Selective Nonsteroidal Anti-Inflammatory Drugs Induce Thymosin β-4 and Alter Actin Cytoskeletal Organization in Human Colorectal Cancer Cells

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ABSTRACT

Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used for their anti-inflammatory effects and have been shown to have chemopreventive effects as well. NSAIDs inhibit cyclooxygenase (COX) activity to exert their anti-inflammatory effects, but it is not clear whether their antitumorigenic ability is through COX inhibition. Using subtractive hybridization, we previously identified a novel member of the transforming growth factor-β superfamily that has antitumorigenic activity from indomethacin-treated HCT-116 human colorectal cancer cells. On further investigation of this library, we now report the identification of a new cDNA corresponding to the thymosin β-4 gene. Thymosin β-4 is a small peptide that is known for its actin-sequestering function, and it is associated with the induction of angiogenesis, accelerated wound healing, and metastatic potential of tumor cells. However, only selective NSAIDs induce thymosin β-4 expression in a time- and concentration-dependent manner. For example, indomethacin and SC-560 [5-(4-chlorophenyl)-1-(4-methoxyphenyl)-3-(trifluoromethyl)-1H-pyrazole] induce thymosin β-4 expression whereas sulindac sulfide does not. We show that selective NSAIDs induce actin cytoskeletal reorganization, a precursory step to many dynamic processes regulating growth and motility including tumorigenesis. This is the first report to link thymosin β-4 induction with NSAIDs. These data suggest that NSAIDs alter the expression of a diverse number of genes and provide new insights into the chemopreventive and biological activity of these drugs.

Nonsteroidal anti-inflammatory drugs (NSAIDs) are most commonly used to treat various inflammatory diseases. NSAIDs are often exploited for their analgesic effects in alleviating swelling, redness, pain of inflammation, fever, and headache. The potent anti-inflammatory action of NSAIDs is widely known to be its inhibition of the cyclooxygenase (COX) enzymes, which are responsible for synthesizing prostaglandins from arachidonic acid, causing inflammation (Hinz and Brune, 2002). Epidemiological studies, animal studies, and in vitro studies involving human colorectal cancer cells indicate NSAIDs possess antitumorigenic activity in colorectal cancer, and to a lesser extent, breast and esophageal cancer (Thun et al., 1993; Taketo, 1998; Gwyn and Sinicrope, 2002; Thun, 2003). Although some data link NSAID chemopreventive activity to COX inhibition (Watson, 1998), other data indicate that such activity may be COX-independent. Early hypotheses centered on COX-2 inhibitory mechanisms. As these mechanisms were investigated, increasing evidence indicated possible prostaglandin-independent pathways, particularly with respect to the induction of apoptosis. For instance, inhibition of COX by NSAIDs may increase the cellular pool of free arachidonic acid by preventing its use as a substrate for prostaglandin synthesis, resulting in the induction of apoptosis (Chan et al., 1998; Cao et al., 2000). Furthermore, it has been shown that the R-enantiomer of the NSAID flurbiprofen, which does not inhibit COX, possesses chemopreventive activity in a mouse model of intestinal polyposis and prostate cancer (Wechter et al., 1997, 2000). Non-COX-expressing colorectal cancer cells have been shown to undergo NSAID-induced apoptosis (Baek et al., 2002; Thun, 2003).
Thus, the proapoptotic activity of NSAIDs may act through both COX-dependent and -independent pathways. Thymosin β-4 is a 4.9-kDa acidic polypeptide found to be ubiquitous in vertebrate cells (Low et al., 1981). Thymosin β-4 was discovered to be a major G-actin binding protein (Safer et al., 1991; Safer and Nachmias, 1994), whose function as a simple passive sequestering protein has been demonstrated in vitro (Sanders et al., 1992) and in vivo (Cassimeris et al., 1992). Thymosin β-4 stimulates tissue remodeling, cell and tissue healing after injury, and cell differentiation by mechanisms that have not been well defined (Grant et al., 1999; Philp et al., 2003). Recently, evidence has been presented implicating thymosin β-4 as a facilitator of tumor metastasis and angiogenesis. Overexpression of thymosin β-4 is associated with an increase in the expression of a known angiogenic factor, vascular endothelial growth factor (Cha et al., 2003). Other studies correlate elevated thymosin β-4 expression with metastasis in colorectal cancer cells (Wang et al., 2003) as well as nonsmall cell lung cancer (Ji et al., 2003). Thus, the expression of thymosin β-4 may stimulate tumor metastasis by activating cell migration and angiogenesis.

