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# Enhanced colitis-associated colon carcinogenesis in a novel *Apc* mutant rat

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**To establish an efficient rat model for colitis-associated colorectal cancer, azoxymethane and dextran sodium sulfate (AOM/DSS)-induced colon carcinogenesis was applied to a novel adenomatous polyposis coli (*Apc*) mutant, the Kyoto Apc Delta (KAD) rat. The KAD rat was derived from ethylnitrosourea mutagenesis and harbors a nonsense mutation in the *Apc* gene (S2523X). The truncated APC of the KAD rat was deduced to lack part of the basic domain, an EB1-binding domain, and a PDZ domain, but retained an intact β-catenin binding region. KAD rats, homozygous for the *Apc* mutation on a genetic background of the F344 rat, showed no spontaneous tumors in the gastrointestinal tract. At 5 weeks of age, male KAD rats were given a single subcutaneous administration of AOM (20 mg/kg, bodyweight). One week later, they were given DSS (2% in drinking water) for 1 week. At week 15, the incidence and multiplicity of colon tumors developed in the KAD rat were remarkably severe compared with those in the F344 rat: 100 versus 50% in incidence and 10.7 ± 3.5 versus 0.8 ± 1.0 in multiplicity. KAD tumors were dominantly distributed in the rectum and distal colon, resembling human colorectal cancer. Accumulation of β-catenin protein and frequent β-catenin mutations were prominent features of KAD colon tumors. To our knowledge, AOM/DSS-induced colon carcinogenesis using the KAD rat is the most efficient to induce colon tumors in the rat, and therefore would be available as an excellent model for human colitis-associated CRC. (*Cancer Sci* 2009; 100: 2022–2027)**

Colorectal cancer (CRC) is one of the leading causes of cancer deaths in the world. Globally, the CRC mortality was 639 000 in 2004.[1] Chronic inflammation has been identified as a potential risk factor for CRC. Clinical studies have shown that inflammatory bowel disease, such as Crohn's disease[2] and ulcerative colitis,[3] elevates the risk of CRC.

Animal experiments are assumed to simulate or at least provide plausible pathophysiological mechanisms of various diseases, including cancer and chronic inflammatory disorders. For inflammation-related CRC, a two-stage colitis-related mouse colon carcinogenesis model was recently established.[4] In this model, colon carcinogenesis is initiated with carcinogens and then dextran sodium sulfate (DSS), which can induce colonic mucosal inflammation resembling the histopathology of ulcerative colitis, is used as a tumor-promoting agent. Colon carcinogenesis initiated with carcinogens such as azoxymethane (AOM),[4] 1,2-dimethylhydrazine (DMH),[5] and heterocyclic amines[6] is effectively promoted in combination with DSS.

The two-stage colitis-related model has been applied to a rat colon carcinogenesis study. Similar to mice, DSS also promotes DMH-induced[7] and AOM-induced[8]colon carcinogenesis in the F344 rat. These rat models can be utilized to investigate the pathogenesis of colitis-related colon carcinogenesis and to detect carcinogenesis modifiers.[7,8] For more effective colorectal carcinogenesis, however, a novel model, in which much more and larger tumors are induced in a shorter experiment period, is required. It would be preferable to obtain a large volume of tumors, sufficient to be identifiable on macroscopic observation, because this would be an advantage in testing chemotherapeutic efficacy of anticancer drugs as well as chemoprevention ability of anti-inflammatory drugs.

One possible idea to enhance the AOM/DSS model is deficiency of the tumor suppressor adenomatous polyposis coli (*Apc*) gene, which plays a significant role in tumor development in the gut,[9] for example, AOM enhances colorectal carcinogenesis in $Apc^{min/+}$ mice that carry a germline mutation in the *Apc* gene and develop multiple polyps in the intestine.[10] Furthermore, DSS strongly promotes colorectal carcinogenesis in $Apc^{min/+}$ mice.[11] These findings prompted us to examine AOM/DSS-induced colon carcinogenesis in an *Apc* mutant rat. In the rat, an *Apc*-deficient Pirc rat is available.[12] The Pirc rat carries a nonsense mutation in the *Apc* gene and the resultant truncated APC (Δ1137) lacks the β-catenin binding region. The Pirc rat develops multiple tumors with a distribution between the colon and small intestine. The average number of colonic polyps is 8 ± 3 in Pirc rats aged 4–6 months, and most of them are adenomas. *N*-ethyl-*N*-nitrosourea (ENU) treatment enhances colonic polyps, but it takes more than 7 months to obtain them.[12] However, a carcinogenesis test with AOM alone or AOM/DSS has not yet been done in the Pirc rats.

