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Increased susceptibility of chronic ulcerative colitis-induced carcinoma development in DNA repair enzyme *Ogg1* deficient mice†

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Abstract

Ogg1 DNA repair enzyme recognizes and excises oxidative stress-caused 8-hydroxyl-deoxyguanosine (8-OHdG) from GC base-pairs. Ogg1 knockout mice are phenotypically normal, but exhibit elevated levels of 8-OHdG in nuclear and mitochondrial DNA, as well as moderately elevated mutagenesis and spontaneous lung tumors and UV-induced skin tumors. To elucidate the mechanistic role of inflammation-caused oxidative stress in carcinogenesis, the development of chronic ulcerative colitis (UC)-induced carcinoma in Ogg1 knockout mice was studied using a dextran sulfate sodium (DSS)-induced UC model without the use of a carcinogen. Ogg1 (−/−), Ogg1 (+/−), and wild type C57BL/6 mice were subjected to long-term, cyclic DSS treatment to induce chronic UC and carcinogenesis. In wild type C57BL/6 control mice after 15 cycles of DSS treatment, colorectal adenocarcinoma incidence was 24.1% (7/29 mice), with a tumor volume of 27.9 ± 5.2 mm³. Ogg1 (−/−) mice showed significantly increased adenocarcinoma development in the colon with a tumor incidence of 57.1% (12 of 21 mice, $P < 0.05$) and a tumor volume of 35.1 ± 6.1 mm³. Ogg1 mice (+/−) also exhibited significantly increased tumor development in the colon with a tumor incidence of 50.0% (13/26 mice) and a tumor volume of 29.1 ± 7.2 mm³. Histopathologic analyses revealed that colorectal tumors were well-differentiated tubular adenocarcinomas or mucinous carcinoma and adjacent colonic mucosa showed mild to moderate chronic UC. Using immunohistochemical approaches, Ogg1 (−/−) and (+/−) mice exhibited similar numbers and staining intensities of macrophages in UC areas as seen in Ogg1 (+/+) mice, but markedly increased numbers and staining intensities of 8-OHdG positive inflammatory and epithelial cells. These results provide important evidence on the relationship between inflammation-caused oxidative stress, DNA repair enzyme Ogg1, and carcinogenesis.

Keywords

ulcerative colitis; carcinogenesis; Ogg1; oxidative stress

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INTRODUCTION

Chronic inflammation is one of the most important risk factors contributing to human cancer [1]. Ulcerative colitis (UC) is an idiopathic chronic inflammatory bowel disease associated with the development of cancer [2–4]. Several aspects of the chronic inflammatory process, including the overproduction of reactive oxygen and nitrogen species (RONS), excessive arachidonic acid metabolites, and immunity system dysfunction, may contribute to increased cancer risk [5], in which the role of inflammation-caused oxidative cellular damage in carcinogenesis has been discussed extensively [6–9]. However, direct experimental evidence linking oxidative stress to carcinogenesis has been lacking.

Various biological reactions are known to generate RONS, including the superoxide anion, hydrogen peroxide and hydroxyl radicals, nitric oxide, and peroxynitrite [10–12]. Superoxide plays a critical role by directly causing cellular injury or by taking part in the formation of highly reactive hydroxyl radicals and peroxynitrite radicals. The increased activities and accumulation of leukocytes (neutrophils and macrophages) during the inflammatory process result in greatly enhanced generation of RONS [13][14]. In UC patients, abundant neutrophil and macrophage infiltration into the local inflamed mucosa contributes to an increase in the production of RONS, and inflammatory cells may also carry iron to the inflamed area, which further enhances oxidative damage [13][15–17]. RONS have been shown to cause DNA strand breaks (which facilitate chromosomal translocation), oxidative modification of DNA bases [e.g., 8-hydroxydeoxyguanine (8-OHdG)], deamination of 5-methylcytosine, lipid peroxidation, protein oxidation, and altered gene expression [6–9].

