Colitis-accelerated colorectal cancer and metabolic dysregulation in a mouse model

Yuyan Gao1,2, Xin Li1, Ming Yang3, Qi Zhao1, Xiaolong Liu4, Guangyu Wang5, Xiaolin Lu1, Qi Wu6, Jin Wu6, Yannme Yang7, Yue Yang5 and Yanqiao Zhang1,5,8

1Department of Gastrointestinal Medical Oncology, The Affiliated Tumor Hospital of Harbin Medical University, Harbin, Heilongjiang 150040, China; 2Department of Geriatrics, The Fourth Affiliated Hospital, Harbin Medical University, Harbin, Heilongjiang 150001, China; 3Pathology Department and 4Geriatric Oncology Department, The Affiliated Tumor Hospital of Harbin Medical University, Harbin, Heilongjiang 150040, China and 5Cancer Research Institute, Harbin Medical University, Harbin, Heilongjiang 150040, China

*To whom correspondence should be addressed. No. 150, Haping Road, Harbin, Heilongjiang Province 150040, China. Tel/Fax: +86 0451 86298222; Email: yanqiao_zhang@126.com

The connection between inflammation and colorectal cancer (CRC) has been well recognized, and numerous related molecular mechanisms have been uncovered. To gain further insight, we used BALB/c mice treated with azoxymethane (AOM) and dextran sulfate sodium salt (DSS) to establish a colitis-associated CRC model recapitulating tubulovillous adenoma with high-grade dysplasia at week 14. We evaluated the mice in four groups: a control group fed a standard diet; a group given DSS, in which we observed no tumor or dysplasia; a group given AOM, in which we observed few dysplastic foci despite repeated administrations of the carcinogen and a group given both AOM and DSS, in which our observations agreed with those of other studies that found accelerated colorectal carcinogenesis following DSS-induced colitis. We examined the messenger RNA and micro RNA (miRNA) expression profiles of the four groups. In colitis-associated CRC, we observed the dysregulation of many pathways, including the upregulation of Wnt signaling and CRC pathways and the downregulation of apoptosis. Also, most differentially expressed genes were significantly enriched in metabolic rather than immune/inflammation pathways/processes. Additionally, we demonstrated that the expression of several important miRNAs involved in both the inflammatory response and metabolism was dramatically altered during colitis-associated CRC. Gene network analysis and gene profile analysis confirmed a close relationship between metabolic and inflammatory genes in colitis-associated CRC. Thus, our study may provide a framework for identifying metabolic genes as targets of novel molecular-based therapies against CRC.

Introduction

Colorectal cancer (CRC) has been recognized as the third most common cancer and the fourth most frequent cause of cancer death, with more than 1 million new cases each year worldwide (1). The primary cause of CRC is thought to be environmental exposure, with <20% of cases associated with hereditary factors (2). Growing evidence indicates that CRC can be induced by persistent inflammation of the colon (3,4).

Identifying the underlying molecular mechanisms of inflammation that promote CRC is the objective of much research, with studies reporting critical roles for activated NF-κB and STAT3, both of which act as non-classical oncogenes in CRC and other cancers (5,6). Also found to promote CRC are inflammation-related factors, such as interleukin (IL)-1β and tumor necrosis factor (TNF) α, and dysfunction in inflammation pathways, such as the IL-6 pathway and nucleotide-binding oligomerization domain receptor signaling (7–9).

To further elucidate the association between colitis and carcinogenesis, we performed a comprehensive evaluation of the genomic profile of CRC. We chose to study a BALB/c mouse model because it provides a relatively homogeneous genetic background and allows for the control of environmental factors and the application of a standard, randomized experimental design. Recent CRC studies in mice have focused on drug intervention in transgenic and gene knockout animals (10). High-throughput microarray analysis, however, has rarely been used to assess mouse models. We employed high-throughput detection techniques to analyze the colon tissue samples from mice randomized to three treatment groups and one control group and assessed messenger RNA (mRNA) miRNAs, micro RNA (miRNA) microarrays and the results of quantitative reverse transcription–polymerase chain reaction (qRT–PCR). We then conducted extensive computational analyses to identify the expression data that best revealed the association between CRC and inflammation.

