Bacteria-Induced Intestinal Cancer in Mice with Disrupted Gpx1 and Gpx2 Genes

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ABSTRACT

Two glutathione peroxidase (GPX) isozymes, GPX-1 and GPX-2 (GPX-G1), are the major enzymes that reduce hydroperoxides in intestinal epithelium. We have previously demonstrated that targeted disruption of both the Gpx1 and Gpx2 genes (GPX-DKO) results in a high incidence of ileocolitis in mice raised under conventional conditions, which include the harboring of Helicobacter species [non-specific-pathogen-free (non-SPF) conditions]. In this study, we have characterized GPX-DKO mice that have microflora-associated intestinal cancers, which are correlated with increased intestinal pathology/inflammation. We found that GPX-DKO mice raised under germ-free conditions have virtually no pathology or tumors. After colonizing germ-free mice with commensal microflora without any known pathogens (SPF), <9% of GPX-DKO mice develop tumors in the ileum or the colon. However, about one-fourth of GPX-DKO mice raised under non-SPF conditions from birth or transferred from SPF conditions at weaning have predominantly ileal tumors. Nearly 30% of tumors are cancerous; most are invasive adenocarcinomas and a few signet-ring cell carcinomas. On the basis of these results, we conclude that GPX-DKO mice are highly susceptible to bacteria-associated inflammation and cancer. The sensitivity exhibited in these mice suggests that peroxidative stress plays an important role in ileal and colonic pathology and inflammation, which can lead to tumorigenesis.

INTRODUCTION

Enteric microflora begin to colonize the gut at birth and affect development and maintenance of the mucosal immune response and epithelial cell functions (1). Recently, commensal bacteria have emerged as cofactors in the development of ileocolitis and intestinal malignancies. Human inflammatory bowel disease (IBD), which includes Crohn’s disease and ulcerative colitis, increases cancer risk by 20–30-fold in patients with prolonged IBD histories (2). Many mouse models have been generated to facilitate studying the etiology, prevention, and treatment of IBD and gastrointestinal cancer associated with IBD. The essential role of enteric microflora in ileocolitis-associated cancer has been established in several of these mouse idiopathic IBD models with defects in the immune response. Interleukin (IL)-2 KO, IL-10 KO, T-cell receptor β/p53 KO, or tumor growth factor β1/Rag-2 KO mice, when maintained under germ-free (GF) conditions, fail to develop severe inflammation, as well as small intestinal or colonic cancers that often occur when those animals are raised under conventional housing conditions (3–6). Because microflora can modulate epithelial cell signaling for immune reactions (7–9), this may explain the susceptibility of these immune-compromised mice to the development of IBD. Because GPX-DKO mice have an intact immune system at the outset of these studies, the role of microflora on the subsequent development of any pathological condition in these mice was unclear.

Glutathione peroxidases (GPX) are a family of four selenium-dependent antioxidant enzymes in mice (five in humans; Ref. 10) that reduce H2O2 and organic hydroperoxides by oxidizing glutathione. Taken together, the ubiquitous GPX-1 and epithelial-specific GPX-2 contribute nearly all glutathione-dependent H2O2-reducing activity in the intestinal epithelium (11). We have previously reported that GPX-DKO mice, with targeted disruption of Gpx1 and Gpx2 genes, exhibit ileocolitis between 2 and 7 weeks of age, which is accompanied by accumulation of lipid hydroperoxides, weight loss, and proctitis (12). Because some colitis models such as Mdr1-KO mice do not appear to develop cancer (13, 14), we set out to determine whether GPX-DKO mice would develop intestinal cancer. Because most mouse IBD and ileocolitis-associated cancer models are either immunodeficient or defective in membrane proteins that affect epithelial barrier integrity (15, 16), showing that intracellular GPX activity could prevent microflora-induced ileocolitis and cancer would strengthen the notion that peroxidative stress is one basis for the pathogenesis of inflammation-associated cancer. Although elevated reactive oxygen and nitrogen species are recognized as an integral part of the pathophysiology of IBD, there is little evidence to specify the precise role of hydroperoxides in IBD pathology (17, 18). Demonstrating that epithelial GPX activity could inhibit both IBD and IBD-associated cancers might set the stage for the prevention of IBD-related cancers with inhibitors of the major hydroperoxide-generating enzymes that reside in the mucosal epithelium or inflammatory cells.

