Published OnlineFirst June 22, 2010; DOI: 10.1158/1940-6207.CAPR-09-0110

Research Article

Cancer Prevention Research

Profiling Lipoxygenase Metabolism in Specific Steps of Colorectal Tumorigenesis

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Abstract

Lipoxygenases (LOX) are key enzymes for the oxidative metabolism of polyunsaturated fatty acids into biologically active products. Clinical data on comparative levels of various LOX products in tumorigenesis are lacking. Therefore, we examined the profiles of several LOX products (5-LOX, 12-LOX, 15-LOX-1, and 15-LOX-2) by liquid chromatography/tandem mass spectrometry in the major steps of colorectal tumorigenesis (normal, polyp, and cancer) in a clinical study of 125 subjects (49 with normal colon, 36 with colorectal polyps, and 40 with colorectal cancer) who underwent prospective colorectal biopsies to control for various potential confounding factors (e.g., diet, medications). Mean 13-hydroxyoctadecadienoic acid (13-HODE) levels were significantly higher in normal colon [mean, 36.11 ng/mg protein; 95% confidence interval (95% CI), 31.56-40.67] than in paired colorectal cancer mucosa (mean, 27.01 ng/mg protein; 95% CI, 22.00-32.02; $P = 0.0002$), and in normal colon (mean, 37.15 ng/mg protein; 95% CI, 31.95-42.34) than in paired colorectal polyp mucosa (mean, 28.07 ng/mg protein; 95% CI, 23.66-32.48; $P < 0.001$). Mean 13-HODE levels, however, were similar between the left (mean, 37.15 ng/mg protein; 95% CI, 31.95-42.35) and the right normal colon (mean, 32.46 ng/mg protein; 95% CI, 27.95-36.98; $P = 0.09$). No significant differences with regard to 12- or 15-hydroxyeicosatetraenoic acid or leukotriene B_4 levels were detected between normal, polyp, and cancer mucosae. 15-LOX-1 inhibited interleukin-1β expression. This study establishes that reduced 13-HODE levels are a specific alteration in the LOX product profile associated with human colorectal tumorigenesis. *Cancer Prev Res; 3(7); 829–38. ©2010 AACR.*

Introduction

Lipoxygenases (LOX) are key enzymes in the oxidative metabolism of polyunsaturated fatty acids, particularly arachidonic and linoleic acids, into products that can influence cell signaling, structure, and metabolism (1). Preclinical and limited clinical data suggest that products of LOXs, especially of 5-LOX, 12-LOX, 15-LOX-1, and 15-LOX-2, have differential roles in relation to human tumorigenesis (2–4). Up to the present, reported comparisons between levels of various LOX products in humans during different stages of multistep tumorigenesis have been limited to single LOX products in retrospectively collected surgical samples primarily from cancer patients (5–7). These studies were also limited by a lack of information on potential factors such as the LOX substrates linoleic and arachidonic acid, nutritional elements that modulate LOX activity (e.g., calcium, which is necessary for 15-LOX-1 activation; ref. 8), and medications [e.g., nonsteroidal anti-inflammatory drugs (NSAID); refs. 7, 9, 10] that could have confounded LOX product measurements. Furthermore, tumorigenesis, especially colorectal tumorigenesis, is a multistep process (11), and no study reported to date has directly compared LOX product levels between the different steps of tumorigenesis.

Mass spectrometry is an emerging technology that allows sensitive, specific, and simultaneous measurements of various LOX products and thus provides a LOX product profile of human tissues (12–14). The current clinical study examined the LOX product profile of each major step of colonic tumorigenesis, from normal colons/rectums to polyps to cancer, in prospectively collected biopsy samples of colonic mucosa, which allowed us to control for potential confounding factors.

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Note: Supplementary data for this article are available at Cancer Prevention Research Online (http://cancerprevres.aacrjournals.org/).

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doi: 10.1158/1940-6207.CAPR-09-0110

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Materials and Methods

Clinical samples

Colonic biopsy specimens were collected during colorectal endoscopic procedures after obtaining written informed consent from participating patients. Study patients were selected from among patients seen at outpatient gastrointestinal clinics at The University of Texas M.D. Anderson Cancer Center and other hospitals within the Texas Medical Center (Gastroenterology Section at Baylor College of Medicine, an outpatient gastrointestinal endoscopy unit affiliated with St. Luke's Hospital, and the Michael E. DeBakey VA Medical Center) for colorectal cancer screening and for the follow-up and management of colorectal cancers. This study was approved by the institutional review board at each participating institution.

Our study involved a total of 125 patients divided into three groups: 49 subjects with normal colon, 36 with colonic polyps, and 40 with colorectal cancer. The colorectal cancer group patients' biopsies were obtained from colorectal cancers and from normal-appearing mucosa at least 10 cm from the cancer. The colorectal polyp group included patients with no history of colorectal cancer. Biopsies were obtained from the colorectal polyps and from normal-appearing mucosa at least 10 cm form the polyp. The normal-colon group included patients with no history of colorectal cancer or polyps and who had a normal colonoscopic examination at the time of biopsy. In this normal-colon group, two sets of biopsies of the colonic mucosa were obtained, one from the left and one from the right colon.

Subjects in all groups were between 45 and 85 years old, had no history of hereditary colon cancer (familial colorectal polyposis syndrome, hereditary nonpolyposis colon cancer syndrome, or family history of one or more first-degree relatives with colon cancer), and were U.S. citizens or permanent residents (to reduce the potential for large variability in risk factors such as dietary habits if international patients were included; refs. 15, 16). Patients were excluded if they had a history of inflammatory bowel disease, had received chemotherapy within 4 weeks before the colonoscopy, had participated in a chemopreventive study during the month before the colonoscopy, had a history of bleeding diathesis, had a history of another active cancer within 5 years before enrollment (except for nonmelanoma skin cancer), were taking warfarin, or were taking anti-inflammatory medications (e.g., nonsteroidal agents, aspirin, sulfasalazine) within 1 week of the colonoscopies. Biopsies were collected between 2001 and 2006. All tissue samples were fresh frozen and stored at −80°C until the time of laboratory analyses.

