Retinoids Suppress Epidermal Growth Factor-induced Transcription of Cyclooxygenase-2 in Human Oral Squamous Carcinoma Cells

Juan R. Mestre, Kotha Subbaramaiah, Peter G. Sacks, Stimson P. Schantz, Tadashi Tanabe, Hiroyasu Inoue, and Andrew J. Dannenberg

Department of Medicine, The New York Hospital-Cornell Medical Center and Anne Fisher Nutrition Center at Strang Cancer Prevention Center, New York, New York 10021 [K.S.; A.J.D.]; Head and Neck Service, Department of Surgery, Memorial Sloan Kettering Cancer Center, New York, New York 10021 [J.R.M., P.G.S., S.P.S.]; and Department of Pharmacology, National Cardiovascular Center Research Institute, Osaka, Japan [T.T., H.I.]

ABSTRACT

Cyclooxygenase-2 (Cox-2), the inducible form of cyclooxygenase, is up-regulated in tumors and transformed cells. Because this enzyme catalyzes the formation of prostaglandins from arachidonic acid, chemopreventive strategies that suppress its expression could be useful for preventing cancer. We investigated whether retinoids suppressed basal expression of Cox-2 or EGF-mediated induction of Cox-2 in human oral squamous carcinoma cells. Treatment with retinoids [all-trans-retinoic acid (all-trans-RA), 9-cis-RA, 13-cis-RA, and retinyl acetate] suppressed both basal levels of Cox-2 and EGF-mediated induction of Cox-2 protein and synthesis of prostaglandin E2. Retinoids also suppressed the induction of Cox-2 mRNA by EGF. Transient transfection experiments showed that EGF caused about a 100% increase in Cox-2 promoter activity, an effect that was suppressed by retinoids. Levels of epidermal growth factor receptor were unaffected by retinoids. Epidermal growth factor caused a nearly 10-fold increase in mitogen-activated protein kinase activity; this effect was not blocked by retinoids.

INTRODUCTION

Increased levels of prostaglandins have been detected in multiple epithelial cancers including those of head and neck origin (1-4). Prostaglandins, such as PGE2, affect cell proliferation and inhibit the immune response to malignant cells; therefore, overproduction of prostaglandins could favor malignant growth (5). Two Cox enzymes designated Cox-1 and Cox-2, encoded by separate genes, catalyze the synthesis of prostaglandins. Cox-1 is thought to be a housekeeping gene with essentially constant levels of expression, whereas Cox-2 is an early-response gene that, like c-fos and c-jun, is induced rapidly by growth factors, tumor promoters, oncogenes, and carcinogens (6-12). The different responses of Cox-1 and Cox-2 reflect, in part, differences in the regulatory elements in the 5'-flanking region of the two genes (13).

Because cyclooxygenases catalyze the formation of prostaglandins from arachidonic acid, compounds that inhibit the activity or expression of Cox should be chemoprotective. Epidemiological studies have shown, in fact, that chronic intake of NSAIDs, which inhibit Cox activity, reduce the incidence of colon cancer by as much as 40%. NSAIDs also are known to protect against mammary, esophageal, oral, and colon cancer in experimental animals (14-18); and sulindac, an NSAID, decreases the number and size of polyps in 6 knockout mice, which is a model for human familial adenomatous polyposis (19). Additional evidence of a critical link between Cox and tumorigenesis was provided recently by Oshima et al. (20), who showed that a Cox-2 null mutation was associated with a marked reduction in the number and size of intestinal polyps in APC-/- knockout mice, which is a model for human familial adenomatous polyposis. Treatment with a novel selective inhibitor of Cox-2 also reduced the number of polyps in APC-/- mice (20).

Deregulated signaling through the EGFR pathway is recognized to be an early event in the development of head and neck cancers (21). EGF, a ligand of the EGFR, induces Cox-2 (22), which may contribute to the increased levels of prostaglandins in head and neck tumors (2). Retinoids, a group of naturally occurring and synthetic analogues of vitamin A, suppress carcinogenesis in various epithelial tissues including the oral cavity (23-25). Because retinoids are chemoprotective and inhibitors of prostaglandin synthesis, e.g., NSAIDs, possess anticancer properties, we wondered whether retinoids would suppress basal expression of Cox-2 or EGF-mediated induction of Cox-2. In the present work, we provide evidence that retinoids inhibit both basal expression of Cox-2 and EGF-mediated induction of Cox-2 transcription and prostaglandin production in human squamous carcinoma cells.

