Omega-3 Fatty Acid Is a Potential Preventive Agent for Recurrent Colon Cancer

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Abstract

Increasing evidence supports the contention that many malignancies, including sporadic colorectal cancer, are driven by the self-renewing, chemotherapy-resistant cancer stem/stem-like cells (CSC/CSLC), underscoring the need for improved preventive and therapeutic strategies targeting CSCs/CSLCs. Omega-3 polyunsaturated fatty acids (ω-3 PUFA), have been reported to inhibit the growth of primary tumors, but their potential as a preventive agent for recurring cancers is unexplored. The primary objectives of this investigation are (i) to examine whether eicosapentaenoic acid (EPA; one of the ω-3 PUFA) synergizes with FuOx (5-FU+Oxaliplatin), the backbone of colon cancer chemotherapy, and (ii) whether EPA by itself or in combination with conventional chemotherapy prevents the recurrence of colon cancer via eliminating/suppressing CSCs/CSLCs. FuOx-resistant (chemoresistant; CR) colon cancer cells, highly enriched in CSCs, were used for this study. Although EPA alone was effective, combination of EPA and FuOx was more potent in (i) inhibiting cell growth, colonosphere formation, and sphere-forming frequency, (ii) increasing sphere disintegration, (iii) suppressing the growth of SCID mice xenografts of CR colon cancer cells, and (iv) decreasing proinflammatory metabolites in mice. In addition, EPA + FuOx caused a reduction in CSC/CSLC population. The growth reduction by this regimen is the result of increased apoptosis as evidenced by PARP cleavage. Furthermore, increased pPTEN, decreased pAkt, normalization of β-catenin expression, localization, and transcriptional activity by EPA suggests a role for the PTEN–Akt axis and Wnt signaling in regulating this process. Our data suggest that EPA by itself or in combination with FuOx could be an effective preventive strategy for recurring colorectal cancer. Cancer Prev Res; 7(11); 1138–48. ©2014 AACR.

Introduction

Cancer stem/stem-like cells (CSC/CSLC), that are self-renewing undifferentiated cells, are thought to be one of the leading causes of cancer recurrence. In the colon, they are identified by specific surface epitopes such as CD44, CD166, CD133, and ESA (epithelial-specific antigen; refs. 1, 2). Like normal stem cells, CSCs/CSLCs grow slowly and are more likely to survive chemotherapy than other tumor cells (2–5). This is exemplified by the observation that oxaliplatin treatment of colon cancer boosts the abundance of CSCs by more than 10 times (3). We have also reported that although exposure of colon cancer HCT-116 or HT-29 cells to FuOx (5-FU+Oxaliplatin) inhibits their growth, the same treatment leads to enrichment of CSC/CSLC phenotype (4, 5). These chemoresistant cells show an increased colonosphere formation, Wnt–β-catenin signaling, EGFR signaling, increased expression of miR21, and decreased miR145 (6, 7).

Omega 3- and 6-polyunsaturated fatty acids (ω-3 and ω-6 PUFA) are substantial components of the diet, comprising about 7% to 10% of daily energy intake in U.S. adults (reviewed in ref. 8). A meta-analysis by the World Cancer Research Fund and the American Institute for Cancer Research in 2007 reported that although no definitive correlations could be drawn, there was suggestive evidence that dietary fish (main source of ω-3 PUFAs) intake protects against colorectal cancer risk in humans (9). Additional support came from clinical observations (10, 11), suggesting its significance as a chemopreventive agent.

The current investigation examines the potential of ω-3 PUFA as an effective preventive agent for recurrent colon tumors that are reported to be enriched in CSCs/CSLCs. Two main ω-3 PUFAs, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have been isolated from fish...
oil. Recent evidence has demonstrated that EPA and DHA reduce inflammation in humans (12, 13) and may have antineoplastic properties (14–16). Animal studies have revealed that EPA and, to a lesser extent, DHA reduced VEGF expression and microvessel formation (17). Recently, Fan and colleagues (18) demonstrated a stimulatory role of ω-6 PUFA–derived PGE2 on Lgr5+ stem cell population in the colonic crypts. In contrast, ω-3 PUFA derived PGE3 had diminished ability to support stem cell expansion (18). Hawcroft and colleagues recently showed an inhibition of liver metastasis in mice that received dietary EPA (19). However, there are no reports on the antineoplastic activity of this PUFA on recurrent colon cancer. The current investigation was undertaken to examine the preventive and therapeutic potential of EPA alone or when administered together with the conventional chemotherapy on chemotherapy-resistant colon cancer HT-29 and HCT-116 cells. Herein, we report that EPA alone or in combination with FuOx could be effective in prevention of recurrent colon cancer.