Our laboratory has studied NSAID-induced gene expression in cell culture to identify possible COX-independent mechanisms of action (Baek et al., 2001b). Using suppression subtractive hybridization, we recently reported the identification of a cDNA-designated NSAID-activated gene (NAG-1) from an indomethacin-induced library of human colorectal cancer cells devoid of COX activity (Baek et al., 2001a,b). We report another cDNA from the same library, corresponding to thymosin β-4 after treatment with diffuse NSAIDs may help explain the efficacy of some NSAIDs versus others in regard to their chemopreventive effects.

Materials and Methods

Cell Lines and Reagents. Cell lines in this study were purchased from American Type Culture Collection (Manassas, VA). HCT-116, human colorectal carcinoma cells, were maintained in McCoy's 5A medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. Cells were cultured at 37°C under a humidified atmosphere of 5% CO2 in air. PGE2 was purchased from BIOMOL Research Laboratories (Plymouth Meeting, PA). Indomethacin, naproxen, ibuprofen, piroxicam, and diclofenac were purchased from Sigma-Aldrich (St. Louis, MO). Sulindac sulfide, SC-560, and aspirin were purchased from Cayman Chemical (Ann Arbor, MI). DPU [5,5-dimethyl-3-(3-fluorophenyl)-4-(4-methylsulfonyl)phenyl-2(H)-furanone] was obtained from Merck (Whitehouse Station, NJ). All NSAIDs were dissolved in dimethyl sulfoxide (DMSO) and stored at −20°C prior to treatments.

Identification of Thymosin β-4 from an Indomethacin (INDO)-Induced cDNA Library in HCT-116 Cells. Isolation of mRNA from either indomethacin- (100 μM) or vehicle-treated (0.2% DMSO) cells was performed using a poly(A) spin mRNA isolation kit (New England Biolabs, Beverly, MA). INDO+ and INDO− cDNA libraries were constructed using the cDNA subtraction kit (BD Biosciences Clontech, Palo Alto, CA) according to the manufacturer's protocol, as previously described (Baek et al., 2001b). A 170-base pair fragment was isolated from the library and designated as INDO15. Homology searches were carried out using BLAST programs through E-mail servers at the National Center for Biotechnology Information.

The sequence of this fragment matched identically with a sequence in the 3′-untranslated region of thymosin β-4 mRNA (GenBank accession no. M17733). The cDNA corresponding to the full-length mRNA sequence for thymosin β-4 was obtained by reverse transcription-PCR using the sense strand 5′-TCGTAATCTCTGCCTCCCTGCT-TCGGCTTTCC-3′ and the antisense strand 5′-CTGTCGTCGCCCAC-CCCACTTCTTCCACCCAC-5′ primers.

