Molecular Genetics of Ulcerative Colitis-associated Colon Cancer in the Interleukin 2- and \( \beta_2 \)-Microglobulin-deficient Mouse

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ABSTRACT

Mice deficient in \( \beta_2 \)-microglobulin and interleukin 2 (IL-2null) spontaneously develop colon cancer in the setting of chronic ulcerative colitis (UC). We investigated mutations of the Apc and p53 genes and microsatellite instability in colon adenocarcinomas arising in this model. Mutations of the Apc and p53 genes in the regions corresponding to mutation hot spots in human colorectal cancer were determined by sequencing in 11 colonic adenocarcinomas. Microsatellite instability was determined in matched normal and neoplastic DNA at five loci. All 11 adenocarcinomas harbored Apc mutations. Of these 11 tumors, 5 harbored truncating mutations. A total of 67 Apc mutations were found in these 11 tumors; 59 were missense mutations, whereas 8 were frameshift or nonsense mutations. Six of the 11 adenocarcinomas harbored p53 mutations. A total of seven p53 mutations were found in these 11 tumors; all mutations were transitions, 4 of which were C\( \rightarrow \)T transitions occurring in codon 229 at cytosine-guanine dinucleotides. Nine adenocarcinomas exhibited microsatellite instability in at least one of the five loci examined; 1 tumor had microsatellite instability in two loci. Molecular genetics, as well as clinical features, of colon cancer in the \( \beta_2 \)-mnull × IL-2null mice are similar to those of human UC-associated colorectal cancer. As such, this model appears to be an excellent animal model to study UC-associated colorectal carcinogenesis.

INTRODUCTION

Chronic UC is associated with a 10- to 40-fold increased risk of developing CRC compared with the general population (1). Recently, population-based, large, prospective studies have confirmed this association, although the actual magnitude of risk has been shown to be less than that observed from retrospective analyses (2). Molecular alterations accompanying the histological progression of the normal colonic mucosa to dysplasia and to cancer in chronic UC have just begun to be elucidated; UC-associated CRC appears to develop through a molecular pathway slightly different from that involved in sporadic colorectal carcinogenesis (3, 4). Point mutations and allelic loss of the APC gene have been reported in UC-related dysplasia and cancer (5, 6), although a recent study suggests that sporadic colorectal carcinogenesis (3, 4), alterations in p53 appear to be an earlier event in UC-associated colorectal carcinogenesis (5, 8–14). p53 mutations have also been observed in noncancerous colon tissue from patients with chronic UC (15, 16). In UC-associated dysplasia and cancer, K-ras mutations are either common or unusual, depending on the study referenced (6, 9, 10, 17–20). Point mutations in one of the mismatch repair genes, MSH2 (21), and microsatellite instability, the hallmark of DNA replication error defects, have been found in UC-associated dysplasia and cancer (22–24) and even in nonneoplastic mucosa (24, 25).

Currently available animal models to study UC-associated CRC include chemical carcinogen, transgenic, and genetic knockout models (26). Mice deficient in IL-2 (IL-2null) have been found to develop wasting syndrome with colonic inflammation resembling UC (27). Approximately 50% of the IL-2null mice die within the first 9 weeks because of severe anemia (27). The remainder develop diarrhea, colitis, and anemia accompanied by a systemic wasting disease, resulting in death usually within 6 months (27). None of these mice surviving beyond 6 months have been observed to develop CRC (27). Previously, mice deficient in CD8+ T cells and MHC class I expression as a result of a targeted mutation in the \( \beta_2 \)-m gene (\( \beta_2 \)-mnull) were bred with IL-2null mice to generate double knockout mice (\( \beta_2 \)-mnull × IL-2null; Ref. 28). The \( \beta_2 \)-mnull × IL-2null mice develop pancolitis as severe as seen in the IL-2null mice (28). However, in contrast to the IL-2null mice, the \( \beta_2 \)-mnull × IL-2null mice appear less systemically ill (less wasting and anemic) and survive beyond 6 months, suggesting a milder overall disease (28). Most of these mice show signs of diarrhea and several develop rectal prolapse (28). At times, some of these mice appear ill with signs of diarrhea and wasting, especially between 8 and 12 weeks, and then recover with normal stools, weight gain, and normal appearance, suggesting disease flare followed by remission from colitis (28). Histologically, 75% of these mice have mild to moderate colonic inflammation restricted to the mucosa, and ~25% have no inflammation at the time of necropsy (28). Recently, it has been shown that 32% of the \( \beta_2 \)-mnull × IL-2null mice develop adenocarcinoma in the proximal half of the colon between 6 and 12 months (29). No tumors have been observed in mice <6 months of age, suggesting that adenocarcinomas arose only after a prolonged period of colonic inflammation (29). All of the tumors are well to moderately differentiated adenocarcinomas invading into or through the muscularis propria (29). More recently, the \( \beta_2 \)-mnull × IL-2null mice have been observed to develop low- and high-grade dysplasia (30). Therefore, it appears that the \( \beta_2 \)-mnull × IL-2null mice are an excellent animal model to study UC-associated colorectal carcinogenesis.