Our original non-specific-pathogen-free (non-SPF) GPX-DKO mouse colony harbors several enterohepatic Helicobacter species such as H. hepaticus, which is widely spread in rodents (19). Although H. hepaticus was originally identified as causing hepatitis and hepatocellular tumors in A/Jcr mice, its primary site of colonization is in the intestine. Colonization of H. hepaticus to SPF mice causes ileocolitis and colon cancer in immune-deficient animals such as nude mice, IL-10 KO, and T-cell deficient mice (20–22). However, H. hepaticus only induces mild or no colitis in SPF and immune competent wild-type C57BL/6 and Mdr1-KO mice (19, 23). In this article, we address whether specific microflora are essential for ileocolitis and its associated cancer in GPX-DKO mice by comparing the extent of ileocolitis and cancer incidences in mice harboring non-SPF, SPF, or no enteric microflora.

MATERIALS AND METHODS

Animals. GPX-DKO mice were generated by mating Gpx1tm1Ysh/tm1Ysh (Gp1-KO) and Gpx2tm2Coh/tm2Coh (Gp2-KO) mice. Both lines were on a mixed C57BL/6 and 129SvJ or 129Sv mixed background, as we have reported previously (12). The original colony harbors Helicobacter species, including H. hepaticus, evaluated by PCR on fecal samples (Missouri University Research Animal Diagnostic Laboratory). A GF GPX-DKO colony was established by neonatal transfer at the Gnotobiotic Laboratory of the University of Wisconsin-Madison. Upon the closure of the University of Wisconsin-Madison gnotobiotic facility, the GF colony was transitioned aseptically to the Gnotobiotic Laboratory kindly provided by Kathryn A. Eaton, DVM, Ph.D., at Ohio State University-Columbus for an additional 5 months. To establish a SPF colony, we transferred GF mice from University of Wisconsin.

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Madison to the City of Hope Animal Resources Center and naturally colonized the mice with bacteria by handling and feeding a nonirradiated diet (catalogue no. 5001 Laboratory Rodent Diet; Purina Mills, Inc., Richmond, IN) for 1 month, then switching to an irradiated diet. The non-SPF colony was fed with nonirradiated Laboratory Rodent Diet with 6% fat (catalogue no. 5001). Breeders in SPF and non-SPF colonies were fed, respectively, with irradiated and nonirradiated Mouse Diet 9F containing 9% fat (catalogue no. 5020) to maintain health. The original non-SPF colony and the SPF colony were kept in separate rooms and maintained in polycarbonate microisolator cages. The GF colony was fed with Autoclavable Rodent Diet with 6% fat (catalogue no. 5010). All mice had free access to food and water. The City of Hope Research Animal Care Committee approved the housing and care for our mouse colonies.

Tumor Analysis. Six non-SPF mice > 5 months of age were found dead or appeared moribund and were thus euthanized for cause. Four of these mice had tumors. The remaining asymptomatic mice not warranting euthanasia were thus examined after 5 months of age to evaluate tumor prevalence. We visually examined the entire length of the small and large intestine of every animal sacrificed for any abnormal growth or tumor formation. In most cases, we excised tumors and made Swiss rolls on the remaining ileum and colon to avoid missing small lesions (24). All tissue samples were fixed in 10% formalin in phosphate buffer and then processed for sectioning. Most sections were stained with H&E for histological analysis. Only those lesions that had dysplastic histology (as defined below) examined by our staff pathologists were scored as tumors. Goblet cells were stained with Alcian Blue and counterstained with Nuclear Fast Red.

The tumors were analyzed for the stages of cancer progression described by Riddel et al. (2). Because of the nature of the GPX-DKO intestinal epithelium, which always had focal inflammation or pathology in the presence of luminal microflora, we did not include hyperplastic epithelium, which only showed crypt distortion or enlarged and branched glands as seen in polyps, as tumors. We scored tumors with low-grade dysplasia and high-grade dysplasia as precancerous lesions; cancers included both invasive carcinomas and signet-ring cell malignancies. The criteria for low-grade dysplasia were hyperplasia with cytological abnormalities, primarily loss of nuclear polarity, marked stratification of nuclei, nuclear hyperchromatism, and cellular and nuclear pleomorphism. High-grade dysplasia was scored when dysplastic epithelium extended to the apical surface or involved a large area. High-grade dysplasia also included carcinoma in situ, which has neoplastic epithelium forming a complex cribriform pattern (back-to-back glands with no intervening stroma) yet without evidence of invasion into the muscularis mucosa. Invasive carcinoma was scored when the atypical glands were seen in the muscularis mucosa, serosal adipose tissue, or muscle layers.