RNA Isolation and Northern Blot Analysis. When 70 to 80% confluence was obtained on 150-mm plates, the cells were treated at indicated concentrations and times with different compounds or DMSO in the absence of serum. Total RNAs were isolated using TRIzol reagents (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. For Northern blot analysis, 10 μg of total RNA was denatured by incubating at 55°C for 15 min and then electrophoresed in a 1.4% agarose gel containing 2.2 M formaldehyde. RNA was subsequently transferred to a Hybond-N membrane (Amersham Biosciences Inc., Piscataway, NJ). After fixing the membrane by UV, the blots were prehybridized in hybridization solution (Rapid-hyb buffer; Amersham Biosciences Inc.) for at least 1 h at 65°C followed by hybridization with cDNA labeled with [α-32P]dCTP by random primer extension (DECAprimeII kit; Ambion, Austin, TX). The probe used was either INDO15 or full-length thymosin β-4 fragment obtained by reverse transcriptase-PCR. After 1 h of incubation at 65°C, the blots were washed once with 1× standard saline sodium citrate/0.1% SDS at room temperature and four times with 0.1× standard saline sodium citrate/0.1% SDS at 65°C. Equivalent loading of RNA samples was confirmed by 18S RNA, and mRNA abundance was estimated by intensities of the hybridization bands of autoradiographs using Scion Image (Scion Corporation, Frederick, MD).

Analysis of Thymosin β-4 Peptide Content by High-Pressure Liquid Chromatography. HCT-116 cells were grown to 80% confluence in 150-mm plates and treated with different compounds or DMSO for 24 h in the absence of serum. Cells were washed one time with cold PBS, scraped, and collected in 20 mM Tris-HCl buffer (pH 7.4) containing protease inhibitors (1 μg/ml leupeptin and pepstatin and 0.5 mM phenylmethylsulfonyl fluoride). Cells were sonicated, and lysates were then centrifuged at 110,000g for 60 min at 4°C. Aliquots (100 μl) of the resulting supernatants were subjected to reverse-phase HPLC to determine cellular thymosin β-4 content. Analysis was performed using a C18 Ultrasound ODS column (5 μm, 4.6 × 250 mm; Beckman Coulter, Fullerton, CA). Solvent A was 0.1% trifluoroacetic acid in water; solvent B was 0.08% trifluoroacetic acid in acetonitrile. The resolving gradient was 10 to 55% B in 12 min at 2 ml/min. Proteins were measured at 220 nm. Thymosin β-4 standard was generously provided by Dr. Ewald Hannappel (University Erlangen-Nuremberg, Erlangen, Germany).

Immunofluorescence. HCT-116 cells were seeded onto polyllysine-coated glass coverslips in 35-mm sterile plastic dishes with 2 ml of medium and 10% serum. After sufficient attachment, cells were treated with various NSAIDs for 24 h in serum-free medium. After treatment, medium was removed and cells were washed twice with PBS. Cells were fixed with 3.5% formaldehyde, then rinsed twice with PBS, and permeabilized by treatment with 0.1% Triton X-100 for 2 min. For actin staining, cells were incubated with 1.5 μM TRITC-labeled phalloidin for 45 min and then washed twice with PBS. Fluorescent images were visualized using a Zeiss confocal microscope at 30× magnification.

Results

Identification of Thymosin β-4 as an Indomethacin-Induced Gene in HCT-116 Cells. To identify and isolate inducible genes by NSAIDs, we performed suppression subtractive hybridization utilizing the human colorectal adenocarcinoma cell line HCT-116, which does not express either COX-1 or COX-2 (Baek et al., 2002b). We have had success using this method to pinpoint altered levels of gene expres-
sion (Baek et al., 2001b). In this report, we have further
analyzed this library and found several additional genes
induced by indomethacin. As shown in Table 1, several
genes were isolated that are induced by indomethacin. Among
those, INDO15 was identified as thymosin β-4 and was of
particular interest because it induces angiogenesis, which
acts against the expected antitumorigenic effect of NSAIDs.
Subsequently, the full-length mRNA of thymosin β-4 was
isolated using reverse transcriptase-PCR as described under
Materials and Methods. As shown in Fig. 1, INDO15 (black
bar) represents a clone from the subtractive library. The open
reading frame encoding the functional 43-amino acid peptide
is shown in the gray bar.