8-OHdG is the most prevalent oxidative DNA lesion, and is commonly used as a marker of oxidative DNA damage [18][19]. 8-OHdG can base pair with adenine and potentially lead to GC to TA transversions. 8-OHdG is removed from DNA primarily by the action of the base excision repair system [20]. The *Ogg1* gene, located at chromosome 3p26.2, encodes a 32 kD DNA glycosylase (DNA repair enzyme) that recognizes and excises 8-OHdG from GC base-pairs [21]. Mice lacking *Ogg1* activity as a result of gene knockout are phenotypically normal, but exhibit elevated levels of 8-OHdG in nuclear and mitochondrial DNA, as well as moderately elevated mutagenesis, predominately in the form of GC to TA transversions [22–24]. Increased development of spontaneous lung tumor and UV-induced skin tumor have been reported in *Ogg1* knockout mice. However, the effects of *Ogg1* deficiency on UC, and UC-induced DNA damage and cancer development, have not been studied.

The oral administration of dextran sulfate sodium (DSS) to rodents induces colonic inflammation that is clinically and histologically similar to human UC [25]. Like the human disease, DSS-induced chronic UC is complicated by the development of colorectal dysplasia and adenocarcinoma [26–29]. We have shown that dietary iron supplementation enhances colorectal carcinoma development in the DSS model, an effect that is associated with increases in nitrotyrosine formation and iNOS expression by colonic inflammatory cells [29]. In addition, the antioxidant *N*-acetylcysteine had an inhibitory effect on nitrotyrosine formation and colorectal carcinogenesis in mice with DSS-induced and iron-enhanced UC carcinogenesis model [30]. These results suggest that inflammation-induced nitro-oxidative stress participates in inflammatory process and inflammation induced carcinogenesis.

In order to study the mechanistic roles of inflammation-caused nitro-oxidative stress in UC-induced carcinogenesis, UC-induced colorectal carcinoma development and its related inflammation and oxidative-stress were determined in *ogg1*-deficient mice with DSS-induced chronic UC.

MATERIALS AND METHODS

Animals

Ogg1 knockout mice are generated by the lab of Dr. T. Lindahl [22], and kindly provided by Dr. T. Lindahl and Dr. C. Walters (University of Texas at San Antonio). Six-week-old wild-type C57BL/6 mice were purchased from The Jackson Laboratory (Bar Harbor, ME). The genotypes were confirmed by polymerase chain reaction of tail DNA using specific oligonucleotide primers (primer sequences: Wild-type: 5'-ACTCAGGACCTTCAGAAGAGCA-3' Mutant: 5'-GATGGATACTTTCTCGGCAGG-3' Common: 5'-GAGACACATCTTGCACCCAGC-3'). The mice were housed five per cage under pathogen-free conditions in the facilities of Laboratory Animal Services, Rutgers University and Northwestern University. Body weights and food consumption were monitored every other week for the duration of the experiment.

Chemicals and Diet

DSS (MW:~40,000) was purchased from ICN Pharmaceuticals, Inc. (Costa Mesa, CA). Mayer's hematoxylin solution, eosin Y solution, and 3, 3'-diaminobenzidine (DAB) were from Sigma Chemical Co. (St. Louis, MO). Avidin/biotinylated horseradish peroxidase macromolecular complex kits were from Vector Laboratories (Burlingame, CA). Polyclonal rat anti-Mac3 was from BD Pharmingen, Cat #: 550292 (San Diego, CA) and monoclonal anti-8-OHdG antibody N45.1 was generated from Dr. Toyokuni's lab. AIN76A diet was purchased from Research Diets, Inc. (New Brunswick, NJ).

Animal Experiments

In a long-term study of chronic UC and associated colorectal carcinogenesis, *Ogg*^{-/-} mice were randomized into four groups. Group 1 (*Ogg*^{+/+}), Group 2 (*Ogg*^{+/-}), and Group 3 (*Ogg*^{-/-}) were administered water throughout the experiment as negative controls (*n* = 5 mice per group). Group 4 (*Ogg*^{+/+}), Group 5 (*Ogg*^{+/-}), and Group 6 (*Ogg*^{-/-}) mice (*n* = 25 mice per group) were subjected to cyclic DSS treatment [25][29][30]. One DSS "cycle" consisted of 7 d of 1% DSS (w/v, in deionized water) administration through the drinking fluid (DSS period), followed by 10 d of ordinary tap water administration (recovery period). The negative control groups were given deionized water during the DSS period and ordinary tap water during the recovery period. This 17-d period is defined as 1 DSS "cycle." The mice were subjected to 15 consecutive DSS cycles. Starting 2 d before the start of the experiment and continuing for the duration of the experiment, all groups were given a modified AIN76A diet. Cyclic administration of DSS is to mimic the disease flare-up and quiescence process in UC patients. The mice were sacrificed at the end of the 15th DSS cycle (d 255). Mice were sacrificed prior to the end of the experiment period if they exhibited significant weight loss (greater than 20%), excessive rectal bleeding, and loss of activity. Mice sacrificed prior to the completion of 15 DSS cycles were excluded from the results of the carcinogenesis experiment. To determine if there is difference in the acute response of *Ogg*1 (-/-) mice to the colonic ulceration and inflammation, five mice from each group were sacrificed after one DSS cycle treatment.

