Modulation of Inducible Nitric Oxide Synthase and Related Proinflammatory Genes by the Omega-3 Fatty Acid Docosahexaenoic Acid in Human Colon Cancer Cells

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ABSTRACT

Epidemiological and preclinical studies demonstrate that consumption of diets high in omega-3 polyunsaturated fatty acids reduces the risk of colon cancer. Inhibition of colon carcinogenesis by omega-3 polyunsaturated fatty acids is mediated through modulation of more than one signaling pathway that alters the expression of genes involved in colon cancer growth. In our earlier studies on global gene expression with cDNA microarrays, we have shown that treatment of CaCo-2 colon cancer cells with docosahexaenoic acid (DHA) down-regulated the prostaglandin family of genes, as well as cyclooxygenase 2 expression and several cell cycle-related genes, whereas it up-regulated caspases 5, 8, 9, and 10 that are associated with apoptosis. It is known that nitric oxide activates the cyclooxygenase 2 enzyme, which plays a pivotal role in the progression of colon cancer via prostaglandin synthesis and angiogenesis. The present study was undertaken to examine the multifaceted role of DHA in the expression of inducible nitric oxide synthase (iNOS) and of related proinflammatory genes, as those have been shown to play a role in tumor progression. In addition, we aimed to identify associated target genes by DNA microarray, reverse transcription-PCR analysis, and cellular localization of iNOS expression in CaCo-2 cells. Results of this study demonstrate that treatment with DHA down-regulates iNOS in parallel with a differential expression and down-regulation of IFNs, cyclic GMP, and nuclear factor κB isoforms. More importantly, our findings clearly demonstrate the up-regulation of cyclin-dependent kinase inhibitors p21(34) and p27, differentiation-associated genes such as alkaline phosphatases, and neuronal differentiation factors. These findings strongly suggest that the antitumor activity of DHA may be attributed, at least in part, to an effect on iNOS regulatory genes. In addition, our results indicate the presence of specific gene expression profiles in human colon cancer that can be used as molecular targets for chemopreventive agents.

INTRODUCTION

Cancer of the colon is one of the leading causes of deaths in both men and women in Western countries, including the United States, where ~148,300 new cases of colorectal cancer and 56,600 related deaths are expected for the year 2002 (1). Epidemiological studies have provided evidence that high intake of saturated fat and/or animal fat increases the risk of colon cancer, and that diets rich in omega-3 PUFAs3 (fish oils) reduce the risk of colon cancer development (2, 3). Caygill et al. (2) reported an inverse association of consumption of fish and fish oil with colon cancer. Studies in our laboratory and elsewhere have provided convincing evidence that diets rich in omega-3 PUFAs reduce the risk of chemically induced colon carcinogenesis compared with diets high in omega-6 PUFAs and/or saturated fatty acids. This suggests that the composition of the ingested fat is critical to colon cancer risk (4–7). In addition, laboratory animal assays have indicated that the influence of these omega-3 PUFAs is exerted foremost during the postinitiation phase of colon carcinogenesis (5). In a Phase II clinical trial of patients with colon polyps, dietary fish oil supplements have, in fact, inhibited cell proliferation in the colonic mucosa (8).

With regard to mode of action of different types of fat in colon carcinogenesis, dietary fish oil decreases the concentration of secondary bile acids in the colon as compared with diets high in omega-6 PUFAs and saturated fats (9). Secondary bile acids have been shown to increase cell proliferation and to act as colon tumor promoters (10, 11). However, the cellular and molecular mechanisms by which omega-3 PUFAs inhibit colon carcinogenesis and reduce the growth of tumor cells remain poorly understood. Preclinical evidence demonstrated that several dietary components could influence the pathways involved in cell proliferation and differentiation (12, 13). Our earlier studies demonstrated that omega-3 fatty acids are capable modulating a panel of cell cycle and apoptosis-regulating genes in tumors (14). Much attention has been given recently to endogenous factors that appear to be responsible for tumor cell growth, metastasis, and invasion. Identifying whether such endogenous factors are modulated by omega-3 PUFAs should lead to a better understanding of the processes of tumor cell progression, and would also provide new strategies for developing nutritional and chemopreventive agents that specifically suppress these processes.

