Role of ω-3 polyunsaturated fatty acids on cyclooxygenase-2 metabolism in brain-metastatic melanoma

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Abstract Cyclooxygenase-2 (COX-2) is important in the progression of epithelial tumors. Evidence indicates that ω-6 PUFAs such as arachidonic acid (AA) promote the growth of tumor cells; however, ω-3 fatty acids [eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)] inhibit tumor cell proliferation. We investigated the effects of ω-3 PUFA on the expression and function of COX-2 in 70W, a human melanoma cell line that metastasizes to the brain in nude mice. We show that *1*) tumor necrosis factor-α upregulates the expression of both COX-2 mRNA and prostaglandin E_2 (PGE_2) production, and *2*) ω-3 and ω-6 PUFA regulate COX-2 mRNA expression and PGE_2 production. AA increased COX-2 mRNA expression and prostaglandin production in ω-6-stimulated 70W cells. Conversely, COX-2 mRNA expression decreased in cells incubated with EPA or DHA. AA increased Matrigel™ invasion 2.4-fold, whereas EPA or DHA did not. Additionally, PGE_2 increased in vitro invasion 2.5-fold, whereas exposure to PGE_3 significantly decreased invasion. Our results demonstrate that incubation of 70W cells with either AA or PGE_2 increased invasiveness, whereas incubation with EPA or DHA downregulated both COX-2 mRNA and protein expression, with a subsequent decrease in Matrigel™ invasion. Taken together, these results indicate that ω-3 PUFA regulate COX-2-mediated invasion in brain-metastatic melanoma.— Denkins, Y., D. Kempf, M. Ferniz, S. Nileshwar, and D. Marchetti. **Role of ω-3 polyunsaturated fatty acids on cyclooxygenase-2 metabolism in brain-metastatic melanoma. *J. Lipid Res.* 2005.** 46: **1278–1284.**

Supplementary key words arachidonic acid • brain melanoma • cancer • docosahexaenoic acid • eicosapentaenoic acid • polyunsaturated fatty acids • prostaglandin E_2 • prostaglandin E_3 • ω-6 polyunsaturated fatty acids

There is increasing evidence that constitutive expression of cyclooxygenase-2 (COX-2) plays a role in the development and progression of malignant epithelial tumors (1–4). COX-2 is an inducible enzyme involved in the production of prostaglandins during inflammation (1). Overexpression of prostaglandins of the E_2 series is associated with a variety of illnesses, including skin cancer, and is linked with a poor prognosis (1, 5). Importantly, COX-2 expression in malignant melanoma has been associated with the regulation of tumor invasion. For example, Denkert et al. (2) showed that 93% of the primary melanomas investigated expressed COX-2 mRNA, whereas adjacent normal epithelium and benign nevi were negative.

Downregulation of COX-2 expression with nonsteroidal anti-inflammatory drugs (NSAIDs) has shown promise in anticancer therapy by reducing tumor burden, decreasing the risk in a number of tumor types (3, 4, 6), and effectively inhibiting the in vitro invasion of metastatic cells (2). The inverse relationship between prostaglandin E_2 (PGE_2) expression in brain cancers and prognosis has led investigators to explore the effect of NSAIDs in these neoplasms. For example, in vitro growth inhibition of a human glioma cell line was obtained with exposure to *N*-(2-cyclohexyloxy-4-nitrophenyl)methanesulfonamide (NS-398), an NSAID that works posttranscriptionally on COX-2 protein (7). PGE_2-producing melanoma cell lines have been shown to reduce production of the prostaglandin when treated with COX-2 inhibitors (2). Moreover, although inhibition of COX-2 failed to reduce the proliferation of malignant melanoma lines, a 50% to 68% reduction in Matrigel™ invasion, an indicator of malignant cell invasion capability, was demonstrated (2). Nevertheless, adverse events associated with the use of NSAIDS are severe and can cause life-threatening complications.

Both epidemiological and laboratory studies have recently shown that the long-chain ω-3 PUFAs found in fish oil have antitumor activity relative to a number of cancer types (8–12). Mechanisms to explain these findings include the competition of ω-3 with ω-6 fatty acids as substrates for COX-2 and the subsequent generation of anti-inflammatory and antimitogenic prostaglandins. Epidemiological

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Abbreviations: AA, arachidonic acid; COX-2, cyclooxygenase-2; DHA, docosahexaenoic acid; EIA, enzyme immunoassay; EPA, eicosapentaenoic acid; NSAID, nonsteroidal anti-inflammatory drugs; NS-398, *N*-(2-cyclohexyloxy-4-nitrophenyl)methanesulfonamide; PGE_2, prostaglandin E_2; TNF-α, tumor necrosis factor-α.