Tissue Preparation and Histopathological Evaluation

After sacrificing the mice by CO₂ asphyxiation, the colons were perfused with normal saline, removed, and opened longitudinally. The numbers and positions of colon tumors were recorded, and three perpendicular diameter measurements were obtained for each tumor using calipers. Tumor volume was determined using the equation $V = 4/3\pi r^3$, where *r* was the average tumor radius obtained from the three diameter measurements. The colons were then fixed in 10% formalin for 24 h, routinely processed, and embedded in paraffin as a "Swiss roll". Serial tissue sections (5 μm) were made and stained with hematoxylin and eosin (H & E) for

histopathological and immunohistochemical analyses. Colorectal tumors were analyzed by light microscopy and classified as colorectal adenocarcinoma according to established criteria [29]. The tumors were further categorized as tubular well-differentiated adenocarcinoma or mucinous well-differentiated adenocarcinoma based on the glandular architecture and presence or absence of mucin lakes. Colorectal dysplasia was also analyzed based on the established criteria of architectural and cytologic atypia. The Chronic UC was analyzed based on inflammation severity, ulceration formation and its extent, and epithelial hyperplasia. The criteria for the scoring of these parameters have been described previously [29][31].

Immunohistochemistry

Immunohistochemical staining was performed using the avidin-biotin-peroxidase complex method as described previously [30]. In brief, endogenous peroxidase activity was quenched in paraffin embedded tissue sections with 1% H₂O₂. Antigen was retrieved by pretreatment with citrate buffer (Antigen Unmasking Solution, Vector Laboratories) in a microwave oven. In addition, the RNase (10 µg/mL in PBS) treatment (at 37°C for 1 h) was performed for 8-OHdG staining. Non-specific protein-protein interactions were blocked with diluted normal serum, then the slides were incubated with a primary antibody (5 µg/mL for anti-mouse Mac-3 antibody, BD Bioscience, San Jose, CA, Cat #: 550292, or 10 µg/mL for anti-8-OHdG N45.1 antibody), followed by the appropriate biotinylated secondary antibody and the ABC complex for 45 min each. Diaminobenzidine was used as the chromagen. Slides were washed thoroughly between incubations with PBS. Negative controls were established by replacing the primary antibody with PBS and normal serum. Positive staining was indicated by the presence of brown-colored precipitate.

Statistical Analysis

The data were expressed as the mean ± SE. Statistical significance was tested using SigmaStat Version 1.01 software (Jandel Scientific, San Rafael, CA). Tumor incidence was analyzed using the Chi-square test. Normally distributed data were analyzed using Student's t-test, and nonparametric data, such as tumor multiplicity, were analyzed using the Mann-Whitney Rank Sum test. *P* values less than 0.05 were taken to be statistically significant.

RESULTS

Body Weight, Food Consumption, and Mortality

In the long-term animal experiment, the average body weight of *Ogg1*^{-/-}, *Ogg1*^{+/-} mice in control groups did not differ from that of their *Ogg1*^{+/+} counterparts at the end of the experiment (mean ± SE, 22.3 ± 0.6, 21.9 ± 0.9 vs. 22.8 ± 0.5 g, respectively). The average body weights for DSS-treated *Ogg1*^{-/-}, *Ogg1*^{+/-}, *Ogg1*^{+/+} mice at the end of the experiment were 22.8 ± 0.5, 21.1 ± 0.9, and 21.8 ± 0.4 g, respectively, which was not statistically significantly different from each other or from the negative control groups. Similarly, there were no differences in average food consumption among the four groups (data not shown).