Indomethacin Induces Thymosin β-4 mRNA in HCT-
116 Cells in a Dose- and Time-Dependent Manner. To
confirm our subtractive hybridization data, we measured
thymosin β-4 expression after indomethacin treatment using
Northern analysis. Both dose-response and time course anal-
yses were conducted. The thymosin β-4 mRNA expression
was dependent on indomethacin concentration, with a signif-
ificant increase in expression at 10 μM (Fig. 2A), the highest
increase in expression at 50 μM, followed by a decrease in
expression at 100 μM. Thymosin β-4 expression increased
with duration of indomethacin treatment (100 μM) with a
2-fold increase in expression at 1 h. The highest mRNA
expression was observed after 48 h of treatment (Fig. 2B).
Thus, indomethacin induced thymosin β-4 mRNA in a con-
centration- and time-dependent manner.

Other NSAIDs Affect the Induction of Thymosin β-4
in HCT-116 Cells. After verifying thymosin β-4 induction
via indomethacin treatment, HCT-116 cells were treated
similarly with other NSAIDs to investigate thymosin β-4
induction. The other NSAIDs used were DFU (100 μM),
sulindac sulfide (30 μM), SC-560 (25 μM), and aspirin (1000
μM), all over a 24-h period (Fig. 3). Indomethacin and SC-560
induced thymosin β-4 mRNA expression whereas sulindac
sulfide and aspirin did not affect thymosin β-4 levels. DFU
(100 μM) treatment slightly down-regulated thymosin β-4
expression (data not shown). SC-560 induced thymosin β-4
expression on the order of 3- to 4-fold and is significantly
greater expression compared with indomethacin. We also
examined NAG-1, a known antitumorigenic protein, induced
by some NSAIDs (Baek et al., 2001b). As shown in Fig. 3,
NAG-1 was induced by indomethacin, sulindac sulfide, and
SC-560, which is consistent with previous results (Baek et
al., 2002b). Interestingly, indomethacin and SC-560 induced
both thymosin β-4 and NAG-1 whereas sulindac sulfide in-
duced only NAG-1. Other NSAIDs (ibuprofen, diclofenac, pi-
roxicam, and naproxen) were also examined for thymosin β-4
induction. It was found that ibuprofen significantly induced
thymosin β-4 expression (2-fold) but not other NSAIDs (data
not shown).

PGE2 Effect on Thymosin β-4 Expression. The involve-
ment of prostaglandins in the development of human cancer
has been known for long time. PGE2 is the major arachidonic
acid metabolite of the COX pathway. We treated HCT-116
cells with PGE2 and examined thymosin β-4 expression. As
shown in Fig. 4, Northern analysis revealed that treatment
with a broad range of PGE2 concentrations did not alter
thymosin β-4 expression, providing evidence that thymosin
β-4 expression is prostaglandin independent. Next, different
concentrations of PGE2 were added to INDO-treated-HCT-
116 cells to examine if thymosin β-4 expression is altered in
the presence of PGE2. As shown in Fig. 4, thymosin β-4 is still
induced by indomethacin, suggesting thymosin β-4 induction
by NSAIDs is prostaglandin independent. We have previ-
ously shown that NAG-1 is not induced at this concentration
of PGE2 (Baek et al., 2002b). Taken together with previous
data, PGE2 did not alter thymosin β-4 or NAG-1 expression
in HCT-116 cells.

TABLE 1
Indo-induced genes from HCT-116 cells
 Messenger RNAs were isolated from INDO-treated (100 μM) or vehicle-treated (0.2% DMSO) HCT-116 cells. Suppression subtractive hybridization was used to construct the INDO(+) and INDO(−) libraries. The clone “INDO15” is homologous with the sequence for human thymosin β-4.