The present study investigated molecular genetics of UC-associated colon cancer arising in this murine model. In particular, we studied mutations of the Apc and p53 genes and microsatellite instability, three commonly observed molecular alterations in human UC-associated and sporadic CRCs.

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The abbreviations used are: UC, ulcerative colitis; CRC, colorectal cancer; APC, adenomatous polyposis coli; IL-2, interleukin-2; \( \beta_2 \)-m, \( \beta_2 \)-microglobulin; dNTP, deoxynucleotide triphosphate; MCR, mutation cluster region.

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MATERIALS AND METHODS

Tissue Samples

Eleven histologically confirmed colonic adenocarcinomas from 11 β-mnull × IL-2mnull mice were analyzed in the present study. The detailed protocol of the study where 11 of 34 (32%) β-mnull × IL-2mnull mice developed moderately differentiated adenocarcinomas invading into or through the muscularis propria in the proximal half of the colon between 6 and 12 months of age has been published previously (29). The β-mnull × IL-2mnull mice were generated on a C57BL/6 × 129/OLA or 129/Sv mixed background (29). All macroscopically identifiable tumors were harvested at the time of necropsy, fixed in 10% buffered formalin, and embedded in paraffin in a standard fashion. Five-μm-thick sections were cut and mounted on microscope slides for staining with H&E using standard techniques.

DNA Extraction

Areas corresponding to histologically confirmed adenocarcinomas on H&E staining were marked on matched unstained slides. DNA from each adenocarcinoma was extracted as crude preparations using proteinase K lysis mix (10 mM Tris-HCl (pH 8.0), 0.5% Tween 20, and 1 mg/ml proteinase K) as described previously (31). Care was taken to avoid contamination from adjacent nonneoplastic tissues. The sections were homogenized in the lysis mix and digested for 1 h at 65°C, followed by 10 min at 95°C. Extracted DNA was stored at −20°C until subsequent analyses. DNA from the adjacent nonneoplastic colonic mucosa was extracted from areas corresponding to normal histology on H&E section from matched unstained slides in a similar fashion (31). DNA from the liver (negative control), snap-frozen at the time of sacrifice and stored at −70°C, was extracted by a standard technique using a lysis buffer containing proteinase K, followed by phenol, chloroform, and isooamyl alcohol organic extraction (32).

Mutation Analyses

Apc Gene. A 2738-bp region, between nucleotides 2020 and 4758 in exon 15 of the Apc gene, including a region designated as the MCR in human CRC (nucleotides 3906–4589), was amplified by PCR using three pairs of exon primers to generate three overlapping segments (segment A, nucleotides 2020–2996; segment B, nucleotides 2863–3925; segment C, nucleotides 3829–4758) as described previously (31). About 60% of the somatic mutations of the Apc gene in human CRC are clustered in a 500-bp region in the MCR (33). The primer sequences, which contain flanking sequences of EcoRI and XhoI restriction sites to facilitate subcloning into a vector, were constructed based on the published murine Apc cdna sequence (Ref. 34; GenBank accession no. M88127) and synthesized by the ACGT Corp. (Toronto, Ontario, Canada). The sequences of the primers were as follows: segment A, 5'-ACACTCTAATTGCAACTTCAAAAAGGACAGGAAGC-3' (sense) and 5'-ACACTCATTAGGTTGCGCCCTTCTTACATCTC-3' (antisense); segment B, 5'-TCTAGGAGAAACCCCTGTGAGTACCTC-3' (sense) and 5'-ACACCTCTGGAGTTGTTGTCGACATCC-3' (antisense); and segment C, 5'-TCTAGGAAAACTCAACAGAGAGCAGATC-3' (sense) and 5'-ACACCTGAGTCTACACCTCTTGTGAGG-3' (antisense).