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and clinical evidence has also demonstrated that ω-3 PUFAs inhibit the promotion and progression of tumorigenesis. However, to date, no evidence has been presented that describes the involvement of these fatty acids in the invasion of brain-metastatic melanoma.

The objective of this study was to investigate the effects of ω-3 PUFAs on the invasive capability of brain-metastatic melanoma cells as they relate to COX-2 regulation. We provide first-time evidence of *1*) COX-2 expression in human brain-metastatic melanoma cells, and *2*) PGE_2 and PGE_3 effects on their invasive mechanisms.

MATERIALS AND METHODS

Materials

Arachidonic acid (AA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) were purchased from Sigma-Aldrich (St. Louis, MO), NuCheck Prep (Elysian, MN), and Cayman Chemicals, Inc. (Ann Arbor, MI), respectively. Tumor necrosis factor-α (TNF-α) and NS-398, a COX-2 inhibitor, were purchased from Calbiochem (La Jolla, CA). COX-2 monoclonal antibody, the PGE_2 enzyme immunoassay (EIA) monoclonal kit, PGE_2, and PGE_3 were purchased from Cayman Chemicals, Inc. Criterion gels used for Western blots were purchased from Bio-Rad Laboratories (Hercules, CA). The anti-COX-2 antibody and the goat anti-mouse HRP antibody were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). DMEM medium was purchased from Gibco (Grand Island, NY), FBS from Hyclone Laboratories (Logan, UT), and BSA and IGEPAL™ CA-630 from Sigma-Aldrich. Transwell cell culture chambers were purchased from Corning Incorporated Life Sciences (Acton, MA), and Matrigel™ was obtained from BD Biosciences Discovery Labware (Bedford, MA). All other chemicals used were reagent grade or better.

Tissue culture

Early-passage melanoma cells (70W line) with high metastatic propensity compared with the parental line MeWo were maintained as monolayer cultures in a 1:1 (v/v) mixture of DMEM/F-12 supplemented with 10% FBS (v/v). Cells were maintained at 37°C in a humidified 5% CO_2/95% air atmosphere (v/v) and passaged using trypsin-EDTA before reaching confluence. Cells were incubated in serum-free medium for 24 h before adding fatty acids, cytokines, NS-398, or prostaglandins. Fatty acids, prostaglandins, and NS-398 were dissolved in DMSO before being added to serum-free DMEM and 0.1% BSA. DMSO was included in the serum-free medium during cell incubation with reagents.

Isolation of RNA, RT-PCR, and quantitative PCR

Total RNA was isolated from cells using the RNeasy kit (Qiagen, Inc., Valencia, CA). Protein assay was done using the bicinchoninic acid assay (Pierce, Inc., Rockford, IL). Reverse transcription was performed using oligo-dT primers according to the manufacturer's instructions (Promega, Inc., Madison, WI), and PCR was performed for 35 cycles of denaturation at 94°C for 30 s, annealing at 56°C for 30 s, and at 72°C for 1 min using a thermal cycler (ABI 9600; Applied Biosystems, Inc., Foster City, CA). The following specific primers were used: COX-2 forward (5′-GCCTTCTCTAACCTCTCC-3′) and reverse (5′-CTGATGCGTGAAGTGC-3′) and control GAPDH forward (5′-AGCCACATCGCTCAGAACAC-3′) and reverse (5′-GAGGCATTGCTGATGATCTTG-3′). PCR products were subsequently resolved on a 1.5% agarose gel and visualized by ethidium bromide staining using a Versadoc imaging system (Bio-Rad Laboratories).

Real-time quantitative polymerase chain reaction for COX-2 and GAPDH as the housekeeping gene was performed using the ABI PRISM 7700 Sequence Detection System (Applied Biosystems). Amplification was carried out in a reaction volume (27.5 μl) that, in addition to the cDNA solution (2.5 μl) from the RT reaction and water, contained 2× TaqMan Mastermix (Applied Biosystems), COX-2 (900 nM) or GAPDH (50 nM) primer, and probe (200 nM). Amplification was performed in MicroAmp optical tubes using the standard amplification protocol recommended by the manufacturer (2 min at 50°C, 10 min at 95°C, 40 cycles of 15 s at 95°C, and 1 min at 60°C). Recorded C_T (average cycle threshold) values for COX-2 were normalized with corresponding C_T values for GAPDH mRNA, resulting in values termed ΔC_T. The average ΔC_T from melanoma cells was subtracted from the ΔC_T values of normal cells, resulting in $\Delta\Delta C_T$ values, which were used to calculate fold difference in COX-2 expression by the formula $F = 2^{\Delta\Delta C_T}$.