Liquid chromatography/tandem mass spectrometry measurements of levels of LOX products

Samples were subjected to extraction similar to procedures previously published (13, 17). Briefly, each frozen biopsy tissue sample was cut into approximately 1 × 1- to 2-mm stripes. Samples were transferred to sealed microcentrifuge tubes to which 500 μL of ice-cold tissue homogenization buffer were added (17). The sample was homogenized by an Ultrasonic Processor (Misonix) at 0°C for 3.5 minutes × 2 with 1-minute rest in between and then centrifuged at 10,000 rpm for 5 minutes at 4°C. A 400-μL aliquot of the supernatant was transferred to a glass tube; 600 μL of PBS buffer [containing 1 mmol/L EDTA and 1% butylated hydroxytoluene and 10 μL of deuterated prostaglandin E_2; 5-, 12-, or 15-hydroxyeicosatetraenoic acid (5-, 12-, 15-HETE); leukotriene B_4 (LTB_4); or 13-hydroxyoctadecadienoic acid (13-HODE; 1 μg/mL)] were added and samples were acidified with 0.5 N HCl to pH 3.2 to 3.3. Lipid product was extracted by adding 2 mL of ethyl acetate and vortexing for 30 seconds followed by centrifugation at 2,000 rpm for 5 minutes at 4°C. The upper organic layer was collected, extraction was repeated for two more times, and the organic phases from three extractions were pooled and then evaporated to dryness on ice under a stream of nitrogen. Samples were reconstituted in 100 μL of methanol/ammonium acetate buffer (10 mmol/L at pH 8.5; 70:30, v/v) before liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis. The protein concentration was determined by a Bradford protein assay (Bio-Rad). LC-MS/MS analyses were done using a Quattro Ultima tandem mass spectrometer (Micromass) equipped with an Agilent HP 1100 binary-pump HPLC inlet as described previously (13).

Measuring intakes of nutritional, mineral, and vitamin supplements and medications

Dietary intake and alcohol intake were assessed with a semiquantitative food frequency questionnaire (FFQ). This 137 item self-administered FFQ elicited usual intake over the past 6 months. Martinez et al. have described the details of this FFQ and its measurement characteristics (18). FFQ data were entered into the Food Frequency Data Entry and Analysis Program[10] and analyzed for 49 macronutrients and micronutrients as well as individual fatty acids using nutrient and gram weight information from the Food Intake Analysis System[11] USDA Survey Nutrient Data Base (U.S. Department of Agriculture, Agricultural Research Service. 1997. ON: Nutrient Database for Individual Intake Surveys. 1994-96 Continuing Survey of Food Intakes by Individuals and 1994-96 Diet and Health Knowledge Survey. CD-ROM). Intakes of hormonal replacement therapy, vitamins, and other nutritional supplements were measured by using a medication questionnaire.

RNA extraction and quantitative reverse transcription-PCR analyses

Total RNA was extracted from cells using TRI reagent (Molecular Research Center, Inc.; ref. 19). The integrity of

[10] Food Frequency Data Entry and Analysis Program (FFDEAP) [computer program]. Version 2.0. Houston, TX: The University of Texas Health Science Center at Houston, School of Public Health; 1995.

[11] Food Intake Analysis System (FIAS) [computer program]. Version 3.0. Houston, TX: The University of Texas Health Science Center at Houston, School of Public Health; 1996.

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total RNA was verified on an Agilent 2100 Bioanalyzer using the RNA 6000 Nano LabChip kit (Agilent Technologies). Extracted mRNA samples of adequate RNA quality (RNA integrity number ≥8) from paired tissues were available from 11 patients with colorectal cancer. RNA was reverse transcribed and then measured quantitatively by quantitative reverse transcription-PCR (RT-PCR) using a comparative C_t method, as described previously (19). Primers and probes for human interleukin-1β (IL-1β; assay ID: HS00174097_m1), 15-LOX-1 (HS00609608_m1), and human HPRT1 (4326321E; internal control for cell line expression studies) were purchased from Applied Biosystems. β-Amyloid was the internal control for human colon tissue samples because of its similar expression levels in normal and cancer tissues of the colon (20). β-Amyloid primers (forward primer, 5′-ctcatgccatctttgaccga-3′; reverse primer, 5′-gggcatcaacaggctcaact-3′) were purchased from Sigma, and the β-amyloid 5′-end FAM-labeled probe (5′-gttcagcctggacgatctccagc-3′) was purchased from Integrated DNA Technologies.

LoVo colon cancer cell transfection with adenoviral vectors

LoVo colon cancer cells (directly obtained from the American Type Culture Collection) were cultured and transfected with modified 5/3 adenoviral vectors that express either 15-LOX-1 (Ad-15-LOX-1) or luciferase (Ad-luciferase) at 200 viral particles per cell as described previously (21). Transfected cells were harvested at 24 and 48 hours and processed for 15-LOX-1 and IL-1β mRNA expression measurements by quantitative RT-PCR.

Western blot analyses

As previously described (21), cell lysate proteins were subjected to Western blot analyses using a solution of rabbit polyclonal antibody to human 15-LOX-1 (1:2,000 dilution) and IL-1β (1:200; Abcam).

Statistical methods

Fisher's exact tests were used to determine the association between the disease status and categorical variables. ANOVA was used to compare age, body mass index (BMI), and each of the continuous energy adjusted nutritional variables among three disease statuses. 13-HODE, 12-HETE, and 15-HETE were compared within each group with paired *t* test, whereas LTB_4 levels were compared with a sign test because of non-normal distributions secondary to undetectable levels in large numbers of subjects in all three disease categories. Continuous nutrient variables were energy adjusted using a regression method (22). Multinomial logistic regression analyses were used to determine the association of disease status and each of the medication intake and nutritional variables after adjusting gender effect. We performed a one-way ANOVA for analyses involving single factors and more than two groups.