Materials and methods

Establishment of the CRC model

We followed a published protocol (11) to establish mouse models using two drugs: azoxymethane (AOM) and dextran sulfate sodium salt (DSS). AOM is a major CRC-inducing agent that causes O6-methylguanine formation in rodents (13). DSS has been used in repeated administrations to induce chronic inflammation (14). A total of 48 BALB/c female mice between 5 and 7 weeks of age were acclimated for 7 days with boiled tap water and a basic diet (provided by the Cancer Research Institute of Harbin Medical University). All the mice were kept in a temperature-controlled (20–22°C) environment with a 12 h light–dark cycle (lights on at 7 a.m.) with regular food intake. They were randomly divided into the following four groups: (i) AOM/DSS group (colitis-associated CRC group), (ii) AOM group (drug-induced CRC group), (iii) DSS group (colitis group) and (iv) untreated control group. The differential treatment by group is detailed here and summarized in Supplementary Figure 1, available at Carcinogenesis Online. In the AOM/DSS group the mice received an intraperitoneal injection of 12.5 mg/kg AOM (Sigma–Aldrich, Milwaukee, WI) on day 1. Subsequently, the animals were given a regular diet and water for 5 days, followed by water with 2.5% DSS (MP Biomedicals, Santa Ana, CA) for 5 days and then rest for 16 days. This administration of DSS in the drinking water was repeated for two more cycles. In the DSS group, the mice were subjected to 2.5% DSS in the drinking water for three cycles. In the AOM group, the mice received an intraperitoneal injection of 10 mg/kg AOM once a week for 4 weeks. The control group was maintained on a basal diet and water. All mice were killed on day 100. The experimental procedures and the animal use and care protocols were approved by the Committee on the Ethical Use of Animals of the Cancer Research Institute at Harbin Medical University.

Tissue sampling and histopathologic evaluation

The entire colon from the cecum to the anus was removed, and a 4 cm segment of the left colon, where CRC and colitis occur frequently in this model, was used for the tissue samples and to assess histological changes. The epithelial tissue from the AOM, DSS and control groups was scraped using a slide and kept in TRIzol reagent (Invitrogen, Carlsbad, CA). The neoplasm samples from the AOM/DSS group were clipped using ophthalmic scissors and kept in RNA storage solution (Tiangen, Peking, China). The colon tissue adjacent to the sampling site was fixed and embedded for histological analysis.

Real-time qRT–PCR

Three RNA samples from each group were reverse transcribed to complementary DNA using Superscript II (Invitrogen). Real-time qRT–PCR was performed with the Prism 7900 Sequence Detection System (ABI, Foster City, CA) to detect gene expression levels, as described by Wang et al. (15). The delta-delta Ct method was used to determine the target gene mRNA levels related to those of glyceraldehyde 3-phosphate dehydrogenase. One-way analysis of variance followed by Fisher’s least significant difference post hoc test was performed using SPSS 13.0 (Statistical Product and Service Solutions, Chicago, IL), and the results were presented as the mean ± the standard error of
the mean. The primer sequences are listed in Supplementary Table 1, available at Carcinogenesis Online.

Gene expression profiling and bioinformatic analysis
Total RNA was extracted and mRNA expression was measured using the Whole Mouse Genome Microarray Kit, 4x44K (Agilent Technologies, Santa Clara, CA), which contained more than 41,000 mouse transcripts. Profiling of miRNA expression was performed using Agilent mouse miRNA (8x60K) v16.0. Three samples for each group were used to detect mRNA and miRNA expression, a process that was completed by Shanghai Biochip Co., Ltd. (Shanghai, China), according to the manufacturer’s instructions.

The GeneSpring GX 11.5.1 software package (Agilent Technologies) was used to perform principal component analysis (PCA), identify differentially expressed genes (DEGs), analyze heat maps and Venn diagrams and normalize the data with the quantile method (16). PCA was used to summarize gene expression changes between groups. The DEGs were identified using analysis of variance followed by Tukey’s honestly significant difference post hoc test. Genes that met the following analytic criteria were considered differentially expressed: (i) not labeled as ‘absent’ in two or more groups, (ii) the two-sided significance was \( P < 0.05 \) and (iii) the false discovery rate was \( < 0.05 \).

Functional annotation and analysis
Cellular pathway and functional data were retrieved from the Kyoto Encyclopedia of Genes and Genomes (KEGG) and the Gene Ontology (GO) database, respectively (17,18). Both the pathway and GO term enrichment analyses were performed using the Gene Set Analysis Toolkit, version 2 (19). The GO biological network was assessed using the Cytoscape software platform plugin BiNGO (20). The enrichment significance of KEGG pathways and GO terms was tested using hypergeometric analysis and corrected using the Bonferroni method \( (P < 0.01) \) (21). For the GO hierarchy, non-significantly enriched terms are not shown.