**Materials and Methods**

**Cell lines and reagents**

Human colon cancer cells HT-29 and HCT-116 were obtained from the American Type Culture Collection (ATCC). They were expanded and frozen in aliquots. Fresh aliquots were used every 6 to 7 months; therefore, the cell lines were not authenticated again. The cells were maintained in DMEM as reported (5, 20). FuOx-resistant (chemoresistant; CR) cells were generated as described earlier (5, 6, 21) in our laboratory by exposing the cells to 14 consecutive cycles of exposure to increasing concentrations of 5-FU and oxaliplatin. Unless otherwise stated, the CR cells were cultured in medium containing 2× FuOx (50 μmol/L 5-FU and 1.25 μmol/L oxaliplatin).

**Determination of cell growth and interaction between EPA and FuOx**

Cell growth was assessed by mitochondrial-dependent reduction of 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT; Sigma) to formazan as described previously (22). Briefly, the cells (5 × 10³) were seeded in triplicates onto 96-well culture dishes and subsequent cycles of exposure to increasing concentrations of EPA and/or FuOx for 48 hours to determine the synergism between EPA and FuOx.

**Colonosphere formation and disintegration**

Formation of colonospheres and their disintegration in response to EPA and/or FuOx were carried out according to our standard protocols described previously (5, 23). Briefly, cells were pretreated with EPA and/or FuOx for 72 hours, subsequently plated at a concentration of 100, 10, and 1 cell per well, and incubated for 8 days. The frequency of sphere formation was determined using ELDA webtool at http://bioinf.wehi.edu.au/software/elda.

**Flow cytometry**

After 48-hour incubation in the absence (control) or presence of EPA and/or FuOx, CR-HT-29 colon cancer cells were subjected to direct immunofluorescence staining with PE-Cy7- or PerCP-Cy5–conjugated anti-human CD44 and/or CD166 antibody followed by flow cytometric analyses using a FACSDiva (BD Biosciences) at the MICR core of Karmanos Cancer Institute of Wayne State University as described previously (5). The cells stained with IgG2b (isotype-negative control) served as gating control. The proportion of CD44+CD166−/low cells was determined on the basis of fluorescence intensity spectra.

**Indirect immunofluorescence**

The cells were seeded at a density of 25,000 per chamber in an 8-chamber slide. After treatment with EPA + FuOx for 48 hours, the cells were fixed, permeabilized, and processed for indirect immunofluorescence as described (25) using appropriate antibodies. Stained cells were observed under an Olympus 1×71 microscope supporting a Hamamatsu 1394 ORCA-ERA video camera and the images were stored using Slidebook Digital Microscopy Software (Intelligent Imaging Innovations). For controls, the primary antibody was omitted.

**Western blot analysis**

Western blots were performed according to our standard protocol (26). Briefly, cell lysates containing 25 or 50 μg protein were separated by SDS-polyacrylamide gel electrophoresis, transferred onto a polyvinylidene difluoride (PVDF) membrane (Millipore) and subjected to Western blot analysis with the recommended dilution of primary and appropriate secondary antibody conjugated to IR Dye 680 or IR Dye 800 (Molecular Probes). The membranes were scanned by Odyssey Infrared Imaging System (LI-COR Biosciences) to locate the respective bands. β-Actin or GAPDH was used as a loading control.