Results

Clinical characteristics of the study population

The three diseases groups had no significant differences in age or ethnic background (Table 1). Gender distribution was significantly different among the three groups, with the proportion of male to female patients markedly higher in the polyp disease group as compared with the normal or cancer group (Table 1). This difference resulted from the high number of polyp patients recruited through the Michael E. DeBakey VA Medical Center. BMI was significantly higher in the polyp group as compared with the cancer group, a difference possibly explained by secondary weight

Table 1. Demographic characteristics and colon disease status groups

Variable	Colon disease status	*n*	Mean (SD)	Median	Min	Max	*P**
Age	Normal	49	59.35 (6.70)	58.00	50.00	75.00	0.16
	Polyp	36	61.44 (6.56)	60.50	50.00	73.00	
	Cancer	40	62.35 (9.19)	63.00	45.00	80.00	
BMI	Normal	49	28.03 (5.97)	27.12	19.58	47.74	0.008
	Polyp	36	30.42 (5.71)	30.75	19.20	47.55	
	Cancer	40	26.30 (5.27)	25.92	12.40	38.21	
Race		White	African American	Asian	Hispanic		0.08
	Normal	43	5	1	0		
	Polyp	30	2	0	4		
	Cancer	29	4	2	5		
Gender		Male	Female				<0.0001
	Normal	18	31				
	Polyp	31	5				
	Cancer	23	17				

*P values by one-way ANOVA for age and BMI and by Fisher's exact test for race and gender. All statistical tests were two-sided.

Published OnlineFirst June 22, 2010; DOI: 10.1158/1940-6207.CAPR-09-0110

loss because of cancer. Medication intake including NSAIDs was similar among the three groups (Table 2). Nutrient intake with or without energy adjustment was not statistically different between the disease groups (Table 3). In the multinomial logistic regression analyses with gender adjustment, the only significant association found between medication and nutritional intake variables and disease status had a higher odds ratio of developing polyp than cancer for the subject taking NSAIDs (odds ratio, 4.81; $P = 0.014$).

Table 2. Comparison of medication intake among the colon disease status groups

Variables	Disease group			*P**
	Normal	Polyp	Cancer	
Cholesterol medication				0.37
No	40	27	35	
Yes	9	9	5	
Cardiac medication				0.34
No	25	13	20	
Yes	24	23	20	
Other medications				0.48
No	15	14	17	
Yes	34	22	23	
NSAIDs				0.09
No	37	24	35	
Yes	12	12	5	

*P values by Fisher's exact test; all statistical tests were two-sided.

LOX metabolism in normal and cancer mucosae of colorectal cancer patients

13-HODE mean levels were significantly higher in normal [mean, 36.11 ng/mg protein; 95% confidence interval (95% CI), 31.56-40.67 ng/mg protein] than in cancer mucosa (mean, 27.01 ng/mg protein; 95% CI, 22.00-32.02 ng/mg protein; $P = 0.0002$; Fig. 1A). The ratio of normal to cancer mucosa was less than 1 in 31 of 40 (78%) subjects (mean cancer to normal ratio, 0.8; 95% CI, 0.68-0.91). In contrast, 15-HETE levels were similar between normal (mean, 6.21; 95% CI, 4.61-7.82) and cancerous mucosa (mean, 5.93; 95% CI, 4.18-7.68; $P = 0.58$; Fig. 1B). 12-HETE levels were similar between normal (mean, 2.71; 95% CI, 1.91-3.52) and cancerous mucosa (mean, 2.79; 95% CI, 1.97-3.61; $P = 0.82$; Fig. 1C). LTB_4 levels were below detectable levels in normal mucosa in 27 of 39 (69%) and in cancerous mucosa in 24 of 39 (62%) of subjects. The levels in subjects with detectable LTB_4 levels were low and similar between normal (mean, 0.24; 95% CI, 0.06-0.43 ng/mg protein) and cancerous mucosa (mean, 0.4; 95% CI, 0.14-0.65 ng/mg protein; $P = 0.5$; Fig. 1D).

