

Targeted Metabolomics Identifies the Cytochrome P450 Monooxygenase Eicosanoid Pathway as a Novel Therapeutic Target of Colon Tumorigenesis



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Abstract

Colon cancer is the third most common cancer and the second leading cause of cancer-related death in the United States, emphasizing the need for the discovery of new cellular targets. Using a metabolomics approach, we report here that epoxyoxygenated fatty acids (EpFA), which are eicosanoid metabolites produced by cytochrome P450 (CYP) monooxygenases, were increased in both the plasma and colon of azoxymethane (AOM)/dextran sodium sulfate (DSS)-induced colon cancer mice. CYP monooxygenases were overexpressed in colon tumor tissues and colon cancer cells. Pharmacologic inhibition or genetic ablation of CYP monooxygenases suppressed AOM/DSS-induced colon tumorigenesis *in vivo*. In addition, treatment with 12,13-

epoxyoctadecenoic acid (EpOME), which is a metabolite of CYP monooxygenase produced from linoleic acid, increased cytokine production and JNK phosphorylation *in vitro* and exacerbated AOM/DSS-induced colon tumorigenesis *in vivo*. Together, these results demonstrate that the previously unappreciated CYP monooxygenase pathway is upregulated in colon cancer, contributes to its pathogenesis, and could be therapeutically explored for preventing or treating colon cancer.

Significance: This study finds that the previously unappreciated CYP monooxygenase eicosanoid pathway is deregulated in colon cancer and contributes to colon tumorigenesis.

Introduction

Colon cancer is the third most common cancer and the second leading cause of cancer-related deaths in the United States (1). It is important to discover novel cellular targets that are crucial in the pathogenesis of colon cancer, which could facilitate the development of mechanism-based strategies to reduce the risks of colon cancer. Eicosanoids, which are endogenous lipid-signaling molecules produced from enzymatic metabolism of arachidonic acid

(ARA, 20:4 ω -6), play essential roles in inflammation and have recently been implicated in cancer (2, 3). There are three major pathways involved in biosynthesis of eicosanoids: cyclooxygenases (COX) producing prostaglandins and thromboxanes, lipoxygenases (LOX) producing leukotrienes and hydroxyl fatty acids, and cytochrome P450 (CYP) monooxygenases producing epoxyoxygenated fatty acids (EpFA; refs. 4, 5). Besides ARA, other polyunsaturated fatty acids, such as linoleic acid (LA, 18:2 ω -6),

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α -linolenic acid (ALA, 18:3 ω -3), and docosahexaenoic acid (DHA, 22:6 ω -3), are also efficient alternative substrates for these metabolizing enzymes, which convert them to the corresponding fatty acid metabolites (4, 5). As a result, the enzymatic metabolism of polyunsaturated fatty acids leads to formation of a large array of eicosanoid metabolites, many of which act as autocrine or paracrine mediators to regulate inflammation and hemostasis (4, 5).

Previous research has shown that certain eicosanoid metabolites are deregulated in colon cancer and contribute to its pathogenesis (6). Notably, the most prominent cancer-associated eicosanoids are prostaglandins, which are produced by COX-2 that is overexpressed in most human colon cancer samples (3). Guo and colleagues reported the increased ARA and COX-produced metabolite prostaglandin A₂ in the plasma of *Apc*^{min/+} mice (7). Clinical and epidemiologic studies support that pharmacologic inhibitors of COX-2 are highly effective for preventing colon cancer (3). However, the gastrointestinal and cardiovascular toxicities induced by COX-2 inhibitors have limited their clinical applications (8). Besides COX-2, the roles of other eicosanoid pathways in colon tumorigenesis are not well understood (6). It is important to discover novel eicosanoid signaling pathways involved in colon tumorigenesis.

Most previous studies to investigate the roles of eicosanoids in colon tumorigenesis have only studied single or limited number of eicosanoid metabolite(s); few systematic analyses have been carried out, hampering our understanding of the roles of eicosanoid signaling in colon tumorigenesis (6). To discover novel eicosanoid metabolites and pathways involved in colon tumorigenesis, in this study, we use a LC/MS-MS-based targeted metabolomics to systematically profile eicosanoids in a well-established azoxymethane (AOM)/dextran sulfate sodium (DSS)-induced colon cancer model in C57BL/6 mice, then use pharmacologic and genetic approaches to validate the functional roles of the identified enzymatic target and its metabolite in colon tumorigenesis.

Materials and Methods

Animal experiment

The animal experiments were conducted in accordance with the protocols approved by the Institutional Animal Care and Use Committee of the University of Massachusetts Amherst (protocol number: 2017-0019) and NIH/National Institute of Environmental Health Sciences (protocol number: 05-18).

Animal protocol 1: AOM/DSS-induced colon tumorigenesis in mice

C57BL/6 male mice (age, 6 weeks) were purchased from Charles River and maintained on a modified AIN-93G diet (containing 10% fat, corn oil was the only source of fat content, diet composition was described in Supplementary Table S1) for 3 weeks, then the mice were divided into two groups: (i) the mice in the AOM/DSS group were treated with 10 mg/kg AOM (Sigma-Aldrich) via intraperitoneal injection, and at week 1 post the AOM injection, they were given 2% DSS (36–50 kDa, MP Biomedicals) in drinking water for 1 week; and (ii) the mice in control group were maintained on the same diet without AOM/DSS treatment. At week 9.5 post the AOM injection, the mice were sacrificed to harvest plasma and colon tissues for analysis. For analysis

of colon tumor, colon tissues were cut open longitudinally, washed in PBS, and inspected under a dissection microscope. The size of the tumor was determined by the following formula: tumor size = $\pi \times d^2/4$, where d is the diameter of each tumor. The methods for biochemical analyses are described in Supplementary Materials and Methods, and the sequence of RT-PCR primers are listed in Supplementary Table S2.