Network and miRNA target analysis
To obtain comprehensive interactions between mouse genes, the gene interaction network (GIN) data were integrated from three widely used interaction databases: IntAct (http://www.ebi.ac.uk/intact) (22), 12D (http://ophid.utoronto.ca/ophidv2.20/4/) (23) and MINT (http://mint.bio.uniroma2.it/mint/) (24). A subnet constructed by the interactions of DEGs was visualized using Cytoscape for further analysis (25). The enrichment of metabolic genes, transcription factors and immune response-related genes in this subnet was evaluated using the hypergeometric test for all genes in the GIN as background. To evaluate the significance of an interaction tendency between DEGs with similar expression changes, 1000 random networks were generated by the edge-swapping method (26). The edge-swapping method randomly rewired the edges between nodes in the GIN, thus preserving the node degree and allowing us to compare networks affected by only the affinity between different genes in the GIN. With a sufficient number of random networks, an empirical \( P \) value of the interaction tendency between genes was obtained.

miRNA gene regulation data were obtained from miRNA target databases using miRanda and TargetScan prediction software (27) and visualized using Cytoscape. The target enrichment in the aforementioned subnet was evaluated with the hypergeometric test using all targets in the two data sets as the background.

Results

Chronic colitis accelerated CRC
Six of 11 mice in the AOM/DSS group developed a rectocele and high tumor burden, whereas none of the mice in the remaining three groups developed a rectocele (Supplementary Figure 2A, available at Carcinogenesis Online). Mild chronic active colitis was observed in both the AOM/DSS and DSS groups, whereas no overt inflammation was observed in the AOM group. Because of tissue changes associated with colitis chronicity, the colon lengths in the AOM/DSS and DSS groups were significantly shorter compared with those of the control group \( (P < 0.05) \), whereas the colons in the AOM group did not demonstrate any significant change in length (Supplementary Figure 2B and C, available at Carcinogenesis Online).

Tumors within the distal colons at 4.5 cm from the anuses were observed with stenosis in eight AOM/DSS group mice, whereas no tumor was detected in the remaining groups. Microscopic examination showed numerous focally coalescing, polyloid tumors with tubular adenoma- or tubulovillous adenoma-like growth patterns and ranging from <1 mm to as large as 4.5 mm in size. High-grade dysplasia was focally identified. Of note, these observations have been reported elsewhere in the literature as adenocarcinomas in similar mouse colitis-associated cancer models (10). However, only a minute focus of invasion into the submucosa was seen in one mouse in the AOM/DSS group (the minimal requirement for colon adenocarcinoma in humans). No metastatic disease was detected, and much smaller polyloid tumors were observed to be sparsely distributed within the mucosa of the remaining proximal colons in the AOM/DSS group.

In contrast, the colons from the AOM group revealed thickened intestinal walls. Moreover, occasional small tubular adenomas and dysplastic foci were identified, despite the higher doses of AOM used in this group (see Materials and methods). The colons from the DSS group also revealed thickened intestinal walls, mild crypt distortion and focal mild-to-moderate chronic active colitis within the mucosa that occasionally extended to the muscularis mucosa. No tumor formation was observed in the DSS group (Supplementary Figure 2D and E, available at Carcinogenesis Online).

Comparing gene expression profiles among the four treatment groups
DEGs may provide clues as to how inflammation promotes CRC, thus we sought to examine and compare the gene expression profiles between the experimental groups using microarray analysis followed by a large-scale analysis. First, we compared the gene expression profiles between groups using PCA and a hierarchical clustering algorithm. This showed clear separation of the samples from the different groups in the three-dimensional PCA space, especially for the AOM/DSS group samples (Figure 1A). As expected, the distance from either the AOM or DSS group to the control group was smaller than the distance from the AOM/DSS group to the control group. We observed similar results for the DEG profiles when we used analysis of variance (Figure 1B). We found similarities in many DEGs between the AOM/DSS and AOM groups. Compared with the gene expression profile of the control group, we found far greater differences in the profiles of the AOM group than in the profiles of the DSS group. We used Venn diagrams to show the number of DEGs in the AOM/DSS group, AOM group and DSS group relative to that of the control group and the overlap between groups (Figure 1C–E). As shown in the Venn diagrams, despite a large number of overlapping DEGs, ~50% of the DEGs in the AOM/DSS group were not observed in the AOM or DSS group, which suggests that a large number of genes may be activated or repressed in colitis-associated cancer.

