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ORIGINAL ARTICLE

Retrospective Study

Expression profile of polyunsaturated fatty acids in colorectal cancer

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Abstract

AIM: To investigate the relationship between the metabolism of polyunsaturated fatty acids (PUFAs) and tumor-associated factors for predicting the outcome of colorectal carcinoma (CRC) in Chinese patients.

METHODS: Fresh-frozen malignant and normal tissues from 82 Chinese patients with CRC were analyzed for PUFA composition using gas-liquid chromatography. The levels of vascular endothelial growth factor (VEGF), cyclooxygenase-2 (COX-2), prostaglandin E2 and platelet-derived growth factor (PDGF) were measured by enzyme-linked immunosorbent assay, and the levels of VEGF, p53 and Ki-67 were measured by immunohistochemistry.

RESULTS: In malignant tissue, compared with normal tissue, the levels of total ω-6 PUFAs (24.64% ± 3.41% *vs* 26.77% ± 3.37%, $P = 0.00$) and linoleic acid (LA) (15.46% ± 3.51% *vs* 18.30% ± 2.83%, $P < 0.01$) were lower, whereas the levels of total ω-3 PUFAs (1.58% ± 0.74% *vs* 1.35% ± 0.60%, $P < 0.01$) and dihomo-gamma-linolenic acid (DGLA) (1.32% ± 0.69% *vs* 0.85% ± 0.29%, $P < 0.01$) were significantly higher. The ratios of arachidonic acid (AA)/LA (0.53 ± 0.22 *vs* 0.42 ± 0.19, $P < 0.01$) and AA/total ω-6 PUFAs (0.31 ± 0.09 *vs* 0.27 ± 0.10, $P < 0.01$) were also significantly higher in malignant tissue. The levels of PDGF (353.10 ± 148.85 pg/mL *vs* 286.09 ± 104.91 pg/mL, $P < 0.01$), COX-2 (125.21 ± 70.29 ng/mL *vs* 67.06 ± 42.22 ng/mL, $P < 0.01$) and VEGF (357.11 ± 128.76 pg/mL *vs* 211.38 ± 99.47 pg/mL, $P < 0.01$) were also higher in malignant tissue compared to normal tissue. COX-2 was inversely correlated with LA ($R = -0.3244$, $P < 0.05$) and positively correlated with AA/total ω-6 PUFAs ($R = 0.3083$, $P < 0.05$) and AA/LA ($R = 0.3001$, $P < 0.05$). The tissue level of LA was highest in poorly differentiated tumors (19.9% ± 6.3%, $P < 0.05$), while the ratio of AA/ω-3 PUFAs was lowest in these tumors (10.8 ± 2.6, $P < 0.05$). In VEGF-positive tumors, the level of LA was higher (16.2% ± 3.7% *vs* 13.9% ± 2.7%, $P < 0.01$), while the AA/ω-3PUFA, AA/ω-6 PUFA, and AA/LA ratios were lower than in VEGF-negative

tumors (5.0 ± 1.8 *vs* 6.7 ± 3.3, 0.30 ± 0.09 *vs* 0.34 ± 0.09, 0.50 ± 0.21 *vs* 0.61 ± 0.21, $P < 0.01$).

CONCLUSION: The metabolism of PUFAs may play an important role in the evolution of inflammation-driven tumorigenesis in CRC and may be considered a potential marker for prognosis.

Key words: Fatty acids; Unsaturated; Clinicopathologic; Colorectal neoplasms; Carcinogenesis

Core tip: Colorectal cancer (CRC) is the third most lethal malignancy worldwide. Both ω-3 and ω-6 polyunsaturated fatty acids (PUFAs) are important constituents of cell membranes. In this study, we investigated the relationship between the metabolism of PUFAs and tumor-associated factors for predicting the outcome of CRC in Chinese patients. The identification of modifiable risk factors for CRC is needed to develop interventions and to expand our understanding of this disease. This research shows that the metabolism of PUFAs may play an important role in the evolution of inflammation-driven tumorigenesis in CRC.

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INTRODUCTION

Colorectal cancer (CRC), one of the most common causes of cancer-related deaths in industrialized countries, is highly correlated with a Western-style diet characterized by a lower intake of vitamins and fiber and a higher intake of meats, fats, and ω-6 polyunsaturated fatty acids (PUFAs) relative to ω-3 PUFAs[1-3]. PUFAs are of particular interest due to their potential roles in inflammation-driven colorectal carcinogenesis[4].

