Impaired Self-Renewal and Increased Colitis and Dysplastic Lesions in Colonic Mucosa of AKR1B8-Deficient Mice

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Abstract

Purpose: Ulcerative colitis and colitis-associated colorectal cancer (CAC) is a serious health issue, but etiopathological factors remain unclear. Aldo-keto reductase 1B10 (AKR1B10) is specifically expressed in the colonic epithelium, but downregulated in colorectal cancer. This study was aimed to investigate the etiopathogenic role of AKR1B10 in ulcerative colitis and CAC.

Experimental Design: Ulcerative colitis and CAC biopsies (paraffin-embedded sections) and frozen tissues were collected to examine AKR1B10 expression. Aldo-keto reductase 1B8 (the ortholog of human AKR1B10) knockout (AKR1B8−/−) mice were produced to estimate its role in the susceptibility and severity of chronic colitis and associated dysplastic lesions, induced by dextran sulfate sodium (DSS) at a low dose (2%). Genome-wide exome sequencing was used to profile DNA damage in DSS-induced colitis and tumors.

Results: AKR1B10 expression was markedly diminished in over 90% of ulcerative colitis and CAC tissues. AKR1B8 deficiency led to reduced lipid synthesis from butyrate and diminished proliferation of colonic epithelial cells. The DSS-treated AKR1B8−/− mice demonstrated impaired injury repair of colonic epithelium and more severe bleeding, inflammation, and ulceration. These AKR1B8−/− mice had more severe oxidative stress and DNA damage, and dysplasias were more frequent and at a higher grade in the AKR1B8−/− mice than in wild-type mice. Palpable masses were seen in the AKR1B8−/− mice only, not in wild-type.

Conclusion: AKR1B8 is a critical protein in the proliferation and injury repair of the colonic epithelium and in the pathogenesis of ulcerative colitis and CAC, being a new etiopathogenic factor of these diseases. Clin Cancer Res; 1–11. ©2014 AACR.

Introduction

Ulcerative colitis (ulcerative colitis) is a chronic inflammatory disease of the colon characterized by mucosal inflammation (1). The current model for the pathogenesis of ulcerative colitis is that in the colonic mucosa, there is an inappropriately robust immune response to commensal bacteria. This is driven at least in part by genetic defects or susceptibility in the host although no single gene has been found to be either necessary or sufficient for the development of ulcerative colitis (2, 3).

Long-standing ulcerative colitis is associated with an increased incidence of colorectal cancer (4, 5). The pathogenesis of this colitis-associated cancer (CAC) has some similarities and some differences when compared with the pathogenesis of sporadic colorectal cancer. Colorectal cancers, either sporadic or CAC, develop from a dysplastic precursor lesion. In sporadic colorectal cancer, the dysplastic precursor is usually an adenomatous polypl, a discrete focus of neoplasia; in contrast, CAC develops from dysplastic lesions that can be polyplid, flat, localized, or multifocal (6, 7). Like sporadic colorectal cancer, CAC occurs through mutations of somatic cells followed by clonal expansion (5).