We have recently produced a rat mutant archive consisting of cryopreserved sperm derived from ∼5000 ENU-mutagenized male rats and corresponding DNA samples. The mutant archive is estimated to store ∼2 million mutations, sufficient to find several mutations in a particular gene of interest.[13]

We present here a novel homozygous *Apc* mutant rat strain, the Kyoto Apc Delta (KAD) rat, from the rat ENU-mutant archive. KAD rats harbor a nonsense mutation in exon 15 that results in premature termination at codon 2523 of the serine residue of APC protein. KAD rats are viable and show no spontaneous tumors in the small intestine or colorectum. Treatment with AOM/DSS revealed an increased susceptibility of KAD rats to colitis-associated colon carcinogenesis in a 15-week experimental period. Also, the development of colorectal tumors can be tracked by endoscopic observation. AOM/DSS-induced colon carcinogenesis in the KAD rats will provide a novel rat model for investigating colitis-related colon carcinogenesis, identifying xenobiotics with modifying effects, and evaluating anticancer drug candidates.

## Materials and Methods

**Establishment of KAD rat strain.** A total of 1735 DNA samples from ENU-mutagenized F344/NSlc rats from the Kyoto

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University Rat Mutant Archive (KURMA) were screened with seven sets of primers (Table S1). These primers were designed to amplify exons 9, 11, 14, or 15 of the rat *Apc* gene. Approximately 6.27 Mb of genomic DNA was screened. Rats carrying the *Apc* mutation were recovered by intracytoplasmic sperm injection.[13] Male rats carrying the *Apc* mutation were backcrossed five times with female F344/NSlc rats to remove latent mutations induced by ENU.

**Western blotting.** Proteins were prepared from the brainstems of KAD and control F344/NSlc rats at 5 weeks of age. Western blotting and signal detection were carried out as described.[14] Antibodies against the N terminus of APC (H-290; Santa Cruz Biotechnology, Santa Cruz, CA, USA), the C terminus of APC (C-20; Santa Cruz Biotechnology), and β-actin (AC-40; Sigma-Aldrich Japan, Tokyo, Japan) were used. Secondary antibodies against rabbit IgG (NA934; GE Healthcare Bio-Sciences, Tokyo, Japan) and mouse IgG (NA931; GE Healthcare Bio-Sciences) were used.

**Carcinogenesis test.** Colon carcinogenic tests were carried out as described.[4] Briefly, male KAD rats ($n$ = 17) were divided into three experimental and control groups. Group 1 ($n$ = 6) was given a single subcutaneous injection of AOM (20 mg/kg bodyweight) at 5 weeks of age. Starting 1 week after the AOM injection, animals were given 2% DSS in drinking water for 7 days and then no further treatment for 13 weeks. Groups 2 ($n$ = 5) and 3 ($n$ = 3) were given AOM alone and DSS alone, respectively. Group 4 ($n$ = 3) was untreated. Male F344/NSlc rats ($n$ = 6) were treated with AOM followed by DSS (group 5) and were controls of group 1. All rats were maintained under the conditions of humidity (50 ± 10%), light (14 : 10 h L : D cycle), and temperature (24 ± 2°C) at the Institute of Laboratory Animals, Graduate School of Medicine, Kyoto University. At 15 weeks after the AOM injection, they were killed by cervical dislocation under anesthesia with isoflurane (Forane; Abbott Japan, Tokyo, Japan). All experimental procedures were approved by the Animal Research Committee of Kyoto University and were carried out according to the Regulation on Animal Experimentation at Kyoto University.

**Histopathology and immunohistochemistry.** At autopsy, the colorectum of the rats was resected, washed with PBS, and opened longitudinally along the main axis. After careful macroscopic inspection, tumors and the colonic mucosa were dissected and processed for histopathological examination with hematoxylin–eosin staining. Immunohistochemical staining of β-catenin was carried out as described previously.[15]

**Endoscopic observation and biopsy.** Endoscopic observations were carried out every week after the 8 weeks of the carcinogenesis tests. Anesthesia was administrated through the regulated flow of isoflurane vapor (2%) through a nose cone. The colon was flushed with a tap water enema. The endoscope (BF TYPE 3C40; Olympus, Tokyo, Japan) was inserted into the colon and endoscopic images were acquired. A tumor specimen was biopsied under microscopic observation.