Chronic UC-Induced Colorectal Carcinogenesis in *Ogg1*-Deficient Mice

Ogg1 (-/-) and (+/-) mice were used in comparison with wild-type C57BL/6 mice to determine the roles of *Ogg1* gene in the development of chronic UC-induced colorectal carcinoma. *Ogg1* (-/-), *Ogg1* (+/-), and wild type C57BL/6 mice were subjected to long-term, cyclic DSS treatment to induce chronic UC and its induced carcinogenesis. *Ogg1* (-/-) and (+/-) mice and wild-type C57BL/6 mice in negative control groups did not develop inflammation and colorectal tumors or tumors in other organs during the long-term experiment. As shown in Table 1, mice with homozygous deficiency *Ogg1* (-/-) showed significantly increased adenocarcinoma development in the colon and markedly increased tumor volume and tumor

multiplicity. Heterozygous *Ogg1* (+/-) mice also exhibited significantly increased tumor development in the colon with moderately increased tumor volume.

The large intestine and tumors from all of the mice in each group were examined in serial H&E stained slides histopathologically and the tumors were classified as well- to moderately-differentiated tubular adenocarcinoma or mucinous carcinoma. Morphologically, as shown in Figure 1A and B, well- to moderately-differentiated tubular adenocarcinoma was characterized as polypoid growth pattern (Figure 1A), carcinoma cells formed glandular structure as either tubular or cribriform architecture and invaded into lamina propria and even into muscularis propria (Figure 1B). Cytologically, tumor cells exhibited increased nuclear/cytoplasm ratio, atypical and hyperchromasia nuclei, frequently seen mitosis (Figure 1B). Mucinous carcinoma was identified as more than 75% carcinoma formed mucinous tumor nests and invasive growth pattern (invasion into muscularis propria, even into serosa), as seen in Figure 1C; cytologically, mucinous tumor nest lined by atypical carcinoma cells or presented as acellular mucinous lake (Figure 1D). As shown in Table 2, there was statistically significant increase in the development of mucinous carcinoma in *Ogg1*^{-/-} mice as compared to wild type *Ogg1*^{+/+} mice and in Heterozygous *Ogg1*^{+/-} mice.

Similar to human UC-associated dysplasia, low and high grade dysplastic lesions were frequently identified in long-standing DSS-induced chronic UC in mice. As shown in Figure 1E–H, dysplastic lesion was identified in the background of hyperplastic mucosa (Figure 1E), adjacent to ulcer (Figure 1F–G) and chronic inflamed flat mucosa (Figure 1H). Due to extensive chronic inflammation-caused regenerative hyperplastic epithelial change which often mixed with low-grade dysplasia, as shown in Figure 1E, low grade dysplasia was difficult to be determined semi-quantitatively. We have just analyzed the foci of high grade dysplasia (as seen in Figure 1F–H). Significantly increased number of foci of high grade dysplasia (5.2 ± 2.7 foci) in DSS-induced UC in *Ogg1*^{-/-} mice was found as compared to *Ogg1*^{+/+} and *Ogg1*^{+/-} mice (3.4 ± 1.7 and 3.4 ± 1.5 , respectively; $P = 0.007$, Student *t*-test).

Grading of Acute and Chronic UC in *Ogg1*^{+/+}, *Ogg1*^{+/-} and *Ogg1*^{-/-} Mice

Colorectal inflammation was analyzed histopathologically in noncancerous mucosa based on inflammatory severity, extent of ulceration, and epithelial hyperplasia [29][31]. As compared to non-DSS treated animals that presented normal morphology of the colon (Figure 2A), DSS-induced chronic UC exhibited as glandular distortion, crypt dropout, and mixed chronic inflammatory cell infiltration including lymphocyte, plasma cell and macrophage, and inflammatory cells mainly located in the crypt dropout basal area of the mucosa called basal lymphoplasmacytosis (Figure 2B). Active inflammation in chronic UC was characterized as erosion and ulcer formation. Most of the animals presented small mucosal erosion and ulcer (Figure 2C), and less than 5% of the animals in each group showed extensive large ulcer identified as ulcers involved in more than 10% colonic mucosa (Figure 2D). *Ogg1*^{+/+} and *Ogg1*^{-/-} mice exhibited similar inflammatory activity as seen in *Ogg1*^{+/-} mice. Most of animals showed chronic UC limited to colonic mucosa with focal erosion and ulceration and with mild to moderate inflammatory activity which was determined mainly based on the extent of ulcer formation (mild, <5% of ulcer in the colon; moderate, 5–10%, and severe, >10%). Hyperplastic epithelial changes were frequently observed in the epithelia adjacent to ulcer.