Many of the cellular defects associated with the development of sporadic colorectal cancer are also seen with CAC. Frequencies of chromosomal instability and microsatellite instability are similar in both (8). However, progression of sporadic colorectal cancer differs from CAC in the timing and frequency of these alterations. For example, loss of adenomatous polyplosis coli (APC) function is an early event in the progression of sporadic colorectal cancer, but is less frequent and occurs later in the development of CAC. In contrast, the loss of p53 function is an important early step in progression of CAC, but occurs later in the development of sporadic colorectal cancer (9–11).
CAC progresses from no dysplasia to low-grade dysplasia to high-grade dysplasia to carcinoma. This progression is primarily driven by somatic mutations that develop in response to chronic inflammation (5, 11). Although it is clear that somatic mutations seen in CAC are related to chronic inflammation, it is not clear how this chronic colonic inflammation leads to the somatic mutations. Inflammatory oxidative stress is a critical factor in the pathogenesis of CAC (10, 12, 13). Overloaded reactive oxygen species (ROS) target a wide range of macromolecules including proteins, DNA and lipids. ROS cause protein dysfunction by interacting with susceptible amino acid residues (14) and induce DNA mutations and breaks by forming 8-oxo-deoxyguanosine (8-oxodG) adducts (13). Free radical-mediated oxidative stress also induces lipid peroxidation and biomembrane damage (15). Lipid peroxides are electrophilic carbonyl compounds and induce lipid peroxidation and biomembrane damage (13). Lipid peroxides are electrophilic carbonyl compounds and are highly cytotoxic and genotoxic (16–18). By interaction with proteins and DNA, peroxides form covalently modified protein or DNA adducts, leading to protein dysfunction and DNA mutations and breaks. Therefore, cellular and DNA damage induced by oxidative stress provides a mechanistic basis for events, including epithelial damage, genetic instability, and gene mutations which drive the severity of ulcerative colitis and its progression to cancer.

Aldo-keto reductase 1B10 (AKR1B10), also known as aldose reductase-like-1 (ARL-1), is a multifunctional protein (19). It functions as a monomeric NADPH-dependent enzyme that efficiently reduces cytotoxic and carcinogenic α, β-unsaturated carbon compounds to less toxic alcoholic forms, protecting host cells from carbonyl damage (19–21). AKR1B10 also functions as an important regulator of de novo fatty acid/lipid synthesis. Through physical association, AKR1B10 prevents the ubiquitination and proteasomal degradation of acetyl-CoA carboxylase-α (ACCA), a rate-limiting enzyme in the de novo fatty acid synthesis, thus promoting fatty acid synthesis (22, 23). Aldo-keto reductase 1B8 (AKR1B8) is the ortholog of human AKR1B10 in mouse (24). AKR1B10 and AKR1B8 are both primarily expressed in the colon and small intestine and have similar functions in carbonyl detoxification and lipid biosynthesis (19, 24). In the intestine, AKR1B10 eliminates carbonyl compounds and thus plays a protective role to the colonic mucosa. AKR1B10 may also promote fatty acid/lipid synthesis in the colonic mucosa and thus facilitate the constant renewal of cryptic cells. The loss of AKR1B10 might be expected to increase the severity of ulcerative colitis and DNA instability, thus accelerating the development of dysplasia in patients with ulcerative colitis. In this study, we addressed the question of whether the AKR1B10 plays a role in blocking the development of dysplasia in ulcerative colitis. To address this question, we produced an AKR1B8-deficient (AKR1B8−/−) mouse strain and studied dextran sulfate sodium (DSS)-induced colitis and CAC. Our results showed an important role for AKR1B8 in the pathogenesis of ulcerative colitis and CAC.

Materials and Methods

Ethics statement

Institutional Review Board (IRB) protocols were approved by Springfield Committee for Research Involving Human subjects. Animal protocols were approved by Southern Illinois University School of Medicine Laboratory Animal Care and Use Committee (LACUC; Springfield, IL).

Human sample procurement

Two groups of human specimens were procured with approved IRB protocols. One is paraffin-embedded sections of biopsies (n = 79) from Department of Pathology at Memorial Medical Center, Springfield, IL. These sections were used for IHC of AKR1B10 expression. The other is the frozen surgical specimens procured from the Tissue Bank in Simmons Cancer Institute at the Southern Illinois University. These frozen specimens were used for Western blot analysis and RT-PCR analyses of AKR1B10 expression.

