inflammation was evident in the lesions shown. The role of bacterial flora in lesion development was not described.

### Carcinogen-Treated Rodents

The discovery that 1,2-dimethylhydrazine is a potent and specific colorectal carcinogen in rodents led to experimental studies on the initiation and progression of nonfamilial forms of CRC.[68] 1,2-Dimethylhydrazine is a procarcinogen requiring metabolism into the ultimate carcinogen, the alkylating ion methyldiazonium.[69] Azoxymethane (AOM), an intermediate in the metabolism of 1,2-dimethylhydrazine, is also a colorectal-specific procarcinogen. 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 dysplastic lesions in treated rodents is similar to colonic adenomas in humans (Figure 7*A*). Neoplastic crypts may contain abundant apoptotic cellular debris, and increases in inflammatory cells in the lamina propria are often seen in the adenomas. Larger adenomas contain crypts with complex cribriform architecture corresponding to high-grade dysplasia or intramucosal carcinoma in human specimens. Some tumors in AOM-treated rodents show a propensity for early invasion of the fibrovascular stroma of the adenoma stalk, even in small lesions. Adenocarcinomas may be confined to the submucosa or, as in rats, may extend through the colonic wall (Figure 7*B*) with peritoneal spread and metastasis to regional lymph nodes. Metastatic tumors in rats tend to be poorly differentiated, having solid and mucinous characteristics.[70] Mucinous and signet-ring cell adenocarcinomas are reported in

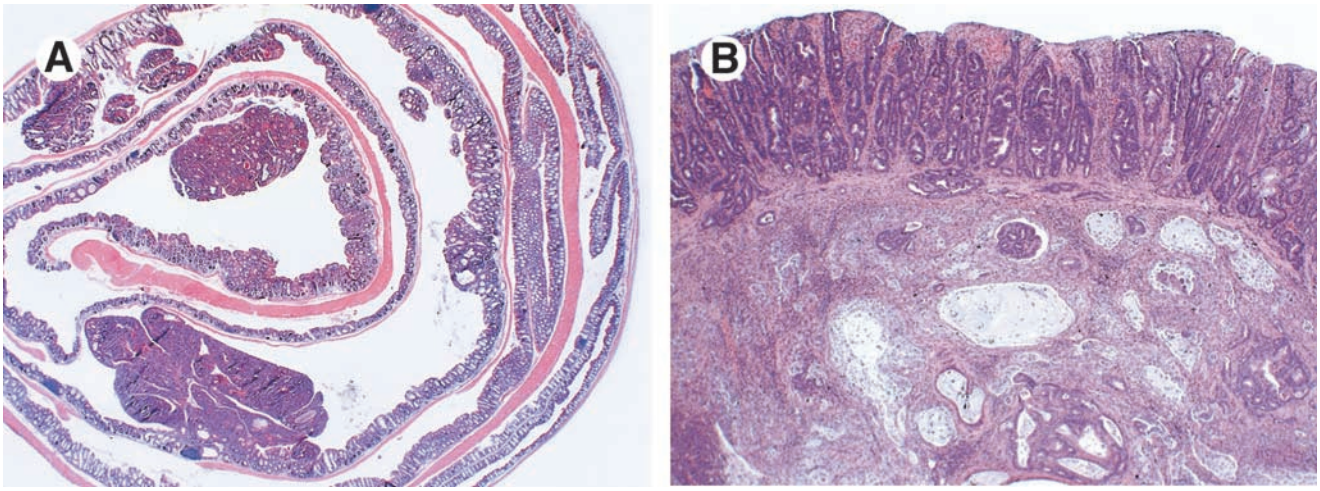


**Figure 7.** (*A*) Multiple polyps in the colon of an AOM-treated mouse. (*B*) Carcinoma with severe desmoplasia in the colon of an AOM-treated mouse.

AOM-treated rats but not in mice. AOM-treated mice are a potential model of metastasis.[71] Molecular changes in the tumors include mutations in β-catenin[72] and p53.[73] Other carcinogens used to induce gastrointestinal tumors in rodents include *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine,[74] *N*-ethyl-*N*′-nitro-*N*-nitrosoguanidine, 1,2-dimethylhydrazine,[75] 2-amino-3,4-dimethylimidazo[4,5-f]quinoline,[76] and *N*-methyl-*N*-nitrosourea.[27] Others identified in 2-year safety bioassays include capsaicin, captafol, captan, hydrogen peroxide, and *N*-(trichloromethylthio)phthalimide.[77]