Animal protocol 2: AOM/DSS-induced colon tumorigenesis in *Cyp2c*^{+/+}, *Cyp2c*^{+/-}, and *Cyp2c*^{-/-} mice

Littermate wild-type *Cyp2c*^{+/+} mice, heterozygous *Cyp2c*^{+/-} mice, and knockout *Cyp2c*^{-/-} mice (background on C57BL/6; age, 6 weeks) were treated with 10 mg/kg AOM via intraperitoneal injection. At week 1 post the AOM injection, they were given 2% DSS in drinking water for 1 week. During the whole experiment, the mice were maintained on mouse chow. At week 9.5 post the AOM injection, the mice were sacrificed to collect blood and colon tissues for analysis.

Animal protocol 3: effects of EpOME on AOM/DSS-induced colon tumorigenesis

C57BL/6 male mice (age, 6 weeks) were treated with 10 mg/kg AOM via intraperitoneal injection. At week 1 post the AOM injection, they were given 2% DSS in drinking water for 1 week. At week 5 post the AOM injection, the mice were subcutaneously implanted with osmotic mini-pumps (Durect, model 1004), which contained 12,13-epoxyoctadecenoic acid (12,13-EpOME; Cayman, dose = 2 mg/kg/day) or vehicle (a 1:1 vol/vol mixed solution of DMSO and PEG 400). During the whole experiment, the mice were maintained on mouse chow. At week 9 post the AOM injection, the mice were sacrificed to collect blood and colon tissues for analysis.

Animal protocol 4: effects of 12,13-EpOME on MC38 primary tumor growth in mice

C57BL/6 male mice (age = 6 weeks) were subcutaneously implanted with osmotic mini-pumps (Durect, model 1004), which contained vehicle (a 1:1 vol/vol mixed solution of DMSO and PEG 400) or 12,13-EpOMEs (dose = 2 mg/kg/day). After one week of mini-pump implantation, 4×10^5 MC38 colon cancer cells (purchased from Kerablast, catalog number ENH204, cell line authentication, and *Mycoplasma* testing have been verified by company) in 100 μ L PBS were subcutaneously injected into each mouse to initiate primary tumor growth. Tumor size was measured using caliper. At the end of the experiment, the tumors were dissected, weighed, and subjected to biochemical analysis.

Animal protocol 5: effects of CYP inhibitors (SKF-525A and clotrimazole) on AOM/DSS-induced colon tumorigenesis in mice

C57BL/6 male mice (age, 6 weeks; maintained on mouse chow) were treated with 10 mg/kg AOM via intraperitoneal injection. At week 1 post the AOM injection, they were given 2% DSS in drinking water for 1 week. After the AOM/DSS stimulation, the mouse diet was changed to a modified AIN-93G diet containing 200-ppm SKF-525A or clotrimazole (Alfa Aesar) dissolved in polyethylene glycol 400 (PEG 400, Millipore) as vehicle (0.1% in diet, v/w), or vehicle alone for 6 weeks. The diets were freshly prepared and changed every 2 to 3 days. At week 8 post the AOM injection, the mice were sacrificed to collect blood and colon tissues for analysis.

Statistical analysis

Data are expressed as mean \pm SEM. Shapiro–Wilk test was used to verify the normality of data and Levene's mean test was used to assess equal variance of data. Statistical comparison of two groups was performed using either Student *t* test or Wilcoxon–Mann–Whitney test (when normality test is failed). Comparison of three groups was analyzed by either parametric one-way ANOVA or nonparametric one-way ANOVA (Kruskal–Wallis test by ranks, used when normality test is failed) followed by Dunn's *post hoc* test. All of these data analysis was performed by using SigmaPlot software (Systat Software, Inc). *P* values less than 0.05 are reported as statistically significant. Gene expression data of colorectal adenocarcinoma matched tumor and nontumor were derived from The Cancer Genome Atlas (TCGA) database through Firebrowse (<http://firebrowse.org/>).

Results

CYP monooxygenase-produced eicosanoid metabolites are elevated in the plasma and colon of AOM/DSS-induced colon cancer mice

To our knowledge, a metabolomics-based approach to systematically profile eicosanoids in colon cancer has not been attempted (6). In an effort to better understand the roles of eicosanoids in colon tumorigenesis, we used a LC/MS-MS–based targeted metabolomics to compare the profiles of eicosanoids in the plasma and colon of control healthy mice (not treated with AOM/DSS) and colon cancer mice (treated with AOM/DSS; Fig. 1A). In agreement with previous studies of the AOM/DSS model (9), 100% of the AOM/DSS-treated mice developed colon tumors (Fig. 1B), with increased expressions of proinflammatory genes (*Il6*, *Tnf α* , *Mcp-1*, and *Cox-2*) in the colon tissues (Fig. 1C).

LC/MS-MS detected 42 metabolites in the plasma and 56 metabolites in the colon (see complete LC/MS-MS result in Supplementary Tables S3 and S4). Among the detected metabolites, only CYP monooxygenase–produced EpFAs are significantly increased in both the plasma and colon tissues of the AOM/DSS-induced colon cancer mice (Fig. 1D–G). Indeed, the concentrations of an array of EpFAs, including 9,10- and 12,13-EpOME produced from LA (Fig. 1D), 8,9-, 11,12-, and 14,15-epoxy eicosatrienoic acids (EET) from ARA (Fig. 1E), 9,10- and 15,16-epoxyoctadecadienoic acid (EpODE) from ALA (Fig. 1F), and 19,20-epoxydocosapentaenoic acid (EDP) from DHA (Fig. 1G), are increased in the plasma and colon of the tumor-bearing mice.