qRT–PCR to confirm microarray expression levels
Six genes were selected for further validation by qRT–PCR, including three genes (ARG1, ALOX12 and LPL) involved in basal metabolism, two genes (TNFα and MMP9) associated with inflammation and the important oncogene STAT3. The genes related to basal metabolism, ARG1, ALOX12 and LPL, were highly expressed in the AOM/DSS group and have recently been implicated in the inflammatory response (28–30). TNFα and MMP9 were also highly expressed in the AOM/DSS group. The oncogene STAT3 did not show a significant change in expression according to the microarray data but was selected for validation because it is persistently activated in the tumor microenvironment and is thought to induce cancer (31). We selected five additional genes at random as a control to test the consistency between the microarray and qRT–PCR data. The microarray results confirmed our qRT–PCR observations in 10 of the 11 cases (Figure 2 and Supplementary Figure 3, available at Carcinogenesis Online). STAT3 was the only exception, as significant changes in this gene were not demonstrated in the microarray data, although increased
and decreased levels were observed in the AOM/DSS and AOM groups, respectively. This difference was likely due to the increased sensitivity of qRT-PCR compared with high-throughput microarray analysis. High STAT3 expression is consistent with our understanding of this gene’s function in inflammation-associated tumors.

Fig. 1. Global analysis of gene expression profiles in the four models. (A) PCA of gene expression patterns. (B) Hierarchical clustering analysis of the four groups. Overlap of DEGs between groups for (C) all DEGs, (D) upregulated DEGs and (E) downregulated DEGs (figure in color available at Carcinogenesis Online).
To investigate biological processes that were significantly altered in the AOM/DSS group compared with the AOM group, we performed KEGG pathway and GO term enrichment analyses using the Gene Set Analysis Toolkit, V2. These analyses revealed a total of 27 significantly enriched canonical pathways (Table I). To further clarify the specific function of each canonical pathway, we performed the same enrichment analyses using upregulated and downregulated genes separately to obtain 6 activated pathways, 16 inhibited pathways, 4 signaling pathways enriched for both up- and downregulated genes and 1 pathway enriched for neither. Not surprisingly, Wnt signaling and CRC pathways were activated during colorectal carcinogenesis, whereas the apoptosis pathway was negatively regulated, as commonly seen in sporadic CRC. Other pathways related to carcinogenesis were also dysregulated; for example, we observed upregulation of the ubiquitin and spliceosome pathways and downregulation of the vascular endothelial growth factor (VEGF) signaling pathway.

We found most enriched pathways to be related to cellular metabolism and only two to be related to immune system function. Additionally, almost all of the enriched metabolic pathways, which were mainly associated with the metabolism of drugs, lipids and amino acids, were inhibited as a result of colorectal carcinogenesis. The specific DEGs enriched for the cytochrome P450 pathway, the arginine and proline metabolic pathway and the fatty acid metabolic pathway are shown in Supplementary Table 2, available at Carcinogenesis Online. These data demonstrated that most of the downregulated DEGs were associated with metabolic pathways. Specifically, genes involved in multiple important metabolic pathways and VEGF-associated signaling pathways, such as cyp4a10 and aldh1b1, were dramatically downregulated.

GO enrichment analysis confirmed the results of the KEGG pathway analysis, suggesting that DEGs were mainly enriched for metabolic processes, such as those related to the metabolism of RNA, proteins and other macromolecule substances (Figure 3A). The GO and KEGG enrichment analyses suggested that metabolic processes were significantly different between the AOM/DSS and AOM groups. Therefore, we concluded that metabolic disturbances play an important role in promoting the progression of CRC that is triggered by inflammation.