PGE_2 EIAs

70W cells were grown to 70% confluence in tissue culture dishes (100 mm). After a 24 h incubation with serum-free DMEM/F-12 medium, cells were incubated with various concentrations of AA, EPA, or DHA in DMEM and 0.1% BSA for 24 h. Aliquots of the conditioned medium were collected and stored at −80°C until assayed. EIAs were performed using the conditioned media and the PGE_2 EIA kit (Cayman Chemical, Inc.) as recommended by the manufacturer. PGE_2 levels are expressed as picograms per cell number.

Western blot analyses

Cells to be analyzed for COX-2 protein expression were plated on 100 mm dishes and incubated in serum-free medium at 37°C for 24 h. Cells were then incubated with COX-2 and with various concentrations of AA, EPA, or DHA in DMEM and 0.1% BSA. Twenty-four hours later, they were released using trypsin, centrifuged at 300 rpm for 5 min, and resuspended in lysis buffer [TBS (pH 7.4) containing Triton X-100 (0.5%), leupeptin (10 μg/ml), pepstatin (10 μg/ml), and PMSF (0.2 mM)]. Cells were vortexed and lysed on ice for 10 min. They were then centrifuged for 10 min at 13,000 rpm at 4°C, and the supernatant was collected. Protein concentration was determined, and protein samples (40–60 μg) were heated to 100°C for 5 min with Laemmli sample buffer (13) and separated on a 10% Criterion gel (Tris-HCl; Bio-Rad Laboratories). Gels were transferred to a polyvinylidene difluoride (PVDF) membrane (Pierce Endogen, Inc., Rockford, IL) and incubated for 1 h in a blocking reagent [3% (w/v) nonfat dry milk, 0.5% (w/v) BSA, and 0.3% (v/v) Tween 20 in PBS, pH 7.5]. COX-2 was labeled using an anti-COX-2 antibody (1:500 dilution; Santa Cruz Biotechnology, Inc.). Membranes were incubated with either antibody in 3% (w/v) nonfat dry milk and 0.5% (w/v) BSA for 18 h. They were then washed with 0.5% IGEPAL (CA-630; Sigma-Aldrich) in Tris buffer (20 mM Tris and 150 mM NaCl, pH 7.4) for 1 h, changing the solution six to eight times. Polyvinylidene difluoride membranes were then incubated with secondary antibodies in 3% (w/v) nonfat dry milk and 0.5% (w/v) BSA for 30 min. Goat anti-mouse HRP antibody was used for COX-2 (1:10,000 dilution; Bio-Rad Laboratories). Membranes were washed and developed using the Super-signal West Femto Maximum Sensitivity Substrate (Pierce Endogen). Labeling was detected using the Versadoc imaging system (Bio-Rad Laboratories).

Matrigel™ invasion assays

Cell invasion (72 h) was assayed using Transwell cell culture chambers (12 μm pore size, 12 mm diameter; Corning Incorporated Life Sciences) coated with artificial basement membrane or Matrigel™ (diluted 1:30 with cold DMEM/F-12, 100 μl final coating volume) as described previously (14). To quantify cell in-

vasion, noninvasive cells were removed from the upper chamber using cotton swabs, and invasive cells present on filters in the lower chambers were visualized using the Diff-Quick Stain Kit (Kokusai Shiyaku, Kobe, Japan) according to the manufacturer's instructions, photographed with a Sony digital camera at fixed focus (Sony, Inc., Tokyo, Japan) using a Zeiss Axiovert 25 microscope (Zeiss Microimaging, Inc., Thornwood, NY), and counted.

Statistical analysis

Comparisons between groups were made using the Student's *t*-test. A difference between treatment groups of $P < 0.05$ was considered significant.