AKR1B8−/− mouse generation and DSS treatments

Heterozygous AKR1B8-mutant C57BL/6 mice were generated in Deltagen Research Laboratory. In the animal facility at Southern Illinois University School of Medicine, the heterozygous pairs were used to generate homozygous AKR1B8−/− and littermate wild-type (WT) mice for experimental studies. Animals were housed at 24°C±0.5°C, 50%±10% humidity, and 12 hours of light from 8:00 am to 8:00 pm with free access to regular commercial diet and tap water. Chronic colitis in mice was induced using 2% DSS (35,000–50,000 kDa; MP Biomedicals) for four cycles (Supplementary Fig. S3; refs. 25, 26). Each cycle consisted of 7 days of DSS in drinking water, followed by 14 days of DSS-free water. Animals were monitored daily for body weight, occult and gross rectal bleeding, and diarrhea for disease activity (Supplementary Table S4). At indicated time points, mice were euthanized and the entire large intestine (cecum, colon, and rectum) was excised and measured. The colons were fixed in 10% formaldehyde buffer for transverse sections or “Swiss rolls,” or were frozen in liquid nitrogen and stored at −80°C. Palpable tumors were counted, measured, and processed for paraffin blocks or snap frozen.

Histologic evaluations

“Swiss rolls” were sectioned at 4 μm and stained with hematoxylin and eosin (H&E). Crypt length was defined as cell number per crypt by counting 50 integrated crypts per mouse. Inflammation and neoplastic lesions were evaluated blindly by an experienced investigator and a pathologist. Inflammation was graded from 0 to 4 upon histopathology of colonic mucosa (Supplementary Table S5). Neoplastic lesions were estimated and counted as described in Supplementary Table S6.
IHC
Paraffin-embedded sections were deparaffinized using a standard technique. Antigens were retrieved in 10 mmol/L citrate buffer (pH 6.0) and endogenous peroxidase was blocked in 3% H2O2. IHC was carried out using a staining kit (Life Technologies).

BrdUrd labeling assays
Mice received a single intraperitoneal injection of a mixture of bromodeoxyuridine (BrdUrd, 120 mg/kg) and 5-fluoro-2-deoxyuridine (12 mg/kg). At 1, 24, and 48 hours after injection, respectively, animals were euthanized, and the whole colons were collected and fixed in formalin for paraffin embedding and IHC. Number and location of BrdUrd-positive cells in integrated crypts were assessed.

Western blot analysis
Colonic mucosa was scraped, washed by PBS, and homogenized in 50 µL Roche buffer on ice. Homogenates were cleared up by centrifugation at 4°C, 14,000 g for 10 minutes. Proteins (50–100 µg) in supernatants were separated on 10% to 12% SDS-PAGE and blotted onto nitrocellulose membrane (Bio-Rad) for immunoprobing as previously described (22).

Real-time RT-PCR
Total RNA was extracted from colon mucosa using TRIzol (Invitrogen), and reversely transcribed. The cDNA was used as templates for real-time RT-PCR with SYBR green qPCR mixture following the manufacturer’s protocol (BioSciences Inc.). Gene-specific primer sequences are listed in Supplementary Table S7.

ELISA
Colonic mucosa was minced with surgical scissors and homogenized in ice-cold PBS, followed by centrifugation at 4°C, 10,000 g for 15 minutes. ELISA kits for IL-1β, II-6, and IFNγ (R&D System) were used to measure these protein levels following the manufacturer’s instructions.

Reactive oxygen species
ROS in colonic mucosa were estimated using an antioxidant assay kit (Cayman Chemical Company) following the instruction of the manufacturer.

Lipid peroxidation
Lipid peroxides were measured using a Lipid Peroxidation Microplate Assay Kit (Oxford Biomedical Research), following the instruction of manufacturer.

Lipid synthesis
Radiochemicals 14C-butyrate (54 mCi/mmol) was purchased from Moravek Biochemicals, Inc. Colonocytes were isolated as previously described (27), and incubated with 5 to 10 µCi of 14C-butyrate in 5 to 10 ml medium for 2 hours. Cells were collected for lipid extraction and radioactivity tests using a scintillation counter (Beckman), as previously described (22).