**Isolation of RNA and quantitative polymerase chain reaction analysis**

Total RNA was extracted from CR cells using the TRIzol reagent (Invitrogen) according to the manufacturer’s instructions. RNA concentration was measured using a NanoDrop 2000C spectrophotometer. Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) was performed using the GeneAmp RNA PCR Kit (Applied Biosystems). Briefly, 1 μg of purified RNA was reverse transcribed (5, 27). For quantitative PCR amplification, 5 μL of 1:10 diluted cDNA was amplified with SYBR Green Quantitative PCR Master Mix (Applied Biosystems) using the following PCR primers: CK20...
forward: 5’-TGAAGAGCTCGAAGTCGA-3’ and reverse: 5’-GAAGCTCGACGCGATGT-3’; β-catenin: forward 5’-ATACCAACCCACTTGGCGAGC-3’; reverse 5’-GGAAGGTC-TCCCTGGGACTC-3’; sequences for stem-cell markers were reported earlier (27). Reactions were carried out in triplicates as described previously (5).

TCF/LEF transcriptional activity

The activation of transcription factor TCF/LEF was evaluated by using the Cignal TCF/LEF Reporter Assay Kit (SA Biosciences) as described (7, 23). The cells were grown to 70% to 80% confluence and cotransfected with TCF/LEF reporter constructs using Lipofectamine 2000 transfection reagent (Invitrogen) according to the manufacturer’s instructions. After 16 to 24 hours, the cells were trypsinized, seeded into 12 wells of a 96-well plate in DMEM containing 10% FBS in the presence of EPA and/or FuOx. After 2 days of incubation, they were collected and analyzed for TCF/LEF activity using a Dual-Luciferase Assay Kit (Promega Biosciences) following the manufacturer’s instructions as described (7, 23). Activity of TCF/LEF was calculated in relation to positive control.

Tumor growth in SCID mice

All animal experiments were performed according to the Wayne State University’s Institutional Animal Care and Use Committee (IACUC) approved protocol #A02-02-13. Animal Welfare Assurance #A3310-01.

Tumors were generated in 4-week-old female SCID mice (Taconic Laboratory) by s.c. injections of 1 × 10^6 CR HCT-116 or CR HT-29 cells suspended in 100 μL Matrigel on either side. To study the chemopreventive efficacy of EPA, animals were given EPA (250 mg/kg in sesame oil) by oral gavage 7 days before inoculation of chemoresistance colon cancer cells. The dose of EPA was selected on the basis of previous studies (17, 28, 29). To study the therapeutic effectiveness, EPA was administered 7 days after inoculation of the cells. EPA treatment was continued for 4 weeks every day for 5 days a week (Monday to Friday). The animals in this group (control and EPA group) were also injected i.p. with a mixture of 25 mg/kg 5-FU and 2 mg/kg oxaliplatin (FuOx) once a week for 3 weeks. Tumor volumes were calculated as described previously (20, 23). Mice were monitored regularly. At the end of treatment period, all animals were sacrificed, blood was collected immediately from the heart in a tube containing 50 μL of 80 mmol/L EDTA, centrifuged, and saved at −70°C. The tumors were harvested and tumor aliquots were frozen for RNA isolation, fixed in 10% buffered formalin or immediately digested with enzymes for single-cell isolation (20, 23).

Eicosanomic analysis

Mass spectrometry–based eicosanomic analysis for eicosanoids derived from both arachidonic acid and EPA was performed on the plasma extracts collected from mice as described earlier (30, 31). Briefly, plasma samples were spiked with a mixture of internal standards (5 ng each of PGE1-d4, LTB4-d4, and 15-HETE-d5), diluted with methanol to 15%, and applied to C18 solid phase extraction cartridges, washed sequentially with 15% methanol in water and hexane, followed by elution of the eicosanoids with methanol containing 1% formic acid. The eluates were evaporated to dryness and reconstituted in HPLC mobile phase for LC/MS analysis.

Eicosanomic analysis was performed by LC/MS using Luna C18 column (3 μm, 2 × 150 mm; Phenomenex) for HPLC resolution of the eicosanoids and detected by QTRAP5500 mass analyzer (ABSCIEX) using optimized conditions for each eicosanoid by Multiple Reaction Monitoring (MRM) method as described before (31). LC/MS chromatograms were analyzed by MultiQuant (ABSCIEX) for quantitation of each eicosanoid and normalized to the internal standard signal. Under the standard conditions of the method, the detection limits for most of the eicosanoids were <2 pg on the column with a signal/ratio of 3.