RESULTS

COX-2 mRNA expression and in vitro invasion in brain-metastatic melanoma

To determine whether in vitro invasion correlated with COX-2 expression (15), we compared the highly aggressive brain-metastatic melanoma 70W cell line (16, 17) with its parental non-brain-metastatic MeWo counterpart. COX-2 mRNA expression from these cells revealed that the *cox-2* gene is highly expressed in the 70W line compared with MeWo (**Fig. 1A**).

To assess whether COX-2 mRNA expression in 70W cells was regulated by TNF-α, a known upregulator of COX-2 in other cell types (7), we incubated the cells with a low (2 ng/ml) or high (20 ng/ml) concentration of TNF-α for 24 h. Compared with no TNF-α-exposed cells, RT-PCR of 70W mRNA indicated that COX-2 expression is upregulated by high concentrations but not by low concentrations of TNF-α (Fig. 1B).

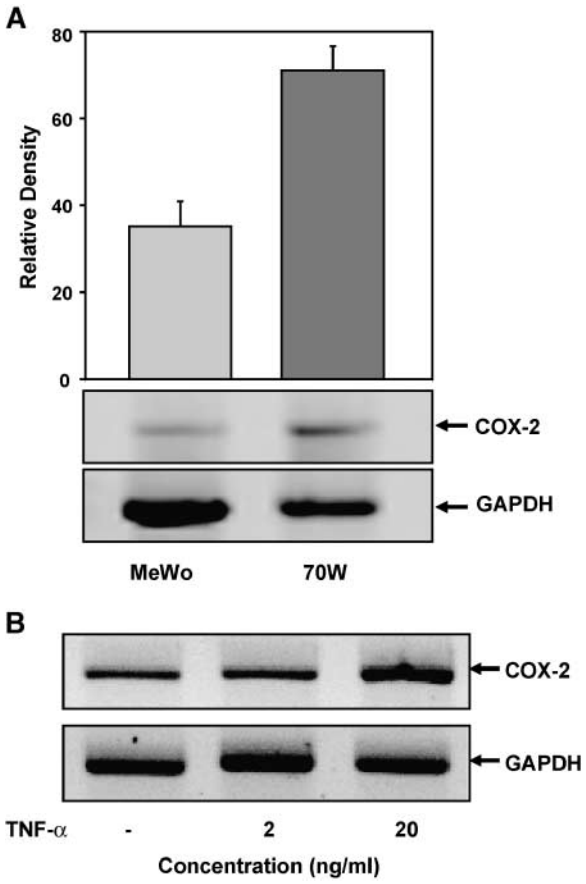

Fig. 1. Cyclooxygenase-2 (COX-2) mRNA expression in brain-metastatic melanoma. Comparison of COX-2 mRNA expression in nonmetastatic MeWo cells and highly metastatic 70W cells (A) and further induction of COX-2 mRNA expression by tumor necrosis factor-α (TNF-α) in 70W cells (B). Serum-deprived 70W cells (lane 1) were incubated with TNF-α (2 ng/ml, lane 2 or 20 ng/ml, lane 3) for 24 h. Results of a reverse transcriptase PCR dose-response assay of a 70W mRNA preparation showed that a 24 h incubation of cells yielded a significant increase in COX-2 expression with high-dose TNF-α. Error bars represent standard deviation.

Prostaglandin production is regulated by TNF-α and NS-398 in brain-metastatic melanoma

To evaluate the involvement of COX-2 in the production of prostaglandins in brain-metastatic 70W cells, cells were incubated with or without NS-398 (50 μM), a specific COX-2 inhibitor (15), in the presence of either a low or a high dose of TNF-α (2 or 20 ng/ml, respectively). PGE_2 (19.8 ng/ml) produced by 70W cells alone was decreased by 38% (PGE_2 produced was 12.2 ng/ml) with the addition of NS-398 (**Fig. 2**). TNF-α stimulated 70W cells in a dose-dependent manner, resulting in 7- and 30-fold increases in PGE_2 production in low versus high TNF-α-stimulated cells, respectively. When incubated with NS-398, production of PGE_2 decreased in low versus high TNF-α-exposed cells by 91.2% and 98.8%, respectively (Fig. 2).

Western blot analysis showed that TNF-α upregulated COX-2 protein levels in 70W cells compared with controls (**Fig. 3**). NS-398 is known to act directly on the protein to inhibit COX-2 enzyme activity (18). Our Western blot analysis demonstrated that NS-398 does not reduce protein levels in either control or TNF-α-stimulated cells. Altogether, this indicated that the increase in COX-2 message generated by incubation with TNF-α correlated with increased enzyme levels and subsequent augmentation of PGE_2 production. Moreover, reduced PGE_2 production after incubation with NS-398 demonstrated that the upregulation of COX-2 by TNF-α produced functional enzyme in these cells.