MATERIALS AND METHODS

Materials. DMEM/nutrient mixture F-12 (DMEM/F-12), Opti-MEM, EGF, MCB, and FBS were from Life Technologies, Inc. (Grand Island, NY). Retinoids, sodium arachidonate, Lowry protein assay kits, and monoclonal anti-EGFR (clone F4) were from Sigma Chemical Co. (St. Louis, MO). Enzyme immunoassay reagents for PGE2, assays were from Cayman Co. (Ann Arbor, MI). [32P]CTP and [32P]ATP were from DuPont-NEN (Boston, MA). pFx-3, the cationic lipid used for transfections, was from Invitrogen (San Diego, CA). Random-primer kits were from Boehringer-Mannheim Biochemicals (Indianapolis, IN). Nitrocellulose membranes were from Schleicher & Schuell (Keene, NH). Plasmid DNA was prepared using a Qiagen DNA purification kit (Chatsworth, CA). The 18S rRNA cDNA was from Ambion, Inc. (Austin, TX). Western blotting detection reagents were from Promega Corp. (Madison, WI).

Cell Line. 1483 squamous carcinoma cells have been described previously (26). Cells were maintained in a 1:1 mixture of DMEM/F-12 supplemented with 10% FBS and 50 μg/ml gentamicin. Cells were grown to 70% confluence, trypsinized with 0.05% trypsin-2 mM EDTA, and plated for experimental use in DMEM/F-12 medium without FBS unless stated otherwise. Treatment with vehicle (0.01% DMSO), retinoids, or EGF was always carried out under serum-free conditions.

PGE2 Production. Cells were plated in 100-mm dishes at 106 cells/dish and allowed to attach for 24 h. The DMEM/F-12 medium was then replaced with fresh DMEM/F-12 and retinoids or vehicle (0.01% DMSO). Twenty-four hours later, cells were harvested to assess basal production of PGE2, or the culture medium was replaced with fresh medium with or without EGF (1 ng/ml) for 5 h. Subsequently, lysates were prepared by treating cells with lysis buffer consisting of 150 mM NaCl, 100 mM Tris-buffered saline, 1% Tween 20, 50 mM diethyldithiocarbamate, 1 mM EDTA, and 1 mM phenylmethylsulfonyl fluoride. Lysates were sonicated twice for 20 s on ice and centrifuged at 10,000 × g for 10 min to sediment the particulate material. The protein concentration of the supernatant was measured by the method of Lowry et al. (27). To determine production of PGE2, 10 μg of protein were incubated in 2
ml of HEPES-buffered saline containing 100 μM sodium arachidonate at 37°C for 4 min. This length of treatment led to maximal production of PGE2. Fifty μl were then removed for determination of PGE2 by enzyme immunoassay (11).

MAP Kinase Assay. To assess MAP kinase activity, we performed in-gel assays, where pretranslational proteins were released in gels polymerized in the presence of MBP as a substrate (28). These conditions have been used previously to detect MAP kinase activity (29). Briefly, cells were plated in 100-mm dishes and then treated for 24 h with fresh DMEM/F-12 medium containing vehicle (0.01% DMSO) or retinoids, as described above. To activate MAP kinase, cells were then treated with fresh medium containing EGF (1 ng/ml) for 30 min. Cell lysates were prepared as described above, and samples containing 40 μg of protein were then used for SDS-polyacrylamide (10%) gel electrophoresis under reducing conditions in gels containing 0.5 mg/ml MBP. After electrophoresis, the gel was denatured in 8 M urea in buffer A [50 mM Tris (pH 8) and 5 mM β-mercaptoethanol] and renatured in buffer A and 0.04% Tween 20. The kinase activity was assayed by incubating the gel with 100 μCi of [γ-32P]ATP and 10 μM of unlabeled ATP in 30 mM Tris (pH 7.4), 10 mM MgCl2, and 2 mM MnCl2 for 30 min. The reaction was terminated by washing the gel in a solution containing 1% sodium PP1 and 5% trichloroacetic acid. The gel was subjected to autoradiography. MAP kinase activity was assessed by densitometric measurement of the M, 42,000 band that corresponds to the in-gel phosphorylated MBP substrate for MAP kinases.