Immunohistochemical Evaluation of Macrophage-Mediated Inflammation and 8-OHdG Formation

To determine the role of inflammatory cells, particularly the macrophages as key cells in producing nitro-oxidative stress and cytokines, Mac3-labeled macrophages were analyzed immunohistochemically. In negative water control groups in both *Ogg1*^{+/+} and *Ogg1*^{-/-} mice, scattered Mac3-labeled macrophages were identified in the lamina propria of colonic mucosa,

as shown in Figure 3A and D. In DSS-induced chronic UC in wild-type *Ogg1*^{+/+} mice, accumulation of intense Mac3-stained macrophages was observed in chronic inflamed (Figure 3B) and ulcerative (Figure 3C) mucosa. Similar staining intensity and number of Mac3-stained macrophages were found in chronic inflamed (Figure 3E) and ulcerative (Figure 3F) mucosa in *Ogg1*^{-/-} mice. In addition, similar staining intensity and number of Mac3-stained macrophages as seen in *Ogg1*^{+/+} and *Ogg1*^{-/-} mice were also found in noninflamed mucosa and chronic inflamed mucosa in *Ogg1*^{+/-} mice.

To determine if there is difference in the acute response of the *Ogg1*(-/-) mice in colonic ulceration and inflammatory process, one DSS-treated animal experiment was performed. The results showed that at d 17 after 7 d 1% DSS treatment, all of the mice including *Ogg1*(-/-), *Ogg1*(+/-), and *Ogg1*(+/+) (*n* = 5 for each strain) exhibited mild to moderate inflammatory activity with 5–10% extent ulcer formation and markedly regenerative hyperplastic epithelial changes in the colon. There is no difference among *Ogg1*(-/-), *Ogg1*(+/-), and *Ogg1*(+/+) mice.

8-OHdG formation was determined immunohistochemically. In non-DSS-treated control mice, scattered 8-OHdG-positive colonic epithelial cells (Figure 4A) were observed in *Ogg1*^{+/+} mice, and extensively 8-OHdG-positive colonic epithelial cells were found in *Ogg1*^{+/-} (Figure 4D) and *Ogg1*^{-/-} (Figure 4G) mice, but more intense staining of 8-OHdG in colonic epithelial cells was observed in *Ogg1*^{-/-} (Figure 4G) mice. In DSS-induced chronic UC, a few 8-OHdG-stained inflammatory cells and colonic epithelial cells were observed in chronic inflamed (Figure 4B) and ulcerative (Figure 4C) mucosa in *Ogg1*^{+/+} mice. Extensively and intense stained 8-OHdG-positive colonic epithelial cells and inflammatory cells were identified in chronic inflamed (Figure 4H) and ulcerative (Figure 4I) mucosa in *Ogg1*^{-/-} mice. Interestingly, similar staining intensity and number of 8-OHdG-positive colonic epithelial cells and inflammatory cells as seen in *Ogg1*^{-/-} mice were also found in chronic inflamed (Figure 4E) and ulcerative (Figure 4F) mucosa in *Ogg1*^{+/-} mice.

In addition, the similar staining intensity of 8-OHdG in the tumor cells including both tubular and mucinous adenocarcinomas has been observed as seen in adjacent non-neoplastic colonic mucosa in *Ogg1*^{+/+}, *Ogg1*^{+/-}, and *Ogg1*^{-/-} mice.

DISCUSSION

Long-standing inflammation is the most important risk factor for cancer development, in which inflammation-caused oxidative stress is a leading biologic event involved in its associated carcinogenesis. 8-OHdG formation is the most common DNA adduct caused by oxidative stress and commonly induces GC to TA transversion mutation. Such mutation patterns have frequently been observed in inflammation-induced cancer, such as inflammatory bowel disease. There are three base-excision repair genes acted synergistically to prevent mutagenesis caused by 8-OHdG, including *Ogg1*, *MTH1*, and *MYH*. *Ogg1* is key enzyme for detecting and removing 8-OHdG incorporated into the DNA. In the present study, we have first demonstrated that *Ogg1* deficient mice are statistically significantly susceptible to chronic UC-induced colorectal dysplasia and carcinoma development without use of carcinogen. In humans, UC-induced carcinogenesis involves a morphological progression from inflamed and hyperplastic epithelia to dysplasia and adenocarcinoma [32], and the majority of UC-associated cancers are mucinous adenocarcinomas [33][34]. The present findings of UC-induced colorectal dysplasia and high frequency of mucinous carcinoma in *Ogg1* deficient mice indicates that it is the most mimicked mouse model of human UC-associated carcinogenesis.