Each 5.0 μl of DNA sample was amplified by PCR in a 50-μl volume containing 350 ng of each primer, 0.25 mM each dNTP, PCR buffer (Qiagen, Mississauga, Ontario, Canada), 1.5 mM MgCl₂, and 2 units of HotStart Taq DNA polymerase (Qiagen). After hot start PCR at 95°C for 5 min, 35 cycles of denaturation (95°C) for 1 min, annealing (54°C) for 1 min, and extension (72°C) for 1 min were performed in a thermal cycler (PTC-200 DNA Engine; MJ Research). All PCR amplifications included a 10-min extension at 72°C after cycle 35.

Subcloning and Sequencing. The PCR products for the Apc (segments A-C) and p53 (exons 5–8) genes were gel purified using the Qiaex II Agarose Gel Extraction kit (Qiagen) according to the manufacturer’s protocol, re-extracted, and dissolved in 50 μl of double-distilled H₂O. The PCR products were then subcloned into pBlueScript II KS(+) vector (Stratagene, Cambridge, United Kingdom) at EcoRI and XhoI sites. Sequencing was performed on a total of 2 clones from each PCR product using the Dideoxy Terminator Label Cycle Sequencing kit (Applied Biosystems, Foster City, CA) and an Applied Biosystems 373 Sequencer (Applied Biosystems) as described previously (31). The Apc and p53 sequences thus generated were analyzed against the GenBank sequences [Apc, accession no. M88127 (34); p53, accession nos. X01237 and K01700 (36)] using the DNASIS software program (Hitachi, San Diego, CA). Mutations were confirmed by sequencing the opposite strand as well as sequencing 1 independent clone from each of three to five independently performed PCR reactions. In total, therefore, 2 clones from the initial PCR reaction and 3–5 independent clones from three to five separate PCR reactions were sequenced per tumor. Only those mutations consistently present in all of the sequencing analyses were considered to be real mutations. Liver and nonneoplastic colonic mucosal DNA from each mouse harboring colonic adenocarcinoma were PCR amplified under the same conditions for tumor DNA, and a total of 2 clones from each PCR reaction were sequenced initially, followed by sequencing 1 independent clone from a separately performed PCR reaction as described above.

Microsatellite Instability Assay

Microsatellite instability was detected by comparison of electrophoretic mobility of amplified nonneoplastic and neoplastic colonic DNA from each mouse harboring colonic adenocarcinoma using primers from five loci on mouse chromosomes 6 (D6Mit68), 7 (D7Mit91), 10 (D10Mit22), 18 (D18Mit149), and 19 (D19Mit56) as described previously (Research Genetics, Huntsville, AL; Refs. 32, 37). Each 3.0 μl of DNA sample was amplified by PCR in a 15-μl volume containing 0.4 μM of each primer, 0.20 mM each dNTP, 0.033 mM [α-32P]dATP (New England Nuclear, Boston, MA), PCR buffer (Life Technologies, Inc., Gaithersburg, MD), 1.5 mM MgCl₂, and 1 unit of Taq DNA polymerase (Life Technologies, Inc.). All reactions were overlaid with 10 μl of mineral oil. After hot start PCR at 95°C for 5 min, 40 cycles of denaturation (95°C) for 1 s, annealing (58°C) for 20 s, and extension (72°C) for 20 s were performed in a thermal cycler (PTC-200 DNA Engine; MJ Research). All PCR amplifications included a 10-min extension at 72°C after cycle 40. A 4-μl aliquot of the PCR products was mixed with formamide dye mix (2 μl), denatured at 95°C for 3 min and electrophoresed on 6% polyacrylamide gels under denaturing conditions for 2 h. Gels were dried and exposed to X-ray film for 16 h. A positive case was confirmed in two independently performed PCR reactions.

RESULTS

Apc Mutations. A total of 67 mutations were found in the 2738-bp region in exon 15 of the Apc gene from all of the 11 adenocarcinomas analyzed (mutation frequency of 100%; Table 1). On average, each adenocarcinoma harbored 6.1 mutations (Table 1). In contrast, no mutations were observed in the same region in the adjacent nonneoplastic colonic mucosal and liver DNA from all of the 11 animals harboring colonic adenocarcinomas. Fifty-nine of the 67 mutations (88%) were single base substitutions resulting in missense mutations, whereas the remaining 8 mutations (12%) were frameshift or nonsense mutations resulting in truncation of the Apc protein (Table 1).
Five of the 11 tumors (45%) harbored frameshift or nonsense mutations (Table 1). Six of the 8 truncating mutations were frameshifts (3 deletions and 3 insertions), and 2 were point mutations creating a nonsense codon. Of the 59 missense mutations, 5 (8%) were transversions and 54 (92%) were transitions (Table 1). Of the 54 transitions, 27 (50%) were A:T→G:C, 14 (26%) T:A→C:G, 8 (15%) G:C→A:T, and 5 (9%) C:G→T:A (Table 1). No C:G→T:A transitions at cytosine-guanine dinucleotides (CpG) were observed. Seventeen of the 67 mutations (25%) were located within the MCR (Table 1). In contrast, 54% of the observed mutations (36 of 67) were located in a 791-bp region between nucleotides 3020 and 3811 in segment B upstream of the MCR (Table 1).