Western Blotting. SDS-PAGE was performed under reducing conditions on 10% polyacrylamide gels as described by Laemmli (30). The resolved proteins were transferred onto nitrocellulose sheets as detailed by Towbin et al. (31). The nitrocellulose membrane was then incubated with a rabbit polyclonal anti-Cox-2 antiserum that was raised against the unique 18-amino acid sequence from the COOH-terminal portion of Cox-2 (32). Nitrocellulose membranes were also probed with a polyclonal anti-Cox-1 antiserum that was a generous gift of Dr. Kenneth Wu (University of Texas, Houston, TX) and a mouse monoclonal antibody to EGF. The membranes were subsequently probed with goat anti-rabbit or goat anti-mouse antibodies conjugated to alkaline phosphatase and developed as described previously (11). A computer densitometer (Molecular Dynamics, Sunnyvale, CA) was used to determine the density of the bands.

Northern Blotting. Cells were plated in 100-mm dishes at a density of 2 × 106 cells/dish and allowed to attach for 24 h prior to experiments. To prepare total cellular RNA, cell monolayers were washed and then directly lysed in 4 ml guanidinium isothiocyanate solution. RNA was then isolated by phenol-chloroform extraction according to Chomczynski and Sacchi (33). For Northern blots, 6 μg of total cellular RNA per lane were electrophoresed in formaldehyde-containing 1.2% agarose gels and transferred to nylon-supported membranes. After baking, membranes were prehybridized overnight and then hybridized in a solution containing 50% formamide, 5× sodium chloride-sodium phosphate-EDTA buffer (SSPE), 5× Denhardt’s solution, 0.1% SDS, and 100 μg/ml single-stranded salmon sperm DNA. Hybridization was carried out for 24 h at 42°C with a radiolabeled human Cox-2 cDNA probe. The human Cox-2 CDNA was generously provided by Dr. Stephen Prescott (University of Utah, Salt Lake City, UT). After hybridization, membranes were washed for 20 min at room temperature in 2× SSPE-0.1% SDS, twice for 20 min in the same solution at 55°C, and twice for 20 min in 0.1× SSPE-0.1% SDS at 55°C. Washed membranes were then subjected to autoradiography. To verify equivalency of RNA loading in the different lanes, the blot was stripped of radioactivity and rehybridized to determine the levels of 18S rRNA. Cox-2 and 18S rRNA probes were labeled with [32P]dCTP by random priming. The signal level of the bands for Cox-2 and 18S rRNA was quantified by densitometry. The values for Cox-2 were normalized for the level of 18S rRNA in each lane.

Transient Transfection Assays. 1483 cells were seeded at a density of 8 × 103 cells/well in six-well dishes and grown to 30–40% confluence in DMEM/F-12 containing 10% FBS. For each well, 2 μg of Cox-2 promoter construct (~1432/+59) was transfected into 1483 cells using pFx-3 at a 1:12 ratio of DNA:lipid as per the manufacturer’s instructions. This plasmid contains 1432 bases 5′ of the Cox-2 transcription start site ligated to luciferase (13). After transfection, cells were treated with DMEM/F-12 containing 1 μM retinoid or vehicle (0.01% DMSO). Twenty-four h later, this medium was replaced with DMEM/F-12 with or without EGF (1 ng/ml). Luciferase activity was measured in cellular extract 6 h later.

Luciferase activity was measured as follows. Each well was washed twice with PBS. Two hundred μl of 1× lysis buffer (Analytical Luminescence Laboratories, San Diego, CA) was added to each well for 30 min. Lysate was centrifuged for 5 min at 4°C. The supernatant was used to assay luciferase activity and protein concentration. Luciferase activity was measured using a Monolight 2010 lumimeter (Analytical Luminescence Laboratories) according to the manufacturer’s instructions. Luciferase activities were normalized to protein concentrations.

Statistics. Comparisons between groups were made by the Student’s t test. A difference between groups of P < 0.05 was considered significant.