Statistical analysis

Unless otherwise stated, data were expressed as mean ± SD. Where applicable, the results were compared by using the unpaired, two-tailed Student t test, as implemented by Excel 2007 (Microsoft). P values smaller than 0.05 were considered statistically significant.

Results

EPA synergizes with FuOx

The data obtained from synergy analysis of EPA and/or FuOx-treated CR HT-29 cells revealed that cells treated with the combined dosage are 6.05 times (P = 0.009) more likely to die than those treated with FuOx alone. This was calculated by the difference in intercepts of the groups in a logistic regression model with data from EPA and FuOx each alone, or in combination (Fig. 1A), assuming a combined slope. The data clearly show synergism between the two.

EPA or EPA + FuOx treatment inhibits stem-cell characteristics in CR cells

To analyze whether EPA and/or FuOx would affect the properties of colon CSCs/CSLCs, the ability of CR HT-29 and CR HCT-116 cells to form colonospheres was examined. ELDA performed on CR HT-29 cells demonstrated that the frequency to form colonospheres was 139-fold higher in control group, compared with 20 μmol/L EPA and/or FuOx–treated cells (Fig. 1B).

With respect to formation of colonospheres, EPA alone at 10 and 20 μmol/L caused a significant inhibition in CR HCT-116 cells, which was further exacerbated when combined with FuOx (Fig. 2A and B). Interestingly, CR HT-29 cells appeared to be more resistant to the EPA at 10 μmol/L, but in the presence of combination treatment, a significant inhibition in colonosphere formation was observed. EPA at 20 μmol/L was found to be as effective as the combinatorial treatment.

In addition, the combined treatment of EPA and FuOx also induced disintegration of colonospheres, especially in CR HCT-116 cells. We observed that combined treatments were significantly more effective than FuOx or EPA alone at
untreated controls contained 5.12% CD44 (Fig. 3Aa–Af). While the cells in response to 10 μmol/L EPA and FuOx regulation any more than FuOx alone in CR HT-29 cells (Fig. 2D), strengthening our observation that EPA/FuOx combinatory treatment inhibits the growth of CSCs/CSC-LCs.

Quantitative real-time PCR performed on RNA isolated from tumor as well as from the CR HT-29 cells showed a significant increase in CK20 mRNA levels in EPA + FuOx-treated tumors and cells, indicating an increased number of differentiated cells (Fig. 4E).

The EPA + FuOx–mediated growth inhibition could be due to induction of apoptosis as Western blot analysis of the EPA + FuOx–treated CR HT-29 cells showed an increased PARP cleavage (Fig. 5A).

**EPA + FuOx treatment regulates β-catenin activity via PTEN–Akt axis**

To elucidate the regulatory mechanism(s) for EPA and FuOx-mediated inhibition of growth and development of CSCs in chemoresistant colon cancer cells in vivo and in vitro, we examined the localization and transcriptional activity of β-catenin, which is known to be activated in colorectal cancer (32) and plays a critical role in maintaining the growth and functional properties of colon CSCs (7).

Indirect immunofluorescence staining showed β-catenin to be primarily localized on the cell membrane in normal HT-29 cells (Fig. 5B top, long arrows). In contrast, CR HT-29 cells showed nuclear localization of β-catenin in a number of cells (Fig. 5B middle, short arrow). However, in EPA/FuOx–treated CR HT-29 cells, β-catenin was found to be mainly localized on the cell membrane (Fig. 5B bottom). Western blot analysis showed a decreased expression of β-catenin as well as its target proteins c-myc and cyclin D1 in EPA + FuOx–treated cells after 24 and 48 hours (Fig. 5C).
EPA + FuOx treatment also caused approximately 40% reduction in the transcriptional activity of TCF/LEF in CR cells (Fig. 5D). These data indicate that EPA + FuOx treatment inhibits the nuclear localization of β-catenin, thus preventing its transcriptional activity. In addition, decreased levels of ATP-binding cassette (ABC) transporter ABCG-2 protein, which is involved with drug efflux (3, 33), indicate reduced drug resistance in EPA/FuOx–treated cells (Fig. 5C).