Next-generation sequencing
Genomic DNA was extracted with a standard method from mucosa and tumors. Genome-wide DNA mutations were analyzed by next-generation sequencing (Otogenetics Corporation) with a minimum of 30× average coverage and paired-end 100 base-pair (bp) reads. Exome coverage reports were provided for quality control (Supplementary Table S1). DNA variants were identified using the platform provided by DNAexus (https://dnanexus.com). Nucleotide-Level-Variations in AKR1B8+/− and WT mouse genome were identified by variations from a reference genome.

Statistical analyses
Statistical analyses were carried out with Prism 4 (GraphPad Software). Variance test, Student t test, or one-way ANOVA test, as appropriate, was used to compare the difference between AKR1B8+/− and WT groups with P < 0.05 as statistical significance.

Results

AKR1B10 expression is diminished in human ulcerative colitis and CAC
We examined the cellular distribution of AKR1B10 in the normal colon and found that it is specifically present in the mature epithelial cells (Fig. 1A, left). In contrast, AKR1B10 was significantly reduced in 45.6% (36/79) of ulcerative colitis tissues and undetectable in 50.6% (40/79; Fig. 1A, middle). Furthermore, in six associated CAC in this collection, AKR1B10 was absent in five tumors (83.3%; Fig. 1A, right) and was weakly positive in the other. We further examined AKR1B10 expression in frozen specimens and found that AKR1B10 protein (Fig. 1B) and mRNA (Fig. 1C) were decreased or undetectable in 77.3% (17/22) of ulcerative colitis tissues. Together these data suggest that AKR1B10 expression in ulcerative colitis and CAC is lost or significantly decreased.

AKR1B8+/− mice have defects in crypt development and self-renewal of the colon
AKR1B8, the ortholog of human AKR1B10 in the mouse, is primarily expressed in the colon (24). To clarify the role of AKR1B10 in ulcerative colitis and CAC, we produced an AKR1B8 knockout (−/−) mouse strain by replacing 25 nucleotides (GCA GCA ACC ATG GCC ACC TTC GTG G) around the ATG translational start site with a LacZ/Neo cassette (a selection marker; Supplementary Fig. S1A). This replacement leads to a 5 amino acid deletion and downstream frame-shifting. The AKR1B8 mutant was confirmed by Southern blot (Supplementary Fig. S1B) and AKR1B8 expression at mRNA (Supplementary Fig. S1C) and protein levels (Supplementary Fig. S1D–S1E). AKR1B8 disruption in mice had no evident effect on general appearance, body weight, and reproductive. The mutant AKR1B8 allele displayed a typical Mendelian dissemination, indicating that AKR1B8 is not an essential gene for embryo development and survival. The expression of other aldo-keto reductase isofoms in the colon of AKR1B8−/− mice, such as AKR1B3, AKR1B7, and AKR1C12-14, was not significantly altered except for AKR1B7 in the DSS-treated mucosa (Supplementary Fig. S2A and S2B).

Histologic assessments demonstrated that AKR1B8 abrogation significantly altered the colonic crypts. The colonic crypts were notably shorter in AKR1B8+/− mice than in WT mice (Fig. 2A and Supplementary Fig. S3A). On average, the distal colon had 25 ± 2.4 cells/crypt in WT mice, but only 19 ± 4.0 cells/crypt in age-matched AKR1B8+/− animals (n = 30, P < 0.01). Proliferating cell nuclear antigen (PCNA)-positive cryptic cells were remarkably reduced to 36.6% in AKR1B8−/− crypts versus 49.7% in the WT
AKR1B8 deficiency leads to susceptibility and severity of colitis induced by DSS