COX-2 mRNA is modulated by ω-3 and ω-6 PUFAs in brain-metastatic melanoma

Regulation of COX-2 mRNA expression occurs in a number of tumor types incubated with PUFAs (18). To determine the effect of ω-3 and ω-6 PUFAs on COX-2 mRNA expression, 70W cells, preincubated with serum-free medium, were subsequently exposed to AA, EPA, or DHA (50 μM each) for 24 h. COX-2 mRNA expression increased significantly above control levels in cells incubated with AA (**Fig. 4**). Conversely, COX-2 mRNA expression decreased in cells incubated with EPA or DHA (50 μM each; Fig. 4). These data show that COX-2 expression may be regulated at the gene level by ω-3 and ω-6 PUFAs. Furthermore, quantitative PCR analysis of the cDNA revealed that the relative copy number of mRNA from COX-2 increased ~3-fold in cells incubated with AA compared with control cells and in cells incubated with EPA or DHA (data not shown).

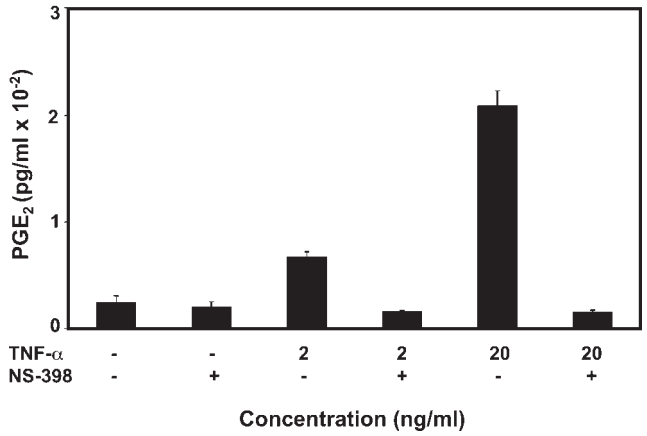

Fig. 2. Prostaglandin E_2 (PGE_2) production following incubation with TNF-α or *N*-(2-cyclohexyloxy-4-nitrophenyl)methanesulfonamide (NS-398) in 70W cells. Comparison of 24 h PGE_2 production of serum-starved 70W cells subsequently incubated with TNF-α (2 or 20 ng/ml) with PGE_2 production of 70W cells incubated with NS-398 (50 μM) with and without TNF-α (2 or 20 ng/ml). The positive control was serum-starved cells, and the negative control was serum-starved cells incubated with NS-398. Results show that TNF-α upregulates COX-2 production of PGE_2 in 70W cells. Exposure to NS-398 selectively inhibits PGE_2 production at both TNF-α concentrations. Error bars represent standard deviation.

COX-2-mediated PGE_2 and PGE_3 production in PUFA-exposed 70W cells

Both ω-3 and ω-6 PUFAs serve as substrates for COX-2 (19). The use of the ω-3 PUFA EPA produces PGE_3, whereas the use of the ω-6 PUFA AA as a substrate produces PGE_2. The anticancer effects of ω-3 PUFAs have been linked to the inhibition of COX-2 activity and, consequently, to reduced levels of PGE_2 production both in vivo and in vitro (20, 21). Examination of media from cells incubated for 24 h with AA, EPA, or DHA for the presence of PGE_2 showed a correlative increase in COX-2 activity. Cells preincubated with AA had more than a 50-fold increase in PGE_2 activity compared with control cells ($P = 0.007$) (**Fig. 5**). This increase was 12 and 22 times greater than

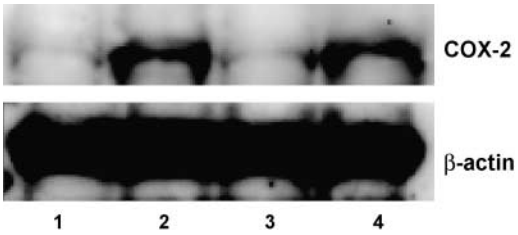

Fig. 3. Expression of COX-2 protein in 70W cells after TNF-α and NS-398 exposure. Serum-starved 70W cells (control; lane 1) were incubated with TNF-α (20 nM; lane 2), NS-398 (50 μM; lane 3), or TNF-α and NS-398 (20 nM and 50 μM, respectively; lane 4) for 24 h as reported in Materials and Methods. Western blot analysis was performed as described (see Materials and Methods). COX-2 protein expression of cells exposed to TNF-α was significantly greater than in both control cells and cells exposed to NS-398. NS-398 did not reduce protein levels in either control or TNF-α-stimulated cells. Expression of β-actin was used for the standardization of protein levels. Results from one of three independent experiments are shown.