Although the precise mechanism(s) for EPA + FuOx–mediated changes in β-catenin is not fully understood, we hypothesized that PTEN–Akt signaling may play a role in regulating this process. EGFR, which is known to be activated in colorectal cancer leads to induction of the PTEN–Akt axis (27). In addition, we have reported that miR21-mediated induction of colon CSCs is associated with downregulation and decreased phosphorylation of PTEN (decreased activation), leading to activation of Akt (34, 35). Figure 4E demonstrates a marked increase in pPTEN and decreased pAkt levels over the corresponding controls in response to 20 µmol/L EPA + FuOx (Fig. 5E). The dose of 10 µmol/L EPA + FuOx also caused reduction in the activated (phosphorylated) form of Akt (Fig. 5E). The fact that EPA + FuOx activates PTEN resulting in the decreased Akt activity suggests that the PTEN–Akt axis is involved in modulating Wnt–β-catenin signaling.

**EPA + FuOx treatment reduces the levels of proinflammatory metabolites in SCID mice**

Both arachidonic acid (AA) and EPA are metabolized in vivo by the cyclooxygenase, lipoxygenase, and epoxygenase pathways to prostaglandins, hydroxy fatty acids as well as leukotrienes, and epoxy fatty acids, respectively. To assess the PUFA metabolic changes in the animals treated with EPA, we analyzed the plasma eicosanomic profiles of both treated and untreated animals by LC/MS. The method included all possible metabolites of AA and EPA from all the three enzymatic pathways. Table 1 shows the detected metabolites of both fatty acids in plasma. The results show a lower concentration of AA metabolites in EPA + FuOx–treated animals compared with control FuOx-treated mice and a significantly lower concentration of inflammatory mediators, LTB4 (and its metabolite 12-Oxo LTB4) and PGE2 (as well as its metabolites, 13,14-dihydro-15-keto PGE2 and bicyclo PGE2) in EPA + FuOx–treated animals. Interestingly, a majority of EPA metabolites shows similar plasma concentrations between the two groups, despite the fact that EPA is a significantly
poorer substrate to the metabolic enzymes compared with AA (36).

**Discussion**

The main objective of the current investigation was to study the efficacy of EPA as an inhibitor of recurrent colorectal cancer growth and to determine whether EPA in combination with FuOx would be more effective than either agent/regimen alone.

The preventive and therapeutic efficacy of a combination of EPA and DHA or each PUFA alone has been demonstrated in multiple preclinical studies using a variety of rodent models of early-stage colorectal cancer (37). These studies have consistently demonstrated reduction in colorectal cancer incidence (reviewed in ref. 37). Our data demonstrate for the first time that EPA acts synergistically with FuOx to markedly inhibit the growth of chemoresistant colon cancer cells that form bulk of the recurrent tumor. Although the underlying cause for tumor recurrence is not fully understood, one of the reasons is thought to be the presence of CSCs/CSLCs that are resistant to conventional chemotherapy and retain limitless potential to regenerate (1–3, 38). The resistance of CSCs/CSLCs to therapy has been attributed to a multitude of factors, including increased expression of drug transporters and intracellular detoxification enzymes, upregulation of antiapoptotic proteins, increased efficiency of DNA repair, and alterations in cell kinetics (39). As the CR HT-29 and CR HCT-116 cells exhibit increased stem-like characteristics, as evidenced by increased colonosphere formation, increased drug efflux, an elevated expression of CSC/CSLC markers, higher tumorigenic potential in SCID mice, an increased Wnt–β-catenin and EGFR signaling (4–7), they provide a suitable model to study the efficacy of EPA and/or FuOx in inhibiting recurring colon cancer.