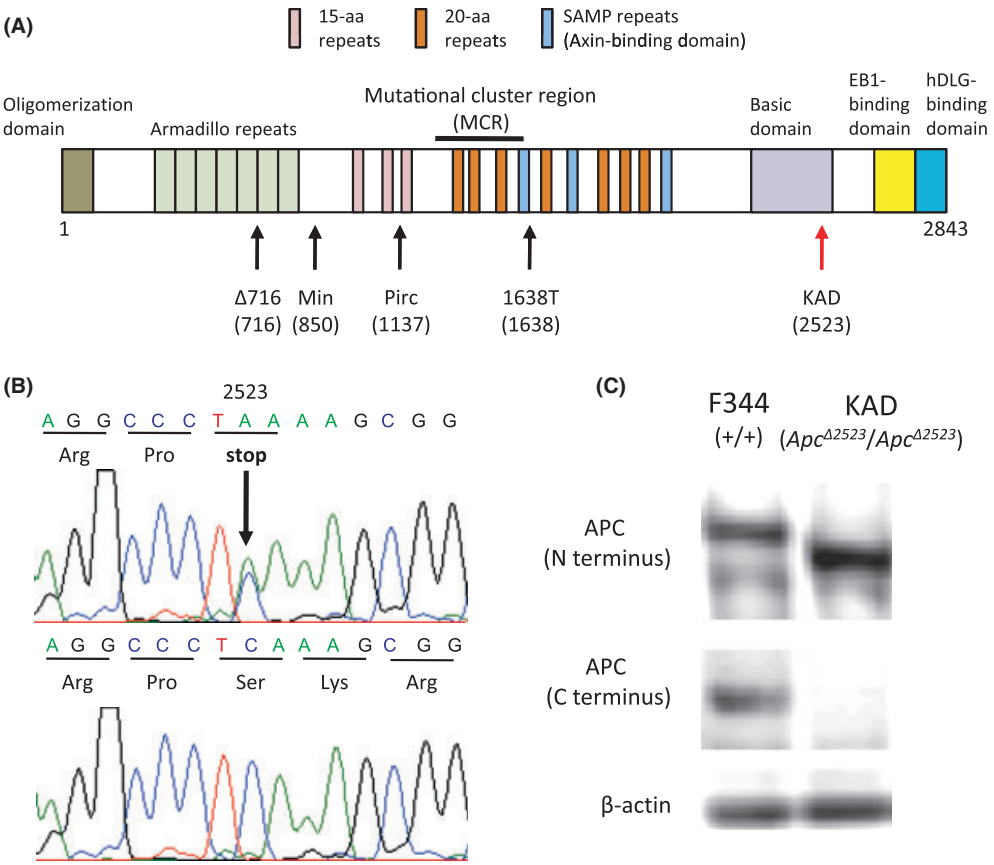


**Fig. 1.** Establishment of the Kyoto Apc Delta (KAD) rat strain. (A) Schematic diagram that shows multiple domains of full-length adenomatous polyposis coli (APC). Black arrows indicate orthologous locations of truncating mutations in mouse and rat models. The nonsense mutation in the KAD rat is indicated by a red arrow. (B) Sequence trace of a founder rat showing heterozygosity for C-to-A transversion (arrow) at nucleotide 7621 of the *Apc* gene (upper) compared with wild-type littermates (lower). The mutation generated a premature stop codon (TAA) at the 2523 amino acid position of APC. (C) Western blot analysis of APC in KAD and control F344/NSlc rats. Proteins extracted from the brains of F344 (+/+) and KAD ($Apc^{\Delta 2523}/Apc^{\Delta 2523}$) rats were hybridized with anti-N terminus of APC (top), anti-C terminus of APC (middle) antibodies. β-Actin was used as an internal control (bottom). In KAD rats, smaller APC protein was detected with the anti-N terminus APC antibody than F344 rats, and no signal was detected with the anti-C terminus APC antibody.

**Mutation detection.** Mutations of the β-catenin (*Catnnb1*) or K-ras (*Kras*) genes in tumors were screened by direct sequencing. Genomic DNA was extracted from tissues stored in RNAlater (Applied Biosystems, Inc., Carlsbad, CA, USA). PCR primers were designed to amplify mutational hot spots detected in the AOM-induced colon tumors.[16] The nucleotide sequences of primers were as follows: rCatnnb1-F, GCTGACCTCATGGAGTTGGA and rCatnnb1-R, GCTACTTGCTCTTGCGTGAA; rKras-F, TGAATTCAGAATGCCTTAGAGTTTT and rKras-R, GCACCGATGGTTCCCTATTA. DNA sequencing was carried out as described previously.[17]