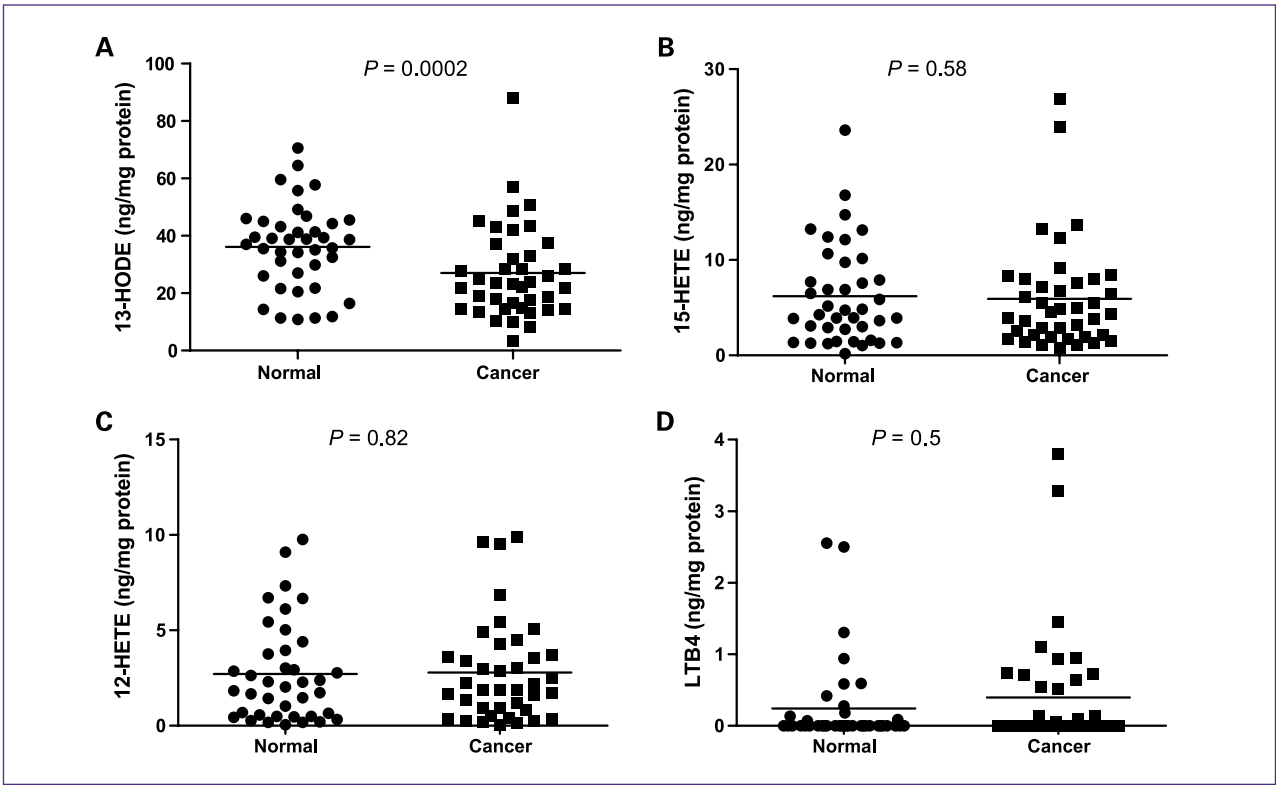

Fig. 1. LOX product levels in subjects with colorectal cancer. Biopsies of paired normal and cancer mucosae were analyzed for 13-HODE (A), 15-HETE (B), 12-HETE (C), and LTB_4 (D) by LC-MS/MS. Values from each subject are depicted in the dot plots. Lines represent the mean values for groups.

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Table 3. Energy-adjusted nutrient intake by disease group

Covariable	Disease group	*n*	Mean (SD)	Median	Min	Max	*P**
Adj_Saturated_Fat (g)	Normal	49	25.30 (6.13)	25.52	9.66	53.92	0.34
	Polyp	36	26.10 (5.53)	26.20	15.59	41.71	
	Cancer	40	24.17 (5.42)	24.71	12.20	40.26	
Adj_Polyunsat_Fat (g)	Normal	49	16.39 (5.04)	16.91	−6.90	28.13	0.59
	Polyp	36	17.34 (5.14)	16.35	0.05	29.78	
	Cancer	40	16.40 (3.84)	16.80	3.45	24.14	
Adj_Monounsat_Fat (g)	Normal	49	28.69 (5.81)	28.81	12.15	46.25	0.42
	Polyp	36	29.87 (6.29)	29.29	12.97	42.72	
	Cancer	40	28.17 (5.12)	29.30	15.83	39.44	
Adj_Dietary_Fiber (g)	Normal	49	19.96 (6.29)	19.39	4.17	37.50	0.98
	Polyp	36	20.16 (5.68)	18.70	10.79	35.60	
	Cancer	40	19.86 (7.00)	18.57	3.28	38.04	
Adj_Alcohol (g)	Normal	49	6.34 (11.99)	2.11	−3.93	66.50	0.22
	Polyp	36	6.56 (15.92)	1.89	−16.13	77.27	
	Cancer	40	12.95 (27.85)	2.78	−4.83	153.45	
Adj_Folate (μg)	Normal	49	469.83 (158.23)	482.19	−133.07	829.41	0.57
	Polyp	36	506.39 (163.85)	488.07	151.22	971.62	
	Cancer	40	483.58 (149.11)	497.22	41.88	861.31	
Adj_Calcium (mg)	Normal	49	974.83 (461.02)	868.60	515.94	3153.33	0.37
	Polyp	36	852.13 (312.79)	834.35	301.46	2104.49	
	Cancer	40	903.12 (390.26)	853.44	10.49	2118.69	
Adj_Linoleic (g)	Normal	49	14.49 (4.56)	14.97	−7.07	24.66	0.61
	Polyp	36	15.36 (4.68)	14.52	−0.88	26.35	
	Cancer	40	14.55 (3.43)	14.89	2.93	21.15	
Adj_Arachidonic (g)	Normal	49	0.14 (0.09)	0.15	−0.11	0.32	0.43
	Polyp	36	0.16 (0.10)	0.15	−0.09	0.48	
	Cancer	40	0.14 (0.07)	0.16	−0.04	0.28	
Adj_Linolenic (g)	Normal	49	1.47 (0.40)	1.46	0.17	2.74	0.64
	Polyp	36	1.54 (0.43)	1.46	0.83	2.77	
	Cancer	40	1.46 (0.38)	1.48	0.20	2.54	
Adj_EPA (g)	Normal	49	0.03 (0.03)	0.03	−0.02	0.16	0.99
	Polyp	36	0.03 (0.03)	0.03	−0.01	0.12	
	Cancer	40	0.03 (0.02)	0.03	−0.02	0.08	
Adj_DHA (g)	Normal	49	0.09 (0.07)	0.10	−0.07	0.29	0.97
	Polyp	36	0.09 (0.07)	0.08	−0.06	0.27	
	Cancer	40	0.09 (0.05)	0.09	−0.05	0.20	

*P values by one-way ANOVA; all statistical tests were two-sided.