Preclinical model assays indicate that dietary fish oil inhibits COX-2 activity and enhances apoptosis in colon tumors (7), Overexpression of the COX-2 gene in colon epithelial cells leads to altered adhesion properties and resistance to apoptosis (14). Although the above studies are indirectly supporting the anticancer properties of the omega-3 fatty acid DHA (Fig. 1), a thorough understanding of the pathways that are involved in the mechanism of tumorigenesis is necessary to fully assess the chemopreventive efficacy of this agent against colon cancer. In this connection we have demonstrated recently by cDNA microarray and RT-PCR analyses that in CaCo-2 cells DHA alters several proinflammatory genes (15). Studies in our laboratory and elsewhere support the hypothesis that COX-2 regulation is influenced by various exogenous factors including NO (16–19). Remarkably, several studies have demonstrated that colonic tumors in laboratory animals, and colonic adenomas and adenosarcomas in humans have increased activities and/or expression of iNOS when compared with the levels in adjacent non-neoplastic mucosa (19–22). High levels of iNOS may increase the invasiveness and metastatic potential of human colon cancer (23). It is also known that NO can damage DNA either directly or indirectly by several mechanisms including interference with DNA repair. NO can also cause post-translational modifications of proinflammatory cytokines that may lead to tumor initiation and promotion (24, 25). Therefore, it is possible that sustained high levels of NO generated by iNOS can produce various kinds of damage. In chronic conditions, such damage leads to an accumulation of gene mutations, including mutation of the tumor suppressor gene p53, and alterations in cellular functions (26).

Taken together, these observations suggest that COX-2, iNOS, and...
other proinflammatory genes may play a critical role in colon carcinogenesis.

In view of the significance of omega-3 PUFAs, including DHA, in colon carcinogenesis, and involvement of COX-2 and iNOS in colon tumor progression, the current study was designed to explore the mechanisms of chemopreventive efficacy of DHA, specifically on modulation of iNOS and other proinflammatory genes with respect to apoptosis and cell differentiation. First, we determined the effect of DHA on colon cancer cell growth, apoptosis, and DNA fragmentation, and then its effect on iNOS expression by nuclear localization with iNOS-specific antibodies and by Western blot for the iNOS proteins. Also, we identified COX-2- and iNOS-activating proinflammatory genes, and their levels of expression in DHA-treated CaCo-2 cells using cDNA microarray analysis. Although NO functions as a mediator in the inflammatory processes while it also is a physiologically important signaling molecule in virtually every tissue in the body, we addressed here the question of whether DHA could indeed induce cell death via down-regulation of iNOS expression and/or by modulating sets of genes involved in apoptosis and differentiation.

MATERIALS AND METHODS

Cell Growth and DHA Treatment. CaCo-2 cells were grown under cell culture conditions and maintained by serial passage in RPMI 1640 containing 10% FBS. For stimulation, DHA (Cayman, Ann Arbor, MI) was dissolved in DMSO, and cells were treated with 5 μg/ml of DHA in the cell culture medium for 48 h. Control cultures were treated with DMSO alone and were processed similarly.

RNA Isolation and Probe Preparation for Microarray Analysis. Untreated CaCo-2 cells and those treated with DHA for 48 h were collected, and total RNA was isolated using Trizol reagent and Qiagen columns (Life Technologies, Inc. Rockville, MD and Qiagen, Valencia, CA, respectively). One control probe (untreated CaCo-2 cells) and one test probe (DHA-treated CaCo-2 cells) were made independently for microarray hybridization. RNA from the untreated cells was labeled with Cy3 and used as the control probe. RNA from DHA-treated CaCo-2 cells was labeled separately with Cy5 and was used as the test probe. The reverse transcription reaction was carried out, and the labeled probes were washed with 70% and 95% ethanol, respectively, and were stored at −20°C for additional hybridization. Hybridizations were carried out as described earlier (15).

Human Oligonucleotide Array. The impact of DHA on gene expression profiles was performed using Clontech Human Atlas Glass Arrays. Each gene on Atlas Glass Arrays is represented by a “long oligo,” an 80-bp fragment, which has 70% homology to any entry in GenBank that combines the high hybridization efficiency of a DNA fragment with the ability of a short oligonucleotide to distinguish between homologous genes. Atlas Glass 3.8 microarrays contain 3800 carefully selected, well-characterized genes to provide high-quality, reliable expression data from many biological pathways. Briefly, the genes on the array include a number of functional categories of genes and transcription factors relevant to this study.