Our current observation that EPA causes a marked reduction in colonosphere formation by CR HT-29 and CR HCT-116 cells, which is further exacerbated by the combination of EPA and FuOx, suggests that this regimen not only

![Figure 3. Changes in CSC phenotype and expression of CSC markers CR HT-29 cells in response to EPA and/or FuOx. A, flow cytometric analysis of CD44$^+$ and CD166$^-$ phenotype (a–e); f, graphical representation of a–e; bars, mean of three readings ± SD. * $P < 0.005. B$, qRT-PCR showing mRNA levels of CSC and differentiation markers normalized to β-actin. Bars, mean of three readings ± SD. † $P < 0.005.
marked reduction in the proportion of CD44
without/CD166low CSC phenotype in EPA/FuOx–treated CR HT-29 cells and a
reduced expression of stem-cell markers, CD44, ALDH1, CD133, and β-catenin. Yang and colleagues recently
reported that a combination of EPA and DHA exerts a direct
antiproliferative and proapoptotic effect on the CSLCs using
SW620 colon cancer cell line and increases their sensitivity
to 5-FU (40). Likewise, our data show that although EPA is
effective in inhibiting growth of CSC/CSLC-enriched chemo-
resistant cells, the combination is even more effective.

An increased expression and activity of Akt has been
reported as an essential factor for cell survival during car-
cinogenesis (41). Aberrant Akt activation is the result of
PI3K and PTEN downstream signaling (42), and, in turn,
affects various pathways, including inactivation of GSK-3β,
resulting in dysregulated canonical Wnt–β-catenin signal-
ing (43, 44). Dysregulation of the Wnt–β-catenin pathway
has been reported to play a pivotal role in the development and progression of colorectal cancer. Translocation of
β-catenin to the nucleus activates the transcription of its
target genes like cyclin D1, c-myc, MMP-7, MT1-MMP,
axin-1, etc. (45–47). We have reported that the Wnt–β-cate-
nin pathway also plays a crucial role in regulating the growth
and maintenance of colon CSCs (7). Although the precise
mechanism by which EPA + FuOx inhibits the growth of chemotherapy-resistant colorectal cancer is not known, our current
observation that EPA/FuOx greatly reduces β-catenin tran-
scriptional activity suggests a crucial role for Wnt–β-catenin signaling in regulating this event. Furthermore, the fact that
EPA + FuOx activates PTEN resulting in decreased Akt
activity suggests that the PTEN–Akt axis is involved in
modulating Wnt–β-catenin signaling. Additional support
comes from the observation that the combination of EPA and FuOx also decreases cyclin D1 and c-myc expression,
two downstream effector molecules of the Wnt–β-catenin
signaling pathway, that are known to be involved in regu-
lation of cell proliferation. In addition, the combination of
EPA and FuOx was found to reduce the expression of ABCG-
2 protein, which has been implicated as a key regulator in
the maintenance of stem cells in various human cancer cell
lines (4, 33, 48) and contributes toward resistance to che-
motherapy (3, 33). Downregulation of ABCG-2 in EPA/
FuOx–treated cells indicates a reduction in drug efflux
capability resulting in its sensitization to chemotherapy.
An increased apoptosis in EPA/FuOx–treated cells as indi-
cated by reduced PARP cleavage further confirms the efficacy of combination therapy on viability of CR cells. These data
strongly suggest that EPA/FuOx treatment could be used to
target stem cell–enriched recurrent colorectal cancer.
Data generated from our in vivo studies using SCID mice xenograft model of colon cancer also support the in vitro observations. When SCID mice bearing xenografts of CR HCT-116 and CR HT-29 cells were administered with either EPA or EPA + FuOx, the tumor growth was greatly reduced. In fact, no significant increase in growth of xenografts by CR HT-29 was observed following administration of EPA and FuOx. Xenografts formed by CR HCT-116 cells were decreased by at least 50% following EPA or the combinatorial treatment. The fact that EPA by itself reduced the growth indicates its chemopreventive efficacy. This is supported by EPA’s ability to inhibit colonosphere formation in vitro, indicating decreased number of CSCs/CSLCs. The fact that these changes were more apparent following combination treatment, also suggests that EPA could be used for preventive as well as therapeutic purposes.

The reduction in tumor growth could be attributed to decrease in tumor cell proliferation, as evidenced by decreased PCNA staining in the treated xenograft (data not shown). Furthermore, our observation that the expression of CK-20, a marker of differentiation, is greatly increased in cells isolated from SCID xenografts of chemoresistant cells following EPA/FuOx treatment strongly suggests that the current combination therapy induces differentiation leading to increased sensitivity to the combination of EPA/FuOx treatment strategy.