Mammals are unable to synthesize ω-3 and ω-6 PUFAs, thus they must be obtained through the diet. These PUFAs are vital constituents of cell membranes. The cell membrane fatty acid compositions of both normal and neoplastic tissues are affected by the fatty acid content of the diet[5], which may affect membrane properties, such as permeability or lipid packing, gene expression, transcription factor activity, signal transduction, and the activity of specific proteins, such as protein kinase C and ornithine decarboxylase[6-8]. ω-3 PUFAs [eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and α-linolenic acid (ALA)], which are found in fish and seed oils, have anti-inflammatory and anticarcinogenic effects on the colon, whereas ω-6 PUFAs [linoleic acid (LA) and arachidonic acid (AA)], which are found in commercially popular oils and animal products, have adverse effects[7,9,10].

We investigated the metabolism of PUFAs and the correlation with tumor-associated factors in colorectal tissue and normal tissue obtained from the same CRC patient. In particular, we investigated the relationship between PUFA tissue levels and clinicopathologic parameters of CRC and evaluated their significance in predicting the outcome of CRC. The identification of modifiable risk factors for CRC is needed to develop effective interventions and to expand our understanding of the disease.

MATERIALS AND METHODS

Subjects

Between September 2010 and September 2011, radical CRC specimens were obtained from 82 patients. The study protocol was approved by the Medical Ethical Committee of Chinese PLA General Hospital. Written informed consent was obtained from each subject before inclusion in this study. Patients were eligible for the study if they had CRC that was histologically proven, newly diagnosed, and untreated. Exclusion criteria included a previous history of malignant disease, previous anti-cancer treatment, and the presence of diabetes, which was defined as treatment with insulin, oral antidiabetics, or a special diet. At the time of the study, patients were hospitalized for medical tests or to receive anti-cancer treatment such as surgery, chemotherapy or radiation. Mean alcohol consumption and smoking behavior over the previous 6 mo were recorded using a questionnaire. Overall, 82 patients were diagnosed with CRC. Of these patients, 31 were female and 51 were male (average age, 60 years; range, 29 to 83 years). This research was approved and supported by the Chinese PLA General Hospital and presented no ethical conflicts.

Sample collection

All CRC specimens were macroscopically assessed from suspected benign and malignant areas. A scraped cytology specimen was taken for confirmation of either benign or malignant histology in each specimen. Colorectal tissue, which otherwise would have been discarded, was obtained after surgical resection of the colorectum. Cancerous and corresponding adjacent normal tissues (at least 5 cm away from the malignant site) were dissected from full-thickness colorectal samples weighing 2.0-3.0 g. Each sample was divided into three parts. One part was subjected to routine histopathological evaluation, and the others were immediately frozen in liquid nitrogen after surgical resection and maintained at -70 ℃ until final analysis.

Table 1 Antibodies, working dilutions and sources

Antibody	Dilution	Source
VEGF	1:40	Santa Cruz Biotechnology, Inc.
Ki-67	1:100	Dako North America. Inc.
p53	1:160	Santa Cruz Biotechnology, Inc.

VEGF: Vascular endothelial growth factor.

Gas-liquid chromatography

Fatty acid methyl esters were prepared according to a previously described method[11]. An aliquot of tissue homogenate (< 50 μL) in a glass methylation tube was mixed with 1 mL of hexane and 1 mL of 14% BF_3/MeOH reagent. After the sample was blanketed with nitrogen, the mixture was heated at 100 ℃ for 1 h, cooled to room temperature and methyl esters extracted in the hexane phase following the addition of 1 mL of H_2O. The samples were centrifuged for 1 min, and the upper hexane layer was removed and concentrated under nitrogen. Fatty acid methyl esters were analyzed by gas chromatography using a fully automated GC-2010 Plus Gas Chromatography analyzer (SHIMADZU Corporation, Kyoto, Japan). The chromatography utilized an Omegawax 250 capillary column (30 m × 0.25 mm I.D.). Peaks were identified by comparison with fatty acid standards (Nu-chek-Prep, Elysian, MN, United States), and the area and percentage for each resolved peak were analyzed using a Perkin-Elmer M1 integrator.

The PUFA composition in these tissues was determined by gas-liquid chromatography on a capillary column. The relative amount of each PUFA (% of total fatty acids) was quantified by integrating the area under the peak and dividing the result by the total area of all fatty acids.