Scanning and Image Analysis. Microarray slides were scanned using an Axon GenePix 4000A scanner (Axon Instruments, Foster City, CA). This is a nonconfocal scanning instrument containing two lasers that excite cyanine dyes at appropriate wavelengths, 635 nm for Cy5 and 532 nm for Cy3, respectively, with high-resolution (10 μm pixel size) photo multiplier tubes that detect fluorochrome emission. The photo multiplier tube levels of the two channels at 635 nm and 532 nm were balanced (100–1000 V) to limit the number of saturated pixels for generating a gray scale TIFF image file. The microarray images were analyzed using GenPix Pro-3.0 software. The microarray data sets and color images were generated on Microsoft Excel spreadsheet sheets and JPEG images, respectively. The GeneSpring bioinformatics software package (Silicon Genetics, Inc.) was used to explore the microarray data sets generated from this study for multivariate analysis.

Validation of Gene Expression by RT-PCR. Because RT-PCR of mRNA provides maximum sensitivity, a standardized measurement of expressed genes was carried out by a semiquantitative RT-PCR. The RT-PCR used 33 cycles for selected gene-specific primer sequences. All of the templates were initially denatured for 2 min at 94°C, and the amplification of the amplicon was extended at a final extension temperature of 72°C for 7 min. A separate set of RT-PCR reactions with an increasing amount of mRNA was carried out, if necessary, to show a linear increase in the band intensity of the amplified PCR product. PCR amplification with glyceraldehyde-3-phosphate dehydrogenase was used as an internal control.

Cellular Localization of iNOS. In this study, we have used nuclear staining of colon cancer cells for detecting iNOS-positive cells by immunofluorescence technique based on published results of Fehr et al. (27) indicating that the receptors of certain cytokines signal through STAT proteins. Receptor occupation and dimerization induce phosphorylation of STATs. Activated STATs dimerize and translocate to the nucleus where they increase the expression of transcription factor IRF-1, which binds to specific DNA elements in the iNOS gene promoter region to increase iNOS gene expression. We have detected this gene by nuclear staining as described here. CaCo-2 cells with or without 48 h of DHA treatment were fixed in 10% formalin and pretreated with 0.1% Triton X-100 and 2× HCl at 37°C for 10 min. They were then treated with 0.1 M sodium borate for 5 min and washed with PBS three times. Immunofluorescence detection of iNOS-positive cells was visualized with anti-iNOS antibody (Cayman) followed by rhodamine conjugated with goat-antimouse IgG. An epifluorescence microscope (AX-70; Olympus, Tokyo, Japan) was used for detection of iNOS-positive cells. The positively stained cells were quantified with Image Pro plus software (Media Cybernetics, Silver Spring, MD).

Western Blot Analysis for iNOS Expression. CaCo-2 cells treated with DHA (10−3 M) for 48 h were harvested by trypsinization. Cellular protein was isolated with protein extraction buffer containing 150 mM NaCl, 10 mM Tris (pH 7.2), 5 mM EDTA, 0.1% Triton X-100, 5% glycerol, and 2% SDS in addition to a mixture of protease inhibitors (Boehringer Mannheim, GmbH, Germany). Equal amounts of protein (50 μg/lane) were fractioned on 10% SDS-PAGE gels and transferred to polyvinylidene difluoride membranes. The Western blot procedure was carried out as described earlier (26). The antibody used for Western blotting was iNOS polyclonal antisera (Cayman). The reactive reaction band for iNOS expression was detected using chemiluminescence detection reagents (ECL; Amersham). Densitometric analysis of the protein bands was performed with the software Gel-Pro Analyzer (Media Cybernetics).

Apoptosis Detection and DNA Fragmentation Analysis. CaCo-2 cells without or with DHA treatment were stained with DAPI for nuclear staining and then scanned for characteristic changes in the nuclear material. This indicated convoluted budding and bleeding of the membrane, chromatin aggregation, and nuclear and cytoplasmic condensation pertaining to apoptosis. DNA fragmentation analysis was carried out using methods described earlier (26). Briefly, CaCo-2 cells without or with 48-h-DHA treatment were harvested by trypsinizing, and were suspended in 1-ml cell lysis buffer. The cell lysate was incubated at 55°C for 4–6 h. Cells were again treated with RNase (10 μg/ml) for 1 h at 37°C. The supernatant was collected, and DNA was extracted with phenol-chloroform. This procedure was repeated two or more times to obtain a clear aqueous phase that was then ethanol-precipitated and centrifuged. The pellet was then air dried and resuspended in 18 μl of distilled water. The final concentration of DNA was determined by UV absorbency at 260 nm. DNA (10 μg/ml) was electrophoresed on 1.8% agarose gels containing ethidium bromide (1 μg/ml). DHA-induced DNA fragmentation was confirmed by the appearance of internucleosomal cleavage, and the banding pattern as DNA ladder was photographed immediately.