LOX metabolism in normal and polyp mucosae of colorectal polyp patients

13-HODE mean levels were significantly higher in normal (mean, 37.15; 95% CI, 31.95-42.34 ng/mg protein) than in polyp mucosa (mean, 28.07; 95% CI, 23.66-32.48; $P < 0.001$; Fig. 2A). The ratio of normal to polyp mucosa was less than 1 in 28 of 36 (78%) subjects (mean polyp to normal ratio, 0.79; 95% CI, 0.69-0.90). In contrast, 15-HETE levels were similar between normal (mean, 6.75; 95% CI, 4.98-8.53) and polyp mucosa (mean, 6.00; 95% CI, 4.72-7.29; $P = 0.35$; Fig. 2B). 12-HETE levels were similar between normal (mean, 2.44; 95% CI, 1.8-3.09) and polyp mucosa (mean, 2.58; 95% CI, 1.86-3.29; $P = 0.73$; Fig. 2C). LTB_4 levels were below detectable levels in normal mucosa in 25 of 37 (68%) and in polyp mucosa in 24 of 37 (65%) of subjects. The levels in subjects with detectable LTB_4 levels were low and similar between normal (mean, 0.34; 95% CI, 0.14-0.55 ng/mg protein) and polyp mucosa (mean, 0.26; 95% CI, 0.11-0.41 ng/mg protein; $P = 0.3$; Fig. 2D).

LOX metabolism in the colonic mucosae of subjects with normal colons

13-HODE mean levels were not significantly different between the left (mean, 37.15; 95% CI, 31.95-42.35) and the right normal colonic mucosa (mean, 32.46; 95%

Published OnlineFirst June 22, 2010; DOI: 10.1158/1940-6207.CAPR-09-0110

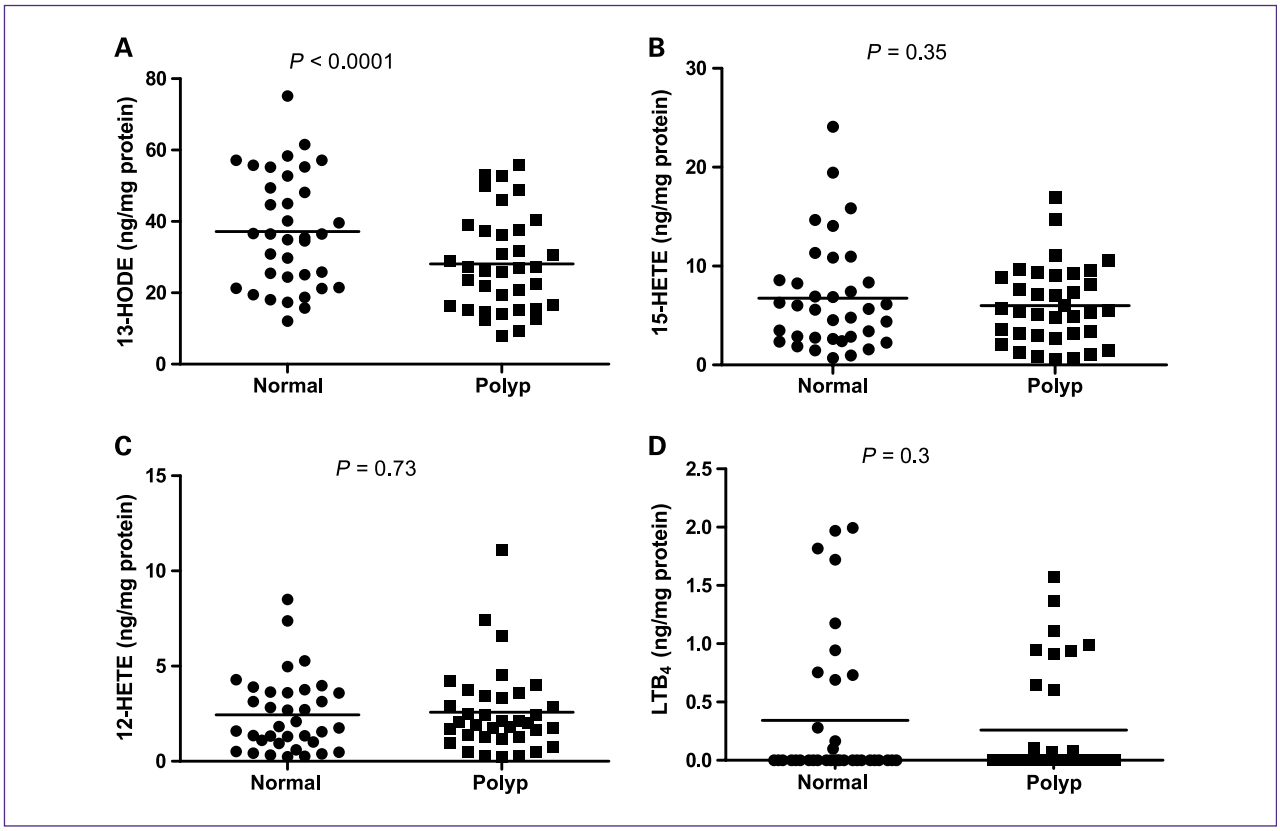

Fig. 2. LOX product levels in subjects with colorectal polyps. Biopsies of paired normal and polyp mucosae were analyzed for 13-HODE (A), 15-HETE (B), 12-HETE (C), and LTB_4 (D) by LC-MS/MS. Values from each subject are depicted in the dot plots. Lines represent the mean values for groups.

CI, 27.95-36.98; $P = 0.09$; Fig. 3A). 15-HETE levels were also similar between the left (mean, 8.23; 95% CI, 6.85-9.61) and the right normal colonic mucosa (mean, 7.87; 95% CI, 6.6-9.15; $P = 0.6053$; Fig. 3B). 12-HETE levels were similar between the left (mean, 3.37; 95% CI, 2.32-4.43) and the right normal colonic mucosa (mean, 3.06; 95% CI, 2.23-3.9; $P = 0.6154$; Fig. 3C). LTB_4 levels were below detectable levels in left normal colonic mucosa in 29 of 49 (59%) and in right normal colonic mucosa in 30 of 49 (61%) of subjects. The levels in subjects with detectable LTB_4 levels were low and similar between the left (mean, 0.53; 95% CI, 0.29-0.77 ng/mg protein) and cancerous mucosa (mean, 0.64; 95% CI, 0.07-0.1.21 ng/mg protein; $P = 0.44$; Fig. 3D).