Enzyme-linked immunosorbent assay

Tissue samples were analyzed for vascular endothelial growth factor (VEGF), cyclooxygenase-2 (COX-2), prostaglandin E2 (PGE-2) and platelet-derived growth factor (PDGF) expression by enzyme-linked immunosorbent assay (MULTISKAN EX PRIMARY EIA V. 2.3) according to the manufacturer's instructions.

Immunohistochemistry

Immunohistochemistry was performed to study the expression of VEGF, Ki67 (a well-recognized nuclear antigen-specific marker of cellular proliferation which is mainly used to evaluate proliferation activity), and p53. Immunohistochemistry was carried out using a streptavidin-labeled peroxidase (SP) kit in accordance with the manufacturer's instructions. The working dilutions and sources are shown in Table 1. Immunoreactivity was scored based on chromatosis intensity (0 = no pigmentation, 1 = light yellow, 2 = buffy, 3 = brown) and distribution, *i.e.*, the percentage of cell staining (0 = 0%-5%, 1 = 5%-25%, 2 = 2%-50%, 3 = 51%-75%, 4 ⩾ 75%) in high-power fields in series from each slice.

Table 2 Demographic and clinical characteristics of the study subjects

Clinicopathologic parameter	*n* = 82
Sex	
Male	51
Female	31
Age (yr)	
< 60	38
⩾ 60	44
Location	
Colon	44
Rectum	38
Tumor size (cm)	
< 5	42
⩾ 5	40
Differentiation degree	
High	24
Middle	53
Low	5
Clinicopathological stage	
Ⅰ + Ⅱ	35
Ⅲ + Ⅳ	47
Lymph node metastasis	
Negative	40
Positive	42

Statistical analysis

Descriptive statistics are provided in the cross table. Significant differences were calculated using Student's *t*-test and the non-parametric *t*-test. A P value < 0.05 was considered statistically significant. The data were analyzed using the SPSS 17 Software package (SPSS Inc., Chicago, IL, United States).

RESULTS

The demographic and clinical characteristics of the study subjects are presented in Table 2.

Characterization of fatty acid distribution in CRC specimens

The examination of adjacent normal tissue and cancerous tissue from the same subject offers the possibility of investigating fatty acid distribution within the same nutritional status. LA is a type of ω-6 PUFA that can be converted into AA[12]. Our results show that cancerous tissue samples, compared with normal tissue, showed lower levels of total ω-6 PUFAs (24.64% ± 3.41% *vs* 26.77% ± 3.37%, P < 0.01) and LA (15.46% ± 3.51% *vs* 18.30% ± 2.83%, P < 0.01). In contrast, the ratios of AA/LA (0.53 ± 0.22 *vs* 0.42 ± 0.19, P < 0.01) and AA/total ω-6 PUFAs (0.31 ± 0.09 *vs* 0.27 ± 0.10, P < 0.01) were significantly higher in cancerous tissue samples (Table 3), which indicated that a large amount of LA was converted to AA. AA consumption is part of an appropriate inflammatory defense reaction, the goal of which is the restoration

Table 3 Fatty acid composition (by percentage) in colorectal carcinoma cancerous tissue and adjacent normal tissue

Fatty acid	Adjacent normal tissue	Cancerous tissue	*P* value
LA (%)	18.30 ± 2.83	15.46 ± 3.51	0.00
DGLA (%)	0.85 ± 0.29	1.32 ± 0.69	0.00
AA (%)	7.32 ± 2.73	7.62 ± 2.48	0.41
Total ω-6 PUFAs (%)	26.77 ± 3.37	24.64 ± 3.41	0.00
ALA (%)	0.09 ± 0.12	0.10 ± 0.13	0.32
EPA (%)	0.25 ± 0.17	0.24 ± 0.18	0.72
DPA (%)	0.43 ± 0.20	0.49 ± 0.25	0.02
DHA (%)	1.11 ± 0.50	1.33 ± 0.63	0.00
Total ω-3 PUFAs (%)	1.35 ± 0.60	1.58 ± 0.74	0.00
ω-6/ω-3PUFAs	3.88 ± 0.43	4.94 ± 0.55	0.02
AA/LA	0.42 ± 0.19	0.53 ± 0.22	0.00
AA/ω-6 PUFAs	0.27 ± 0.10	0.31 ± 0.09	0.00

LA: Linoleic acid; AA: Arachidonic acid; ALA: α-linolenic acid; EPA: Eicosapentaenoic acid; DHA: Docosahexaenoic acid; PUFAs: Polyunsaturated fatty acids; DGLA: Dihomo-gamma-linolenic acid.