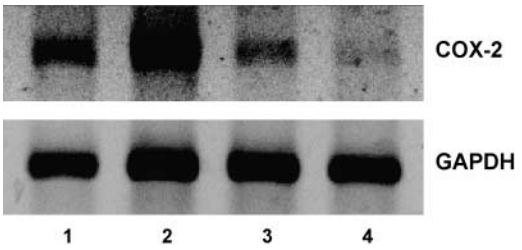

Fig. 4. Expression of COX-2 mRNA in 70W cells after arachidonic acid (AA), eicosapentaenoic acid (EPA), or docosahexaenoic acid (DHA) exposure. Serum-starved 70W cells (control; lane 1) were incubated with AA (lane 2), EPA (lane 3), or DHA (lane 4) (50 μM each) for 24 h as reported in Materials and Methods. mRNA was extracted as described (see Materials and Methods). COX-2 mRNA expression of cells exposed to AA was significantly greater than in both control cells and cells exposed to EPA or DHA. COX-2 expression was downregulated in 70W cells exposed to DHA. Expression of GAPDH was used for the standardization of mRNA levels. Results from one of three independent experiments are shown.

PGE_2 production in EPA and DHA, respectively ($P = 0.009$ and 0.001, respectively) (Fig. 5). These results suggested that PGE_2 was being synthesized in cells exposed to ω-6 PUFAs but not in those exposed to ω-3 PUFAs.

PUFA exposure of 70W cells and invasion

To investigate the effect of ω-3 and ω-6 PUFAs on invasion, we preincubated 70W cells with AA, EPA, or DHA (50 μM for 24 h) before and during an invasion assay (72 h) using Matrigel™ as a coating barrier. Incubation with AA resulted in a significant increase ($P = 0.011$) in invasion compared with controls and cells preincubated with EPA or DHA (50 μM each) (**Fig. 6**).

Finally, to determine the role of PGE_2 and PGE_3 in invasion, 70W cells were exposed to a range of concentrations

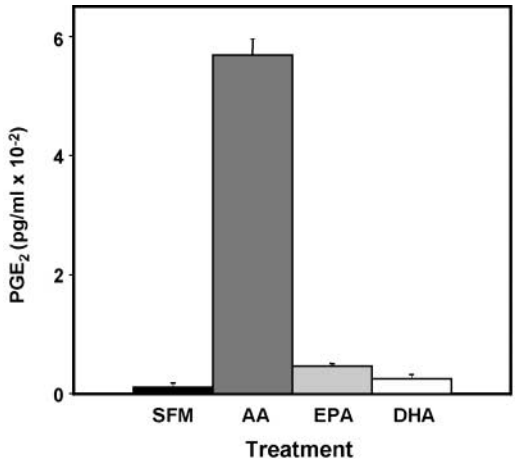

Fig. 5. COX-2 PGE_2 production in PUFA-exposed 70W cells. 70W cells were incubated for 24 h in serum-free medium (SFM) and then incubated with AA, EPA, or DHA (50 μM each) for 24 h. Medium from cells was examined for the presence of PGE_2 as described in Materials and Methods. Incubation of 70W cells with AA resulted in a 50-fold increase compared with control cells ($P = 0.007$). The data shown are representative of three independent experiments. Error bars represent standard deviation.