## Modifiers of Cancer Phenotypes

It became clear during the review of specimens during the meeting that strain, nutrition, and bacterial status, as in other disease processes in mice, were of critical importance in modifying neoplastic development and progression. For example, the incidence of neoplasia in the $Apc^{Min/+}$ mouse is well known to be strain dependent.[7,78–80] A major modifier locus, known as Modifier-of-Min (Mom1), contributes 50% of the phenotypic variance between C57BL/6J and AKR/J mice. Genetic mapping[78] and subsequent molecular analysis of the locus showed that at least 2 genes contribute to the effects of Mom1: the secretory phospholipase A2 gene[81] and a second unidentified gene.[82] Spontaneous mutations, such as Mom2, have also been detected that contribute to tumor modifier effects.[83] $Msh6^{-/-}$ mice on a C57BL/6 background have a higher incidence of adenomas than mice on a 129/Ola × FVB/N background. $Smad3^{-/-}$ mice on a 129/Sv background develop tumors more at an earlier age than mice with the same mutation on a 129/Sv × C57BL/6 background. Additionally, pure 129/Sv-strain $Smad3^{-/-}$ mice maintained in a mouse colony free of *H. hepaticus* do not develop colitis or adenocarcinoma (John Letterio, personal communication, June 2002). These data show a role for modifier genes in the mouse that regulate the development and incidence of adenoma formation.

Diet also influences tumor formation in GEM. The $Apc^{\Delta 716/+}$ mice show, on average, 134 small intestinal adenomas on a low-fat/high-fiber diet and 209 small intestinal adenomas on a high-fat/low-fiber diet. Similar increases were seen in the large bowel, going from 0.71 to 2.0 adenomas with the high-risk diet.[84] Likewise, tumor progression to malignancy is accelerated in $Apc^{1638N/+}$ mice on a western-style diet containing high fat, low calcium, and low vitamin D.[85] These studies show a role for diet on tumor incidence.

### Necropsy Technique and Recommendations for Tissue Handling

Standard necropsy techniques should be used in accordance with several published recommendations.[86,87] Briefly, the intestinal tract is excised in mass and mesenteric attachments cut to allow full extension of the tract. The gastrointestinal tract can be opened for gross examination, depending on the goals of the research study. If so, it is advisable to flush the lumen of the intestinal tract with saline to clean out ingesta and feces or with fixative to prevent autolysis of the mucosa. When examining an opened intestine for lesions, it is important to avoid excessive contact with the surface mucosa because surface damage may occur. Time is critical, because autolysis begins within a few minutes after death. If the gut is opened to count gross lesions, it is important to lay the intestine out flat on paper for fixation. If ACF are to be evaluated, the intestine must be fixed flat immedi-

ately, either between filter papers with weights above or by pinning the tissue to a support such as a paraffin plate or corkboard.

After initial preparation and examination, the intestine can be placed in a container for immersion fixation. The ratio of tissue to fixative is at least 1:10. Selection of fixatives should be optimized for the intent of the study. For routine diagnostic evaluation, 10% neutral-buffered formalin is the fixative of choice. Bouin's fixative is excellent for maintaining morphology, whereas paraformaldehyde is often used for in situ hybridization. A short fixation in 70% ethanol has been found to be preferable for the extraction of messenger RNA.[88]

Once the tissue has been harvested and examined, there are several choices for orientation of the specimen for histologic analysis. Four common techniques can be used. The first is the alignment of the intestine in short longitudinal sections from duodenum to jejunum in sequential order. This method allows large pieces of tissue to be examined and orientation maintained. The second method is a "Swiss roll" technique in which the intestine is rolled in a circle around itself. This has the same benefits as the first procedure but requires less space. The third technique examines tissues in cross section. This provides excellent orientation of the sample but is inefficient for examining the entire specimen. Another approach is to select only lesions detected by gross or stereomicroscopic examination. In this method, the lesion is sectioned through the middle with a sharp blade, and both halves are embedded in a block. This method is excellent for performing histogenesis studies. It is the only technique that provides orientation so that the center of the lesion can be examined. The other techniques, although providing a good mechanism for comparing numbers of tumors, do not allow an absolute count of tumors in the intestine and the lesions are not sectioned through the center in all instances. Multiple sections are required in gut rolls to define the true nature of the neoplasms. Other preparative protocols have been presented in the Colon Cancer Jackson Laboratories Workshop and are available on "Techniques for Modeling Human Intestinal Cancer in Mice."[89]