Table 4 Tumor-associated factors in colorectal carcinoma cancerous tissue and adjacent normal tissue

Tumor-associated factors	Adjacent normal tissue	Cancerous tissue	*P* value
PGE2 (pg/mL)	221.40 ± 100.35	265.93 ± 162.22	0.12
PDGF (pg/mL)	286.09 ± 104.91	353.10 ± 148.85	0.01
VEGF (pg/mL)	211.38 ± 99.47	357.11 ± 128.76	0.00
COX-2 (ng/mL)	67.06 ± 42.22	125.21 ± 70.29	0.00

PGE-2: Prostaglandin E2; PDGF: Platelet-derived growth factor; VEGF: Vascular endothelial growth factor; COX-2: Cyclooxygenase-2.

of a perturbed condition. The present study provides valuable clues to potential biochemical target sites of fatty acid distribution in human CRC.

Tumor-associated factors in CRC tissue and relationships with fatty acids

As seen in our results, cancerous tissue samples showed significantly higher levels of PDGF (353.10 ± 148.85 pg/mL *vs* 286.09 ± 104.91 pg/mL, $P < 0.01$), COX-2 (125.21 ± 70.29 ng/mL *vs* 67.06 ± 42.22 ng/mL, $P < 0.01$) and VEGF (357.11 ± 128.76 pg/mL *vs* 211.38 ± 99.47 pg/mL, $P < 0.01$) than normal tissue samples (Table 4). COX-2 was inversely correlated with LA and positively correlated with the ratios of AA/ω-6 PUFA and AA/LA (Table 5).

Relationship between PUFA tissue levels and clinicopathologic parameters in CRC

Tissue levels of AA in patients who were older than 60 years were significantly lower than those in younger patients (0.17% ± 0.09% *vs* 0.12% ± 0.06%, $P = 0.045$). In patients with a tumor size < 5 cm, the tissue level of EPA was significantly higher (0.29% ± 0.13% *vs* 0.20% ± 0.14%, $P = 0.030$), whereas the ω-6/ω-3 PUFA ratio was lower (10.8 ± 2.6 *vs* 13.2 ± 6.4, $P = 0.031$). In addition, the levels of tissue LA and the ratio of AA/ω-3 PUFAs differed with the degree of tumor differentiation ($P = 0.013$, $P = 0.027$, respectively); tissue LA was highest in poorly differentiated tumors (19.9% ± 6.3%, $P < 0.05$), while the AA/ω-3 PUFA ratio was lowest in these tumors (10.8 ± 2.6, $P < 0.05$). Tissue LA levels were also higher in VEGF-positive than VEGF-negative tumors (16.2% ± 3.7% *vs* 13.9% ± 2.7%, $P = 0.009$), while the ratios of AA/ω-3PUFA, AA/ω-6 PUFA, and AA/LA in VEGF-positive tumors were lower than those in VEGF-negative tumors (5.0 ± 1.8 *vs* 6.7 ± 3.3, $P = 0.004$; 0.30 ± 0.09 *vs* 0.34 ± 0.09, $P = 0.038$; and 0.50 ± 0.21 *vs* 0.61 ± 0.21, $P = 0.030$, respectively). In Ki-67-negative tumors, the LA level was highest (22.5% ± 10.1%, $P = 0.048$). There were no significant differences in the levels of PUFAs according to sex, clinicopathologic stage, lymph node metastasis or expression of p53 (Table 6).

Table 5 Relationships between fatty acid composition and tumor-associated factors in colorectal carcinoma tissue

		COX2	PGE2	PDGF	VEGF
LA	*R*	-0.3244	-0.1310	-0.2758	-0.1495
	P value	0.0316	0.3966	0.0700	0.3327
AA	*R*	0.2101	0.2663	0.1299	0.2448
	P value	0.1711	0.0806	0.4009	0.1093
AA/LA	*R*	0.3001	0.1855	0.1900	0.1892
	P value	0.0478	0.2280	0.2167	0.2188
ω-6 PUFAs	*R*	-0.2121	0.1661	-0.2373	0.0394
	P value	0.1670	0.2813	0.1209	0.7995
AA/ω-6	*R*	0.3083	0.2007	0.2251	0.2098
PUFAs	*P* value	0.0418	0.1915	0.1418	0.1717

LA: Linoleic acid; AA: Arachidonic acid; PUFAs: Polyunsaturated fatty acids; PGE-2: Prostaglandin E2; PDGF: Platelet-derived growth factor; VEGF: Vascular endothelial growth factor; COX-2: Cyclooxygenase-2.