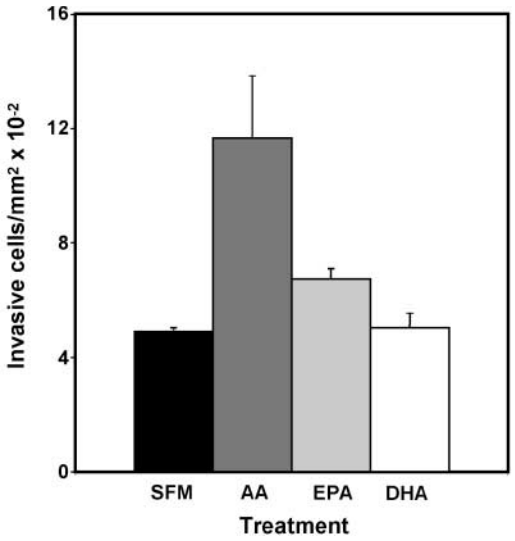

Fig. 6. In vitro invasion by 70W cells after exposure to AA, EPA, or DHA. Effects of AA, EPA, or DHA (50 μM each) on the in vitro invasion of 70W cells were assayed using Transwell cell culture chambers coated with Matrigel™ (see Materials and Methods). Cells were incubated with the fatty acids 24 h before and during a 72 h incubation period on Transwell plates. Invasive cells were visualized using the Diff-Quick Stain Kit according to the manufacturer's instructions (Kokusai Shiyaku). Results show that incubation of 70W cells with AA increased invasion significantly more (P = 0.019) than did either EPA or DHA exposure (P = 0.38 and 0.94, respectively). SFM, serum-free medium. Error bars represent standard deviation.

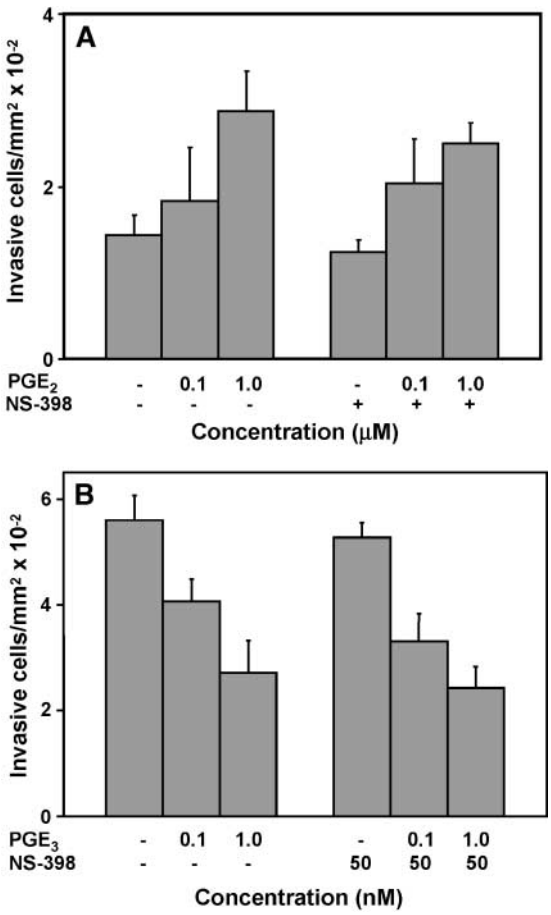

Fig. 7. Effects of PGE_2 and PGE_3 on Matrigel™ invasion of 70W cells. Effects of 0.1 or 1.0 nM PGE_2 (A) and 0.1 or 1.0 μM PGE_3 (B) both with and without NS-398 (50 μM) on invasion of 70W cells were assayed in vitro using Transwell cell culture chambers coated with Matrigel™ (see Materials and Methods). Cells treated with prostaglandins with or without NS-398 were incubated for 60 h at 37°C. Invasive cells were visualized using the Diff-Quick Stain Kit according to the manufacturer's instructions. Results show that PGE_2 significantly increased invasion of 70W cells in a dose-dependent manner (P = 0.005 and 0.001), whereas PGE_3 significantly decreased invasion in a dose-dependent manner (P = 0.021 and 0.029). Results also show that incubation with NS-398 did not change invasion of PGE_2- or PGE_3-treated cells. Furthermore, PGE_2 was more effective in affecting invasion than PGE_3 by an order of magnitude (nanometer versus micrometer). The data shown are representative of three independent experiments. Error bars represent standard deviation.

of PGE_2 or PGE_3 during invasion assays (**Fig. 7**). Incubation with PGE_2 significantly increased the invasion of 70W cells (Fig. 7A), whereas incubation with PGE_3 significantly decreased their invasive values (Fig. 7B). Prostaglandin-treated cells incubated with NS-398 showed no significant difference of invasion (Fig. 7).