We further assessed susceptibility of AKR1B8\(^{-/-}\) mice to colitis induced by a low dose of 2% DSS (Supplementary Fig. S4). Body weight, stool consistency, and gross and occult bleeding were monitored daily during DSS administration and recovery stages to evaluate disease activity index (32). During four cycles of DSS treatment, the overall disease activity index was significantly higher in AKR1B8\(^{-/-}\) mice than in WT (Fig. 3A, n = 15). Occult and gross bleeding (Fig. 3B) and liquid diarrhea occurred in all AKR1B8\(^{-/-}\) mice, but not in WT. On average, the colons of AKR1B8\(^{-/-}\) mice were over 1.5 cm shorter than those of WT animals (Fig. 3C), indicating more severe inflammation. In the AKR1B8\(^{-/-}\) mice, the spleen was enlarged by 2-fold compared with that of WT (Supplementary Fig. S5A). AKR1B8\(^{-/-}\) mice had large and continuous epithelial lesions, such as ulcerations, and intense infiltration of inflammatory cells throughout mucosa, submucosa, and muscle layer of the colon (Fig. 3D and Supplementary Fig. S5B); in contrast, WT mouse colons had minimal epithelial damage. Furthermore, AKR1B8\(^{-/-}\) mice displayed impaired regeneration of the intestinal epithelium. DSS causes epithelial injury; after withdrawal of DSS, the proliferation of colonic epithelial cells is increased (33). In WT mice, a layer of epithelial cells covered the ulcerated area within 2 days after DSS was withdrawn; however, AKR1B8\(^{-/-}\) mice failed to repair the ulcers. As a result, AKR1B8\(^{-/-}\) mice had contiguous focal epithelial cell loss and broader wound surface areas in the colonic epithelium (Fig. 3E, top). The epithelial proliferation in ulcerated and adjacent areas was diminished in AKR1B8\(^{-/-}\) mice, as shown by PCNA expression and BrdUrd labeling (Fig. 3E, middle and bottom). In contrast, increased collagen deposit was seen in the
ulcerated areas of AKR1B8−/− mice (Fig. 3F). These data suggest that AKR1B8 deficiency leads to susceptibility to and severity of colitis and impaired healing of the colonic mucosa.

AKR1B8 deficiency aggravates inflammatory response, oxidative stress, and carbonyl lesions

Cytokines regulate the inflammatory response (3, 34). We performed qRT-PCR for key proinflammatory cytokines (e.g., IL1β, IL6, and IFNγ) in the mucosa (Fig. 4A, top). In the AKR1B8−/− mice, IL1β and IFNγ were upregulated at the baseline (P < 0.05, n = 5). In response to DSS treatment, IL6 and IFNγ were greatly induced in AKR1B8−/− mice compared with WT, indicating an increased inflammatory response. In AKR1B8−/− mucosa, IL1β was also induced by the DSS treatment, but to a lesser compared with that in WT, which may be due to its high basal level. We further measured the protein levels of these cytokines and found that the protein levels were accordingly increased in the colonic mucosa (Fig. 4A, bottom).

Inflammatory oxidative stress is a driving force of cellular damage in ulcerative colitis (12). We measured ROS in the colonic mucosa of AKR1B8−/− and WT mice, and found that the radical levels were significantly higher in AKR1B8−/− mucosa than in WT (Fig. 4B, n = 5). In the AKR1B8−/− mucosa, lipid peroxides were also higher than in WT (Fig. 4C, n = 5), which may be due to increased lipid peroxidation and diminished elimination by AKR1B8. Together these data indicate that AKR1B8 deficiency exaggerates the inflammatory response and oxidative stress and thus exacerbates colitis induced by DSS.