CYP monooxygenases are overexpressed in the colon of AOM/DSS-induced colon cancer mice

To understand the underlying mechanisms behind the elevated concentrations of EpFAs in colon cancer, we studied the expression of enzymes involved in EpFA production and removal. The biosynthesis and degradation of EpFAs involves three enzymatic steps: membrane-bound fatty acids are released by phospholipase A₂ (PLA₂) and related enzymes to generate intracellular free-form fatty acids, which are then metabolized by CYP monooxygenases (predominately CYP2C isoforms) to form EpFAs, which then undergo degradation by soluble epoxide hydrolase (sEH; refs. 5, 10). qRT-PCR showed that the expression of *Pla2g4a* (encoding cytosolic calcium-dependent PLA₂) was unchanged, the expression of several CYP monooxygenases (including mouse *Cyp2c38*, *Cyp2c39*, *Cyp2c65*, and *Cyp2c70*) was increased ($P < 0.05$), and the

expression of *Ephx2* (encoding sEH) was decreased ($P < 0.05$) in colon tissue of mice with colon cancer (Fig. 1H). Together, these results support that there is enhanced biosynthesis and reduced degradation of EpFAs in colon tumors of mice, which contributes to elevated EpFAs in colon cancer.

CYP monooxygenases are overexpressed in human colon cancer cells

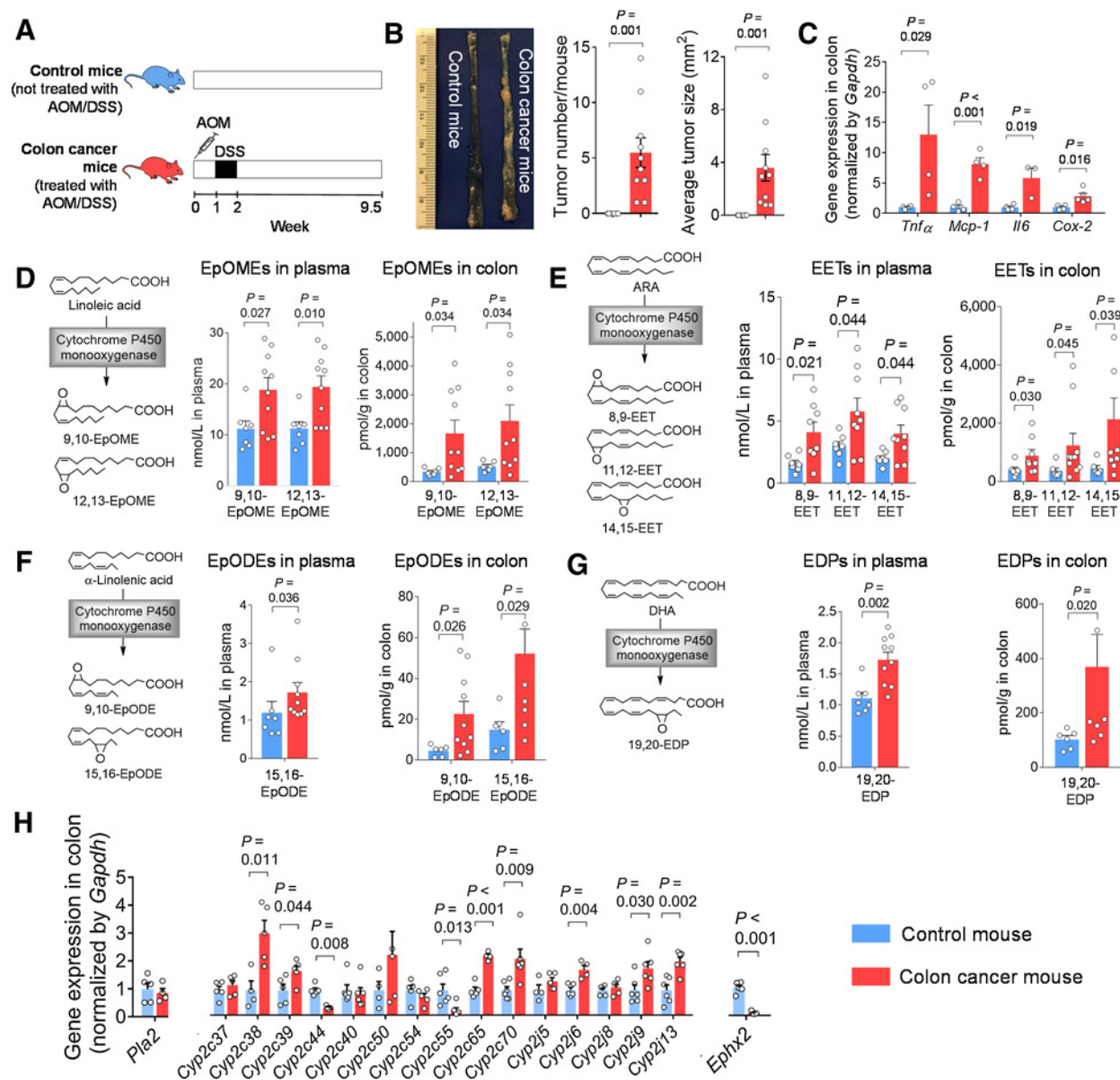
To further validate that CYP monooxygenases are overexpressed in colon cancer, we studied their expressions in human colon cancer cells. Compared with normal human colon cells (CCD-18Co), the gene expression of CYP monooxygenases (human *CYP2C8*, *2C9*, *2C19*, and *2J2*) was significantly ($P < 0.05$) increased in human colon cancer cells (HCT116 and Caco-2; Supplementary Fig. S1A). Consistent with the qRT-PCR result, immunoblotting showed that the protein expression of CYP monooxygenase (using human *CYP2C9* as a marker) was increased in human colon cancer cells (Supplementary Fig. S1B). These results demonstrate that the CYP monooxygenases are overexpressed in human colon cancer cells, which is consistent with the results from animal experiments.