## Summary

The marked diversity in the phenotype of intestinal neoplasia in murine models offers opportunities to model many characteristics of human CRC, including tumor progression, metastasis, gross morphology, and histology. However, the lack of a consistent model of metastasis is of particular concern in developing mouse models of human CRC. AOM-treated mice consistently develop metastasis, but the absence of control by known genetic mutations under carcinogen treatment makes this model system less desirable. The *Smad3*$^{-/-}$, PI(3)K$\gamma^{-/-}$,[90] and Apc$^{1638N/+}$ mutants are the only mouse models reported to develop colonic adenocarcinomas that metastasize to the lymph nodes and liver, which are common metastatic sites of human CRCs. However, there has been difficulty reproducing this observation in similar strains from different laboratories. Neoplastic lesions in mice with specific genetic alterations often do not parallel the phenotype of human cancer. Lastly, the role of intestinal pathogens and their contribution to the inflammatory response and tumor initiation is generally underappreciated. Despite these limitations, mouse models are invaluable in approximating the pathogenesis of human intestinal cancer and thus provide an in vivo platform for identifying therapeutic targets and developing new strategies for prevention and treatment.

## References


1. Hamilton SR, Aaltonen LA, eds. World Health Organization classification of tumours. Pathology and genetics of tumors of the digestive system. Lyon: IARC, 2000.
2. Bird RP. Observation and quantification of aberrant crypts in the murine colon treated with a colon carcinogen: preliminary findings. Cancer Lett 1987;37:147–151.
3. Siu I-M, Robinson DR, Schwartz S, Kung H-J, Pretlow TG, Petersen RB, Pretlow TP. The identification of monoclonality in human aberrant crypt foci. Cancer Res 1999;59:63–66.
4. Pretlow TP, Barrow BJ, Ashton WS, O'Riordan MA, Pretlow TG, Jurcisek JA, Stellato TA. Aberrant crypts: putative preneoplastic foci in human colonic mucosa. Cancer Res 1991;51:1564–1567.
5. Moser AR, Pitot HC, Dove WF. A dominant mutation that predisposes to multiple intestinal neoplasia in the mouse. Science 1990;247:322–324.
6. Moser AR, Shoemaker AR, Connelly CS, Clipson L, Gould KA, Luongo C, Dove WF, Siggers PH, Gardner RL. Homozygosity for the *Min* allele of *Apc* results in disruption of mouse development prior to gastrulation. Dev Dyn 1995;203:422–433.
7. Moser AR, Dove WF, Roth KA, Gordon JI. The min (multiple intestinal neoplasia) mutation: its effect on gut epithelial cell differentiation and interaction with a modifier system. J Cell Biol 1992;116:1517–1526.
8. Halberg RB, Katzung DS, Hoff PD, Moser AR, Cole CE, Lubet RA, Donehower LA, Jacoby RF, Dove WF. Tumorigenesis in the multiple intestinal neoplasia mouse: redundancy of negative regulators and specificity of modifiers. Proc Natl Acad Sci U S A 2000; 97:3461–3466.
9. Shoemaker AR, Haigis KM, Baker SM, Dudley S, Liskay RM, Dove WF. *Mlh1* deficiency enhances several phenotypes of *Apc*$^{Min/+}$ mice. Oncogene 2000;19:2774–2779.
10. Reitmair AH, Cai JC, Bjerknes M, Redston M, Cheng H, Pind MT, Hay K, Mitri A, Bapat BV, Mak TW, Gallinger S. *Msh2* deficiency contributes to accelerated *Apc*-mediated intestinal tumorigenesis. Cancer Res 1996;56:2922–2926.
11. Oshima M, Oshima H, Kitagawa K, Kobayashi M, Itakura C, Taketo M. Loss of *Apc* heterozygosity and abnormal tissue building in nascent intestinal polyps in mice carrying a truncation *Apc* gene. Proc Natl Acad Sci U S A 1995;92:4482–4486.