DISCUSSION

The present work provides first-time evidence that in human brain-metastatic melanoma cells *1*) PGE_3 decreases in vitro invasion, *2*) AA increases in vitro invasion but EPA and DHA do not, and *3*) ω-6 PUFAs increase mRNA expression of COX-2, whereas ω-3 PUFAs do not. Inducible COX-2 has been associated with poor prognosis in a variety of tumor types. Its role in tumor progression has been shown to include increased proliferation (22), inhibition of immunosurveillance and apoptosis (23), and angiogenesis stimulation (24). In the present report, we show that in brain-metastatic melanoma cells (70W), COX-2 expression is modulated by AA much the same as by TNF-α. These cells produced PGE_2 upon exposure to both stimulants (Figs. 2, 5).

That melanoma cells constitutively express COX-2 was first shown by Denkert et al. (2). Those investigators used NS-398, a specific COX-2 protein inhibitor, to demonstrate a significant reduction in PGE_2 production and Matrigel™ invasion in five malignant melanoma cell lines, concluding that COX-2 was involved in tumor cell invasion. In our cell system, COX-2 mRNA expression was similarly downregulated by NS-398, even when the message was upregulated by TNF-α. NS-398 has been shown to work at the protein level to decrease the production of PGE_2 (7). Even though TNF-α increased COX-2 message and PGE_2 production, the addition of NS-398 in 70W cells decreased prostaglandin production. This suggests that they produce functional COX-2 enzyme after TNF-α incubation.

The effect of ω-3 PUFAs on melanoma cells was first addressed by Royce and Martin (25), who demonstrated a dose- and time-dependent decrease in invasiveness and in collagenase IV production after cell treatment with EPA. These investigators also proved that a metabolite of EPA

was less potent than the comparable AA metabolite in restoring collagenase IV production and invasiveness after inhibition of the lipoxygenase pathway (25). Exposure of 70W cells to AA modulated mRNA and PGE_2 expression much as did exposure of cells to TNF-α. Conversely, exposure of cells to DHA or EPA downregulated COX-2 expression and showed trends toward decreasing PGE_2 production, much as did NS-398. This suggests that there are differences in the ω-3 and ω-6 PUFA effects on translation and that there is COX-2 gene inhibition by ω-3 PUFAs.

Downregulation of COX-2 is known to decrease in vitro tumor cell invasion (9–11, 26). We have shown that exposure of 70W cells to ω-3 PUFAs (EPA and DHA) decreased invasion through Matrigel™, whereas incubation with the ω-6 PUFA AA increased it. Moreover, PGE_3 diminished COX-2 mRNA expression (data not shown) and 70W invasiveness through Matrigel™, whereas PGE_2 augmented it. The fact that there was no difference in invasive values after the addition of the COX-2 inhibitor NS-398 demonstrated that exogenously added prostaglandins were responsible for these changes. Others (27) have shown that the 2-series prostaglandins increase the proliferation and invasiveness of cancer cells. However, our laboratory has demonstrated for the first time a direct effect of PGE_3 on the in vitro invasion of cancer cells. Such PGE_3-modulated inhibition of in vitro invasion in human brain-metastatic melanoma cells suggests a mechanism by which in vitro invasion may be manipulated in vivo by dietary ω-3 PUFAs. To this end, several experimental and animal studies have shown that dietary ω-3 PUFA intake is inversely proportional to cancer risk factors (18), with mounting evidence demonstrating inhibitory effects of dietary ω-3 PUFAs on the promotion and progression of carcinogenesis (18, 27, 28). A number of mechanisms have been proposed for the roles of ω-3 PUFAs in reducing cancer risk factors, which include repression of the AA-derived eicosanoid biosynthetic pathway and its effect on the immune response to cancer cells. These mechanisms include modulation of inflammation, cell proliferation, metastasis, and angiogenesis; influence on transcription factor activity, signal transduction, and gene expression (which involves metabolic changes in cell growth and differentiation); and modulation of free radical production. Although there are other possibilities for a reduction of mRNA in ω-3-treated cells, such as message instability, our work supports the theory that the ω-3 PUFA mechanism of action is on gene expression, affecting metabolic changes that, in turn, modulate invasion. This suggests concrete mechanisms by which ω-3 PUFAs could affect carcinogenic risk. Although the relationship between dietary ω-3 PUFAs and carcinogenic risk factors is inconclusive, the overwhelming evidence offered by our work and the investigative work of others suggests that increasing dietary ω-3 PUFAs is beneficial. Experiments are under way in our laboratory to analyze the in vivo mechanisms of these benefits of ω-3 PUFAs.

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