Genetic ablation of CYP monooxygenases suppresses AOM/DSS-induced colon tumorigenesis

To determine the roles of CYP monooxygenases in colon cancer, we tested whether genetic ablation of CYP monooxygenases modulates colon tumorigenesis. To this end, we performed the AOM/DSS-induced colon cancer model in a recently developed *Cyp2c* gene cluster knockout mouse, which has deletions of 14 mouse *Cyp2c* genes, including *Cyp2c29*, *2c37*, *2c38*, *2c39*, *2c40*, *2c50*, *2c54*, *2c55*, *2c65*, *2c66*, *2c67*, *2c68*, *2c69*, and *2c70* (Fig. 2A; ref. 11). Compared with *Cyp2c^{+/+}* mice, the expression of *Cyp2e1* (encoding the enzyme Cyp2e1 to activate the mutagenic activity of AOM; ref. 12) was not changed in *Cyp2c^{+/-}* or *Cyp2c^{-/-}* mice (Supplementary Fig. S2), supporting that it is feasible to perform the AOM/DSS-induced colon tumorigenesis model in these mice.

Compared with AOM/DSS-induced *Cyp2c^{+/+}* mice, the AOM/DSS-induced *Cyp2c^{+/-}* mice had lower tumor number and total tumor burden (Fig. 2B), reduced expression of tumorigenic markers proliferating cell nuclear antigen (PCNA) and active β -catenin (Fig. 2C), and attenuated expression of proinflammatory and protumorigenic genes (*Tnf α* , *Il1 β* , *Axin2*, and *C-myc*), in the colon tumor tissue (Fig. 2D), illustrating reduced colon tumorigenesis. In addition, we found that compared with AOM/DSS-induced *Cyp2c^{+/+}* mice, the colon of AOM/DSS-induced *Cyp2c^{+/-}* mice had lower colonic expression of CYP monooxygenase (using mouse *Cyp2c38* as a marker; Fig. 2D) and reduced colonic concentrations of CYP monooxygenase–produced EpFAs (Fig. 2E; see complete LC-MS/MS result in Supplementary Table S5), supporting the involvement of CYP monooxygenase pathway in colon tumorigenesis.

We should point out that compared with *Cyp2c^{+/+}* mice, *Cyp2c^{+/-}* mice showed minimal signs of basal inflammation, but *Cyp2c^{-/-}* mice had severe basal liver inflammation, with increased expression of proinflammatory genes (*Tnf α* and *Il1 β* ; Supplementary Fig. S3A) and enhanced infiltration of inflammatory cells (Supplementary Fig. S3B) in the liver, as described previously (11). When *Cyp2c^{-/-}* mice were stimulated with AOM/DSS, there was rapid death within 1 to 3 days post the DSS treatment (6 of 8 *Cyp2c^{-/-}* mice died during this period;

**Figure 1.**

CYP monooxygenase-produced eicosanoid metabolites are increased in the plasma and colon of AOM/DSS-induced colon cancer mice. **A**, Scheme of animal experiment. **B**, Quantification of colon tumor in mice ($n = 6$ in control group and $n = 10$ in colon cancer group). **C**, Gene expression of *Tnfα*, *Mcp-1*, *Il6*, and *Cox-2* in colon ($n = 4$ per group). **D–G**, Concentrations of CYP monooxygenase-produced EpFAs in plasma and colon ($n = 6–10$ per group). **H**, Expression of *Pla2*, *Cyp monooxygenases*, and *Ephx2* (encoding sEH) in colon ($n = 4–6$ per group). The results are expressed as mean \pm SEM. The statistical significance of two groups was determined using Student *t* test or Wilcoxon–Mann test.

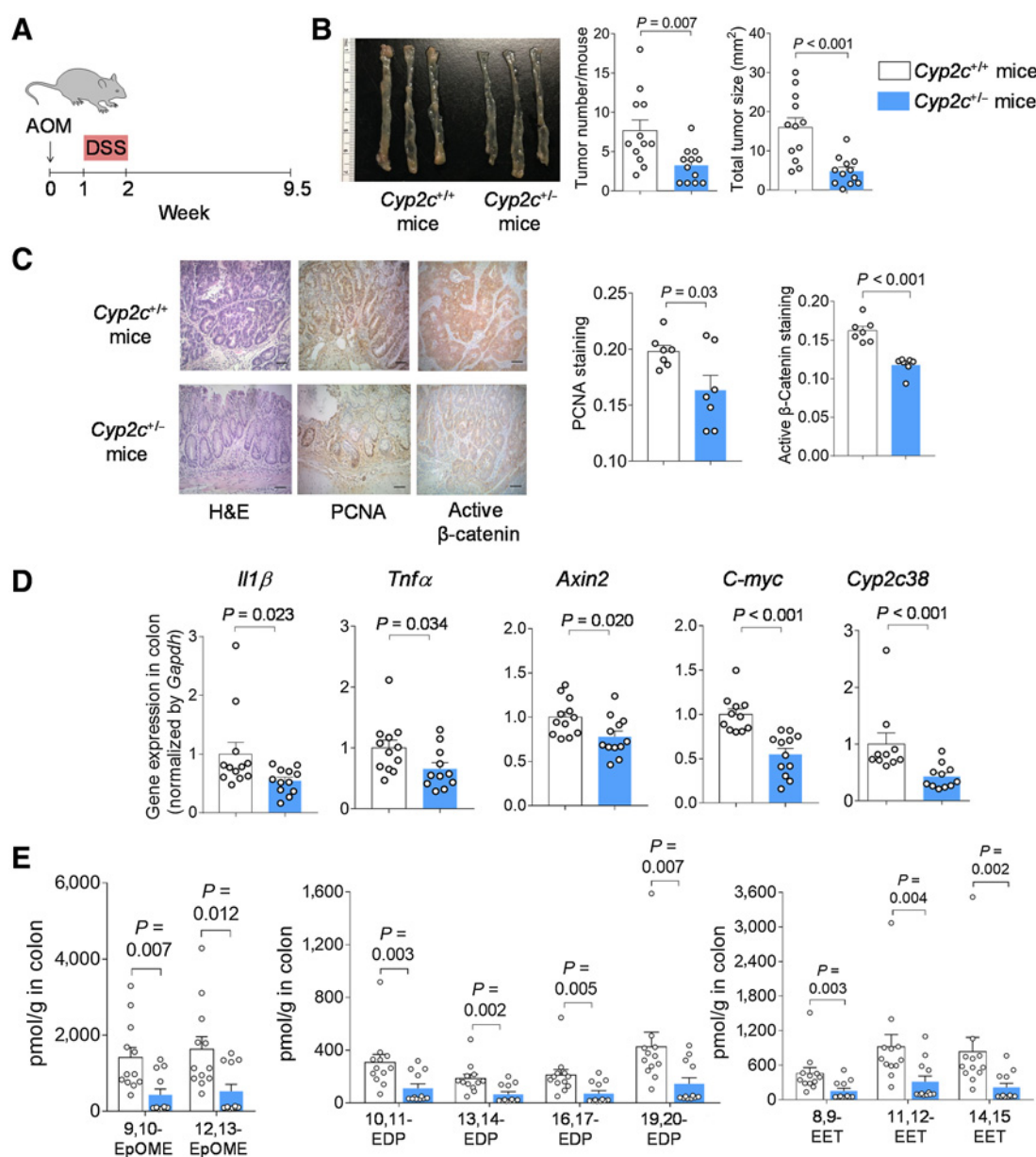
Supplementary Fig. S4), which could be caused by exaggerated inflammatory responses induced by the DSS, making it difficult to use the *Cyp2c^{-/-}* mice to study colon tumorigenesis.