IL-1β and 15-LOX-1 in human colonic tumorigenesis

Among the 11 patients with evaluable mRNA, 15-LOX-1 relative mRNA expression levels were lower in cancer than in paired normal mucosa in 10 patients and were equal in 1 patient (mean cancer-to-normal ratio, 0.18; 95% CI, 0.09-0.39; Fig. 4A; $P = 0.0014$). In contrast, IL-1β relative mRNA levels were higher in 9 of these 11 patients (mean cancer-to-normal ratio, 4.34; 95% CI, 1.98-9.48; Fig. 4B; $P = 0.0043$); the ratios were 0.94 and 0.85 in the other two cases. In LoVo colon cancer cells, Ad-15-LOX-1 viral vector induced 15-LOX-1 expression ($P < 0.0001$; Fig. 4C), which reduced IL-1β mRNA expression by >50% [versus control (Ad-luciferase); $P = 0.014$; Fig. 4D] and IL-1β protein expression (Fig. 4E).

Discussion

This study indicates (*a*) that a reduced level of 13-HODE is a specific alteration in the LOX product profiles of human colorectal polyps and cancer (versus normal colorectal mucosa) and (*b*) that 13-HODE is the predominant component of the LOX product profiles of colorectal normal, polyp, and cancer mucosae.

The reduced 13-HODE level in polyps or cancer was associated with levels of 12-HETE, 15-HETE, or LTB_4, which did not differ significantly between polyps or cancer and normal mucosa. 13-HODE was the predominant LOX product in colonic mucosa, with levels that were several-fold higher in normal, polyp, and cancer patients than the levels of the other LOX products (15-HETE, 12-HETE, and LTB_4). We selected 13-HODE, 12-HETE, 15-HETE, and LTB_4 for measurements in the current study because prior reports support their key roles in tumorigenesis (2). This reduction in 13-HODE establishes the clinical relevance of prior preclinical data showing that the expression of 15-LOX-1, the key enzyme for 13-HODE production, is lost in colorectal cancer cells (6, 23, 24), and 15-LOX-1

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reexpression by antitumorigenic agents such as NSAIDs and histone deacetylase inhibitors, or by adenoviral delivery vectors, inhibits tumorigenesis (9, 10, 12, 21, 25–29). The findings of reduced 13-HODE in colorectal polyps and cancer are consistent with prior reports of 15-LOX-1 downregulation in clinical samples of surgically resected colorectal cancer (6, 7, 30, 31), colorectal polyps (7, 31), and adenomas from a small cohort of familial adenomatous polyposis (FAP) patients ($n = 5$; ref. 12). More importantly, however, the current study is the first to comprehensively examine the full profile of LOX pathways (5-LOX, 12-LOX, 15-LOX-1, and 15-LOX-2) in sporadic colorectal polyps and cancer and to show the relative significance of 15-LOX-1 downregulation to colonic tumorigenesis compared with other LOX pathways. Our very sensitive and specific mass spectrometry methods (12, 13) allowed these simultaneous LOX product measurements and, thus, direct comparisons between them. Prior studies were mostly limited to measuring individual LOX pathways (2, 5–7), and it is difficult to compare various product levels between different studies because of important interstudy differences. The one exception to these individual LOX studies was a very small retrospective study of various LOX profiles in five FAP patients (12). Although the present results agree with those of the prior FAP study findings (12), they provide indispensable confirmation from a much larger study in patients with far more common sporadic colorectal polyps and cancer. Furthermore, the LOX product profiles, including 13-HODE levels, of normal mucosa in subjects without colonic polyps or cancer were unknown before the current study.

The current clinical study is the first prospective analysis of colonic biopsy samples of normal, polyp, or colorectal cancer mucosae collected via the same methods, and the first analysis to adjust for the effects of factors including dietary intake (e.g., of linoleic and arachidonic acid and calcium) and other factors (e.g., NSAID use) known to be potential confounders of measurements of the LOX product profile. In contrast, prior studies were limited by a lack of information about important confounding factors that can influence 13-HODE levels (e.g., NSAIDs and intake of linoleic acid) and questions about the generalizability of data from FAP and the generally advanced stage of the surgically resected sporadic polyps (average diameter of 3 cm; ref. 7). After we adjusted for these potential confounding factors, the 13-HODE level was still lower in polyps and cancer than in paired normal mucosa and colorectal mucosa from subjects with normal colons (Supplementary Fig. S1). Therefore, the reduction in 13-HODE levels was unlikely secondary to lower availability of substrate levels