Logistic regression was used to test the association of cancer prevalence...
with age at sacrifice, sex, parity, and colony conditions, each factor being adjusted for the others. Significant (P < 0.05) likelihood-ratio tests with multiple degrees of freedom were followed by specific contrasts (Wald tests).

**Immunohistochemistry.** Mouse monoclonal anti-β-catenin (1 μg/ml) antibody (BD Transduction Labs) was used to determine cellular localization of β-catenin, which was detected with horseradish peroxidase and 3,3′-diaminobenzidine (Ultravision Detection System; Lab Vision Co., Fremont, CA). Cell proliferation was detected with a BrdUrd immunohistochemistry kit (Oncogene Research Products, San Diego, CA). Mice were injected i.p. with 5′-bromo-2′-deoxyuridine (BrdUrd, 120 mg/kg) and 5′-fluoro-2′-deoxyuridine (12 mg/kg) dissolved in sterile Ringer’s solution. Mice were euthanized 2 h after injection, and the small and large intestines were processed for routine paraffin embedding. Apoptotic cells were detected in situ by a TdT-FragEL DNA Fragmentation Detection Kit (Oncogene) on the paraffin-embedded tissue sections. Rabbit polyclonal anti-myeloperoxidase antibody (7 μg/ml; DakoCytomation, Carpinteria, CA) was used to detect polymorphonuclear neutrophils and monocytes (25). The proliferative index (stained cells/crypt) was determined by counting the number of stained cells in at least seven crypts of the most distorted region of the intestine for each mouse.

**RESULTS**

GPX-DKO mouse intestinal epithelium is highly susceptible to bacteria-induced pathology and inflammation. GF GPX-DKO mice have no pathology or symptoms except after shipping, which induces a temporary increase in crypt apoptosis and proliferation in the ileum (Fig. 1, A and B). When bacterial colonization occurs at birth, the ileal and colonic epithelium always exhibits focal pathology or inflammation throughout the life of the GPX-DKO mice (Fig. 2). The pathology and inflammation was scored in nontumorous areas based on a 17-point system, which includes inflammation as shown by lymphocyte or neutrophil infiltration (0–3 points), mucin depletion (0–2 points), reactive epithelium such as crypt distortion (0–3 points), number of intraepithelial lymphocytes (0–3 points), inflammatory foci (0–3 points), and apoptotic figures (0–3 points; Ref. 12). The pathology/
test). This higher MI in GPX-DKO mice may be necessary to avoid crypt atrophy caused by high levels of apoptosis. We found a lower number of ileal crypts (68 ± 8/cross-section, n = 5) in GPX-DKO mice compared with non-DKO mice (95 ± 19/cross section, n = 5, P = 0.03) at 7–10 weeks of age. This suggests that the crypt loss due to apoptosis may not be fully compensated by a higher MI.

To determine tumor incidence, we euthanized mice primarily between 5 and 9 months of age in non-SPF, SPF, SPF→non-SPF, and GF colonies (Table 1). Because age variation at sacrifice could confound comparisons among the colonies, we adjusted for age variation by using a logistic regression test. We found age at sacrifice was not significantly associated with tumor prevalence (P = 0.3). However, tumor prevalence varied significantly across colony conditions after adjustments for age and sex (P < 0.0001, 3 degrees of freedom, logistic regression test; Table 1). The effect was primarily due to the difference between “the clean conditions” (the GF and SPF) as opposed to “the dirty conditions” (non-SPF and SPF→non-SPF conditions; P = 0.0003). Comparisons within the clean and dirty conditions were not statistically significant (P = 0.15 between GF and SPF, and P = 0.7 between non-SPF and SPF→non-SPF). However, the data are consistent with the hypothesis that the risk of developing a tumor to GPX-DKO mice in SPF conditions was intermediate between that in GF and non-SPF conditions. The tumor rate among females varied significantly with parity adjusted for age and conditions, with non-breeder females having significantly fewer tumors (P = 0.02). Male mice were not statistically distinguishable from either group of females (P > 0.07). No tumors were found under any conditions in mice that had at least one wild-type Gpx1 or Gpx2 allele. These non-DKO