Pharmacologic inhibition of CYP monooxygenases suppresses AOM/DSS-induced colon tumorigenesis

We tested the effects of two different CYP monooxygenase inhibitors, SKF-525A and clotrimazole (13, 14), on AOM/DSS-induced colon tumorigenesis in mice. Because the mutagenic activity of AOM requires metabolic activation by *Cyp2e1* (12),

we initiated treatment of SKF-525A and clotrimazole after the AOM and DSS treatment (see animal experiment scheme in Supplementary Fig. S5A). We found that oral administration of these two inhibitors suppressed AOM/DSS-induced colon tumorigenesis in mice (Supplementary Fig. S5B). Furthermore, treatment with these two inhibitors reduced the expression of PCNA (a marker of cell proliferation), increased expression of cleaved caspase-3 (a marker of cell apoptosis), and decreased expression of proinflammatory and protumorigenic genes (*Tnfα*, *Mcp-1*, *Il6*, *Il1β*, *Ifnγ*, *Axin2*, and *Cox-2*), in colon tumors

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**Figure 2.**

Compared with *Cyp2c^{+/+}* mice, the AOM/DSS-induced colon tumorigenesis is reduced in *Cyp2c^{-/-}* mice. **A**, Scheme of animal experiment. **B**, Quantification of colon tumorigenesis (*n* = 12 per group). **C**, Hematoxylin and eosin (H&E) histology and IHC staining of PCNA and β-catenin in colon (*n* = 7 per group; scale bar, 50 μm). **D**, Expression of proinflammatory and protumorigenic genes, and *Cyp2c38* (using *Cyp2c38* as a marker) in colon (*n* = 10–12 per group). **E**, Concentrations of CYP monooxygenase-produced metabolites in colon (*n* = 12 per group). The results are expressed as mean ± SEM. The statistical significance of two groups was determined using Student *t* test or Wilcoxon–Mann test.

(Supplementary Fig. S5C and S5D). These results support that pharmacologic inhibition of CYP monooxygenases suppressed AOM/DSS-induced colon tumorigenesis.

Treatment with EpOME, but not other CYP monooxygenase metabolites, increases inflammation and JNK phosphorylation in macrophage cells and colon cancer cells

To determine the specific metabolites involved in the colon cancer-enhancing effects of CYP monooxygenases, we studied

the biological actions of CYP monooxygenase metabolites. The ω-6-series CYP metabolites, including EpOMEs produced from LA and EETs produced from ARA, are the most abundant EpFAs in the plasma and tissues (Fig. 1); therefore, we focused on these metabolites. Treatment with 9,10- and/or 12,13-EpOME (concentration = 100 nmol/L) increased the gene expression of proinflammatory cytokines in mouse macrophage RAW 264.7 cells and human colon cancer HCT-116 cells (Fig. 3A–C). In contrast, other types of CYP metabolites, including the

downstream metabolites of EpOMEs termed 9,10- and 12,13-dihydroxyoctadecenoic acid (DiHOME) or CYP metabolites derived from other fatty acids, such as 11,12- and 14,15-EET, had no such effects (Fig. 3A–C).

Because 12,13-EpOME showed the most potent effect to induce inflammation *in vitro*, we further studied this metabolite. Treatment with 12,13-EpOME (concentration = 1–100 nmol/L) increased the gene expression of *Il6* and *Mcp-1* in a dose-dependent manner in RAW 264.7 cells (Fig. 3D). ELISA analysis further validated that 12,13-EpOME increased protein levels of IL6 and MCP-1 in RAW 264.7 cells (Fig. 3E). Consistent with its enhancing effect on inflammation, 12,13-EpOME induced rapid phosphorylation of JNK (Fig. 3F). Similar results were also observed in colon cancer HCT-116 cells (Fig. 3G and H). Together, these results demonstrate that 12,13-EpOME had proinflammatory effects *in vitro*.

Treatment with EpOME exaggerates AOM/DSS-induced colon tumorigenesis *in vivo*

We determined the actions of 12,13-EpOME on colon tumorigenesis *in vivo*. To this end, we stimulated mice with AOM/DSS to induce colon tumors, then treated the mice with 12,13-EpOME or vehicle via Alzet osmotic mini-pumps (Fig. 4A). Compared with vehicle-treated AOM/DSS mice, the 12,13-EpOME-treated AOM/DSS mice had increased tumor number, tumor size, and total tumor burden, illustrating exacerbated colon tumorigenesis (Fig. 4B). Consistent with the increased colon tumorigenesis, treatment with 12,13-EpOME enhanced the infiltration of CD45⁺ and CD45⁺ F4/80⁺ immune cells, increased the expression of proinflammatory and protumorigenic genes (*Tnfa*, *Il1b*, and *Axin2*), and upregulated the expression of tumorigenic markers (PCNA and active β -catenin) in the colon tumor (Fig. 4C–E). We further evaluated the effect of 12,13-EpOME on colon tumorigenesis in a second colon cancer model, the MC38 xenograft model in C57BL/6 mice. We found that 12,13-EpOME had little effect on MC38 primary tumor growth (Supplementary Fig. S6A–S6C), although it increased the expressions of proinflammatory cytokines (*Mcp-1* and *Il6*) in the tumors (Supplementary Fig. S6D).