One consistently downregulated pathway was the fatty acid metabolic pathway (Figure 3B), which plays an important role in cancer development through its involvement in cell growth, cell membrane synthesis and

| Table I. KEGG pathway enrichment analysis in AOM/DSS versus AOM groups |
|------------------|------------------|------------------|------------------|
| **Function**     | **Pathway**      | **Downregulated, P value** | **Upregulated, P value** |
| Metabolism       | Metabolic pathways | 3.28E-41          |                      |
|                  | Drugs (cytochrome P450) | 8.35E-10          |                      |
|                  | Arginine and proline | 2.89E-07          |                      |
|                  | Glycerolipid      | 5.64E-07          |                      |
|                  | Xenobiotic (cytochrome P450) | 5.64E-07    |                      |
|                  | Tryptophan        | 5.10E-06          |                      |
|                  | Propanoate        | 4.32E-05          |                      |
|                  | Fatty acid        | 0.0004            |                      |
|                  | Inositol phosphate| 0.0002            |                      |
|                  | Alanine, aspartate and glutamate | 0.0047    |                      |
|                  | RNA degradation   | 4.24E-09          |                      |
|                  | Ether lipid       |                   |                      |
|                  | Apoptosis         | 8.87E-07          |                      |
|                  | Calcium signaling | 2.00E-07          |                      |
|                  | Endocytosis       | 5.64E-07          |                      |
|                  | ABC transporter   | 2.85E-05          |                      |
|                  | VEGF signaling    | 0.0002            |                      |
|                  | Chemokine signaling | 0.0026        | 0.0081             |
|                  | Adipocytokine signaling | 0.0065    |                      |
|                  | Mitogen-activated protein kinase signaling | 1.46E-07 | 0.0013             |
|                  | Insulin signaling | 5.76E-05          | 0.0008             |
|                  | B-cell receptor signaling | 5.76E-05 | 0.0023             |
|                  | Ubiquitin-mediated proteolysis | 4.47E-10 |                |
|                  | Spliceosome       | 6.40E-07          | 3.09E-05           |
|                  | Cancer            |                   | 4.95E-05           |
|                  | Wnt signaling     |                   | 0.0037             |
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The downregulated genes Adh1, Aldh2 and Cyp4a10 are members of this pathway and are also involved in alcohol and aldehyde metabolism. Published genetic association analyses have revealed that Adh1 is associated with CRC risk (33). A recent study also reported downregulation of the fatty acid metabolic pathway during the transition from colorectal adenoma to carcinoma in humans (34).

Fig. 3. GO annotations for DEGs between AOM/DSS and AOM groups. (A) GO annotation analysis diagram. The radius size is related to the amount of DEGs. The dark orange color is related to significant associations and was calculated according to the $-\log_{10}(P\text{ value})$. Branches with no significant associations were discarded, and only small gray nodes were retained. Pro. represents ‘progress’. (B) Fatty acid metabolic pathway (partial pathway; the same enzymes are involved in β-oxidation processes). Blue and red indicate downregulated and upregulated genes, respectively (figure in color available at Carcinogenesis Online).
Interaction networks of DEGs and miRNAs. (A) Hierarchical clustering algorithm for miRNAs. (B) PCA analysis of miRNAs. (C) Network of DEGs for the AOM/DSS group versus the control group. A rectangle indicates a metabolic gene; red represents upregulated genes; green represents downregulated genes; color intensity is associated with log$_2$(fold change value). (D) Target mRNAs of miR-20a, miR-181a and miR-7b in the network.
Network-based miRNA expression analysis of colitis-associated CRC

PCA of our miRNA microarray data revealed that the distance to the control group was longer from the AOM/DSS group than from the other groups. The small difference between the DSS group and the control group, according to the miRNA data, made it difficult to distinguish these two groups. We note three possible reasons for these results from the miRNA PCA analysis: fewer total miRNA members for analysis compared with total mRNAs, variations in the inflammatory reactions in the colons of the DSS group and low miRNA expression and limitations associated with the miRNA technology. Expression of miRNA for the four groups was detected and clustered based on the expression pattern (Figure 4A and B). Significantly upregulated and downregulated miRNA clusters were observed by comparing the AOM/DSS group to the other groups. These two clusters were enriched for miRNAs known to be involved in the immune response and the development and differentiation of lymphoid or myeloid cells, such as miR-181c, miR-20a, miR-24 and miR-21 in the upregulated cluster and miR-221, miR-181a and miR-7b in the downregulated cluster (35,36). In addition to their association with inflammation, miR-20a and miR-21 are also potential oncomirs and demonstrate increased expression in most tumor types (37). Furthermore, overexpressed miR-20a has been shown to activate autophagy characterized by enhanced T-cell proliferation and survival, and miR-21 expression has been shown to positively correlate with interleukin-6 and STAT3 expression in human colon cancer cells (38). Other studies have also documented an ~10-fold lower level of miR-181a expression in mature T cells during thymocyte maturation, and miR-181a knockdown impaired selection in thymic organ cultures (39), which may explain the observed downregulation of miR-181a in our AOM/DSS group.