## Results

**Establishment of the KAD rat.** A C-to-A point mutation was detected in the DNA archive of KURMA and was predicted to result in premature termination at codon 2523 of the serine residue of the APC protein (Fig. 1A). Rats carrying the mutation were recovered from the corresponding frozen sperm (KURMA sperm archive number: ENU1588) with intracytoplasmic sperm injection.[13] The nonsense mutation (c. 7621C > A, p. Ser2523X) was confirmed in recovered animals, which were $F_1$ hybrids between recipient F344/NSlc and $G_1$ donor animals (Fig. 1B); we therefore named this allele $Apc^{\Delta 2523}$. The deduced APC protein was predicted to lack a part of the basic domain, EB1-binding domain, and PDZ domain (Fig. 1A). Because homozygous Min mice and Pirc rats have been reported to be embryonic lethal,[12,18] we crossed $Apc^{\Delta 2523}$ heterozygous mutants to obtain $Apc^{\Delta 2523}$ homozygotes. Rats homozygous for $Apc^{\Delta 2523}$ were viable and survived almost 2 years. We thus designated the $Apc^{\Delta 2523}$ homozygous strain the KAD rat. Western blot analyses indicated a lack of the C terminus of APC protein in the KAD rat (Fig. 1C). Cellular localization of β-catenin protein was not altered in the colon epithelia of the KAD rat, compared with the F344 rat (data not shown).

**High susceptibility to colitis-associated colon carcinogenesis in the KAD rat.** $Apc^{\Delta 2523}$ homozygous KAD developed no spontaneous tumors in their gastrointestinal tracts even after 20 months of age. We then tried to induce colon tumors in the KAD rats by administrating AOM as a chemical colonic carcinogen and/or DSS as a colitis-inducing agent. The AOM-treated (group 2), DSS-treated (group 3), and non-treated (group 4) KAD rats showed no colon tumors on either macroscopic or microscopic observation. Meanwhile, AOM/DSS-treated KAD rats (group 1) developed multiple colon tumors, of which the incidence, number, and volume could be compared with those of tumors developed in AOM/DSS-treated F344 rats (group 5). Interestingly, the AOM/DSS-treated KAD rats showed a higher incidence of diarrhea than the AOM/DSS-treated F344 rats during a few weeks after the cessation of DSS treatment (Fig. 2).

Macroscopically, all of the AOM/DSS-treated KAD rats showed multiple nodular, polypoid, or caterpillar-like colonic tumors (Fig. 3A), whereas half of the AOM/DSS-treated F344 rats had a few colonic tumors (Fig. 3B). The average number of colorectal tumors in the KAD rat was significantly higher than that of F344 rats (9.5 ± 1.8 vs 1.3 ± 0.8, $P < 0.0001$) (Fig. 4A). The average volume of KAD tumors was not different from that of F344 tumors (33.9 ± 23.0 $mm^3$ vs 10.3 ± 13.7 $mm^3$, $P = 0.38$). Colon tumors that developed in the KAD rats that received AOM and DSS were distributed more prominently in the rectum (4.0 ± 1.5) and distal colon (5.2 ± 1.7) than in the middle colon (0.3 ± 0.5) (Fig. 4B). No tumors were observed in the proximal colon, cecum, or small intestine.

Microscopically, tumors induced in AOM/DSS-treated KAD rats were diagnosed as tubular adenoma (Fig. 5A), well or moderately differentiated tubular adenocarcinoma (Fig. 5B), or signet-ring cell carcinoma (Fig. S1). The multiplicity of adenoma of the KAD rat was significantly higher than that of the F344 rat (7.7 ± 2.9 vs 0.3 ± 0.5, $P < 0.005$) (Fig. 4C). The multiplicity of adenocarcinoma of the KAD rat was also significantly higher than that of the F344 rat (3.0 ± 0.9 vs 0.5 ± 0.8, $P < 0.001$) (Fig. 4C). Twenty-two of 64 colon tumors induced in the KAD rats invaded the submucosa, muscularis propria, or serosa