Discussion

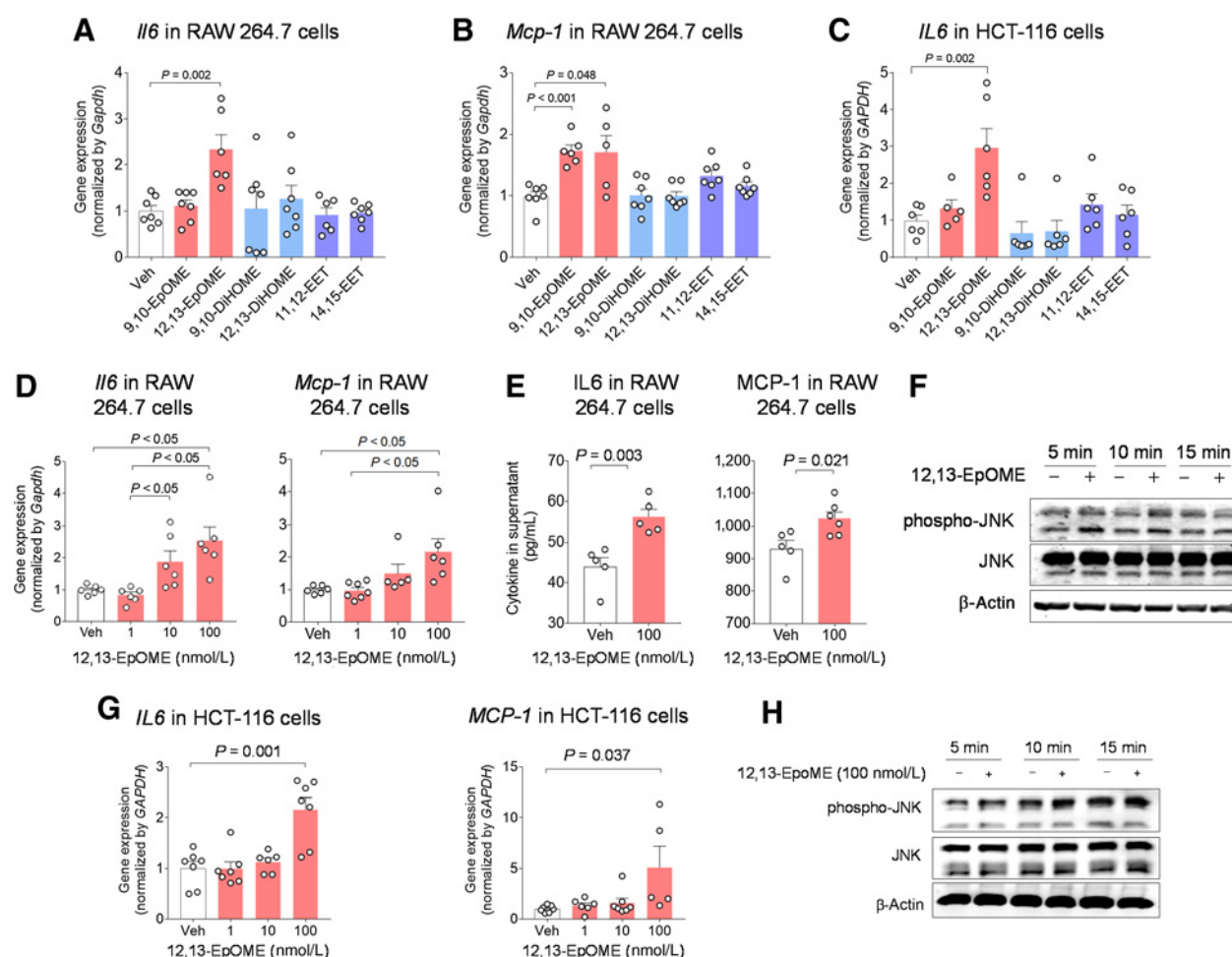
Colon cancer is the third most common cancer and the second leading cause of cancer-related death in United States (1), emphasizing the need for discovery of novel cellular targets, which are crucial in the pathogenesis of colon cancer. Using a LC/MS-MS-based targeted metabolomics, the central finding of our research is that EpFAs, which are eicosanoid metabolites produced by CYP monooxygenases, are significantly elevated in both the circulation and colon tissues of the AOM/DSS-induced colon cancer mice. On the basis of this finding, we further demonstrate that CYP monooxygenases are overexpressed in colon cancer and play critical roles in colon tumorigenesis. Together, our findings demonstrate that the previously unappreciated CYP/EpFA axis is upregulated in colon cancer, contributes to its pathogenesis, and could be therapeutically explored for preventing or treating colon cancer.