RESULTS

Differential Gene Expression Pattern. Genes of which the expressions were altered >2-fold by DHA in CaCo-2 cells are shown in
A red color image of spots represents induced genes, green spots indicate repressed genes. B, scatter plot view of gene expression. Expression intensity Cy5:Cy3 ratios of untreated versus DHA-treated CaCo-2 cells. The ratios (Cy-5:Cy-3) of genes that have ≥2-fold expression are considered induced, and those with ≤0.5-fold expression are considered repressed. Approximately 504 of 3800 genes (13%) were expressed in DHA-treated cells.
Table 1 Impact of DHA on the regulation of proinflammatory genes in CaCo2 cells

<table>
<thead>
<tr>
<th>GenBank accession no.</th>
<th>Coordinate</th>
<th>Gene description Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>NM_000698</td>
<td>A249</td>
<td>Arachidionate 5-lipoxygenase-T1 0.27 ± 0.16</td>
</tr>
<tr>
<td>NM_003960</td>
<td>H2b1</td>
<td>Prostaglandin 12R 0.28 ± 0.13</td>
</tr>
<tr>
<td>NM_001139</td>
<td>A26d</td>
<td>Arachidionate 12-lipoxygenase, R 0.43 ± 0.33</td>
</tr>
<tr>
<td>NM_000955</td>
<td>H2a7</td>
<td>Prostaglandin ER1 0.44 ± 0.11</td>
</tr>
<tr>
<td>NM_001629</td>
<td>A21a</td>
<td>Arachidionate 5-lipoxygenase-AP 0.46 ± 0.09</td>
</tr>
<tr>
<td>NM_001141</td>
<td>A28d</td>
<td>Arachidionate 15-lipoxygenase-T2 0.48 ± 0.15</td>
</tr>
<tr>
<td>NM_000959</td>
<td>H2a9</td>
<td>Prostaglandin F Receptor 0.48 ± 0.09</td>
</tr>
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<td>NM_000956</td>
<td>H2a8</td>
<td>Prostaglandin ER2 0.49 ± 0.16</td>
</tr>
<tr>
<td>NM_000954</td>
<td>K2i2</td>
<td>Prostaglandin D2s 0.59 ± 0.15</td>
</tr>
<tr>
<td>NM_000963</td>
<td>H2b2</td>
<td>Prostaglandin E2s 0.70 ± 0.17</td>
</tr>
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Mean ± SD represent the cy3:cy5 ratios derived from microarray scanned images for DHA-treated versus untreated samples from three independent microarray data sets. Values are shown as fold-up-regulation and down-regulation.
Down-Regulation of iNOS Expression. Immunofluorescence detection based on nuclear positive staining for iNOS expression demonstrated that the number of cells that were positive for iNOS were lower in CaCo-2 cells treated with DHA for 48 h than in the controls (Fig. 5, A–D). Importantly, the lower number of iNOS-positive cells observed in this study is consistent with an increase in the number of apoptotic cells as determined by DAPI staining. To relate the level of iNOS expression to the number of apoptotic cells, iNOS-positive cells and apoptotic cells were quantified. As shown in Fig. 6, iNOS expression was decreased in a dose-dependent manner by 2.5 and 5 μg of DHA, whereas apoptosis was enhanced by 2.5 μg DHA, but no additional enhancement with 5 μg of DHA was observed. Notably, the results from Western blot analysis indicate a dose-dependent inhibition of iNOS expression by DHA (Fig. 7A). Quantification by densitometry analysis of protein bands indicated a 2-fold down-regulation of the iNOS expression by DHA (Fig. 7B). However, a cause and effect relationship between the expression of iNOS and inhibition of apoptosis cannot be drawn from our results.

Down-Regulation of cGMP in DHA-treated CaCo-2 Cells. To determine whether there is any relationship between expression of cGMPs and other DHA-induced genes, a complete in-depth analysis was carried out to assess the expression of several isoforms of cGMP. Interestingly, we identified five clones of cGMP isoforms that were down-regulated by DHA (Fig. 8); however, the functional significance of these isoforms has yet to be determined with respect to iNOS inhibition, and activation of differentiation-inducing genes and related factors that are differentially expressed by DHA (Table 1).