RESULTS

EGF Induces Cox-2 in Oral Epithelial Cells. We investigated whether EGF could induce Cox-2 mRNA. As shown in Fig. 1, EGF caused a dose-dependent increase in levels of Cox-2 mRNA. Peak induction of Cox-2 mRNA was observed when cells were treated with EGF at 1 ng/ml. Western blotting was done to determine whether EGF also induced Cox-2 protein. In the presence of EGF (1 ng/ml), levels of Cox-2 protein were increased for 4.5–24 h (Fig. 2).

Retinoids Suppress Basal Expression of Cox-2 and EGF-mediated Induction of Cox-2. EGF-mediated induction of Cox-2 is associated with approximately a 100% increase in production of PGE2. This effect of EGF was suppressed by treatment with all-trans-RA, 13-cis-RA, retinol acetate, and 9-cis-RA (Fig. 3). Retinoids also inhibited basal production of PGE2 by about 25% (Fig. 4). To determine whether these differences in production of PGE2 could be related to differences in levels of Cox, Western blotting was done. Fig. 5 shows that EGF up-regulated Cox-2 by about 200%. This effect was suppressed by pretreatment with each of the retinoids. In separate experiments, we showed that retinoids inhibited EGF-mediated induction of Cox-2, even when the two agents were administered simultaneously. Retinoids also down-regulated basal levels of Cox-2 by about 50% (Fig. 6). Retinoids did not affect levels of Cox-1 (data not shown). The magnitude of the retinoid-mediated decrease in basal levels of Cox-2 protein was greater than the decline in production of PGE2. This difference is likely to reflect the contribution of a constant level of Cox-1 to PGE2 synthesis.

Changes in amounts of Cox-2 enzyme could reflect altered protein synthesis or degradation. Northern blotting was done to investigate whether retinoids suppressed EGF-mediated induction of Cox-2 via a pretranslational mechanism. Treatment with EGF increased levels of Cox-2 mRNA. This effect was inhibited by 1 μM retinoids (Fig. 7). Retinoids suppressed EGF-mediated induction of Cox-2 mRNA, even when the retinoids and EGF were administered simultaneously (data not shown). As shown in Fig. 8, concentrations of all-trans-RA ranging from 0.01–10 μM blocked EGF-mediated induction of Cox-2.

![Cox-2](image_url)
RETINOLS INHIBIT CYCLOOXYGENASE-2

The precise mechanism(s) by which this autocrine growth pathway promotes cancer is unclear. Our data show that EGF induces Cox-2, which suggests that activation of the EGFR signaling pathway may contribute to the increased levels of prostaglandins found in head and neck cancers (2).

Induction of Cox-2 can potentially predispose to carcinogenesis via several different mechanisms. In extrahepatic tissues in which cytochrome P-450 content is low, such as those of the head and neck (36), Cox is likely to be important for metabolism of carcinogens. Cox converts a broad array of carcinogens, including benzo(a)pyrene-7,8-diol, to reactive metabolites that form DNA adducts (37, 38). The potential importance of this mechanism is highlighted by the recent observation that benzo(a)pyrene-diol epoxide, a mutagen formed by Cox, causes adducts along exons of the p53 gene that correspond to p53 mutational hotspots in lung cancer (39). The metabolism of carcinogens by Cox may be important, therefore, for understanding cancer progression.

To further define the mechanism by which EGF and retinoids modulated Cox-2 expression, transient transfections were performed using a human Cox-2 promoter construct containing 1432 bases of 5'-flanking region DNA ligated to luciferase. Treatment with EGF led to approximately a 100% increase in Cox-2 promoter activity, an effect that was suppressed by retinoids (Fig. 9).

Western blot analysis was performed on the same cell lysates to determine levels of EGFR (Fig. 10B). Retinoids did not decrease EGFR levels, consistent with the lack of effect on MAP kinase activity.

DISCUSSION

Up-regulation of EGFR and its ligands, e.g., transforming growth factor α, occurs early in the development of head and neck cancers (21, 34, 35). The precise mechanism(s) by which this autocrine growth pathway promotes cancer is unclear. Our data show that EGF induces Cox-2, which suggests that activation of the EGFR signaling pathway may contribute to the increased levels of prostaglandins found in head and neck cancers (2).