DISCUSSION

This study is the first to show that fatty acid profiles differ between benign and cancerous tissues within the same CRC patient. In particular, our data show a relationship between the metabolism of PUFAs and tumor-associated factors in Chinese CRC patients. We observed a higher proportion of ω-6 PUFAs (DGLA and AA), higher levels of PDGF, COX-2 and VEGF, and an increased ω-6/ω-3 fatty acid ratio in cancerous tissues compared to adjacent normal tissues. This report may provide a useful foundation for characterizing PUFA metabolism in cancerous tissue.

Nutrition plays a significant role in carcinogenesis and has been recognized as an important environmental factor. The effects of certain dietary components on cancer led to the term "chemoprevention," introduced by Sporn *et al*[13] in the 1970s to describe the pharmacologic ability of dietary factors to either inhibit or promote the development of cancer.

As seen in our results, an increase in LA content in colorectal tissue was associated with poorly differentiated tumors (19.9% ± 6.3%, $P < 0.05$).

Table 6 Relationships between polyunsaturated fatty acid tissue levels and clinicopathologic parameters in colorectal carcinoma

Clinicopathologic parameter	The number of cases	LA (%)	DGLA (%)	AA (%)	ALA (%)	EPA (%)	DPA (%)	DHA (%)	ω-3PUFA (%)	ω-6/ω-3 PUFA	AA/ω-3 PUFA	ω-6 PUFA (%)	AA/ω-6 PUFA	AA/LA
Sex														
Male	51	15.3 ± 2.8	1.37 ± 0.65	0.15 ± 0.08	0.23 ± 0.13	0.24 ± 0.11	0.51 ± 0.18	1.32 ± 0.56	1.56 ± 0.65	12.2 ± 5.5	5.9 ± 2.7	24.9 ± 3.0	0.32 ± 0.10	0.55 ± 0.21
Female	31	16.1 ± 4.5	1.24 ± 0.77	0.13 ± 0.07	0.35 ± 0.18	0.25 ± 0.14	0.47 ± 0.27	1.35 ± 0.74	1.59 ± 0.48	11.6 ± 3.8	4.9 ± 1.8	24.7 ± 3.8	0.29 ± 0.10	0.49 ± 0.23
Age (yr)														
< 60	38	15.8 ± 3.4	1.38 ± 0.80	0.17 ± 0.09[a]	0.23 ± 0.12	0.23 ± 0.11	0.50 ± 0.19	1.34 ± 0.55	1.57 ± 0.63	12.0 ± 4.0	5.6 ± 2.5	25.3 ± 2.9	0.31 ± 0.08	0.53 ± 0.20
⩾ 60	44	15.4 ± 3.6	1.27 ± 0.59	0.12 ± 0.06[a]	0.32 ± 0.16	0.25 ± 0.16	0.48 ± 0.24	1.33 ± 0.71	1.58 ± 0.83	11.9 ± 5.7	5.5 ± 2.4	24.4 ± 3.5	0.31 ± 0.10	0.52 ± 0.23
Location														
Colon	44	15.3 ± 3.3	1.30 ± 0.57	0.14 ± 0.07	0.26 ± 0.15	0.23 ± 0.14	0.50 ± 0.29	1.37 ± 0.72	1.61 ± 0.82	12.3 ± 5.9	5.6 ± 2.5	24.6 ± 3.4	0.32 ± 0.09	0.54 ± 0.22
Rectum	38	15.9 ± 3.8	1.34 ± 0.82	0.15 ± 0.06	0.30 ± 0.12	0.26 ± 0.15	0.48 ± 0.17	1.28 ± 0.53	1.56 ± 0.65	11.6 ± 3.6	5.4 ± 2.4	25.0 ± 3.1	0.30 ± 0.09	0.51 ± 0.21
Tumor size (cm)														
< 5	42	15.5 ± 4.1	1.37 ± 0.78	0.15 ± 0.07	0.31 ± 0.13	0.29 ± 0.13[a]	0.50 ± 0.21	1.32 ± 0.49	1.61 ± 0.59	10.8 ± 2.6[a]	5.0 ± 2.0	24.9 ± 3.5	0.31 ± 0.10	0.54 ± 0.23
⩾ 5	40	15.6 ± 2.8	1.27 ± 0.60	0.14 ± 0.06	0.24 ± 0.12	0.20 ± 0.14[a]	0.48 ± 0.29	1.34 ± 0.76	1.54 ± 0.88	13.2 ± 6.4[a]	6.0 ± 2.8	24.7 ± 3.0	0.31 ± 0.08	0.51 ± 0.20
Differentiation degree														