12. Oshima H, Oshima M, Kobayashi M, Tsutsumi M, Taketo MM. Morphological and molecular processes of polyp formation in *Apc*$^{\Delta 716}$ knockout mice. Cancer Res 1997;57:1644–1649.
13. Fodde R, Edelmann W, Yang K, van Leeuwen C, Carlson C, Renault B, Breukel C, Alt E, Lipkin M, Khan PM, Kucherlapati R. A targeted chain-termination mutation in the mouse Apc gene results in multiple intestinal tumors. Proc Natl Acad Sci U S A 1994;91:8969–8973.
14. Pretlow TP, Edelmann W, Hudson L Jr, Kucherlapati R, Augenlicht LH. Aberrant crypt foci develop spontaneously in *APC*1638 heterozygous mice. Proc Am Assoc Cancer Res 1997;38:126.
15. Smits R, Kartheuser A, Jagmohan-Changur S, Leblanc V, Breukel C, de Vries A, van Kranen H, van Krieken JH, Williamson S, Edelmann W, Kucherlapati R, Khan PM, Fodde R. Loss of *Apc* and the entire chromosome 18 but absence of mutations at the *Ras* and *Tp53* genes in intestinal tumors from Apc1638N, a mouse model for *Apc*-driven carcinogenesis. Carcinogenesis 1997;18: 321–327.
16. Haigis KM, Caya JG, Reichelderfer M, Dove WF. Intestinal adenomas can develop with a stable karyotype and stable microsatellites. Proc Natl Acad Sci U S A 2002;99:8927–8931.
17. Smits R, Kielman MF, Breukel C, Zurcher C, Neufeld K, Jagmohan-Changur S, Hofland N, van Dijk J, White R, Edelmann W, Kucherlapati R, Khan PM, Fodde R. *Apc1638T*: a mouse model delineating critical domains of the adenomatous polyposis coli protein involved in tumorigenesis and development. Genes Dev 1999;13:1309–1321.
18. Quesada CF, Kimata H, Mori M, Nishimura M, Tsuneyoshi T, Baba S. Piroxicam and acarbose as chemopreventive agents for spontaneous intestinal adenomas in *Apc* gene 1309 knockout mice. Jpn J Cancer Res 1998;89:392–396.
19. Sheng H, Shao J, Williams CS, Pereira MA, Taketo MM, Oshima M, Reynolds AB, Washington MK, DuBois RN, Beauchamp RD. Nuclear translocation of β-catenin in hereditary and carcinogen-induced intestinal adenomas. Carcinogenesis 1998;19:543–549.
20. Romagnolo B, Berrebi D, Saadi-Keddoucci S, Porteu A, Pichard A, Peuchmaur M, Vandewalle A, Kahn A, Perret C. Intestinal dysplasia and adenoma in transgenic mice after overexpression of an activated β-catenin. Cancer Res 1999;59:3875–3879.
21. Wong WH, Rubinfeld B, Gordon JI. Effects of forced expression of an NH2-termal truncated β-catenin on mouse intestinal epithelial homeostasis. J Cell Biol 1998;141:765–777.
22. Harada N, Tamai Y, Ishikawa T, Sauer B, Takaku K, Oshima M, Taketo MM. Intestinal polyposis in mice with a dominant stable mutation of the β-*catenin* gene. Eur Mol Biol Org J 1999;21: 5931–5942.
23. Boland CR, Thibodeau SN, Hamilton SR, Sidransky D, Eshleman JR, Burt RW, Meltzer SJ, Rodriguez BM, Fodde R, Ranzani GN, Srivastava S. A National Cancer Institute workshop on microsatellite instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. Cancer Res 1998; 58:5248–5257.
24. Jass JR, Do K-A, Simms LA, Iino H, Wynter C, Pillay SP, Searle J, Radford-Smith G, Young J, Leggett B. Morphology of sporadic colorectal cancer with DNA replication errors. Gut 1998;42:673–679.
25. Lanza G, Gafa R, Matteuzzi M, Santini A. Medullary-type poorly differentiated adenocarcinoma of the large bowel: a distinct clinicopathologic entity characterized by microsatellite instability and improved survival. J Clin Oncol 1999;17:2429–2438.
26. Prolla TA, Baker SM, Harris AC, Tsao JL, Yao X, Bronner CE, Zheng B, Gordon M, Reneker J, Arnheim N, Shibata D, Bradley A, Liskay RM. Tumour susceptibility and spontaneous mutation in mice deficient in Mlh1, Pms1 and Pms2 DNA mismatch repair. Nat Genet 1998;18:276–279.