Eicosanomic analysis of plasma from EPA/FuOx–treated animals offers a possibility of modulating inflammatory response in these animals. Both AA and EPA are metabolized by the same enzymes to highly physiologically active lipid mediators such as prostaglandins, leukotrienes, and hydroxy and epoxy fatty acids. However, metabolites of AA (an ω-6 PUFA) are proinflammatory, whereas those derived from EPA (an ω-3 PUFA) are anti-inflammatory and participate in active resolution of inflammation (49–51). The eicosanomic profile (Table 1) shows a lower concentration of AA metabolites in EPA/FuOx–treated animals. Concentration of about 75% of the AA metabolites detected was less than 50% in EPA/FuOx–treated animals, whereas all EPA metabolites are above 50% or...
nearly equal between the two groups. Although EPA metabolites are expected to be higher in the animals fed with the fatty acid, it has a 2 to 3 fold metabolic disadvantage compared with AA (36). Moreover, the apparent suppression of AA metabolites in EPA treatment is not uniform across all lipid mediators. Although inflammatory mediators such as LTB4 and PGE2 are lower in EPA-treated animals, 5-OxoETE, another neutrophil chemotactic lipid mediator of the 5-lipoxygenase pathway, is similar in both groups. It is noteworthy that 5-OxoETE is increased in cancer cells under oxidative stress (52). On the other hand, the anti-inflammatory lipid mediators of the epoxygenase pathway, e.g., 11(12)-EpETE (53), are elevated upon EPA treatment (Table 1). Although it is difficult to conclude on the limited sample size data presented from this pilot study, the data offer intriguing possibility that EPA treatment alters the balance of pro- and anti-inflammatory lipid mediators toward an improved outcome of chemotherapy.

In summary, the present data indicate the EPA could be a potential preventive and therapeutic treatment modality for recurrent colon cancer, which is known to be highly

Table 1. Concentrations of eicosanoids in control and EPA + FuOx–treated mouse plasma