Induction of Cox-2 can potentially predispose to carcinogenesis via several different mechanisms. In extrahepatic tissues in which cytochrome P-450 content is low, such as those of the head and neck (36), Cox is likely to be important for metabolism of carcinogens. Cox converts a broad array of carcinogens, including benzo(a)pyrene-7,8-diol, to reactive metabolites that form DNA adducts (37, 38). The potential importance of this mechanism is highlighted by the recent observation that benzo(a)pyrene-diol epoxide, a mutagen formed by Cox, causes adducts along exons of the p53 gene that correspond to p53 mutational hotspots in lung cancer (39). The metabolism of carcinogens by Cox may be important, therefore, for understanding cancer progression.

Fig. 2. EGF causes prolonged induction of Cox-2. 1483 cells were treated with vehicle (0.01% DMSO) or EGF (1 ng/ml) for 4.5 h (Lanes 1 and 2), 8 h (Lanes 3 and 4), 14 h (Lanes 5 and 6), or 24 h (Lanes 7 and 8). Odd-numbered lanes represent controls; even-numbered lanes represent EGF-treated cells. Cellular lysate protein (25 μg/lane) was loaded onto a 10% SDS-polyacrylamide gel, electrophoresed and subsequently transferred onto nitrocellulose. Immunoblots were probed with antibody specific for Cox-2. Densitometry values were as follows: Lane 1, 579; Lane 2, 963; Lane 3, 589; Lane 4, 876; Lane 5, 360; Lane 6, 583; Lane 7, 335; and Lane 8, 618.

Fig. 3. Retinoids suppress EGF-mediated increases in production of PGE2. 1483 cells were treated with 1 μM all-trans-RA (ATRA), 13-cis-RA, retinyl acetate, 9-cis-RA, or vehicle (0.01% DMSO). Twenty-four h later, the medium was replaced with DMEM/F-12 with or without EGF (1 ng/ml) for 5 h. Production of PGE2 was determined by enzyme immunoassay as described in "Materials and Methods." Columns, means; bars, SD; n = 3. *, P < 0.01 versus EGF treatment.

Fig. 4. Retinoids inhibit basal production of PGE2. 1483 cells were treated with 1 μM all-trans-RA (ATRA), 13-cis-RA, retinyl acetate, 9-cis-RA, or vehicle (0.01% DMSO). Twenty-four h later, the cells were harvested, and production of PGE2 was determined by enzyme immunoassay as described in "Materials and Methods." Columns, means; bars, SD; n = 4. **, P < 0.05 compared with control treatment; *, P < 0.01 compared with control treatment.

Fig. 5. Retinoids inhibit EGF-mediated induction of Cox-2. 1483 cells were treated with vehicle (0.01% DMSO; Lanes 2 and 3) or 1 μM all-trans-RA (ATRA), 13-cis-RA, retinyl acetate, 9-cis-RA (Lanes 4–7) for 24 h. The medium was replaced with DMEM/F-12 (Lane 2) or DMEM/F-12 and EGF (1 ng/ml; Lanes 3–7) for 5 h. Lane 1, ovine Cox-2 that was used as a standard. Lysate protein (25 μg/lane) was loaded onto a 10% SDS-polyacrylamide gel, electrophoresed and subsequently transferred onto nitrocellulose. Immunoblots were probed with antibody specific for Cox-2. Results of densitometry expressed in arbitrary units were as follows: Lane 2, 24 ± 11; Lane 3, 73 ± 9; Lane 4, 45 ± 3; Lane 5, 48 ± 6; Lane 6, 42 ± 2; Lane 7, 34 ± 2. Values are means ± SD; n = 3. *, P < 0.01 compared with control treatment; **, P < 0.01 compared with control treatment.
3. 281; Lane 4, 133; Lane 5, 178; and Lane 6, 179.

Cellular RNA was isolated. Each lane contained 6 μg of RNA. The Northern blot was effective than either alone in preventing cancer. Enhance processes such as apoptosis and immune surveillance, which amide gel, electrophoresed, and subsequently transferred onto nitrocellulose. Immunoblotting was probed with antibody specific for Cox-2. Results of densitometry in arbitrary units were as follows: Lane 1, 57 ± 6; Lane 2, 33 ± 3; Lane 3, 36 ± 6; Lane 4, 33 ± 3; and Lane 5, 27 ± 9. Values are means ± SD; n = 3. *P < 0.01 compared with control.