27. Qin X, Shibata D, Gerson SL. Heterozygous DNA mismatch repair gene *Pms2*-knockout mice are susceptible to intestinal tumor induction with N-methyl-N-nitrosourea. Carcinogenesis 2000;21: 833–838.
28. Reitmair AH, Redston M, Cai JC, Chuang TCY, Bjerknes M, Cheng H, Hay K, Gallinger S, Bapat B, Mak TW. Spontaneous intestinal carcinomas and skin neoplasms in *Msh*2-deficient mice. Cancer Res 1996;56:3842–3849.
29. Edelmann W, Umar A, Yang K, Heyer J, Kucherlapati M, Lia M, Kneitz B, Avdievich E, Fan K, Wong E, Crouse G, Kunkel T, Lipkin M, Kolodner RD, Kucherlapati R. The DNA mismatch repair genes *Msh3* and *Msh6* cooperate in intestinal tumor suppression. Cancer Res 2000;60:803–807.
30. Edelmann W, Yang K, Umar A, Heyer J, Lau K, Fan K, Liedtke W, Cohen PE, Kane MF, Lipford JR, Yu N, Crouse GF, Pollard JW, Kunkel T, Lipkin M, Kolodner R, Kucherlapati R. Mutation in the mismatch repair gene *Msh6* causes cancer susceptibility. Cell 1997;91:467–477.
31. de Wind N, Dekker M, Claij N, Jansen L, van Klink Y, Radman M, Riggins G, van der Valk M, van't Wout K, te Riele H. HNPCC-like cancer predisposition in mice through simultaneous loss of *Msh3* and *Msh6* mismatch-repair protein functions. Nat Genet 1999;23:359–362.
32. Derynck R, Akhurst RJ, Balmain A. TGF-β signaling in tumor suppression and cancer progression. Nat Genet 2001;29:117–129.
33. Markowitz S, Wang J, Myeroff L, Parsons R, Sun L, Lutterbaugh J, Fan RS, Zborowska E, Kinzler KW, Vogelstein B. Inactivation of the type II TGF-β receptor in colon cancer cells with microsatellite instability. Science 1995;268:1336–1338.
34. Parsons R, Myeroff LL, Liu B, Wilson JK, Markowitz SD, Kinzler KW, Vogelstein B. Microsatellite instability and mutations of the transforming growth factor β II receptor gene in colorectal cancer. Cancer Res 1995;55:5548–5550.
35. Akiyama Y, Iwanaga R, Ishikawa T, Sakamoto K, Nishi N, Nihei Z, Iwama T, Saitoh K, Yuasa Y. Mutations of the transforming growth factor-β type II receptor gene are strongly related to sporadic proximal colon carcinomas with microsatellite instability. Cancer 1996;78:2478–2484.
36. Eppert K, Scherer SW, Ozcelik H, Pirone R, Hoodless P, Kim H, Tsui LC, Bapat B, Gallinger S, Andrulis IL, Thomsen GH, Wrana JL, Attisano L. *MADR2* maps to 18q21 and encodes a TGFβ-regulated MAD-related protein that is functionally mutated in colorectal carcinoma. Cell 1996;86:543–552.
37. Thiagalingam S, Lengauer C, Leach FS, Schutte M, Hahn SA, Overhauser J, Wilson JK, Markowitz S, Hamilton SR, Kern SE, Kinzler KW, Vogelstein B. Evaluation of candidate tumour suppressor genes on chromosome 18 in colorectal cancers. Nat Genet 1996;13:343–346.
38. Takagi Y, Kohmura H, Futamura M, Kida H, Tanemura H, Shimokawa K, Saji S. Somatic alterations of the *DPC4* gene in human colorectal cancers in vivo. Gastroenterology 1996;111: 1369–1372.
39. Shull MM, Ormsby I, Kier AB, Pawlowski S, Diebold RJ, Yin M, Allen R, Sidman C, Proetzel G, Galvin D, Annunziata N, Doetschman T. Targeted disruption of the mouse transforming growth factor-beta 1 gene results in multifocal inflammatory disease. Nature 1962;359:693–699.
40. Engle SJ, Hoying JB, Boivin GP, Ormsby I, Gartside PS, Doetschman T. Transforming growth factor β1 suppresses nonmetastatic colon cancer at an early stage of tumorigenesis. Cancer Res 1999;59:3379–3386.
41. Engle SJ, Ormsby I, Pawlowski S, Boivin GP, Croft J, Balish E, Doetschman T. Elimination of colon cancer in germ-free transforming growth factor beta 1-deficient mice. Cancer Res 2002; 62:6362–6366.