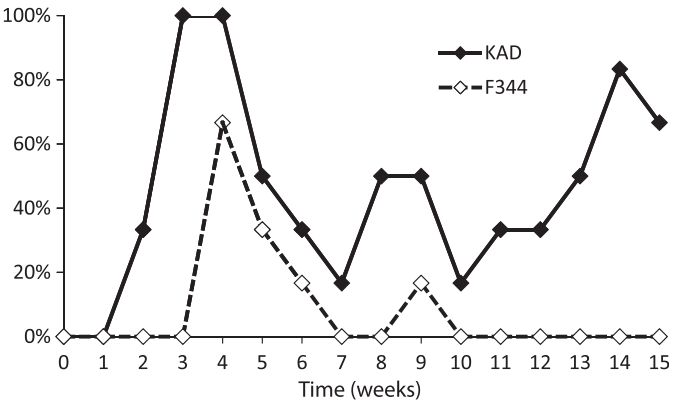


**Fig. 2.** Incidences of diarrhea observed in azoxymethane (AOM)/dextran sodium sulfate (DSS)-treated Kyoto Apc Delta (KAD) and F344 rats. Percentages of rats showing diarrhea in weekly observations are shown. One-week DSS administration is indicated by a grey box. Note that all KAD rats showed diarrhea in week 3, whereas no F344 rats showed diarrhea.

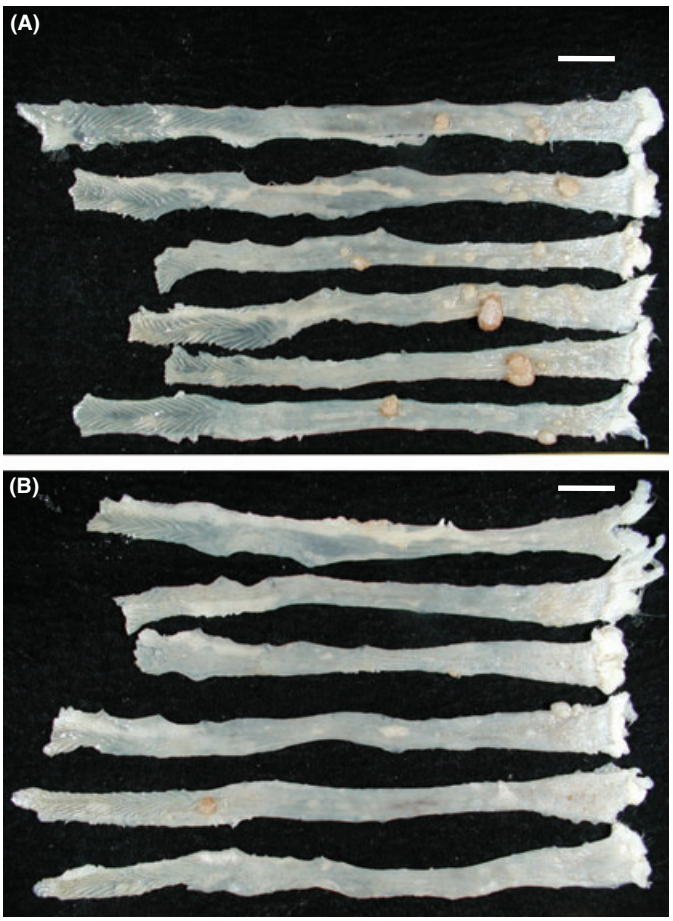


**Fig. 3.** Macroscopic view of large bowels of azoxymethane (AOM)/dextran sodium sulfate (DSS)-treated (A) Kyoto Apc Delta (KAD) and (B) F344 rats. Scale bars = 2 cm.


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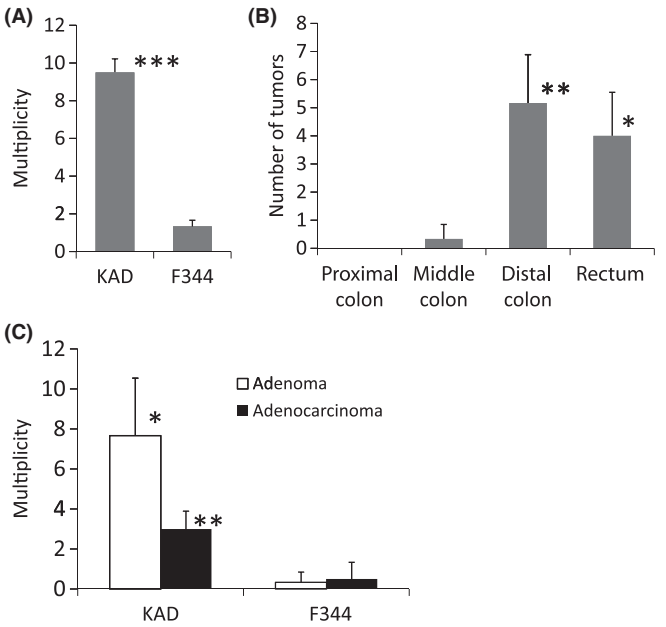


**Fig. 4.** Increased induction of colon tumors in azoxymethane (AOM)/dextran sodium sulfate (DSS)-treated Kyoto Apc Delta (KAD) rats. (A) Multiplicity of tumors observed macroscopically (mean ± SD) at week 15. ***$P < 0.0001$. (B) Distribution of colon tumors in AOM/DSS-treated KAD rats (mean ± SD) at week 15. **Distal colon versus middle colon, $P < 0.001$; *rectum versus middle colon, $P < 0.005$. (C) Multiplicities of adenoma and adenocarcinoma developed in KAD rats were significantly higher than in F344 rats at week 15. *$P < 0.005$, **$P < 0.001$.