To investigate the miRNA–target mRNA correlations, we constructed a GIN and extracted interactions between DEGs from tissues of the AOM/DSS group. Although this analysis was limited by the poor availability of data for mouse gene interaction coverage, we found that important genes demonstrated abundant interactions compared with other genes. By integrating data from three widely used databases, we successfully constructed a mouse GIN with 6951 edges between 2837 genes and extracted a subnet of 145 interactions between 174 important DEGs (Figure 4C). This subnet was significantly enriched for metabolic genes, transcription factors and immune response-related genes ($P = 2 \times 10^{-10}$, $P < 10^{-20}$ and $P = 3.5 \times 10^{-7}$, respectively, by hypergeometric test). The DEG interactions were extensive compared with the poor coverage of the GIN. Additionally, DEGs with similar expression tended to interact with each other (empirical $P = 0.043$ for both upregulated and downregulated genes), reflecting functional coordination between interacting genes in the AOM/DSS model.

Interestingly, we observed that the DEG subnet was significantly enriched for miRNA targets in the upregulated and downregulated miRNA clusters (Figure 4A). As two notable examples, 45 and 41 targets of miR-20a and miR-7b, respectively, were detected in the subnet (Figure 4D, $P = 6.4 \times 10^{-4}$ and $P = 0.0016$, respectively, by hypergeometric test). Most targets of the overexpressed miR-20a in this subnet were downregulated and involved in metabolism, including the metabolic gene Aldh1b1 and the amino acid metabolic gene Maao. On the other hand, many upregulated metabolic genes, such as P1a2g12a, Ikbkb, Gtf2e, Tcf, Eif4e, Nono, Dach1, Alox12, Tgfb1, Ubp1 and Runx1, were targets of the underexpressed miR-7b and miR-181a. Notably, in addition to playing a role in a variety of metabolic pathways of nutrients, such as those involving amino acids and lipids, P1a2g12a is also involved in cancer-related pathways in CRC, such as the VEGF signaling pathway, the high-affinity receptor for the Fc region of immunoglobulin E signaling pathway, the gonadotropin-releasing hormone signaling pathway, the mitogen-activated protein kinase signaling pathway and vascular smooth muscle contraction. These results suggest that the abnormal miRNA expression that silences metabolic genes may also contribute to the progression of colitis-associated CRC.

Discussion

In the present study, we observed that chronic colitis promoted carcinogenesis in the AOM/DSS model. Additionally, cancer-related pathways, including apoptotic and Wnt signaling pathways, were dysregulated during colonic carcinogenesis. Importantly, we observed disturbances in metabolic pathways during the progression of CRC induced by inflammation.

In a recent study, Tang et al. (40) examined mRNA expression profiles of colonic tissues demonstrating inflammation, dysplasia or carcinoma at different times during the establishment of the AOM/DSS model. Similar to our results, their GO term analysis and KEGG pathway analysis revealed changes in metabolic processes and several cancer-related pathways. However, there were several important differences between that study and the current study, which may be attributed to the methods used for mRNA expression analysis. For example, the former study compared AOM/DSS and control groups and emphasized the inflammation coefficient and cancer-causing factors, whereas we compared AOM/DSS and AOM groups and focused on how inflammation accelerates CRC.

In an earlier study, Suzuki et al. (41) established a colitis-associated tumor mouse model using lower doses of AOM and DSS compared with those of the current study (AOM: 10 mg/kg versus 12.5 mg/kg; DSS: 2% only once versus 2.5%, three times, respectively). Also, the tissue samples in our study were collected at later time points (14–15 weeks versus 5 and 10 weeks), and Suzuki et al. did not observe tumors in the AOM/DSS group as we did. Consequently, compared with our data, their data had fewer DEGs. Fang et al. (42) studied the global expression profiles related to acute inflammation at days 0, 2, 4 and 6 in DSS-treated mice and identified 1609 DEGs at days 4 and 6, of which only 501 DEGs were progressively upregulated. The lack of progressive upregulation among the majority of DEGs may have been due to different molecular mechanisms between acute and chronic inflammation, as chronic inflammation has been shown to demonstrate limited carcinogenicity without interactions with other carcinogenic factors. In previous studies, no tumors were seen in mice given DSS alone (11). At week 14 in our study, tumors were observed only in the AOM/DSS group and not in the AOM group, even though six doses of the carcinogen had been administered to the mice in the AOM group and only one dose had been administered to the mice in the AOM/DSS group. In another study that assessed AOM treatment alone, tumors were observed at week 20 (12). These results suggest that interactions between inflammation and carcinogens accelerate CRC. Indeed, no overt invasion or metastasis was noted in the histopathological analysis in both our study and previous studies, which suggests that colitis in this rodent model does not appear to promote tumor cell invasion or metastasis (10).