42. Zhu Y, Richardson JA, Parada LF, Graff JM. *Smad3* mutant mice develop metastatic colorectal cancer. Cell 1998;94:703–714.
43. Yang X, Letterio JJ, Lechleider RJ, Chen L, Hayman R, Gu H, Roberts AB, Deng C. Targeted disruption of SMAD3 results in impaired mucosal immunity and diminished T cell responsiveness to TGF-beta. EMBO J 1999;18:1280–1291.
44. Taketo MM, Takaku K. Gastro-intestinal tumorigenesis in *Smad4* mutant mice. Cytokine Growth Factor Rev 2000;11:147–157.
45. Howe JR, Roth S, Ringold JC, Summers RW, Jarvinen HJ, Sistonen P, Tomlinson IPM, Houlston RS, Bevan S, Mitros FA, Stone EM, Aaltonen LA. Mutations in the SMAD4/DPC4 gene in juvenile polyposis. Science 1998;280:1086–1088.
46. Taketo MM, Takaku K. Gastrointestinal tumorigenesis in Smad4 (Dpc4) mutant mice. Hum Cell 2000;13:85–95.
47. Baker SM, Harris AC, Tsao JL, Flath TH, Bronner CE, Gordon M, Shibata D, Liskay RM. Enhanced intestinal adenomatous polyp formation in *Pms2*$^{-/-}$*;Min* mice. Cancer Res 1998;58:1087–1089.
48. Edelmann W, Yang K, Kuraguchi M, Heyer J, Lia M, Kneitz B, Fan K, Brown AM, Lipkin M, Kucherlapati R. Tumorigenesis in *Mlh1* and *Mlh1/Apc1638N* mutant mice. Cancer Res 1999;59:1301–1307.
49. Mizoguchi A, Mizoguchi E, Saubermann LJ, Higaki K, Blumberg RS, Bhan AK. Limited CD4 T-cell diversity associated with colitis in T-cell receptor mutant mice requires a T helper 2 environment. Gastroenterology 2000;119:983–995.
50. Kullberg MC, Ward JM, Gorelick PL, Caspar P, Hieny S, Cheever A, Jankovic D, Sher A. *Helicobacter hepaticus* triggers colitis in specific-pathogen-free interleukin-10 (IL-10)-deficient mice through an IL12- and gamma interferon-dependent mechanism. Infect Immun 1998;66:5157–5166.
51. Gerrelds IM, van Meeteren ME, Meijssen MAC, Zikjlsra FJ. Interleukin-2-deficient mice: effect on cytokines and inflammatory cells in chronic colonic disease. Dig Dis Sci 2002;47:503–510.
52. Rudolph U, Finegold MJ, Rich SS, Harriman GR, Srinivasan Y, Brabet P, Boulay G, Bradley A, Brinbaumer L. Ulcerative colitis and adenocarcinoma of the colon in $G\alpha_{i2}$-deficient mice. Nat Genet 1995;10:143–150.
53. Schultz M, Tonkonogy SL, Sellon RK, Veltkamp C, Godfrey VL, Kwon J, Grenther WB, Balish E, Horak I, Sartor RB. IL-2-deficient mice raised under germfree conditions develop delayed mild focal intestinal inflammation. Am J Physiol 1999;276:G1461–G1472.
54. Czinn SJ, Zagorski BM, Spencer DM, Levine AD. Superinfection with *Helicobacter hepaticus* does not alter the natural course of colitis in IL10-deficient mice (abstr). Gastroenterology 1998;114:A958.
55. Dieleman LA, Arends A, Onkonogy SL, Goerres MS, Craft DW, Grenther W, Sellon RK, Balish E, Sartor RB. *Helicobacter hepaticus* does not induce or potentiate colitis in interleukin-10-deficient mice. Infect Immun 2000;68:5107–5113.
56. Berg DJ, Davidson N, Kuhn R, Muller W, Menon S, Holland G, Thompson-Snipes L, Leach MW, Rennick D. Enterocolitis and colon cancer in interleukin-10-deficient mice are associated with aberrant cytokine production and CD4+ TH1-like responses. J Clin Invest 1996;98:1010–1020.
57. Shah SA, Simpson SJ, Brown LF. Development of colonic adenocarcinoma in a mouse model of ulcerative colitis. Inflam Bowel Dis 1998;4:196–202.
58. Sturlan S, Oberhuber G, Beinhauer BG, Tichy B, Kappel S, Wang J, Rogy MA. Interleukin-10-deficient mice and inflammatory bowel disease associated cancer development. Carcinogenesis 2001;22:665–671.
59. Harpaz N, Talbot IC. Colorectal cancer in idiopathic inflammatory bowel disease. Semin Diagn Pathol 1996;13:339–347.