AKR1B8 deficiency predisposes mice to colitis-associated tumorigenesis

CAC correlates with extent and severity of inflammation (35, 36). In the AKR1B8−/− mice, masses appeared (20.0%, n = 5) after two cycles of DSS treatment, and after four cycles of DSS treatment, palpable tumors were larger and more frequent (30.0%, n = 10) than in WT. Palpable tumors were not observed in any WT mice (n = 15) after four cycles of DSS. All masses seen in AKR1B8−/− mice were dome shaped and present in the distal colon-rectum (Fig. 5A and Supplementary Fig. S6A and S6B). We stratified the micro-neoplastic lesions in four classes, that is,
hyperplasia, mild dysplasia, moderate/severe dysplasia, and adenoma. As shown in Fig. 5B, the hyperplasia and dysplasia were 2- to 3-fold more frequent in AKR1B8−/− mice than in WT (n = 15, P < 0.05); in particular, the adenomas were five times more frequent at 0.78±0.04/mouse in AKR1B8−/− versus 0.17±0.10/mouse in WT (n = 15, P < 0.01). Approximately 60% of AKR1B8−/− mice had adenomas compared with 13.3% of WT (n = 15, P < 0.01). The neoplasms in AKR1B8−/− mice were of a higher grade and more aggressive, as illustrated by H&E histology (Fig. 5C, top and Supplementary Fig. S6C), PCNA expression (Fig. 5C, middle), and BrdUrd labeling (Fig. 5C, bottom). These data indicate that the AKR1B8 deficiency leads to more severe colitis-associated tumorigenesis.

Large-scale exome sequencing identifies more frequent DNA damage in AKR1B8−/− mouse colons

AKR1B8−/− and WT mice received the same DSS treatment, but gross and micro-tumors were seen earlier and more frequently in AKR1B8−/− mice, suggesting differential DNA damage in these experimental animals. To understand the DNA damage profile that underscores the colitis-associated tumorigenesis, we conducted a genome-wide exome-sequencing analysis in the WT and AKR1B8−/− colitis mucosa and tumors formed in the AKR1B8−/− epithelium. The exome coverage of this deep sequencing was 3-fold more frequent in AKR1B8−/− mice (Supplementary Table S1), and mutant analyses demonstrated that mutations were significantly higher in AKR1B8−/− colitis and tumors than in WT (Fig. 6A and Supplementary Table S2; n = 2, P < 0.001). We further compared the gene mutants among WT colitis, AKR1B8−/− colitis, and tumors, and found that a total of 230 genes were mutated uniquely in AKR1B8−/− colitis, tumors, or both, but not in WT colitis. This includes nonsynonymous point mutations, frameshifts, and stop gain and stop loss. Among the 230 genes, 104 genes were mutated in colitis only, 32 genes were mutated in tumors alone, and 94 genes were mutated in both AKR1B8−/− colitis and tumors. In the 94 genes, 28 genes are oncogenes or tumor suppressors (Supplementary Table S3); using conventional DNA sequencing, we confirmed the mutations of two tumor suppressor genes, DOK1 (37) and MXD1 (38), in AKR1B8−/− colitis and tumors (Fig. 6B). These mutations of oncogenes and tumor suppressor genes may represent the DNA damage that drives the malignant transition of colitis. In addition, nucleotide transition of G:C to A:T (P < 0.05) or A:T to G:C (P < 0.05) were more prevalent in AKR1B8−/− colitis and tumors compared with WT colitis (Fig. 6C), consistent with the oxidative and carbonyl-induced DNA damage (10, 39).

Discussion

AKR1B8 (AKR1B10 in humans) is primarily expressed in epithelial cells of the colon and intestine, but its biologic function in the intestine is unknown. Here, we found that targeted disruption...
of AKR1B8 gene resulted in diminished proliferation of the epithelial cells in the colon. In the DSS model of colitis, mice deficient in AKR1B8 had diminished epithelial repair and suffered more severe inflammation and disease activity and more severe dysplastic lesions. AKR1B8 is a critical protein in the self-renewal and barrier function of the colonic epithelium and in the pathogenesis of ulcerative colitis and CAC.