High	24	15.6 ± 2.6[a]	1.18 ± 0.43	0.14 ± 0.06	0.26 ± 0.12	0.22 ± 0.12	0.46 ± 0.24	1.28 ± 0.72	1.49 ± 0.80	13.5 ± 4.9	6.6 ± 3.2[a]	25.0 ± 3.0	0.31 ± 0.09	0.53 ± 0.22
Middle	53	15.1 ± 3.3[a]	1.41 ± 0.78	0.15 ± 0.07	0.26 ± 0.14	0.26 ± 0.14	0.51 ± 0.26	1.37 ± 0.61	1.62 ± 0.73	11.4 ± 3.9	5.2 ± 1.9[a]	24.4 ± 3.4	0.31 ± 0.08	0.54 ± 0.20
Low	5	19.9 ± 6.3[a]	1.09 ± 0.60	0.15 ± 0.04	0.58 ± 0.20	0.23 ± 0.12	0.46 ± 0.14	1.24 ± 0.54	1.47 ± 0.67	11.4 ± 2.4	4.1 ± 2.0[a]	27.9 ± 2.4	0.25 ± 0.15	0.41 ± 0.32
Clinicopathologic stage														
Ⅰ + Ⅱ	35	14.9 ± 3.0	1.46 ± 0.85	0.15 ± 0.09	0.21 ± 0.13	0.25 ± 0.13	0.52 ± 0.22	1.42 ± 0.55	1.67 ± 0.64	11.2 ± 3.7	5.1 ± 1.9	24.5 ± 3.1	0.32 ± 0.08	0.56 ± 0.22
Ⅲ + Ⅳ	47	16.1 ± 3.8	1.22 ± 0.53	0.14 ± 0.10	0.33 ± 0.18	0.24 ± 0.14	0.47 ± 0.27	1.27 ± 0.69	1.51 ± 0.81	12.6 ± 5.6	5.8 ± 2.7	25.0 ± 3.5	0.30 ± 0.10	0.50 ± 0.22
Lymph node metastasis														
Negative	40	14.9 ± 3.1	1.37 ± 0.83	0.13 ± 0.10	0.21 ± 0.13	0.24 ± 0.14	0.47 ± 0.25	1.32 ± 0.58	1.55 ± 0.66	12.5 ± 5.0	5.7 ± 2.6	24.3 ± 2.9	0.32 ± 0.08	0.56 ± 0.23
Positive	42	16.2 ± 3.8	1.27 ± 0.54	0.15 ± 0.09	0.34 ± 0.14	0.25 ± 0.13	0.51 ± 0.25	1.35 ± 0.69	1.60 ± 0.82	11.5 ± 3.6	5.4 ± 2.2	25.3 ± 3.6	0.30 ± 0.09	0.50 ± 0.21
VEGF														
Positive	57	16.2 ± 3.7[a,b]	1.32 ± 0.67	0.15 ± 0.07	0.30 ± 0.14	0.26 ± 0.13	0.48 ± 0.23	1.34 ± 0.53	1.60 ± 0.64	11.6 ± 3.7	5.0 ± 1.8[b]	25.2 ± 3.0	0.30 ± 0.09[a]	0.50 ± 0.21[a]
Negative	23	13.9 ± 2.7[a,b]	1.33 ± 0.79	0.11 ± 0.05	0.23 ± 0.12	0.22 ± 0.11	0.52 ± 0.30	1.33 ± 0.65	1.54 ± 0.98	12.6 ± 7.1	6.7 ± 3.3[b]	23.7 ± 3.9	0.34 ± 0.09[a]	0.61 ± 0.21[a]
Ki-67														
-	2	22.5 ± 10.1[a]	0.92 ± 0.82	0.04 ± 0.02	1.02 ± 0.55	0.25 ± 0.11[a,b]	0.31 ± 0.02	1.25 ± 0.85	1.49 ± 1.41	10.6 ± 1.3	3.1 ± 2.5	29.9 ± 1.2	0.22 ± 0.28	0.41 ± 0.25
+	4	15.8 ± 2.7[a]	1.50 ± 0.31	0.19 ± 0.07	0.17 ± 0.07	0.26 ± 0.11[a,b]	0.55 ± 0.16	1.79 ± 0.51	2.04 ± 0.63	9.9 ± 2.6	4.5 ± 1.1	26.2 ± 2.3	0.33 ± 0.04	0.56 ± 0.12
++	6	16.3 ± 4.5[a]	1.93 ± 1.51	0.20 ± 0.11	0.30 ± 0.07	0.36 ± 0.17[a,b]	0.55 ± 0.23	1.26 ± 0.89	1.62 ± 0.97	12.4 ± 6.2	5.3 ± 2.3	25.5 ± 2.2	0.28 ± 0.08	0.49 ± 0.28
+++	23	15.9 ± 3.1[a]	1.34 ± 0.56	0.12 ± 0.06	0.28 ± 0.08	0.29 ± 0.13[a,b]	0.50 ± 0.31	1.33 ± 0.68	1.62 ± 0.78	11.7 ± 3.4	5.5 ± 2.5	25.3 ± 2.7	0.31 ± 0.09	0.54 ± 0.23
++++	46	15.0 ± 3.1[a]	1.23 ± 0.60	0.15 ± 0.07	0.25 ± 0.16	0.21 ± 0.12[a,b]	0.48 ± 0.23	1.32 ± 0.58	1.53 ± 0.70	12.1 ± 5.6	5.7 ± 2.5	24.1 ± 3.6	0.31 ± 0.08	0.53 ± 0.20
p53														
+	32	15.6 ± 3.7	1.40 ± 0.84	0.15 ± 0.09	0.25 ± 0.13	0.22 ± 0.13	0.55 ± 0.22	1.46 ± 0.68	1.68 ± 0.79	11.9 ± 6.5	5.5 ± 2.7	25.2 ± 2.8	0.32 ± 0.09	0.55 ± 0.22
++	7	15.2 ± 4.2	1.70 ± 0.86	0.18 ± 0.11	0.23 ± 0.12	0.24 ± 0.09	0.58 ± 0.27	1.32 ± 0.32	1.56 ± 0.41	10.8 ± 2.4	4.9 ± 1.3	24.6 ± 3.0	0.31 ± 0.09	0.55 ± 0.25
+++	28	15.2 ± 3.3	1.27 ± 0.55	0.17 ± 0.11	0.28 ± 0.14	0.26 ± 0.11	0.50 ± 0.24	1.32 ± 0.67	1.58 ± 0.82	11.6 ± 3.9	5.4 ± 2.3	24.2 ± 4.3	0.30 ± 0.07	0.50 ± 0.19