Effect of DHA on Genes Involved in Differentiation. DNA microarray and RT-PCR analysis demonstrated activation of cyclin kinase inhibitor p21Waf1/Cip1 and RXR at the mRNA level as shown in Fig. 3. The RXR is a nuclear receptor that functions as a ligand-activated transcription factor that selectively regulates cell differentiation and proliferation, making these ligands an ideal target for chemoprevention (28). Results from functional analysis of genomics from microarray data consistently indicated activation of several genes involved in cellular differentiation (Table 1; Fig. 9). Our data demonstrate that differential expression of genes involved in differentiation constituted ~0.1% of the total expressed genes. Specifically, up-regulation of alkaline phosphatase P and growth differentiation factors 8 and 10 indicates a potential role for DHA in colonic cell differentiation.

Effect of DHA on IFNs and NFκB. A major inhibitory effect of DHA was observed on expression of IFNs α 5, 10, 21, 16, 14, 13, and γ and β isoforms. However, 25% of the IFNs did not show any remarkable changes (either activation or inhibition) as summarized in Table 1. RT-PCR analyses indicated a 2-fold inactivation of NFκB p65, although not much change could be observed in the transcripts of NFκB p50 in DHA-treated CaCo-2 cells. Repeated experiments using RT-PCR analyses revealed inactivation of iNOS and NFκB p65. It is noteworthy that major changes associated with CaCo-2 cell growth inhibition, inactivation of iNOS, and induction of apoptosis by DHA are consistent with the down-regulation of COX-2, NFκB p65 (Fig. 3), the family of IFNs, and several lipoxygenases as shown in Table 1. Additional studies are in progress to demonstrate the biological significance of differential expression patterns of iNOS, NFκB, and tumor necrosis factor isoforms by DHA.

DISCUSSION

The present study is part of a large-scale investigation on the chemopreventive efficacy of omega-3 PUFAs present in fish oil against colon carcinogenesis. This study was aimed at identifying signaling pathways that regulate colon cancer growth, development, differentiation, and apoptosis. Preclinical studies clearly demonstrate that diets rich in omega-3 PUFAs, including DHA, induce apoptosis, and inhibit COX-2 and iNOS activity in colon tumors (7). Identification of a subset of genes that are modulated by omega-3 PUFAs, including DHA, provides biomarkers for diet intervention studies in humans.

The outcome of this study is of great interest because of its implication for human colon cancer prevention. Earlier, we have demonstrated that DHA inhibits several proinflammatory genes, such as COX-2, and the prostaglandin family of genes in CaCo-2 colon cancer cells (15). The results of the present study clearly demonstrate for the first time that DHA inhibits iNOS expression and expression of associated genes in colon cancer cells. Because iNOS/NO and COX-2/prostaglandins appear to be involved in the pathogenesis of colon cancer (7, 14, 16, 29–32), selective inhibitors of these genes are likely chemopreventive agents. Indeed, our data support the concept that inhibitors of one or both of these inducible enzymes and their target genes are effective chemopreventive agents against colon carcinogenesis in preclinical models (7, 29–31).

Pathophysiological actions are induced by various forms of NO synthase that are mediated not only by free radical oxidants but also by activation of guanylate cyclase, leading to the production of cGMP. It is known that NO or its oxidation product, peroxynitrite, may activate COX-2 activity (33). As discussed earlier, only iNOS produces sustained NO concentrations in the micromolar range, and this inducible form is associated specifically with neoplastic tissue. In addition, NO has been found to post-translationally modify a number of important cellular proteins, including p53, caspases, and DNA repair enzymes (25, 34). Inactivation of iNOS and cyclic GMP by DHA suggests a strong protective mechanism that can abrogate any pathological effects induced by iNOS and cyclic GMP. However, a defined functional mechanism of DHA with respect to cyclic GMP
regulation in colon cancer has yet to be established. The present study also demonstrates an inhibitory effect of DHA on the family of IFNs (Table 1), suggesting its anti-inflammatory properties. IFNs (α and β forms) are implicated in autocrine and paracrine signals critical for induction of murine iNOS (35). Our findings on inactivation of iNOS, and activation of proapoptotic and differentiation-inducing genes are consistent with observations from related studies that indicate an important role for DHA in cellular differentiation and apoptosis (36, 37). A study by Kielar et al. (38) points to the possibility that several proinflammatory factors that activate iNOS could be inactivated by DHA via down-regulation of NFκB and other target genes; however, this needs to be substantiated.