In summary, all colonic adenocarcinomas arising in the \(\beta_{mnull} \times \beta_{Mnull}\) mice harbor multiple Apc mutations in the region corresponding to the mutation hot spot in human CRC. In contrast to human CRC, the majority of these mutations are missense transitions occurring in a unique region upstream of the human MCR.

**p53 Mutations.** A total of 7 mutations in exons 5–8 were found in 6 of the 11 adenocarcinomas analyzed (mutation frequency of 54%; Table 2). No p53 mutations in exons 5–8 were observed in the adjacent nonneoplastic colonic mucosal and liver DNA from all of the 11 animals harboring colonic adenocarcinomas. Five of the 7 mutations (71%) were in exon 5, whereas each of exon 6 and 8 contained 1 mutation. No mutation was detected in exon 7. All mutations were single base substitutions resulting in missense mutations (Table 2). All mutations were transitions: 4 C:G→T:A; 2 A:T→G:C; and 1 G:C→A:T (Table 2). Four of the 7 mutations (57%) were C:G→T:A transitions occurring at nucleotides 688 (codon 229) within CpG sites (Table 2).

**Microsatellite Instability.** A total of 7–9 paired tumor and normal DNA samples was amplified per each of the 5 loci tested (Table 3). No difference in electrophoretic mobility was observed between normal and tumor tissue at three loci (\(D6\ Mit18, D10\ Mit12,\) and \(D18\ Mit14; Table 3). However, microsatellite instability was observed in 7 of the 8 CRCs and 3 of the 7 CRCs analyzed at the \(D7\ Mit91\) and \(D19\ Mit36\) loci, respectively (Table 3; Fig. 1). All tumors exhibiting microsatellite instability at the \(D7\ Mit91\) and \(D19\ Mit36\) loci had the same pattern of contractions and expansions, respectively. This observation suggests that microsatellite instability might play a significant role in UC-associated CRC in this murine model.

**DISCUSSION**

The results of the present study suggest that molecular genetics of CRC arising in the \(\beta_{mnull} \times \beta_{Mnull}\) mice are sufficiently similar to those of human sporadic and UC-associated CRCs, although the mutational spectrum of the Apc gene appears to be different. In the

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<td>3 (all expansions)</td>
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Table 2. Summary of the p53 mutations

Table 3. Summary of microsatellite instability (MSI)

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A: D7Mit91
B: D19Mit36

Fig. 1. Representative autoradiograms of microsatellite instability assay in colonic adenocarcinomas arising from the β,mnull × IL-2null mice. A, D7 Mit91 locus. Colonic adenocarcinomas (T) from samples 874 and 1822A demonstrate a contraction of microsatellite sequences compared with normal colonic mucosa (N), thereby demonstrating microsatellite instability. In contrast, no difference in electrophoretic mobility was observed between normal and tumor tissue in sample 775 (i.e., microsatellite stable). B, D19 Mit36 locus. In contrast to microsatellite stable sample 874, colonic adenocarcinoma from sample 380 demonstrates an expansion of microsatellite sequences compared with normal colonic mucosa (i.e., microsatellite unstable).

In the present analyses, 100% and 54% of CRCs from the β,mnull × IL-2null mice harbor Apc and p53 mutations, respectively, in the regions corresponding to the mutation hot spots in human CRC. Furthermore, a significant portion of CRCs from this murine model demonstrates microsatellite instability at several loci selected in the present analysis, suggesting mismatch repair defects. Clinically, the β,mnull × IL-2null mice spontaneously develop low- and high-grade dysplasia and invasive adenocarcinoma of the colon in the setting of a mild to moderate degree of chronic colonic mucosal inflammation resembling the human situation (29). Thus, clinical and molecular genetic characteristics of this genetically predisposed murine model of UC-associated CRC suggest that the β,mnull × IL-2null mice are an excellent animal model to study UC-associated colorectal carcinogenesis.