![Fig. 3. Histopathology of tumors from GPX-DKO mice. A demonstrates a nonpolypoid tumor mass in the ileum with arrows pointing to the periphery of the 1-cm tumor. B1 and C1 are a signet-ring cell carcinoma (×100) and an invasive adenocarcinoma (×40) stained with H&E. The signet-ring cell carcinoma has abundant Alcian blue-stained mucin with a Nuclear Fast Red counterstain (B2). B3 shows membranous β-catenin localization, and C2 illustrates nuclear accumulation of β-catenin in this invasive cancer (both panels are originally amplified ×200). D correlates the ileal epithelial inflammation/pathology scores with tumor incidence in male non-specific pathogen-free GPX-DKO mice. Ileal histology is shown for mice free of tumors (□) and with ileal tumors (■). Scoring criteria included lymphocytic or neutrophilic infiltration (0–3 points), mucin depletion (0–2 points), reactive epithelium such as crypt distortion (0–3 points), number of intraepithelial lymphocytes (0–3 points), inflammatory foci (0–3 points), and apoptotic figures (0–3 points). A significant difference was noted in scores for tumor-bearing (mean ± SD, 6.1 ± 1.8, n = 16) and nontumor bearing (4.1 ± 3.0, n = 29) mice (P = 0.04, Wilcoxon rank-sum test with continuity correction).]
mice were littermates of GPX-DKO mice and shared identical housing conditions. These observations exclude genetic background, non-intestinal environmental and dietary factors as etiologies for cancer susceptibility in this model.

All tumors were nonpolypoid masses > 3 mm in diameter on inspection (Fig. 3A). Most mice had single tumors, except for a few mice that had 2 tumors each. Ten of 36 (28%) tumors analyzed in the non-SPF colony (from birth) were adenocarcinomas with 8 invasive carcinomas and two signet-ring cell carcinoma (SRCC; Table 2). Two of 4 tumors in the SPF→non-SPF colony were carcinomas with 1 invasive and 1 SRCC. Most of the tumors were located in the distal ileum, except for 1 non-SPF mouse that had a tumor in the distal jejunum. Six mice had colonic tumors; this includes 1 SPF female, 1 SPF→non-SPF male, and 4 SPF male GPX-DKO mice.

Multiple gene mutations have been reported during colorectal carcinogenesis, and mutations in the adenomatous polyposis coli (Apc) gene occur often in both sporadic tumors and with colitis-associated cancers (27–32). The tumor suppressor activity of Apc sequesters the bifunctional β-catenin for degradation. Cells with mutant Apc genes have detectable accumulations of cytoplasmic and nuclear β-catenin (33–35). We stained for β-catenin and used nuclear accumulation, only, as indirect evidence for Apc inactivation because it was difficult to distinguish cytoplasmic staining from background staining. We found that 7 of 14 (50%) low-grade dysplastic adenomas, 19 of 20 (95%) high-grade dysplastic adenomas, and 9 of 13 (69%) adenocarcinomas had nuclear accumulation of β-catenin, including an invasive ileal adenocarcinoma in a GF breeder (Table 2 and Fig. 3). Two SRCCs from the non-SPF colony and 1 from the SPF→non-SPF colony did not show nuclear accumulation of β-catenin (Fig. 3). Signet-ring morphology appeared to be caused by the large amounts of mucin (stained by Alcian Blue) pressing nuclei against the plasma membrane. The only invasive adenocarcinoma that did not have nuclear accumulation of β-catenin was from the SPF→non-SPF colony.

The higher ileal tumor rates in non-SPF mice than SPF and GF mice correlated with higher pathology scores. Among the male non-SPF GPX-DKO mice, we also found that tumor-bearing mice had significantly higher ileal pathology scores than those that were tumor free (Fig. 3). Also, similar ileal scores were found between male (6.0 ± 2.4, n = 29) and female (6.6 ± 2.6, n = 12) GPX-DKO mice regardless of breeding status. However, although similar scores were found in the ileum and colon in the SPF colony, colon tumors were most prevalent.