(Fig. 5C), whereas none of the five colon tumors in F344 rats invaded the submucosa or deeper. Four signet-ring cell carcinomas were observed in the KAD rats. Apart from colonic tumors, colonic dysplasia was observed in all of the rats in groups 1 and 5. The average number of dysplasias in the KAD rats (11.2 ± 8.0) was greater than that of the F344 rats (3.7 ± 4.1), but the difference was insignificant ($P = 0.069$). No dysplastic lesions developed in groups 2–4.

Altered cellular localization of β-catenin protein is frequently observed in AOM- or AOM/DSS-induced colorectal tumors.[4,16] Strong β-catenin expression was seen in the cytoplasm and/or nucleus of adenoma and adenocarcinoma cells (Fig. 5D), which indicated the activation of Wnt signaling in these cells.

**Endoscopic observation and biopsy of colon tumors.** Endoscopic examination was done in the anesthetized KAD rats to determine whether the development of colorectal tumors can be observed without necropsy. We could observe colorectal lesions displaying differences from normal mucosa, including polypoid lesions, as early as the eighth week after AOM administration (Fig. S2A) and could monitor the development of both the number and volume of them during the carcinogenesis test (Fig. S2B). At week 8, the average number of lesions detected by endoscopy was 3.3 ± 1.2. The number of lesions gradually increased with the experimental period and reached 17.3 ± 4.5 at week 14, which was much higher than that of macroscopic observations. Such a discrepancy might be caused by the disappearance of inflammatory polyps at week 14. The biopsy of a tumor specimen under endoscopic observation was successful and the specimen was diagnosed histopathologically (Fig. S2C).

**Highly frequent mutations of the β-catenin gene but no mutation of the K-ras gene in colon tumors.** Mutation of the β-catenin gene in its glycogen synthase kinase (GSK) 3β phosphorylation consensus motif and K-ras mutation at codon 12 are features of AOM-induced rat colon tumors.[15,16] Direct sequencing of PCR products revealed 29 missense mutations in 29 of 39 (74.4%) colon tumors induced by AOM/DSS in KAD rats (Table 1). The mutation spectrum detected in the present study was quite similar to that detected in the AOM-induced rat colon tumors,[16] which indicated that a common molecular pathway to initiate colon carcinogenesis was shared in AOM- and AOM/DSS-treated colons. Meanwhile, no K-ras mutation at codon 12 was detected in the 39 colon tumors.

## Discussion

A two-stage colitis-related colon carcinogenesis model provides a powerful tool for the induction of colon tumors in rats.[7,8] In the current study, to establish a more efficient colon carcinogenesis model, we produced a novel *Apc*-mutant KAD rat. The