60. James R, Erler T, Kazenwadel J. Structure of the murine homeobox gene cdx-2. Expression in embryonic and adult intestinal epithelium. J Biol Chem 1994;269:15229–15237.
61. Suh E, Chen L, Taylor J, Traber PG. A homeodomain protein related to caudal regulates intestine-specific gene transcription. Mol Cell Biol 1994;14:7340–7351.
62. Tamai Y, Nakajima R, Ishikawa T, Takaku K, Seldin MF, Taketo MM. Colonic hamartoma development by anomalous duplication in *Cdx2* knockout mice. Cancer Res 1999;59:2965–2970.
63. Hermiston ML, Gordon JI. Inflammatory bowel disease and adenomas in mice expressing a dominant negative N-cadherin. Science 1995;270:1203–1207.
64. Miyoshi H, Nakau M, Ishikawa TO, Seldin MF, Oshima M, Taketo MM. Gastrointestinal hamartomatous polyposis in lkb1 heterozygous knockout mice. Cancer Res 2002;62:2261–2266.
65. Jishage K, Nezu J, Kawase Y, Iwata T, Watanabe M, Miyoshi A, Ose A, Habu K, Kake T, Kamada N, Ueda O, Kinoshita M, Jenne DE, Shimane M, Suzuki H. Role of *Lkb1*, the causative gene of Peutz-Jegher's syndrome, in embryogenesis and polyposis. Proc Natl Acad Sci U S A 2002;99:8903–8908.
66. Bardeesy N, Sinha M, Hezel AF, Signoretti S, Hathaway NA, Sharpless NE, Loda M, Carrasco DR, DePinho RA. Loss of the *Lkb1* tumour suppressor provokes intestinal polyposis but resistance to transformation. Nature 2002;419:162–167.
67. Velcich A, Yan W, Heyer J, Fragale A, Nicholas C, Viani S, Kucherlapati R, Lipkin M, Yang K, Augenlicht L. Colorectal cancer in mice genetically deficient in the mucin *Muc2*. Science 2002;295:1726–1729.
68. Druckrey H, Preussmann R, Matzkies F, Ivankovic S. Selective production of intestinal cancer in rats by 1,2-dimethylhydrazine. Naturwissenschaften 1967;54:285–286.
69. Fiala ES, Bobotas G, Kulakis C, Weisburger JH. Inhibition of 1,2-dimethylhydrazine metabolism by disulfiram. Xenobiotica 1977;7:5–9.
70. Ward JM. Morphogenesis of chemically induced neoplasms of the colon and small intestine in rats. Lab Invest 1974;30:505–511.
71. Ochiai M, Ubagai T, Kawamori T, Imai H, Sugimura T, Nakagama H. High susceptibility of Scid mice to colon carcinogenesis induced by azoxymethane indicates a possible caretaker role for DNA-dependent protein kinase. Carcinogenesis 2001;22:1551–1555.
72. Takahashi M, Nakatsugi S, Sugimura T, Wakabayashi K. Frequent mutations of the beta-catenin gene in mouse colon tumors induced by azoxymethane. Carcinogenesis 2000;21:1117–1120.
73. Perantoni AO, Rice JM. Mutation patterns in non-ras oncogenes and tumour suppressor genes in experimentally induced tumours. IARC Sci Publ 1999;146:87–122.
74. Schoental R, Bensted JPM. Gastro-intestinal tumours in rats and mice following various routes of administration of N-methyl-N-nitroso-N′-nitroguanidine and N-ethyl-N-nitroso-N′-nitroguanidine. Br J Cancer 1969;23:757–764.
75. Colussi C, Fiumicino S, Giuliani A, Rosini S, Musiani P, Macri C, Potten CS, Crescenzi M, Bignami M. 1,2-Dimethylhydrazine-induced colon carcinoma and lymphoma in msh2(-/-) mice. J Natl Cancer Inst 2001;93:1534–1540.
76. Fujita H, Nagano K, Ochiai M, Ushijima T, Sugimura T, Nagao M, Matsushima T. Difference in target organs in carcinogenesis with a heterocyclic amine, 2-amino-3,4-dimethylimidazo[4,5-f]quinoline, in different strains of mice. Jpn J Cancer Res 1999;90:1203–1206.
77. Gold LS, Manley NB, Slone TH, Ward JM. Compendium of chemical carcinogens by target organ: results of chronic bioassays in rats, mice, hamsters, dogs, and monkeys. Toxicol Pathol 2001;29:639–652.
78. Dietrich WF, Lander ES, Smith JS, Moser AR, Gould KA, Luongo C, Borenstein N, Dove W. Genetic identification of *Mom-1*, a major