[a]$P < 0.05$, [b]$P < 0.01$ between groups. LA: Linoleic acid; AA: Arachidonic acid; ALA: α-linolenic acid; EPA: Eicosapentaenoic acid; DHA: Docosahexaenoic acid; PUFAs: Polyunsaturated fatty acids; VEGF: Vascular endothelial

One explanation for this result is that LA influences cell cycle progression and cancer-related gene expression[14,15]. Indeed, there are several potential mechanisms by which fatty acid metabolism may affect CRC risk.

Three fatty acids comprise the ω-3 PUFAs: ALA, which is found in vegetables, and EPA and DHA, which are found mainly in fish oils. The ability of humans to convert dietary ALA to EPA and DHA is limited and is also influenced by the dietary ω-6/ω-3 PUFA ratio[16]. Lowering the ω-6/ω-3 PUFA ratio is hypothesized to reduce the risk of CRC, as ω-3 fatty acids inhibit the production of proinflammatory, ω-6-derived eicosanoids *via* the cyclooxygenase-2 enzyme[7,17-19]. Some short-term clinical trials and prospective studies have shown that ω-3 fatty acids, particularly DHA and EPA, decrease the levels of both inflammation[20-22] and rectal cell proliferation biomarkers[9,10,23]. ω-3 PUFAs reduce the risk of colorectal neoplasia through the modulation of similar mechanisms such as nonsteroidal anti-inflammatory drugs, which are strong cyclooxygenase inhibitors[24-26]. Our study demonstrated a higher proportion of ω-6 PUFAs (DGLA and AA), along with an increased ω-6/ω-3 fatty acid ratio, in cancerous tissues compared to normal tissues. According to these results and previous results, the ratio of ω-6/ω-3 PUFAs may be useful for the characterization of PUFA metabolism in cancerous tissue.