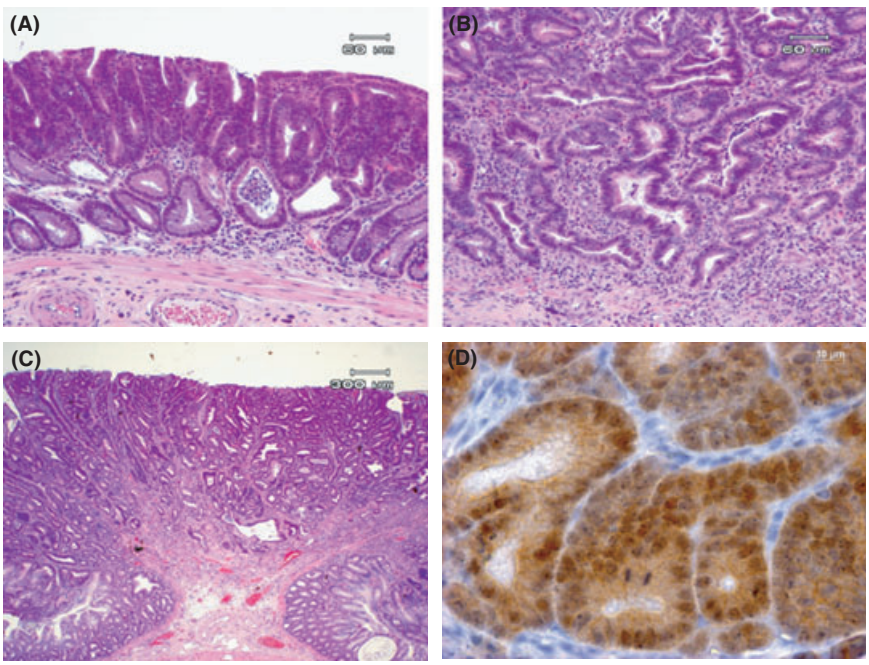


**Fig. 5.** Histopathology of colonic tumors developed in azoxymethane (AOM)/dextran sodium sulfate (DSS)-treated Kyoto Apc Delta (KAD) rats. (A) Tubular adenoma, (B) well-differentiated adenocarcinoma, and (C) moderately differentiated adenocarcinoma invading the submucosa. Hematoxylin–eosin stain. (D) β-Catenin immunohistochemistry in colonic adenocarcinoma.

**Table 1. Mutations in the GSK3β phosphorylation consensus motif of the *Catnnb* gene in colon tumors**

| Mutated codon | Base change | Amino acid substitution† | No. mutations detected |
|---|---|---|---|
| 32 | GAT → AAT | Asp → Asn | 8 |
| 33 | TCT → TTT | Ser → Phe | 4 |
| 34 | GGA → GAA | Gly → Glu | 8 |
| 37 | TCT → TTT | Ser → Phe | 2 |
| 41 | ACC → ATC | Thr → Ile | 4 |
| 44 | CCT → CTT | Pro → Leu | 1 |
| 45 | TCC → TTC | Ser → Phe | 2 |
| Total | | | 29 |

†Serine residues in codons 33, 37, and 45 and the threonine residue in codon 41 are GSK3β phosphorylation sites.

KAD rat harbored a nonsense mutation resulting in the truncated APC protein (Δ2523), in which the β-catenin-binding region was retained. The KAD rat was viable and showed the normal distribution of β-catenin in colon epithelium and no spontaneous colon tumors. These findings suggested that Wnt signal might not be activated in the non-treated colon epithelium of KAD rats. In humans, a subset of attenuated familial adnomatous polyposis harbors C-terminal-truncated APC mutations such as Δ2644 and Δ2663.[19,20] The Δ2644 APC protein failed to activate Wnt signaling,[21] and these patients are rarely related to the occurrence of colonic polyposis, but are responsible for the development of extracolonic lesions, including desmoids, gastric fundic grand hyperplastic polyposis, and osteomas. Although we have not yet observed such extracolonic lesions in the KAD rat, further examinations will allow us to establish the KAD rat as a model for attenuated familial adnomatous polyposis.

The AOM/DSS-treated KAD rat showed colon tumors in 100% incidence, much higher in multiplicity (∼10-fold) and more advanced in malignancy than the control F344 rat. These tumors can be obtained in only a short period of 15 weeks. We therefore concluded that AOM/DSS colon carcinogenesis was extensively enhanced in the KAD rat. This carcinogenesis model has also several advantages over the Pirc rat model. The epithelial malignancy of our model is more significant than the Pirc model: our model could induce adenocarcinomas (multiplicity is 3.0 ± 0.9), whereas most tumors developed in the colon of the Pirc rat were adenomas. This suggests that we could obtain multiple colon tumors in a shorter period (<15 experimental weeks). Next, we can evaluate the effects of potential carcinogens on colon carcinogenesis more strictly, because KAD are free from spontaneous tumors. Additionally, we can prepare tumor-bearing animals in accordance with our needs, which is a major concern in practical studies. It has been thought that an ideal colon carcinogenesis model would involve not only the efficient induction of tumors but also similar tumor characteristics and good availability for clinical application. The colon tumors developed in the KAD rat showed a predominant distribution in the rectum and distal colon and the accumulation of β-catenin protein, similar to human CRC. Furthermore, the tumors induced were large enough to be observed by endoscopy and biopsied tumor specimens were successfully diagnosed. These findings indicate that the KAD colorectal carcinogenesis model has the potential to mimic clinical operations for human CRC. Our results described here strongly suggest that AOM/DSS-induced colon carcinogenesis with the KAD rat model is ideal and provides an excellent tool to investigate basic and clinical studies on colitis-related CRC. For example, this model enables efficient evaluation of the effects of novel anticancer drugs on tumor regression as well as the effects of anti-inflammatory agents on tumor development. Combination with recently developed fluorescence probes that image viable cancer cells[22] would provide clearer images of tumors and further insights into the pathogenesis of CRC.