The two known functions of AKR1B8, that is, control of fatty acid and lipid synthesis and detoxification of carboxyl compounds (24), may both relate to the decreased colonic epithelial proliferation, more severe inflammation, and the increased dysplasia in the DSS model of colitis seen in AKR1B8-deficient mice. Phospholipids are essential building blocks of biomembranes; constant renewal of colonic epithelial cells creates a need for phospholipids for cell growth and proliferation. The colonic epithelium is unique in that it is exposed to high concentrations of short chain fatty acids (SCFA) produced by commensal bacteria in the lumen. Butyric acid from commensal bacteria in the lumen is the major carbon source for lipid synthesis in the colonic epithelium (30). When radiolabeled butyric acid was given to mice deficient in AKR1B8, its incorporation into phospholipids and other complex lipids in the colonic mucosa was diminished when compared with WT mice. It is possible that decreased incorporation of SCFAs into phospholipids contributes to the decreased epithelial proliferation seen in AKR1B8−/− mice.

The intestinal ecosystem consists of epithelium, immune cells, and luminal microflora and contents. Intestinal epithelial cells are maintained in a dynamic balance between cell proliferation, differentiation, and apoptosis to serve as a robust barrier (40). In AKR1B8−/− mice, the defects in epithelial proliferation and maturation may impair the barrier function, leading to susceptibility to DSS-induced colitis. Therefore, DSS at a low dose of 2.0% triggered much more severe inflammation in the AKR1B8−/− mice, leading to serious diarrhea, bleeding, and rigid colonic tubes. Histologically, the AKR1B8-deficient mouse colons showed heavy inflammatory cell infiltration, larger area ulceration, and collagen fiber deposits. Inflammatory cytokines, such as IL1β, IL6, and INFγ, were also increased (Fig. 4A).

Wound healing is the key of ulcerative colitis remission (40, 41). The epithelial cells surrounding the wound bed rapidly

![Figure 4](image-url)

**Figure 4.** Proinflammatory cytokines, oxidative stress, and carbonyl levels in AKR1B8−/− mice. A, expression of cytokines IL1β, IL6, and IFNγ. Top, mRNA levels; bottom, protein levels. Data indicate mean ± SD, n = 5. B, oxidative stress (n = 5 each). C, lipid peroxides (n = 5 each). Statistical significance was tested by the Student t test.
migrate to the denuded area and promptly proliferate to restore the pool of colonocytes (41). The DSS model is marked by epithelial injury during DSS exposure followed by epithelial proliferation when DSS is withdrawn (42, 43). In the DSS treatment, AKR1B8−/− mice experienced severe chronic epithelial damage, but lack of the proper restitution, whereas the ulcerative area in WT mouse was covered by a layer of epithelial cells within 2 days, accompanied with active epithelial proliferation in adjacent areas. The impaired wound healing in the AKR1B8-deficient mice may explain the severity of inflammation triggered by DSS. The impaired proliferative response after DSS withdrawal in the AKR1B8-deficient mice also raises the possibility that their impaired ability to synthesize complex lipids may limit epithelial proliferation in the face of injury. CAC correlates with extent and severity of inflammation (35, 36). Oxidative stress plays a key role in inflammatory cellular and DNA damage in ulcerative colitis. ROS interact with DNA and form 8-oxodG adducts which leads to DNA mutations and breaks (13). In the oxidative stress associated with inflammation, lipid peroxidation mediates the destruction of biomembranes. The electrophilic lipid peroxides also induce cellular and DNA damage. The interaction of peroxide compounds with proteins results in covalent modifications and protein dysfunction (14, 16, 44). The interaction of peroxide compounds with DNA results in DNA mutations and breaks (17, 18). Lipid peroxidation and lipid peroxide-induced injury may aggravate oxidative stress by increasing mitochondrial permeability, forming a vicious cycle. AKR1B8 mediates the detoxification of carbonyl compounds to alcohols. We found evidence for increased oxidative stress and increased lipid peroxidation in the colonic mucosa of AKR1B8−/− mice treated with DSS when compared with mucosa from WT mice treated with DSS. Exome sequencing of colonic mucosal DNA from WT and AKR1B8−/− mice treated with DSS revealed a much higher rate of mutations in the AKR1B8-deficient mucosa. Moreover, the nucleotide transition of G:C to A:T or A:T to G:C and transversion of A:T to C:G were more prevalent in the AKR1B8-deficient mucosa. These changes are consistent with oxidative and carbonyl-induced DNA damage (10, 39). Therefore, the increased inflammatory cytokines and cellular and DNA damage induced by oxidative stress and lipid peroxides provide a mechanism for many of the events that drive the severity of ulcerative colitis and the progression of ulcerative colitis to cancer in this mouse model. It is noteworthy to note that same as literature report (45), p53 and Rb mutations were not evidenced in the colitis and associated tumors in the AKR1B8−/− mice. However, we indeed observed APC mutations in one of the two tested tumors (data not shown), but not in colitis mucosa, which is consistent with literature that APC mutation is less frequent and occurs later in the development of colitis-associated colorectal cancer (46).