Here, we show that CYP monooxygenase-produced EpFAs are increased in both plasma and colon of the AOM/DSS-induced colon cancer mice. A previous study showed that in DSS-induced colitis models, the circulating concentrations of EpFAs were not

changed (15). Together, these results suggest that colon tumor, but not colonic inflammation, induces the CYP monooxygenase pathway. There could be many mechanisms by which the CYP monooxygenases are overexpressed in colon tumors. The expression of CYP monooxygenases has been shown to be elevated by hypoxia (5), which is a common feature of tumor tissues (16). Therefore, the hypoxic tumor microenvironment could contribute to the increased expression of CYP monooxygenases in tumor tissues. To date, the expression patterns of CYP monooxygenases in human colon tumor tissues are not well understood. We analyzed the gene expression of CYP monooxygenases (*CYP2C8*, *CYP2C9*, *CYP2C19*, and *CYP2J2*) in TCGA database, and found that their expressions were not increased in colorectal adenocarcinoma (Supplementary Fig. S7). However, we have to point out that the functions of CYP enzymes are regulated by multiple mechanisms, including transcription, translation, and posttranslational modification (17). Previous research support that the protein expressions of CYP monooxygenases are upregulated in human colon cancer. Enayetallah and colleagues showed that CYP2C9 is detected in 13 of 17 human colon tumor samples, although it is not detected in matched benign samples (18). In addition, recent studies have shown that CYP2W1 is highly expressed in embryonic colon and malignant colon tumors (19, 20), and CYP2J2 is overexpressed in human colon tumors (21). More studies are needed to better understand the changes of CYP monooxygenase pathway in colon tumorigenesis.

Our results support that EpOMEs are critical regulators of colon tumorigenesis. We show that 9,10- and 12,13-EpOME are elevated in the circulation of mice with colon cancer; in addition, 12,13-EpOME has direct and potent effects to induce inflammation *in vitro*, and exacerbate colitis-associated colon tumorigenesis *in vivo*. A better understanding of the roles of EpOMEs in human colon cancer could help to develop EpOMEs as potential biomarkers of colon cancer, which could have important clinical implications. Treatment with 12,13-EpOME increased AOM/DSS-induced colon tumorigenesis, but had little effect on MC38 xenograft tumor growth. There could be multiple mechanisms for the observed results. Transplantation of cancer cells (such as MC38 cells) into immunocompetent hosts induce severe inflammation within the first few days (22), which could obscure the actions of 12,13-EpOME. In addition, the xenograft MC38 model is highly aggressive, which could also contribute to the lack of effects of 12,13-EpOME in this model. Previous studies, performed in other disease models, have shown that EpOMEs have an array of detrimental effects on human health. EpOMEs are elevated in the circulation of patients with severe burns, and are associated with multiple organ failure and adult respiratory distress syndrome in these patients (23–26). In animal studies, treatment with high-dose EpOMEs induced pulmonary edema, lung injury, and cardio-depression (27–29). Together, these results support that EpOMEs could contribute to the pathogenesis of colon cancer, as well as other human diseases.

The tissue concentrations of EpOMEs are, in part, mediated by the levels of LA in membrane phospholipids (5). The consumption of LA, which is highly abundant in vegetable oil products (such as corn, soybean, and canola oils, as well as fried food, salad dressing, and mayonnaise), is very high in western countries (30). Substantial animal experiments showed that a high dietary intake of LA is associated with increased AOM-induced colon tumorigenesis (31–35), but the underlying mechanisms are not well understood. Here, our study showed that EpOMEs have potent

**Figure 3.**

EpOME increases inflammation *in vitro*. **A** and **B**, Effect of EpOMEs, DiHOMEs, and EETs (concentration = 100 nmol/L) on gene expression of *Il6* and *Mcp-1* in mouse macrophage RAW 264.7 cells ($n = 5-7$ per group). **C**, Effect of EpOMEs, DiHOMEs, and EETs (concentration = 100 nmol/L) on gene expression of *IL6* in human colon cancer HCT-116 cells ($n = 5-6$ per group). **D**, Dose-response effect of 12,13-EpOME on gene expression of *Il6* and *Mcp-1* in RAW 264.7 cells ($n = 6-7$ per group). **E**, Effect of 12,13-EpOME on medium concentrations of IL6 and MCP-1 in RAW 264.7 cells ($n = 5-6$ per group). **F**, Effect of 12,13-EpOME on phosphorylation of JNK in RAW 264.7 cells. **G**, Dose-response effect of 12,13-EpOME on gene expression of *IL6* and *MCP-1* in HCT116 cells ($n = 5-7$ per group). **H**, Effect of 12,13-EpOME on phosphorylation of JNK in HCT116 cells. The results are expressed as mean \pm SEM. The statistical significance of two groups was determined using Student *t* test or Wilcoxon-Mann test, and comparison of three groups was determined using one-way ANOVA.

effects to induce inflammation and colon tumorigenesis *in vitro* and *in vivo*, suggesting that EpOMEs could serve as a potential mechanistic linkage between overconsumption of LA and elevated risks of colon cancer. Validation of the roles of EpOMEs involved could help to design human studies to clarify the impact of LA consumption on colon tumorigenesis, which could lead to significant impact for public health.

Using both pharmacologic and genetic approaches, our results support that CYP monooxygenases could be a potential therapeutic target of colon cancer. We showed that pharmacologic inhibition or genetic ablation of CYP monooxygenases suppresses AOM/DSS-induced colon tumorigenesis in mice. The mutagenic activity of AOM requires metabolic activation by Cyp2e1 (12). In our experiments, we used two strategies to minimize the potential impact of inhibition or deletion of CYP monooxygenases on AOM activation: (i) in the pharmacologic inhibition experiment, we initiated the inhibitor treatment (SKF-525A and clotrimazole)

2 weeks post the AOM injection, and (ii) in the genetically engineered mouse experiment, we checked the gene expression of *Cyp2e1* and found that its expression was not altered in *Cyp2c^{+/-}* or *Cyp2c^{-/-}* mice. Together, our results support that inhibition or deletion of CYP monooxygenases suppresses colon tumorigenesis, and this effect is not mediated by impaired AOM activation. This finding is in agreement with our previous report, which showed that compared with WT mice, genetically engineered mice with endothelial overexpression of CYP2C8 monooxygenase (Tie2-CYP2C8 Tr mice) have enhanced xenograft tumor growth of B16F10 melanoma and T241 fibrosarcoma (36). In our experiment, we found that inhibition or deletion of CYP monooxygenases attenuated tumor inflammation, it remains to determine whether the attenuated inflammation is a direct consequence resulted from the inhibition or deletion of CYP monooxygenases, or it is due to reduced colon tumorigenesis. Previous studies