<table>
<thead>
<tr>
<th>Clones</th>
<th>Size (bp)</th>
<th>Highly Homologous Sequences (GenBank Accession No.)</th>
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<tbody>
<tr>
<td>INDO12</td>
<td>300</td>
<td>Unknown</td>
</tr>
<tr>
<td>INDO13</td>
<td>250</td>
<td>Unknown</td>
</tr>
<tr>
<td>INDO14</td>
<td>500</td>
<td>Human bHLHZip transcription factor (XM_032817.3)</td>
</tr>
<tr>
<td>INDO15</td>
<td>170</td>
<td>Human thymosin β4 (BC022857.1)</td>
</tr>
<tr>
<td>INDO18</td>
<td>600</td>
<td>Human bromodomain-containing 7 (XM_003122.6)</td>
</tr>
<tr>
<td>INDO22</td>
<td>250</td>
<td>Unknown</td>
</tr>
<tr>
<td>INDO27</td>
<td>195</td>
<td>Human hypothetical protein FLJ20546 (XM_041599)</td>
</tr>
<tr>
<td>INDO28</td>
<td>314</td>
<td>Human cytochrome c oxidase subunit IV (BC021236)</td>
</tr>
<tr>
<td>INDO29</td>
<td>190</td>
<td>TGF-β (U18242): NAG-1</td>
</tr>
<tr>
<td>INDO33</td>
<td>320</td>
<td>Human heat shock cognate 70 (Y09371)</td>
</tr>
<tr>
<td>INDO34</td>
<td>250</td>
<td>Unknown</td>
</tr>
<tr>
<td>INDO44</td>
<td>180</td>
<td>Unknown</td>
</tr>
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bp, base pairs.

Fig. 1. Schematic diagram for full-length thymosin β-4 mRNA. The gray bar indicates the open reading frame encoding the functional 43-amino acid peptide. The black bar labeled INDO15 indicates the fragment identified by subtractive hybridization from HCT-116 cells.
Indomethacin and SC-560 Induces Thymosin β-4 Peptide in HCT-116 Cells. Upon verifying the up-regulation of thymosin β-4 mRNA by selective NSAIDs, it was now of interest to determine if these NSAIDs had similar effects on thymosin β-4 protein levels. Due to the lack of a suitable antibody for reliable Western analysis, HPLC analysis was used to detect the presence of thymosin β-4 peptide. HCT-116 cells were treated with indomethacin (100 μM), SC-560 (25 μM), and vehicle (DMSO) for 24 h. The cells were lysed and ultracentrifuged. The supernatant was isolated and subjected to HPLC analysis. Thymosin β-4 standard was used to determine the retention time of approximately 8.1 min. Indomethacin-treated cells showed approximately 1.5-fold induction of thymosin β-4 peptide over vehicle levels (Fig. 5) whereas SC-560 treatment showed an induction of approximately 2.2-fold over vehicle peptide levels. These data are consistent with the levels of thymosin β-4 induction shown in our Northern analysis (Fig. 3).

NSAID Treatment Alters Actin Cytoskeleton Organization in HCT-116 Cells. Since thymosin β-4 is known to participate in the organization of the actin skeleton (Otto et al., 2002), we investigated the effects of NSAID treatment on the actin skeleton. HCT-116 cells were treated with two different NSAIDs, DFU and SC-560, the former of which slightly down-regulates thymosin β-4 (data not shown), and the latter of which significantly up-regulates both thymosin β-4 mRNA levels and peptide levels. Cells were stained for actin to visualize filaments and detect possible differences that may correlate with the observed changes in thymosin β-4 levels. SC-560-treated cells showed almost completely diminished actin staining in the cytoplasm of the cells whereas there was greater intensity of staining in the plasma membrane region of the cells compared with vehicle. DFU

Fig. 2. Northern analysis of thymosin β-4 expression in indomethacin-treated HCT-116 cells. A, dose response of thymosin β-4 expression. HCT-116 cells were treated with varying doses of indomethacin for 24 h. B, time point Northern analysis. HCT-116 cells were treated with 100 μM indomethacin for various time points. In both A and B, total RNA (10 μg) was loaded in each lane, transferred onto a nylon membrane, and hybridized with a thymosin β-4 probe. Equal amounts of total RNAs were shown in the bottom.