AKR1B18 is an effective detoxicant of lipid peroxides (24). In the absence of AKR1B8, the colonic mucosa may be unable to efficiently eliminate oxidative lipid peroxides. In the mouse AKR1B protein family, AKR1B7 also has a substrate preference for 4-hydroxynonenal (HNE), a cytotoxic and genotoxic lipid peroxide (47). In the DSS-treated AKR1B8−/− colonic mucosa, AKR1B7 was induced, which may represent a feedback mechanism responding to the carbonyl stress from oxidative lipid peroxidation (e.g., HNE) in the absence of AKR1B8.

The expression of AKR1B10 is known to be increased in many human cancers including breast cancer and lung cancer (48, 49), but is diminished in colorectal cancer (50). We found that AKR1B10 expression is also diminished in ulcerative colitis. We do not know whether the diminished expression of AKR1B10...
occurs before or after the development of colon cancer and ulcerative colitis and therefore, we cannot say whether it may have a causal effect in these conditions. Our studies with the DSS model of colitis in AKR1B8-deficient mice would suggest that in the human colon, the diminished expression of AKR1B10 would result in increased susceptibility to chronic inflammation and dysplasia.

The data present here demonstrate that AKR1B8 plays an essential role in regulating lipid synthesis in the colonic epithelium and in protecting the colonic epithelium from oxidative and carbonyl damage. AKR1B8 deficiency reduces long chain fatty acid/lipid synthesis from butyrate, which diminishes biomembrane assembly and cell proliferation. The AKR1B8 deficiency also leads to accumulation of toxic carbonyl compounds, which, together with oxidative stress, forms a vicious cycle, leading to DNA damage and subsequent dysplastic lesions. AKR1B8 may also affect proinflammatory cytokine expression via a mechanism unknown yet.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
Conception and design: Y. Shen, R. Yan, X. Li, J. Gao, M.-C. Huang, W.F. Stenson, D.-F. Liao, D. Cao
Development of methodology: Y. Shen, R. Yan, X. Li, J. Gao, L. Wan, M.-C. Huang, D. Cao

Figure 6. DNA damage in AKR1B8−/− mice. Genome-wide exome-sequencing analyses of WT and AKR1B8−/− colitis mucosa and tumors (n = 2 each) were conducted as described in Materials and Methods. A, single homozygous nucleotide mutants. B, conventional DNA sequencing of DOX1 and MXD1 tumor suppressor genes, confirming the point mutations. C, transitional and transversional point mutations. *P < 0.05 and **P < 0.01, compared with WT control. Statistical significance was tested by the Student t test. D, hypothetic model of AKR1B8 in colitis and associated dysplastic lesions. AKR1B8 deficiency reduces long chain fatty acid/lipid synthesis from butyrate, which diminishes biomembrane assembly and cell proliferation. The AKR1B8 deficiency also leads to accumulation of toxic carbonyl compounds, which, together with oxidative stress, forms a vicious cycle, leading to DNA damage and subsequent dysplastic lesions. AKR1B8 may also affect proinflammatory cytokine expression via a mechanism unknown yet.

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