In UC patients, inflammation presents a wax and wane process and abundant macrophage infiltration into the local inflamed mucosa (long-standing chronic inflammatory process) which

significantly contributes to an increase in the production of RONS. To mimic the wax and wan inflammatory process, DSS was administered in cyclic pattern and macrophage dominant longstanding chronic inflammation was induced in the colonic mucosa. Although there was similar activity of macrophage-mediated inflammation in the colon in both Ogg1 knockout and wild type mice, marked accumulation of 8-OHdG in both epithelial and inflammatory cells was observed in DSS-induced colitis in both Ogg1^{-/-} and Ogg1^{+/-} mice as compared to wild type Ogg1^{+/+} mice. The most interesting finding is that Ogg1^{-/-} and Ogg1^{+/-} mice exhibit not only accumulation of 8-OHdG but also significantly increased tumor development in the colon as compared to wild type Ogg1^{+/+} mice, indicating that accumulation of 8-OHdG in long-standing DSS-induced chronic UC leads to an increased susceptibility to carcinogenesis.

In humans, the Ogg1 S326C polymorphisms result in decreased repair ability and most genetic epidemiologic studies indicate that the carrier of this gene polymorphism will have a moderately increased risk of colorectal cancer, particularly in younger individuals. In present studies, heterozygous Ogg1^{+/-} mice exhibit accumulation of 8-OHdG and significantly increased colon tumor development. It is reasonable to speculate that low Ogg1 enzyme activity in Ogg1^{+/-} mice is sufficient to lead to carcinogenesis and thus, it provides a mimicking mouse model of the Ogg1 S326C polymorphisms. On the other hand, it is also possible that further genetic damage in remaining allele of Ogg1 gene in Ogg1^{+/-} mice leads to total loss of enzyme activity and carcinogenesis. However, there is no genetic epidemiologic study on Ogg1 S326C polymorphisms and the risk of UC and its related colonic cancer, and further investigation on this aspect is needed.

Increased mucinous carcinoma development in Ogg1^{-/-} mice is another key aspect in this study. Mucinous carcinoma is the predominant carcinoma developed in UC patients. Our previous studies have shown that with dietary iron supplementation there is significantly increased mucinous carcinoma development in DSS-induced long-term chronic UC, suggesting inflammation-caused and iron-enhanced oxidative stress is a major player for mucinous carcinoma development. In the present studies, accumulation of 8-OHdG and development of UC-induced mucinous carcinoma in Ogg1^{-/-} mice provide further experimental evidence of oxidative stress-leading to predominant mucinous carcinoma development, and further mechanism remains to be investigated.

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Abbreviations

UC	ulcerative colitis
RONS	reactive oxygen and nitrogen species
8-OHdG	8-hydroxyl-deoxyguanosine
DSS	dextran sulfate sodium

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Figure 1.

Representative micrographs showing the histopathology of UC-induced colorectal dysplasia and carcinoma in *Ogg1*^{-/-} mice (Scale Bar in yellow line in the photos). (A–D) Representative carcinomas: (A) Low magnification of an invasive tubular adenocarcinoma with polypoid growth pattern; and (B) High magnification (highlighted area in A) of invasive tubular adenocarcinoma showing tubular and cribriform carcinoma invading into stromal tissue. (C) Low magnification of a polypoid tubular adenocarcinoma (open arrow) and two invasive mucinous adenocarcinomas (solid arrow); and (D) Higher magnification micrograph (highlighted area in C) of a mucinous adenocarcinoma showing the mucinous carcinoma nests formed by one and two carcinoma cell layers and adjacent acellular mucin lake. (E–H)

representative dysplasia: (E) low grade dysplasia in the setting of hyperplastic mucosa; (F–H) high grade dysplasia occurring adjacent to ulcer (F,G) and in flat mucosa (H).