In the present study, 100% of the adenocarcinomas analyzed harbored Apc mutations in the 2738-bp region in exon 15 flanking the human MCR. Forty-five % of the adenocarcinomas analyzed had frameshift or nonsense mutations resulting in truncation of the Apc protein. This observation is consistent with a previous study, which reported a truncating mutation frequency of 50% in human UC-associated dysplasia and CRC (6) but contrasts with a much lower reported a truncating mutation frequency of 50% in human UC-associated CRCs (6, 7), and hence the frequency and nature of truncating mutations would have likely come from three to five separately performed PCR reactions, and only those mutations consistently present in all of the confirmatory sequencing were reported; (b) no Apc mutations were detected in liver and adjacent nonneoplastic colonic DNA from each animal harboring tumors with Apc mutations; (c) the analysis of p53 mutations from the same neoplastic tissues demonstrated a “normal” degree of mutations. Recently, Msh2 deficiency has been observed to be associated with a hypermutated state within the same region of the Apc gene in the normal intestinal mucosa from Apc+/−/Msh2−/− mice, which carry a heterozygous germ-line mutation at codon 850 of the Apc gene and a homozygous mutation of the Msh2 mismatch repair gene (41). Furthermore, intestinal adenomas from these mice contained multiple somatic Apc mutations (an average of 10 mutations/tumor) within the same region of the Apc gene, the majority of which were missense mutations (41). Although the exact frequency and nature of microsatellite instability, and hence mismatch repair defects, were not comprehensively analyzed in the present study, a significant portion of CRCs from this murine model demonstrated microsatellite instability at several loci. Future studies are warranted to determine whether mismatch repair defects inherent in UC-associated CRCs in the β,mnull × IL-2null mice are responsible for the hypermutability of the Apc gene observed in the present study. The allelic location of the observed Apc mutations was not determined in the present study. The unusual large number of Apc mutations would have likely come either from multiple mutations on all alleles or from the presence of cells having more than two sets of chromosomes (i.e., polyploidy), or would have resulted from multiclonality. The occurrence of polyploidy has been observed in several human cancers as well as in murine tumors (42–44). The issues of polyploidy and multiclonality in CRCs from this murine model need further clarification in future studies.

In the present study, 54% of the adenocarcinomas analyzed dem
onstrated \( p53 \) mutations in exons 5, 7, and 8. This mutation frequency is comparable with those (33–100%) observed in human UC-associated CRCs (9–11, 13, 14). All mutations were transitional missense mutations resulting in an amino acid substitution. Of particular interest is the finding of C:G \( \rightarrow \) T:A transitions occurring at nucleotide 688 (codon 229) within CpG sites in 4 (57%) of the 7 \( p53 \) mutations. It appears that this site, which corresponds to codon 264 in humans, is a mutation hot spot for UC-associated CRCs in the \( \beta_m^{2\text{null}} \times IL-2^{\text{null}} \) mice. One point mutation at nucleotide 631 (codon 211) corresponds to one of the \( p53 \) mutational hot spots in human sporadic CRCs (i.e., codon 245). Two human studies have also showed that transitional missense mutations are a predominant type of \( p53 \) mutation in UC-associated CRCs in humans (10, 13). Although \( p53 \) mutations were scattered in exons 5–8 in these studies, codons 248 and 282 appear to be mutational hot spots for UC-associated CRCs in humans (10, 13). Deletions in \( p53 \) and mutations in exons 5–8 in the remaining allele are observed in up to 75% of sporadic CRCs in humans (3, 4, 35). Up to 50% of mutations in human sporadic CRCs are C:G \( \rightarrow \) T:A transitions occurring at CpG sites within these exons (35), despite the fact that CpG sequences represent only a very small proportion of the total genomic sequence. The CpG sequence is also the major site for cytosine methylation, suggesting a possible association between methylation and the genesis of \( p53 \) mutations.