Fig. 3. Northern analysis of thymosin β-4 and NAG-1 expression in HCT-116 cells after treatment with various NSAIDs. HCT-116 cells were grown to 80% confluence in 10-cm plates and treated with either vehicle (0.2% DMSO) or NSAIDs in serum-free medium at the indicated concentration. Isolated RNAs were electrophoresed on a 1.4% agarose gel and transferred to a nitrocellulose membrane. Full-length mRNA sequences for thymosin β-4 and NAG-1 were used as probes.

Fig. 4. Effect of PGE2 on thymosin β-4 expression. HCT-116 cells were treated with various concentrations of PGE2 or a combination of PGE2 and indomethacin. Northern analysis was performed, and thymosin β-4 full-length cDNA was used for probe. Equal amounts of total RNAs were shown in the bottom.
appeared to increase actin staining as predicted since DFU appears to down-regulate thymosin \( \beta-4 \) expression. The diminished staining in the cytoplasm of the cells with SC-560 may be attributed to higher levels of thymosin \( \beta-4 \) (Fig. 6).

**Discussion**

Although NSAIDs are inhibitors of COX, one additional mechanism by which these drugs elicit their pharmacological and toxicological responses may be by altering gene expression. Our laboratory has used subtractive hybridization and microarray techniques to identify genes altered by COX inhibitors (Baek et al., 2001b; Bottone et al., 2004). Since our major interest was related to the colon cancer prevention activity of NSAIDs, we used human colorectal cells in culture and indomethacin as a model drug to initially identify target genes. Indomethacin altered the expression of a large number of genes, but we chose to initially focus on a member of the TGF-\( \beta \) superfamily, NAG-1, since this protein indicated possible antitumorigenic activity. As a result of our studies, the antitumorigenic activity of this protein and its regulation by COX inhibitors and other anticancer chemicals has been extensively studied by this laboratory (Baek et al., 2001a,b, 2002a,b, 2003, 2004; Bottone et al., 2002; Wilson et al., 2003).

In this report, we have further characterized the genes altered by indomethacin in human colorectal cells and identified thymosin \( \beta-4 \) as a new target for indomethacin.

We present evidence that only selective NSAIDs induce the thymosin \( \beta-4 \), and, in contrast to NAG-1, thymosin \( \beta-4 \) appears to be a protumorigenic gene. Thymosin \( \beta-4 \) is a 43-amino acid peptide mainly known for its actin monomer-sequestering function. Thymosin \( \beta-4 \) has recently been associated with angiogenesis (Malinda et al., 1997), accelerated wound healing (Frohm et al., 1996; Malinda et al., 1999), and the metastatic potential of tumor cells (Yamamoto et al., 1993; Clark et al., 2000; Huff et al., 2001; Otto et al., 2002), although little is known about the mechanisms by which thymosin \( \beta-4 \) promotes metastasis. The induction of thymosin \( \beta-4 \) mRNA and peptide by selective NSAIDs may result in altered actin cytoskeleton organization. We utilized TRITC-phalloidin staining of filamentous actin in HCT-116 cells after treatment with various NSAIDs. In DFU-treated HCT-116 cells, a slight down-regulation of thymosin \( \beta-4 \) (data not shown) was observed, and the actin staining was brighter than vehicle-treated cells, suggesting a slight increase in actin concentration due to lower expression of thy-

![Fig. 5. Reverse-phase HPLC analysis for thymosin \( \beta-4 \) in HCT-116 cells treated with NSAIDs. Thymosin \( \beta-4 \) standard was dissolved in 0.1% trifluoroacetic acid in water. HCT-116 cells were treated with indomethacin (100 \( \mu \)M) and SC-560 (25 \( \mu \)M) for 24 h. Lysates were ultracentrifuged, and the supernatant was subjected to HPLC as described under Materials and Methods. Thymosin \( \beta-4 \) standard was used to calculate the retention time of thymosin \( \beta-4 \) (approximately 8.1 min).](image)