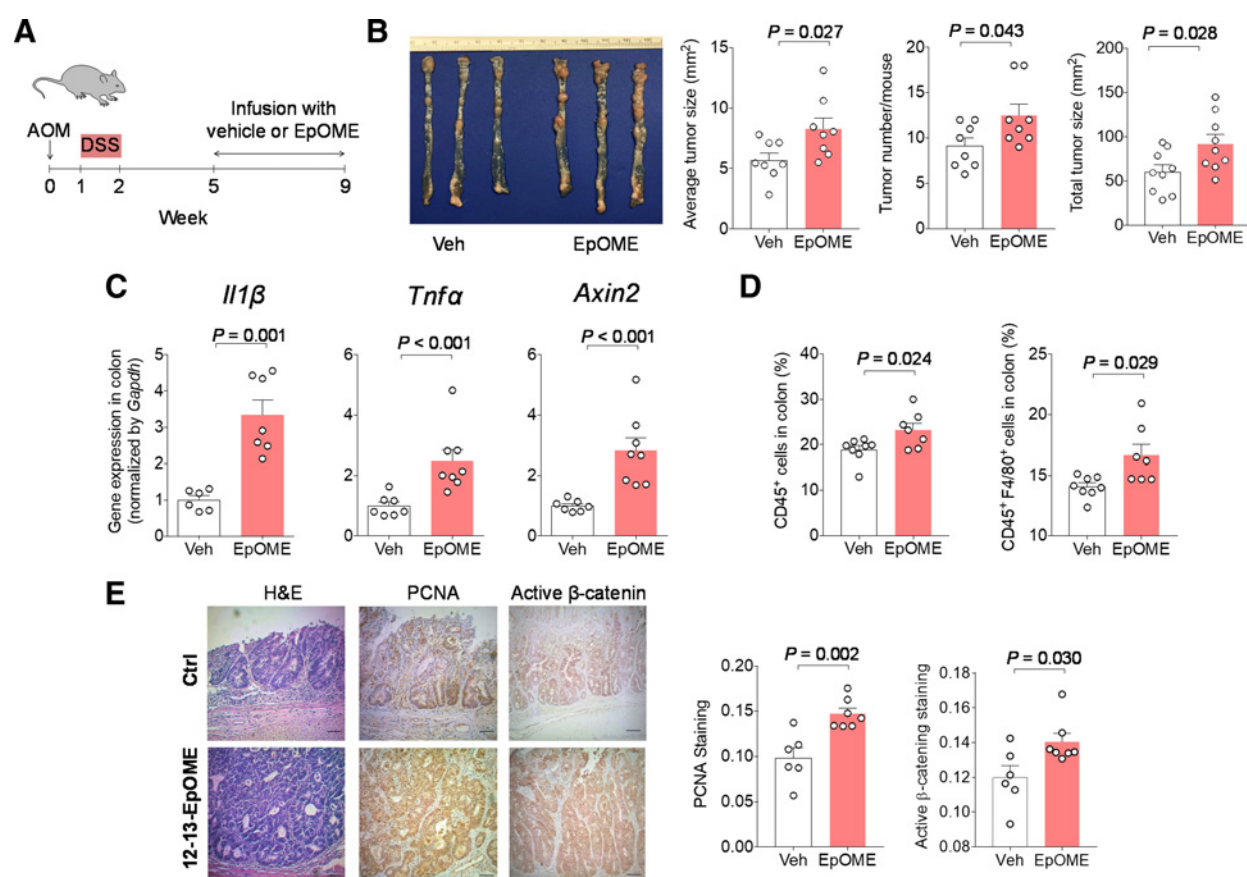


Figure 4.

EpOME exacerbates AOM/DSS-induced colon tumorigenesis *in vivo*. **A**, Scheme of animal experiment to test the effect of 12,13-EpOME (dose, 2 mg/kg/day; administered via mini-pump) on colon tumorigenesis. **B**, Quantification of colon tumorigenesis in mice ($n = 8-9$ per group). **C**, Expression of proinflammatory and protumorigenic genes in colon ($n = 6-8$ per group). **D**, Quantification of CD45⁺ and CD45⁺ F4/80⁺ immune cells in colon ($n = 7-8$ per group). **E**, Hematoxylin and eosin (H&E) histology and IHC staining of PCNA and β-catenin in colon ($n = 6-7$ per group; scale bar, 50 μm). The results are expressed as mean \pm SEM. The statistical significance of two groups was determined using Student *t* test or Wilcoxon-Mann test.

have shown that CYP monooxygenases and their metabolites, EpFAs, have antiinflammatory actions in many disease models (5), whereas the roles of this pathway in tumor inflammation are not well characterized. To better understand the roles of CYP monooxygenases in colon tumorigenesis, it is important to further study the extent to which inhibition or deletion of CYP monooxygenases affects tumorigenesis in other colon cancer models, such as *adenomatous polyposis coli* mutation-induced colon tumorigenesis. Together, these results support that targeting CYP monooxygenases could be a potential strategy to inhibit colon cancer, as well as other types of cancer. Previous studies showed that some FDA-approved drugs are potent inhibitors of CYP monooxygenases (37); these drugs could be repurposed for preventing or treating colon cancer, and novel monooxygenase inhibitors could be developed for human translation. Furthermore, it is important to test how these CYP inhibitors would interact with standard colon cancer chemotherapy drugs to affect colon tumorigenesis.

In conclusion, our study demonstrates that the previously unappreciated CYP monooxygenase pathway is upregulated in colon cancer, contributes to its pathogenesis, and could be

therapeutically explored for preventing or treating colon cancer. A better understanding of its roles in colon tumorigenesis could help to develop novel therapeutic targets or biomarkers of colon cancer, facilitating the development of mechanism-based strategies to reduce the risks of colon cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

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Targeted Metabolomics Identifies the Cytochrome P450 Monooxygenase Eicosanoid Pathway as a Novel Therapeutic Target of Colon Tumorigenesis

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