### Table 2. Histological analysis of intestinal tumors in GPX-DKO mice

<table>
<thead>
<tr>
<th>Colony</th>
<th>Age (months)</th>
<th>n</th>
<th>LG dysplasia</th>
<th>HG dysplasia</th>
<th>Cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-SPF</td>
<td>5–12</td>
<td>36</td>
<td>10 (28%)f</td>
<td>16 (44%)</td>
<td>10 (28%)</td>
</tr>
<tr>
<td>SPF</td>
<td>2–8</td>
<td>6</td>
<td>4 (66%)</td>
<td>2 (33%)</td>
<td>0</td>
</tr>
<tr>
<td>SPF→non-SPF</td>
<td>7</td>
<td>4</td>
<td>0</td>
<td>2 (50%)</td>
<td>2 (50%)</td>
</tr>
<tr>
<td>GF</td>
<td>10</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1 (100%)</td>
</tr>
<tr>
<td>Total</td>
<td>All ages</td>
<td>47</td>
<td>14 (30%)</td>
<td>20 (43%)</td>
<td>13 (28%)</td>
</tr>
<tr>
<td>Nuclear β-catenin</td>
<td>47</td>
<td>7/14 (50%)</td>
<td>19/20 (95%)</td>
<td>9/17 (69%)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>a</th>
<th>n: number of tumor-bearing mice in the colony; LG, low grade; HG, high grade; SPF, specific pathogen-free.</th>
</tr>
</thead>
<tbody>
<tr>
<td>b</td>
<td>38 cancers include 10 invasive adenomas and 3 signet-ring cell carcinomas (SRCC). Three ileal SRCC were found in a 5-month-old and a 6-month-old non-SPF male mouse, as well as a 7-month-old male mouse transferred from SPF to non-SPF at weaning. The number in the parenthesis is the percentage of tumors with nuclear accumulation of β-catenin.</td>
</tr>
<tr>
<td>c</td>
<td>Three ileal SRCC were found in a 5-month-old breeder non-SPF female did not have nuclear accumulation of β-catenin.</td>
</tr>
<tr>
<td>d</td>
<td>One HG-dysplastic tumor from a 10-month-old breeder non-SPF female did not have nuclear accumulation of β-catenin.</td>
</tr>
<tr>
<td>e</td>
<td>Three SRCC and an invasive tumor from a 7-month-old male mouse transferred from SPF to non-SPF at weaning did not have nuclear accumulation of β-catenin.</td>
</tr>
</tbody>
</table>

### DISCUSSION

In this article, we provide the first direct evidence that GPX prevents ileocolitis and intestinal cancer. Elevation of reactive metabolites of oxygen and nitrogen during the inflammatory response are believed to cause some of the intestinal and colonic injury and dysfunction observed in IBD (17). However, most studies have focused on the damaging effect of superoxide and nitric oxide; few studies have examined the effect of hydroperoxides. Some indirect evidence suggests a protective role for Gpx1 and Gpx2 genes against oxidative stress. These data include induction of Gpx1 gene expression in gastric mucosa by *H. pylori* infection (36) and induction of Gpx2 gene expression in intestinal mucosa by commensal bacteria or by γ-irradiation (26, 37). However, elevated GPX gene expression has also been associated with tumorigenesis, presumably because of its antiapoptotic activity (38). Elevated Gpx2 gene expression is observed in squamous cell carcinomas, Barrett’s mucosa, and colorectal adenomas compared with normal tissues (39–41), and overexpression of the Gpx1 gene increases skin cancer risk (42). Therefore, it has been a quandary whether increased GPX activity is anti-inflammatory or procarcinogenic. Our results suggest that GPX can prevent tumorigenesis in intestinal epithelium by its antioxidant activity.

The tumor incidence that we observed in the ilea of GPX-DKO mice is correlated with pathology/inflammation scores, with the highest cancer rate occurring in the non-SPF colony or SPF mice made non-SPF at weaning. However, the colon shows no correlation between the tumor incidence and pathology/inflammation scores. Both non-SPF and SPF colonies have similar pathology/inflammation scores in the colon, but SPF mice have more colonic tumors than non-SPF mice. Also, female non-SPF GPX-DKO mice had similar inflammation scores regardless of their breeding status. These results suggest that inflammatory changes are necessary but not sufficient for tumorigenesis.