Among the informative samples analyzed for microsatellite instability, 9 adenocarcinomas exhibited microsatellite instability in at least one of the five loci examined. One tumor (no. 380) had microsatellite instability in two loci. This suggests that microsatellite instability, and hence mismatch repair defects, may be a significant molecular event in UC-associated CRCs in this murine model. In humans, microsatellite instability has been found in UC-associated dysplasia (8–21%) and cancer (13–21%; Refs. 22–24) and even in nonneoplastic mucosa (16–50%; Refs. 24, 25). Widespread microsatellite instability is observed in the majority of hereditary nonpolyposis CRCs and 15–20% of sporadic CRCs in humans (45). Because not all samples could be amplified in the present analysis and only five loci were examined, the exact frequency and nature of microsatellite instability cannot be ascertained in this study. One interesting observation is that the tumor (no. 380) with microsatellite instability at two loci had 16 \( Apc \) mutations (13 missense, 1 nonsense, and 2 frameshift) compared with other tumors with microsatellite instability at 1 loci (2–9 \( Apc \) mutations/tumor). This suggests that mismatch repair defects might have caused hypermutability in the region of the \( Apc \) gene analyzed in this tumor. This is further supported by prior observations of hypermutability within the same region of the \( Apc \) gene in intestinal adenomas from \( Apc^{+/−} \text{-} Msh2^{−/−} \) mice as described previously (41). Several animal models of UC-associated CRC are currently available (26). The most commonly used animal model of UC-associated CRC is the chemical carcinogen model. In this model, UC is induced in rodents by chemicals (e.g., trinitrophenylsulfonic acid, dextran sulfate sodium, or 1-hydroxyanthraquinone), and \( Apc \) is induced by coadministration of chemical carcinogens (46–48). These models mimic some aspects of the histopathology of the human UC-associated CRCs. However, the use of different strains and species (that may differ appreciably in their relative susceptibility to various agents), different dosing schedules, and different routes of carcinogen administration influence the outcome of such studies. Furthermore, the relatively high dosages of genotoxic chemical carcinogens differ from the natural etiological causes involved in most cases of human sporadic and UC-associated CRCs. Also, the development of CRC arising from relatively acute and severe colitis associated with these chemical carcinogen animal models does not truly reflect that of CRC arising from chronic quiescent UC in humans. Probably, the most serious limitation of the chemical rodent model of UC-associated CRC is the lack of molecular alterations of the \( Apc \), \( K-ras \), and \( p53 \) genes that are commonly implicated in sporadic and UC-associated CRC in humans (49, 50).

To date, several genetically altered animal models of inflammatory bowel disease have been reported to develop adenomas or CRC spontaneously. Sixty % of the 10-locus-deficient mice develop adenocarcinoma in the setting of chronic transmural and segmental inflammation of the colon (51, 52). This model, therefore, may be useful for studying tumorigenesis in Crohn’s disease (51, 52). Interestingly, colonic adenocarcinomas from the IL-10-deficient mice are not associated with mutations in the \( p53 \), \( Apc \), \( Msh2 \), and \( K-ras \) genes and with microsatellite instability (53). The chimeric-transgenic mouse model which expresses a dominant N-cadherin (which is essential for maintaining cell adhesion and for epithelial polarity, migration and normal development) has been found to develop a Crohn’s disease-like inflammatory bowel disease with skip lesions with the development of adenomas in the duodenum and ileum without progression to adenocarcinoma (54). The \( GF_{344} \)-knockout mice develop inflammation limited to the colon, and 31% develop colonic neoplasia in all parts of the colon between 15 and 36 weeks of age (55). However, 75% of the mice die by 28 weeks, which makes long-term studies investigating the effects of environmental factors on tumorigenesis difficult (55). Transgenic rats, expressing the human MHC molecule HLA-B27 alone or in combination with the \( \beta_m \) gene, develop a spontaneous multisystem disease manifested by colitis, arthritis, and skin changes (56). In HLA-B27 transgenic rats on an inbred F344 background, hyperplastic lesions have been observed to evolve in the setting of chronic colitis, with a high frequency of colorectal poly formation and frequent histological progression from adenoma to adenocarcinoma (57). However, molecular genetics of CRC arising in these genetic models are largely unknown at present.

In summary, the \( \beta_m^{2\text{null}} \times IL-2^{\text{null}} \) mouse appears to be an excellent animal model of UC-associated CRC because the clinical features and molecular genetics, except for the mutational spectrum of the \( Apc \) gene, of this genetically predisposed murine model are similar to those of UC-associated CRC in humans. This model provides an excellent opportunity to investigate the effects of environmental and genetic factors on colorectal carcinogenesis associated with chronic UC. Furthermore, this model may be used for chemoprevention with agents such as folate (58, 59), short-chain fatty acids (60), and 5-aminosalicylic acid (61, 62) that appear to be promising in the prevention of the development of UC-associated CRC.

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Molecular Genetics of Ulcerative Colitis-associated Colon Cancer in the Interleukin 2- and \( \beta_2 \)-Microglobulin-deficient Mouse

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