modifier locus affecting *Min*-induced intestinal neoplasia in the mouse. Cell 1993;75:631–639.
79. Gould KA, Dietrich WF, Borenstein N, Lander ES, Dove WF. *Mom1* is a semi-dominant modifier of intestinal adenoma size and multiplicity in *Min*/+ mice. Genetics 1996;144:1769–1776.
80. Shoemaker AR, Moser AR, Midgley CA, Clipson L, Newton MA, Dove WF. A resistant genetic background leading to incomplete penetrance of intestinal neoplasia and reduced loss of heterozygosity in ApcMin/+ mice. Proc Natl Acad Sci U S A 1998;95: 10826–10831.
81. MacPhee M, Chepenik KP, Liddell RA, Nelson KK, Siracusa LD, Buchberg AM. The secretory phospholipase A2 gene is a candidate for the Mom1 locus, a major modifier of $Apc^{Min}$-induced intestinal neoplasia. Cell 1995;81:957–966.
82. Cormier RT, Bilger A, Lillich AJ, Halberg RB, Hong KH, Gould KA, Borenstein N, Lander ES, Dove WF. The Mom1AKR intestinal tumor resistance region consists of Pla2g2a and a locus distal to D4Mit64. Oncogene 2000;19:3182–3192.
83. Silverman KA, Koratkar R, Siracusa LD, Buchberg AM. Identification of the modifier of min 2 (Mom2) locus, a new mutation that influences Apc-induced intestinal neoplasia. Genome Res 2002; 12:88–97.
84. Hioki K, Shivapurkar N, Oshima H, Alabaster O, Oshima M, Taketo MM. Suppression of intestinal polyp development by low-fat and high-fiber diet in $Apc^{\Delta 716}$ knockout mice. Carcinogenesis 1997;18:1863–1865.
85. Yang K, Edelmann W, Fan K, Lau K, Leung D, Newmark H, Kucherlapati R, Lipkin M. Dietary modulation of carcinoma development in a mouse model for human familial adenomatous polyposis. Cancer Res 1998;58:5713–5717.
86. Sundberg JP, Boggess D, eds. Systemic approach to evaluation of mouse mutations. Boca Raton: CRC, 2000:57–90.
87. Feldman DB, Seely JC, eds. Necropsy guide: rodents and the rabbit. Boca Raton: CRC, 1988:51–76.
88. Bird RP, Salo D, Lasko C, Good C. A novel methodological approach to study the level of specific protein and gene expression in aberrant crypt foci putative preneoplastic colonic lesions by Western blotting and RT-PCR. Cancer Lett 1997;116:15–19.
89. Dove WF, Halberg RB, Clipson L, Riegel IL, eds. Techniques for modeling human intestinal cancer in mice. The Jackson Laboratory, October 2000. Available at: http://emice.nci.nih.gov/emice/resources/protocols/organ_site. Accessed December 2002.
90. Sasaki T, Irie-Sasaki J, Horie Y, Bachmaier K, Fata JE, Li M, Suzuki A, Bouchard D, Ho A, Redston M, Gallinger S, Khokha R, Mak TW, Hawkins PT, Stephens L, Scherer SW, Tsao M, Penninger JM. Colorectal carcinomas in mice lacking the catalytic subunit of PI(3)Kγ. Nature 2000;406:897–902.
91. Kuraguchi M, Yang K, Wong E, Avdievich E, Fan K, Kolodner RD, Lipkin M, Brown AMC, Kucherlapati R, Edelmann W. The distinct spectra of tumor-associated *Apc* mutations in mismatch repair-deficient *Apc1638N* mice define the roles of MSH3 and MSH6 in DNA repair and intestinal tumorigenesis. Cancer Res 2001;61: 7934–7942.
92. Papanikolaou A, Wang QS, Delker DA, Rosenberg DW. Azoxymethane-induced colon tumors and aberrant crypt foci in mice of different genetic susceptibility. Cancer Lett 1998;130: 29–34.

**Received June 24, 2002. Accepted December 9, 2002.**

**Address requests for reprints to: Gregory P. Boivin, D.V.M., M.S., Department of Pathology and Laboratory Medicine, University of Cincinnati and Cincinnati Veterans Affairs Medical Center, 231 Albert Sabin Way (ML 0529), Cincinnati, Ohio 45267-0529. e-mail: boivingp@ucmail.uc.edu; fax: (513) 558-2487.**

**Supported by the Mouse Models of Human Cancers Consortium sponsored by the National Cancer Institute.**