Interestingly, metabolic processes but not immune system processes were enriched among the DEGs in the AOM/DSS group. Recently, Liao et al. (43) used AOM to induce precancerous colorectal lesions in male Wistar rats to demonstrate significant metabolic alterations related to glucose and lipid metabolism in urine, sera and colonic tissues.

Furthermore, it has been recognized that in addition to the direct interactions between enzymes or transcriptional factors and
inflammatory-associated genes, lipid metabolic processes also trigger second messengers to promote nuclear receptor expression and initiate specific gene transcription. Indeed, with the exception of the metabolic pathways of fatty acid and L-arginine, which are known to participate in inflammation-triggered carcinogenesis (32), there is little information available regarding the relationship between CRC and other metabolic pathways, such as the cytochrome P450 pathway. We detected several cytochrome P450 pathway members that were downregulated in our model, which corroborates the findings of Suzuki et al. (41) at earlier time points (5 and 10 weeks). This result also suggested that persistent cytochrome P450 inhibition might contribute to CRC formation, as it is known that this enzyme is altered during hepatitis. Furthermore, many cytokines (IL-1α, IL-1β, IL-2, IL-6 and TNFα) are activated by infection, which inhibits cytochrome P450 gene transcription in the liver (44). However, compared with the extensive body of work on hepatic cytochrome P450, little information is available regarding the effects of inflammation on extrahepatic forms of this enzyme. Our study suggests that a mechanism for cytochrome P450 downregulation may also exist in colitis-associated CRC.

Additional studies have recently elucidated the important role of metabolism in carcinogenesis. For example, it was found that patients with metabolic syndrome, including obesity, diabetes, high cholesterol and atherosclerosis, had increased cancer risk, and drugs used for the treatment of these disorders were shown to inhibit tumor growth and transformation (45,46). Furthermore, recent breakthroughs in delineating cancer mechanisms, including the identification of an alternative glycolytic pathway in proliferative cells and the serine synthesis pathway in breast cancer cells (47,48), suggest that metabolic interventions may represent effective antitu-

In summary, the findings of the present study concur with results from published studies by suggesting that appropriate targets for molecular-based therapies may be found in many of the DEGs related to metabolic pathways catalyzed by cytochrome P450: the metabolism of amino acids, fatty acids, lipids, carbohydrates and most drugs. For example, as an enzyme of arginine, ARG1 has been thought to be involved in the proliferation, maturation and differ-
entiation of cells involved in the immune/inflammation responses and the proliferation of malignant cells; therefore, it may be a good therapeutic target. The DEGs Alox12, Alox12e, Alox15, Pla2g2a, Pla2g12a, Ald1l, Aldh1b1 and others, which participate in the metabolism of fatty acids and lipids and are usually involved in both inflammation and malignant cell signaling, may also be appropriate therapeutic targets. Increasing the function of members of the cytochrome P450 family that have lower expression levels in CRC may inhibit the destructive effects of inflammation and thereby limit cancer promotion.

In the current study, we demonstrated that the expression of metabolic genes was significantly altered in the colitis-associated CRC model, including the dysregulation of several important cancer-related pathways and immune/inflammation-related miRNAs associated with the expression of genes involved in metabolism. Despite the limitation that our study lacked detailed in vivo or in vitro experimental validations, our results suggest a correlation between metabolic disturbances and colitis-associated CRC. In future studies, we aim to evaluate key metabolic genes or specific metabolic pathways coupled to oncogenic pathways in an attempt to identify additional carcinogenic mechanisms and effective interventions for inflammation-induced CRC.

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Supplementary material
Supplementary Figures 1–3 and Tables 1–3 and original color Figures 1–3 in color can be found at http://carcin.oxfordjournals.org/

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