Figure 2. Representative micrographs showing the histopathology of DSS-induced chronic UC (Scale Bar in yellow line in the photos): (A) morphologic normal colon in non-DSS treated mice. (B–D) DSS-induced chronic UC with chronic inflammation (B), small ulcer (C), and extensive ulcer formation (D).

Figure 3.

Mac3-labeled macrophages in the colon of non-DSS and DSS-treated *OggI*^{+/+} and *OggI*^{-/-} mice (Scale Bar in black line in the photos). (A,D) Non-DSS treated mice showing scattered mac3-labeled macrophages in the lamina propria of clonic mucosa in *OggI*^{+/+} (A) and *OggI*^{-/-} (D) mice. (B,C) DSS treated *OggI*^{+/+} mice showing an intense infiltration of Mac3-positive macrophages in chronic inflammation mucosa (B) and ulcer area (C). (E,F) DSS treated *OggI*^{-/-} mice showing numerous Mac-3-positive macrophages in chronic inflammation mucosa (E) and ulcer area (F).

Figure 4.

8-OHdG immunostaining in the colons of *Ogg1*^{+/+}, *Ogg1*^{+/-} and *Ogg1*^{-/-} mice (Scale Bar in black line in the photos). (A–C) In the colon of *Ogg1*^{+/+} mice: a few scattered 8-OHdG immunostained-positive cells in non-DSS treated mice (A), an increased number of 8-OHdG immunostained-positive cells with low staining intensity in chronic inflamed mucosa (B) and ulcerative mucosa (C) in DSS treated mice. (D–F) In the colon of *Ogg1*^{+/-} mice: extensive 8-OHdG immunostained-positive colonic epithelial cells with moderate staining intensity in non-DSS treated mice (D), extensive 8-OHdG immunostained-positive cells with high staining intensity in chronic inflamed mucosa (E), and ulcerative mucosa (F) in DSS treated mice. (G–I) In the colon of *Ogg1*^{-/-} mice: extensive 8-OHdG immunostained-positive colonic epithelial cells with highly staining intensity in non-DSS treated mice (G), extensive 8-OHdG immunostained-positive cells with high staining intensity in chronic inflamed mucosa (H), and ulcerative mucosa (I) in DSS treated mice.

Table 1Chronic UC-Induced Carcinoma Development in *Ogg1*^{+/+}, *Ogg1*^{+/-}, and *Ogg1*^{-/-} Mice

Genotype	Total no. of mice	No. of tumor-bearing mice (tumor incidence) ^a	Tumor multiplicity ^b	Tumor volume ^c (mm ³)
<i>Ogg1</i> ^{+/+}	29	7 (24.1%)	1.0 ± 0.0	27.9 ± 5.2
<i>Ogg1</i> ^{+/-}	26	13 (50.0%) ^d	1.0 ± 0.0	29.1 ± 7.2
<i>Ogg1</i> ^{-/-}	21	12 (57.1%) ^d	1.42 ± 0.2	35.1 ± 6.1 ^e

^aTumor incidence = (number of tumor-bearing mice/total number of mice per group) × 100.^bTumor multiplicity = number of tumors/tumor-bearing mouse. Data are expressed as mean ± SE.^cTumor volume = $4/3\pi r^3$; *r*, average tumor radius derived from three perpendicular diameter measurements. Data are expressed as mean ± SE.^d*P* < 0.05 (Mann-Whitney Rank Sum Test).^e*P* < 0.05 (Student *t*-test).

Table 2Histopathologic Analysis of Chronic UC-Induced Colorectal Carcinoma in *Ogg1*^{+/+}, *Ogg1*^{+/-}, and *Ogg1*^{-/-} Mice

Genotype	Total no. of tumor-bearing mice	Total no. of tumor	No. of tubular adenocarcinoma (%)	No. of mucinous adenocarcinoma (%)
<i>Ogg1</i> ^{+/+}	7	7	6 (85.7%)	1 (14.3%)
<i>Ogg1</i> ^{+/-}	13	13	10 (76.9%)	3 (23.1%)
<i>Ogg1</i> ^{-/-}	12	17	8 (47.1%) ^a	9 (52.9%) ^a

^aSignificant difference from wild-type (*Ogg1* +/+) mice, <0.05 (χ^2 -test).