<table>
<thead>
<tr>
<th>Eicosanoid</th>
<th>Control (FuOx)</th>
<th>EPA + FuOx</th>
<th>% of Control</th>
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</thead>
<tbody>
<tr>
<td>6-keto PGF1(_2)(\alpha)</td>
<td>48.4 ± 11.7</td>
<td>20.4 ± 3.8</td>
<td>42</td>
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<tr>
<td>TXB2</td>
<td>78.9 ± 54.9</td>
<td>17.1 ± 0.7</td>
<td>22</td>
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<tr>
<td>PGE2</td>
<td>24.0 ± 8.4</td>
<td>7.7 ± 2.1</td>
<td>32</td>
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<tr>
<td>13,14-dihydro-15-keto PGE2</td>
<td>7.9 ± 5.2</td>
<td>2.2 ± 1.2</td>
<td>27</td>
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<tr>
<td>Bicyclo PGE2</td>
<td>21.1 ± 18.0</td>
<td>5.0 ± 2.8</td>
<td>24</td>
</tr>
<tr>
<td>13,14-dihydro-15-keto PGF2(\alpha)</td>
<td>4.6 ± 2.5</td>
<td>1.8 ± 0.9</td>
<td>39</td>
</tr>
<tr>
<td>13,14-dihydro-15-keto PGD2</td>
<td>9.4 ± 6.9</td>
<td>2.2 ± 1.1</td>
<td>23</td>
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<tr>
<td>PGJ2</td>
<td>65.4 ± 33.2</td>
<td>33.7 ± 17.7</td>
<td>52</td>
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<tr>
<td>5(6)-EpETE</td>
<td>17.2 ± 9.0</td>
<td>5.8 ± 1.9</td>
<td>34</td>
</tr>
<tr>
<td>8(9)-EpETE</td>
<td>17.8 ± 9.9</td>
<td>5.0 ± 1.0</td>
<td>28</td>
</tr>
<tr>
<td>11(12)-EpETE</td>
<td>47.1 ± 22.8</td>
<td>14.8 ± 3.3</td>
<td>31</td>
</tr>
<tr>
<td>11,12-DiHETE</td>
<td>2.8 ± 1.4</td>
<td>1.7 ± 0.8</td>
<td>62</td>
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<tr>
<td>14(15)-EpETE</td>
<td>19.4 ± 16.7</td>
<td>6.4 ± 1.4</td>
<td>33</td>
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<tr>
<td>14,15-DiHETE</td>
<td>4.3 ± 2.9</td>
<td>3.1 ± 1.0</td>
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<td>LTB4</td>
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<td>2.0 ± 0.3</td>
<td>24</td>
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<td>12-Oxo LTB4</td>
<td>4.1 ± 1.4</td>
<td>0.1 ± 0.1</td>
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<td>5-HETE</td>
<td>28.7 ± 9.8</td>
<td>14.9 ± 2.1</td>
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<td>5-oxo ETE</td>
<td>2.7 ± 0.6</td>
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<td>108.8 ± 49.9</td>
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<td>1629.0 ± 855.4</td>
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<td>12-Oxo ETE</td>
<td>5.1 ± 2.5</td>
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<tr>
<td>15-HETE</td>
<td>42.5 ± 20.2</td>
<td>15.4 ± 1.2</td>
<td>36</td>
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<tr>
<td>15-Oxo ETE</td>
<td>1.1 ± 0.4</td>
<td>0.7 ± 0.2</td>
<td>66</td>
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<tr>
<td>8(9)-EpETrE</td>
<td>2.3 ± 1.5</td>
<td>1.2 ± 0.4</td>
<td>53</td>
</tr>
<tr>
<td>11(12)-EpETrE</td>
<td>1.3 ± 1.0</td>
<td>1.4 ± 0.1</td>
<td>110</td>
</tr>
<tr>
<td>14(15)-EpETrE</td>
<td>2.2 ± 1.7</td>
<td>2.0 ± 0.6</td>
<td>90</td>
</tr>
<tr>
<td>17(18)-EpETrE</td>
<td>2.9 ± 1.7</td>
<td>1.6 ± 0.4</td>
<td>55</td>
</tr>
<tr>
<td>5-HEPE</td>
<td>4.3 ± 1.7</td>
<td>3.5 ± 0.6</td>
<td>80</td>
</tr>
<tr>
<td>8-HEPE</td>
<td>4.2 ± 1.6</td>
<td>2.9 ± 0.7</td>
<td>69</td>
</tr>
<tr>
<td>9-HEPE</td>
<td>7.2 ± 3.7</td>
<td>4.0 ± 2.0</td>
<td>55</td>
</tr>
<tr>
<td>11-HEPE</td>
<td>5.0 ± 2.3</td>
<td>3.1 ± 0.4</td>
<td>61</td>
</tr>
<tr>
<td>12-HEPE</td>
<td>112.1 ± 67.9</td>
<td>60.4 ± 40.0</td>
<td>54</td>
</tr>
<tr>
<td>15-HEPE</td>
<td>6.4 ± 2.4</td>
<td>4.0 ± 0.7</td>
<td>63</td>
</tr>
<tr>
<td>18-HEPE</td>
<td>3.9 ± 1.4</td>
<td>3.2 ± 0.6</td>
<td>80</td>
</tr>
</tbody>
</table>

NOTE: All values are ng/mL plasma (mean ± SEM; n = 3). Metabolites of EPA are italicized.
Abbreviations: DiHETE, dihydroxyeicosatrienoic acid; EpETE, epoxyeicosatrienoic acid; EpETrE, epoxyeicosatetraenoic acid; ETE, eicosatrienoic acid; HETE, hydroxyeicosatetraenoic acid; HEPE, hydroxyeicosapentaenoic acid; LT, leukotriene; PG, prostaglandin; TX, thromboxane.
enriched in chemotherapy-resistant CSCs/SLCs, as evidenced by the reduction in stem cell characteristics such as colonosphere formation and increased disintegration, decreased number of CD44+/CD166low cells, decreased expression of stem cell markers, increased CK20 levels, and reduction in drug efflux in vitro and in vivo. These changes are associated with inhibition of the PTEN–Akt axis leading to reduced Wnt–β-catenin signaling, induction of apoptosis, and reduction in the proinflammation markers.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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