Numerous mechanisms have been proposed for the role of fatty acids in carcinogenesis and include modulation of inflammation and cell signaling[7,19]. Fatty acid synthase (FASN), a key enzyme in the saturated fatty acid synthesis pathway, plays an important role in cancer growth and survival as a metabolic oncogene, and its overexpression is common in many cancers[27-29]. In terms of fatty acids in tissues, PUFA compositions are important determinants of the biophysical properties of cell membranes. In addition, a large prospective study reported that PUFAs inhibit some steps in colorectal carcinogenesis[30-32].

AA is the substrate for prostaglandin production mediated by COX activity. Prostaglandins and COX-2 may facilitate colon cancer progression by stimulating cell proliferation and survival, tumor cell invasiveness, and the production of angiogenic agents[33,34]. In colorectal tissue, overexpression of COX-2 is associated with both the invasiveness and the proliferation of malignant cells[35]. VEGF is an essential regulator of normal and abnormal blood vessel growth; this factor regulates both vascular proliferation and permeability and functions as an antiapoptotic factor for newly formed blood vessels, and its expression is associated with poor prognosis in several types of cancer. The function of VEGF in vessel formation is complemented by PDGF, which also indirectly regulates angiogenesis[36]. Clinical and pharmacologic studies have highlighted the importance of eicosanoids and their roles in the occurrence of many cancers[37], and the biosynthesis of eicosanoids depends on the availability of free AA.

There are some limitations in the current study. The primary limitation of this study was that the number of subjects was relatively small. Thus, care must be exercised in the extrapolation of our findings to larger populations of CRC patients. Therefore, further studies with larger samples are needed on this subject.

Our work revealed fatty acid profile differences between cancerous tissues and adjacent normal tissues, which suggests that the fatty acid profile of colorectal tissue is linked to the development of inflammation-driven tumorigenesis and the clinicopathologic parameters of CRC. Therefore, this study may serve as a reference to evaluate the prognosis of CRC through the measurement of PUFAs. These findings provide additional evidence that dietary fat is associated with colorectal tumor carcinogenesis.

COMMENTS

Background

Colorectal cancer (CRC), one of the most common causes of cancer-related deaths in industrialized countries, is highly correlated with a Western-style diet. Nutrition plays a significant role in carcinogenesis and has been recognized as an important environmental factor. Among the studies on environmental factors, recent reports have focused on dietary factors, such as fatty acid levels, and their role in CRC. This study stimulates increased interest in the diet as a modifiable risk factor for cancer. Polyunsaturated fatty acids (PUFAs) are of particular interest due to their potential role in inflammation-driven colorectal carcinogenesis.

Research frontiers

Mammals are unable to synthesize ω-3 and ω-6 PUFAs; thus, these PUFAs must be obtained from the diet. Both ω-3 and ω-6 PUFAs are important constituents of cell membranes, and the cell membrane fatty acid compositions of both normal and neoplastic tissues are affected by the fatty acid content of the diet.

Innovations and breakthroughs

We investigated the metabolism of PUFAs and its correlation with tumor-associated factors in colorectal tissues compared to normal tissues from the same CRC specimens. In particular, we assessed the relationship between PUFA tissue levels and clinicopathologic parameters of CRC and evaluated their significance in terms of patient prognosis. The identification of modifiable risk factors for CRC is needed to develop effective interventions and to expand our understanding of the disease.

Applications

These study results suggest that PUFA levels differ between malignant and benign portions of human radical CRC samples, thus supporting the assumption that PUFAs are involved in colorectal carcinogenesis.

Terminology

PUFAs: Fatty acids are carbon chains with a methyl group at one end and a carboxyl group at the other. Saturated fatty acids contain only carbon-carbon single bonds, whereas unsaturated fatty acids contain one (monounsaturated) or more (polyunsaturated) carbon-carbon double bonds. The dietary PUFAs of interest are the ω-3 and ω-6 PUFAs, so named by the position of the first double bond at the third and sixth carbon from the methyl (u) end, respectively. PUFAs are biologically important, with roles in phospholipid membrane structure and function, as well as cellular signaling and lipid metabolism.

Peer-review

This study investigates the concentration of PUFA in colorectal cancer specimens. The findings are potentially interesting, but the authors should significantly improve the writing.

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