![Fig. 6. F-actin staining in HCT-116 cells treated with NSAIDs. HCT-116 cells grown to 50% confluence and treated with vehicle (0.2% DMSO), DFU (100 \( \mu \)M), or SC-560 (25 \( \mu \)M) for a period of 24 h in serum-free medium. The cells were then fixed and stained with TRITC-labeled phalloidin for F-actin staining and observed by confocal laser microscopy at 30× magnification. Hue has been manually changed from red to green for more sensitive visualization, maintaining relative intensities constant. In the changing process, relative staining intensities remain unchanged.](image)
mosin β-4. However, in SC-560-treated cells, in which strong induction of thymosin β-4 is observed, very significant changes in actin expression and localization were detected. There was very little staining throughout the cytoplasm of the cells. Areas near the plasma membrane showed very intense staining. The lack of staining in the body is consistent with the notion that increased levels of thymosin β-4 would sequester a greater amount of actin in its monomeric form. These data support earlier findings of thymosin β-4 expression and F-actin organization (Kobayashi et al., 2002). The intense staining near the plasma membrane is not well understood but suggests that thymosin β-4 plays a role in cell surface dynamics through actin cytoskeleton reorganization. Understanding this role may be central in understanding how thymosin β-4 promotes metastasis.

In our study of thymosin β-4 induction, several NSAIDs of varying structures including indomethacin, sulindac sulfide, aspirin, and SC-560 were examined for induction of thymosin β-4. Indomethacin and SC-560 showed very strong induction of thymosin β-4 whereas sulindac sulfide and aspirin showed very little to no induction. In contrast, indomethacin, sulindac sulfide, SC-560, and aspirin stimulated the expression of NAG-1. Thus, the pattern of gene expression by NSAIDs is not the same for all NSAIDs and appears to depend on the NSAID under study. This conclusion may help explain the differences in chemopreventive effectiveness of the different NSAIDs. For example, sulindac sulfide and aspirin are well recognized for their chemopreventive activity with colorectal tumors in several in vivo animal models and are very potent inducers of NAG-1. However, they do not increase the expression of thymosin β-4. Thus, the gene expression profile for sulindac sulfide and aspirin favors antitumorigenic activity. In contrast, SC-560, which also has some controversial chemopreventive properties (Cheuk et al., 2002), increases the expression of both NAG-1 and thymosin β-4. Its effectiveness as a preventive drug could be compromised by the expression of thymosin β-4. Thus, differences in gene expression by NSAIDs may influence the different biological activity and could also contribute to differences in the toxic side effects of the different NSAIDs.

NSAIDs are primarily used for their anti-inflammatory activity, and recent data show that thymosin β-4 also possesses anti-inflammatory effects (Young et al., 1999; Sosne et al., 2002). The induction of thymosin β-4 by selective NSAIDs suggests a possible additional mechanism for NSAID anti-inflammatory activity.

In summary, we present the first report linking thymosin β-4 expression with NSAID treatment. Selective NSAIDs induce thymosin β-4, an actin-sequestering protein that is implicated in angiogenesis and tumor metastasis, and its expression may compromise the chemopreventive activity of NSAIDs mediated by NAG-1 and other genes. We propose that NSAIDs have dual targets in cells. First is the well established inhibition of prostaglandin formation by targeting cyclooxygenases whereas the second target is alteration in gene expression (Baek et al., 2002b). Although we have focused on changes in gene expression that occur independent of COX inhibition, we cannot exclude the possibility that changes in gene expression can also be dependent on the formation of prostaglandins and thereby altered by inhibition of COX activity. By advancing the knowledge of NSAID mechanisms, it is our hope that NSAIDs can be more effectively used as therapeutics.

Acknowledgments

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References


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