Our present study also determined whether DHA treatment influences the CaCO-2 cells to undergo differentiation associated with apoptosis as a function of colonic tissue homeostasis. A >2-fold activation of p21(\(^{\text{Waf-1/Cip1}}\)), corresponding with a change in the expression of retinoic acid receptor RXRα, additionally supports our observation on DHA-induced differentiation in colonic epithelial cells.

On the basis of our results, we propose that the mechanism(s) involved in the suppression of colon carcinogenesis by DHA are more likely multiple in nature as shown in Fig. 10. It is very clear that DHA inhibits the iNOS expression at the mRNA and protein levels by reprogramming the expression of several proinflammatory genes that, in turn, might have induced a negative effect on the transcription of
nuclear transcription factor NFκB and IFNs. It is also evident that DHA induces colonic cell differentiation partly through the inhibition of iNOS, and at the same time, by activating cyclin-dependent kinase inhibitor p21, known for its role in mammalian cell differentiation. Importantly, the RXR functions as a ligand-activated transcription factor that modulates cell differentiation, making its ligand an ideal target for chemoprevention (28). Thus, DHA, which acts as an RXR agonist, is a promising, naturally occurring ligand for chemoprevention of colon carcinogenesis.

In summary, we report here for the first time a vast array of DHA-responsive signaling genes and molecules representing more than one signaling pathway involved in colon cancer growth inhibition. The modulation of colon cancer cell growth by DHA is apparently mediated through the inhibition of COX-2 and iNOS expressions, and induction of apoptosis. An inhibitory effect on differential expression of NFκB and tumor necrosis factor receptor isoforms observed in RT-PCR and microarray analysis also suggests a synergistic effect induced by DHA on iNOS regulation. NFκB, which regulates several genes that are involved in the inflammatory process, provides an excellent target for development of new chemopreventive agents such as DHA. The results of this and our earlier study (15) suggest that the molecular targets modulated by DHA may be suitable indicators of effective chemopreventive intervention by selective agents. Our continuing studies may help to identify molecular targets of chemoprevention in colon carcinogenesis. The ability of diet rich in omega-3 PUFAs, including DHA, to modulate several molecular parameters associated with colon carcinogenesis strengthens the concept that a combination of agents targeting various molecular parameters may effectively inhibit colon cancer progression in humans.

Fig. 7. Effect of DHA on iNOS expression. A, Western blot analysis of CaCo-2 cell lysate for iNOS expression after treatment with DHA (2.5 μg/ml and 5.0 μg/ml, respectively) for 48 h. B, densitometric analysis of iNOS protein bands as altered by DHA; bars, ±SD.

Fig. 8. Differential expression of cGMP isoforms. Differential expression is shown as the ratio between DHA-treated versus untreated CaCo-2 cells. More than 2-fold expression is considered up-regulated. cGMP isoforms include: cGMP 5A phosphodiesterase, cGMP 6G phosphodiesterase, cGMP 6C phosphodiesterase, cGMP 6H phosphodiesterase, cGMP-dep.PK type II, cGMP 6D phosphodiesterase, and cGMP 6B phosphodiesterase; bars, ±SD.

Fig. 9. Effect of DHA on levels of expression of genes related to differentiation. Functional analysis of genomics from microarray data demonstrated activation of several genes involved in cellular differentiation.

Fig. 10. Schematic diagram of potential molecular mechanisms of DHA. The illustration presented here depicts the key molecular and cellular events mediated by DHA in inhibiting COX-2 and iNOS target genes. Altered expressions of the above genes at the mRNA and protein levels in CaCo-2 cells after 48 h of DHA treatment were evident from the DNA microarray RT-PCR analysis and Western blot analysis. At the transcription level a simultaneous reprogramming of genes involved in differentiation, such as p21(Waf1/Cip1), p27, and apoptosis by activating caspases (see Table 1) is evident from the present study. Because iNOS inhibition and p21 expression can be both p53-dependent and -independent pathways, there may be multiple pathways for the chemopreventive action of DHA. The cascade of molecular events regulated by DHA shows a unique relationship between proinflammatory genes, including COX-2, iNOS, and differentiation-initiating factors that result in the maintenance of colonic tissue homeostasis.
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REFERENCES

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