In contrast with the AOM/DSS-treated KAD rats, neither AOM-treated nor DSS-treated KAD rats developed colon tumors. It is well known that no colon tumors occurred in the AOM-treated or DSS-treated F344 rats within as short as 15 weeks by the carcinogenesis test.[7,23,24] All AOM/DSS-treated KAD rats developed colon tumors. They also had significant diarrhea for a few weeks after cessation of the DSS exposure. These findings indicate that the KAD rat is susceptible to inflammation provoked by a colitis-inducing agent, DSS, and suggest that severe inflammation of the colon epithelia might be involved in the enhancement of colon carcinogenesis in the KAD rat.

DSS-induced colitis occurs mainly in the distal colon,[25] which is consistent with the predominant distribution of tumors to the distal colon and rectum in the AOM/DSS-treated KAD rat. Additionally, no K-ras mutation was found in the tumors of AOM/DSS-treated KAD rats. K-ras mutation plays a role as a promoter through enhancing COX-2 and iNOS expression in the presence of inflammatory stimuli.[16] However, it is likely that, in our model, DSS enabling the induction of severe inflammation might replace the K-ras mutation. In fact, no mutations of K-ras and a high incidence of substitutions of *Apc* and *p53* genes were found in the colonic tumors induced by a colonic carcinogen, DMH, and a colitis-inducing compound, trinitrobenzene sulfonic acid.[26] These findings may support our idea that inflammation provoked by DSS plays an important role in colon carcinogenesis in the KAD rat, and the C terminus of APC, which is lacking in the KAD rat, might be involved in the effect of DSS on tumor development.

The C terminus of APC, which is lacking in the KAD rat, comprises a 321-amino acid polypeptides and contains a part of the basic domain, EB1-binding domain, and PDZ domain,[27] by which APC interacts with a variety of cytoskeletal proteins, such as microtubules, the microtubule plus end binding protein (EB1), and the mammalian homolog of Discs large.[28–30] With these domains, APC contributes directly and/or indirectly to cell migration, adhesion, chromosome segregation, spindle assembly, and apoptosis in the epithelium of the gut.[31,32] In the DSS colitis model, microbiota alteration, epithelial cell toxicity, increased intestinal permeability, and macrophage activation have been proposed as potential pathogenesis mechanisms of colitis.[33,34] Although so far there is no direct evidence linking these colitis pathogeneses to the functions of APC domains, it is expected that cell migration or adhesion occurring in response to DSS treatment might be disturbed in KAD by the lack of the C-terminal domains. Alternatively, the responses of epithelial cells to cytokines released from macrophages induced by DSS might be altered. Further pathophysiological analysis of the KAD rat colon epithelium would provide insights into the association of the C terminus of APC with colitis. Importantly, other rodent *Apc* mutant models, such as Min mice and Pirc rats, completely lose all protein interaction sites located in the C-terminal half of the protein. Thus, it is very difficult to determine whether the susceptibility to DSS-induced colitis would result from the effects of the C-terminal or central regions of APC.

In summary, we established an enhanced rat AOM/DSS-induced colitis-related colon carcinogenesis model using a novel *Apc* mutant KAD rat. This colon carcinogenesis model system, to our knowledge, is the most effective in the experimental induction of colon tumors and therefore will contribute greatly to promote experimental studies on the pathogenesis, prevention, and treatment of CRC. The KAD rat also provides insights into the involvement of the C terminus of APC in the development of colitis-related CRC.


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## Supporting Information

Additional supporting information may be found in the online version of this article:

**Fig. S1.** Signet-ring cell carcinoma observed in the colon of AOM/DSS-treated KAD rats. Four signet-ring cell carcinomas were observed in AOM/DSS-treated KAD rats (group 1).

**Fig. S2.** Endoscopic observation of KAD colon tumors and biopsy. (A) Endoscopic image of a colon tumor in a KAD rat at week 11. Bleeding from this tumor was found (arrow). (B) Development of colorectal lesions in KAD rats treated with AOM and DSS (group 1). The average numbers of lesions observed by endoscopy were plotted. (C) Microscopic view of a specimen biopsied under endoscopic observation. The specimen was diagnosed as well-differentiated adenocarcinoma. Bar: 60 μm.

**Video S1.** Biopsy of a colorectal tumor induced by AOM/DSS two-stage colitis-related carcinogenesis in the KAD rat.

**Table S1.** Primers used in screening for *Apc* mutation in KURMA ENU-mutagenized DNA archives.

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