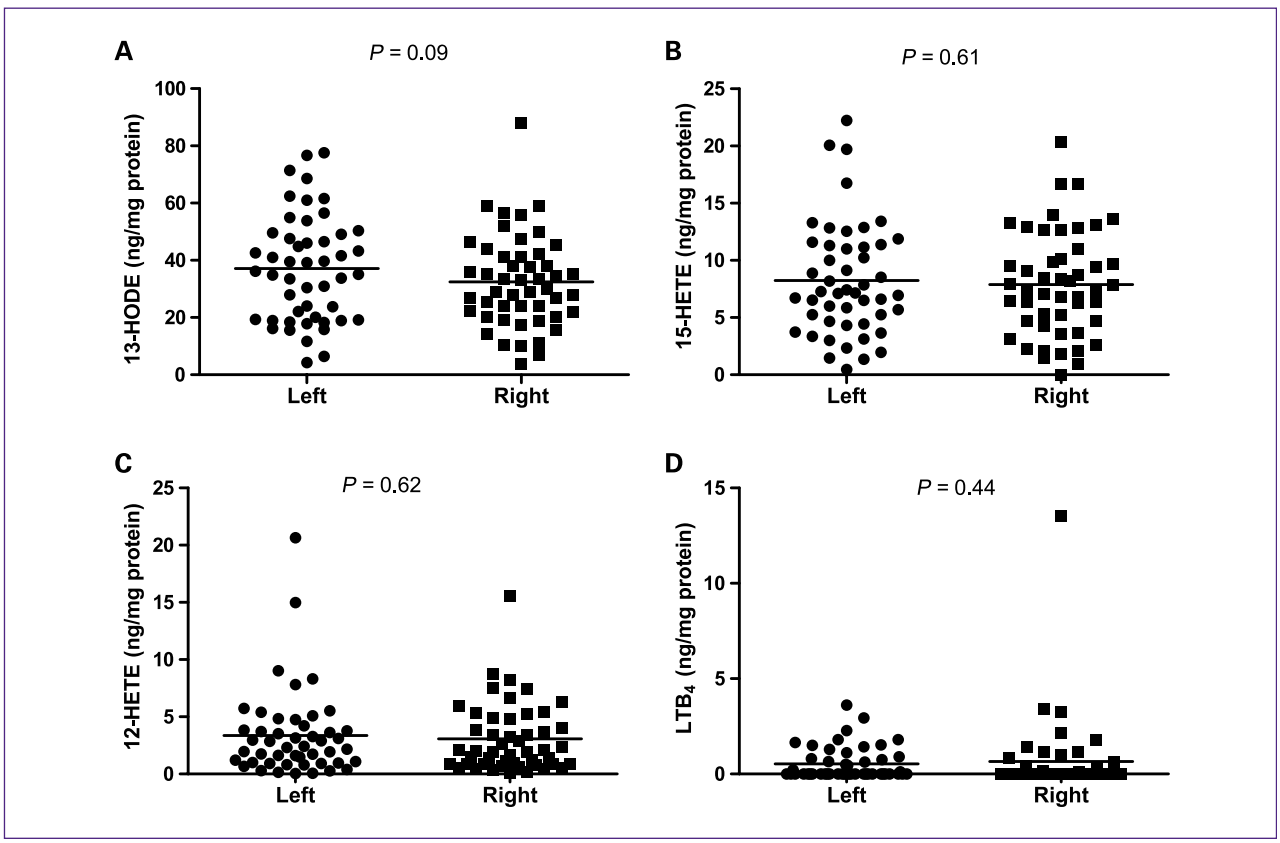

Fig. 3. LOX product levels in subjects with normal colons. Paired left and right colonic mucosal biopsies were analyzed for 13-HODE (A), 15-HETE (B), 12-HETE (C), and LTB_4 (D) by LC-MS/MS. Values from each subject are depicted in the dot plots. Lines represent the mean values for groups.