Although male mice did not have a significantly higher tumor incidence than female mice, breeder females appeared to have a higher tumor incidence than nulliparous females. It has been noted that *H. hepaticus* causes more severe hepatic lesions in male mice, and *H. pylori* causes more severe intestinal-type gastritis and gastric cancer in men and male mice (19, 43, 44). Apparently, those microflora harbored in the non-SPF colony, from which most tumor-bearing mice were derived, did not have as strong an effect on ileal and colonic cancer incidence as *H. pylori* has on gastric cancer. In humans, parity is not recognized as a risk factor for colon cancer. However, it is unclear whether the pregnancy-associated risk in women with IBD can be obscured by decreased numbers of pregnancies due either to reduced fertility or choice and by more preterm births or smaller birth weights (45–47). Although we have found that multiparous female GPX-DKO mice had a significantly higher tumor incidence than nulliparous females because breeder females were on a diet with slightly higher fat content to promote their own and the litters’ health and high-fat diet has been implicated in higher colorectal cancer risk (48), we cannot exclude the possibility that the different cancer rate is skewed by different diets. During normal pregnancy and preeclampsia, there is increased lipid peroxidation in multiple organs, which can increase oxidative DNA damage and gene mutations (49–52). Additional analysis will be needed to better evaluate whether parity increases cancer rate in GPX-DKO mice fed with the same 9% fat diet.

There are multiple sources for bacteria-induced oxidative stress. A few strains of commensal bacteria in *Enterococcus spp.* can generate reactive oxygen species in the lumen and cause inflammation-associated cancer in IL-10 KO mice (53–55). Epithelial cells also respond to bacterial colonization by modulating inflammatory responses (7, 9,
56). Colonization with *H. hepaticus* in A/JCr mice induces oxidative DNA damage in the liver and the expression of a subset of immune-related genes, including γ-IFN in cecal epithelium (57–59). *H. pylori* induces H₂O₂ production and cyclooxygenase-2 expression in gastric epithelial cells (60). Although our results are consistent with the notion that *H. hepaticus* is pathogenic in GPX-DKO mice, additional studies are needed to fulfill Koch’s postulates and to elucidate the molecular mechanism for bacteria-induced ileocolitis.

The tumor pathology in these mice is unique. Although most of tumors were adenomas, among 13 adenocarcinomas, there were only 3 SRCCs. In humans, the occurrence of SRCC in the small intestine or colon is a rare but distinctive event, typically found in younger patients; it carries an adverse prognosis (61, 62). Few murine intestinal cancer models demonstrate signet-ring histology. Among the exceptions are the azoxymethane-induced colorectal cancer model in rats, duodenal polyps of *Smad4*-heterozygous mice, and intestinal tumors in *Smad4*/Ap-c double heterozygous mice (16, 63–65). All 3 SRCCs in our GPX-DKO mice still had membranous β-catenin localization, suggesting that the *Wnt* pathway remains intact. This is consistent with the hypothesis that a set of genes other than those in the *Apc*-β-catenin pathway may be mutated in SRCC (62, 64). This result also suggests that mutations in multiple pathways may occur during tumorigenesis in GPX-DKO mouse intestines.

Mutations in the *Apc* gene are a common early event in sporadic colon cancer. Whether *Apc* gene mutations occur as frequently in colitis-associated cancer is less certain (18). Opposing results have been found in mouse IBD models: IL-2/β2-microglobulin-deficient mice have mutated *Apc* genes in all adenocarcinomas determined by DNA sequence analysis (32), but IL-10 deficient mice do not have *Apc* mutations in all tumors analyzed by immunohistochemistry of APC protein (66). Our results, showing most adenomas and non-SRCC adenocarcinomas have nuclear accumulation of β-catenin, support the notion that mutations in genes in the Wnt pathway occur in our inflammation-associated mouse model.

In summary, we have described a new mouse ileocolitis-associated cancer model. This is the first mouse ileal and colonic cancer model produced by a deficiency in antioxidant enzyme levels. Inflammation and cancer occurring in our GPX-DKO mice provide direct evidence, suggesting that intracellular hydroperoxides, which are reduced by selenium-dependent glutathione peroxidase and induce DNA damage in the liver and the expression of a subset of immune-related genes, including IFN in cecal epithelium (57–59). Colonization with *H. pylori* induces H₂O₂ production and cyclooxygenase-2 expression in gastric epithelial cells (60). Although our results are consistent with the notion that *H. hepaticus* is pathogenic in GPX-DKO mice, additional studies are needed to fulfill Koch’s postulates and to elucidate the molecular mechanism for bacteria-induced ileocolitis.

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