Published OnlineFirst June 22, 2010; DOI: 10.1158/1940-6207.CAPR-09-0110

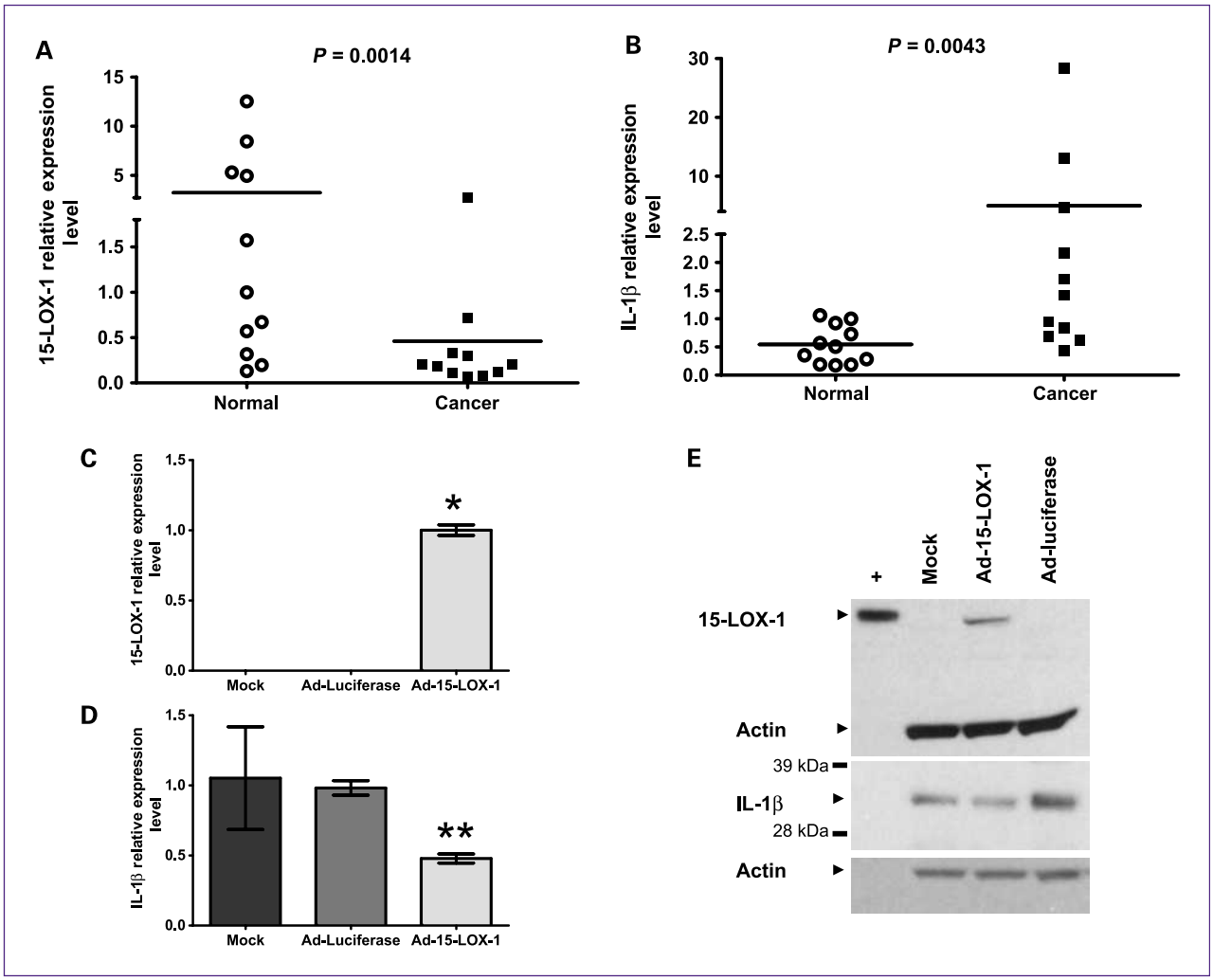

Fig. 4. Effect of 15-LOX-1 on IL-1β expression in colorectal cancer. A and B, expression of 15-LOX-1 and IL-1β in human colonic tumor and normal mucosa. 15-LOX-1 (A) and IL-1β (B) mRNA expression levels were measured by quantitative RT-PCR in paired colonic normal and tumor mucosa samples from colorectal cancer patients. Each value depicts the mean of triplicate measurements from each subject. Lines represent group mean values. C and D, effects of 15-LOX-1 expression on IL-1β expression in colon cancer cells. LoVo colon cancer cells were transfected with either a 15-LOX-1 adenoviral vector that expresses 15-LOX-1 (Ad-15-LOX-1) or with the same vector but replacing 15-LOX-1 cDNA with luciferase cDNA (Ad-luciferase; control vector). C, cells were harvested 24 h after transfection and 15-LOX-1 was measured by quantitative RT-PCR. D, cells were harvested 48 h after transfection, and IL-1β mRNA was measured by quantitative real-time PCR. Values are mean ± SD of triplicate experiments (*, $P < 0.0001$; **, $P = 0.014$, ANOVA). E, cell lysates were collected 48 h after transfection and were processed for 15-LOX-1 and IL-1β protein expression. +, a positive control for 15-LOX-1.

of linoleic acid and more likely was secondary to down-regulated 15-LOX-1 expression (refs. 6, 7, 24, 30, 31 and current report; Fig. 4A). Reduced 13-HODE in the present study also was unrelated to a familial or hereditary syndrome because such patients were excluded. Furthermore, the reduced 13-HODE level was unrelated to variability in colonic biopsy sites because 13-HODE levels were similar in the right and left sides of normal colons in this study.

Linoleic acid is thought to be the most abundant polyunsaturated fatty acid in western diets (32). The current study confirmed this notion in finding that the dietary intake of linoleic acid was higher than that of arachidonic and other polyunsaturated fatty acids. Therefore, the predominance of 13-HODE, which is the main oxidative product of linoleic acid, over 12-HETE and 15-HETE and LTB_4, which are products of arachidonic acid, is possibly related to substrate availability. Further studies in other human organs will help to determine whether the profile of LOX products is organ specific. The current study used undissected colorectal biopsies, which likely contained variable proportions of epithelial and subepithelial tissues. Although variable proportions of epithelial and subepithelial tissues may have influenced LOX product measurements, both subepithelial and epithelial tissues are thought to contribute to polyunsaturated fatty acid oxidative metabolism (33). Future studies potentially should examine the contribution of each compartment to LOX product levels.

Published OnlineFirst June 22, 2010; DOI: 10.1158/1940-6207.CAPR-09-0110

Emerging data increasingly support a mechanistic link between inflammation and cancer (34), especially in the case of colonic tumorigenesis (35). IL-1β is a major proinflammatory cytokine that contributes to the pathogenesis of human colitis (36) and tumorigenesis (37–40). Based on prior reports suggesting anti-inflammatory effects for 15-LOX-1 (41, 42), we examined the relationship between 15-LOX-1 and IL-1β. Our results showed for the first time the relationship between 15-LOX-1 downregulation and IL-1β upregulation in human colon cancer. Our *in vitro* studies of 15-LOX-1 expression in human LoVo colon cancer cells show the mechanistic significance of this association, as 15-LOX-1 expression downregulated IL-1β expression in human colon cancer cells *in vitro*. These findings further support the proposed anti-inflammatory and antitumorigenic roles of 15-LOX-1 in colorectal carcinogenesis.

The current study shows that the LOX product profile can be detected in biopsies of colonic mucosa and that a reduced 13-HODE level is a specific alteration in the LOX product profiles of colorectal polyp and cancer mucosae. We believe that these results support future study of the utility of altered 13-HODE as a biomarker of colorectal tumorigenesis and of the effects of molecular-targeted approaches to preserve 15-LOX-1 expression for colorectal cancer chemoprevention.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank Drs. Madhukar Kaw, Harish Gagneja, Sandeep Lahoti, Patrick Lynch, Norio Fukami, Robert S. Bresalier, Gulchin Ergun, and David Graham for assistance with clinical sample accrual. We also thank Drs. Yuaqing Wu and Xu L. Yang for their technical assistance with the *in vitro* studies.

Grant Support

I. Shureiqi was supported by the K07 grant CA86970 and R01 grants CA106577 and CA137213 from the National Cancer Institute, NIH, Department of Health and Human Services, a Caroline Wiess Law Endowment for Cancer Prevention grant, and a National Colorectal Cancer Research Alliance grant.

Received 06/04/2009; revised 01/22/2010; accepted 02/15/2010; published OnlineFirst 06/22/2010.

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Published OnlineFirst June 22, 2010; DOI: 10.1158/1940-6207.CAPR-09-0110

Cancer Prevention Research

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Cancer Prev Res 2010;3:829-838. Published OnlineFirst June 22, 2010.