The high incidence of cancer among tobacco smokers. Additionally, Cox may predispose to carcinogenesis via mechanisms other than activation of carcinogens. Prostaglandins formed by Cox impair immune surveillance and the killing of malignant cells (40). Overexpression of Cox-2 inhibits apoptosis (41), which could prolong the survival of cells containing damaged DNA. Consequently, compounds that inhibit Cox may decrease the formation of mutagens and enhance processes such as apoptosis and immune surveillance, which tend to destroy initiated cells. Indeed, it is noteworthy that retinoids and NSAIDs inhibit both Cox-mediated metabolism of arachidonic acid and carcinogenesis.

Cox possesses both cyclooxygenase and peroxidase functions. The peroxidase function contributes to the activation of procarcinogens (38). NSAIDs inhibit the cyclooxygenase but not the peroxidase activity of Cox, which potentially limits the effectiveness of this therapy. Because transcription of Cox-2 is enhanced in transformed cells (11, 42), these cells may synthesize functional enzyme despite NSAID treatment. Our results demonstrate, however, the potential of retinoids to suppress basal levels of Cox-2 and EGF-mediated induction of Cox-2. This is important because agents that down-regulate levels of Cox-2 will inhibit both the peroxidase and Cox activities of the enzyme. In the future, it will be important to determine whether combining agents that suppress the transcription of Cox-2, e.g., retinoid, with agents that inhibit Cox activity, e.g., NSAID, is more effective than either alone in preventing cancer.

Retinoids are effective in treating oral leukoplakia (24) and preventing second primary malignancies in patients with a history of head and neck cancer (25). The basis for these effects is uncertain, although RA down-regulates EGFR and transforming growth factor α (43), inhibits cellular proliferation (44), and induces apoptosis (45). In fact, retinoids were recently reported to induce apoptosis in the 1483 cell line used in this study (45). Cyclooxygenases and their end products inhibit apoptosis (41, 46). The results of this study suggest that one possible way that retinoids induce programmed cell death is by suppressing the expression of Cox-2. Additionally, chronic inflammation increases the risk of epithelial malignancy (47). Our data suggest that retinoids may be anti-inflammatory, at least in part, by suppressing the expression of Cox-2 and prostaglandin synthesis. It is possible, therefore, that the anti-inflammatory properties of retinoids also contribute to their chemopreventive activity.

EGF activates the tyrosine kinase activity of its receptor and initiates a signaling cascade, resulting in the transactivation of target genes. Retinoids down-regulate the expression of certain genes by blocking this signaling pathway. For example, retinoids decrease levels of EGFR and thereby block EGF-mediated transcription of ornithine decarboxylase in keratinocytes (48). In our study, EGF induced a nearly 10-fold increase in MAP kinase activity; this response was not blocked by pretreatment with retinoids. As expected

![Figure 6. Basal levels of Cox-2 are down-regulated by retinoids. 1483 cells were treated with vehicle (0.01% DMSO; Lane 1 and 2) or 1 μM all-trans-RA, 13-cis-RA, retinyl acetate, or 9-cis-RA for 24 h. Lysate protein (25 μg/lane) was loaded onto a 10% SDS-polyacrylamide gel, electrophoresed, and subsequently transferred onto nitrocellulose. Immunoblots were probed with antibody specific for Cox-2. Results of densitometry in arbitrary units were as follows: Lane 1, 57 ± 6; Lane 2, 33 ± 3; Lane 3, 36 ± 6; Lane 4, 33 ± 3; and Lane 5, 27 ± 9. Values are means ± SD; n = 3. *P < 0.01 compared with control.](image1)

![Figure 7. Retinoids inhibit EGF-mediated induction of Cox-2 mRNA. 1483 cells were treated with vehicle (0.01% DMSO; Lanes 1 and 2) or a range of concentrations of all-trans-RA (0.01, 0.1, 1, or 10 μM; Lanes 3-6) for 24 h. The medium was replaced with DMEM/F-12 (Lane 1) or DMEM/F-12 and EGF (1 ng/ml; Lanes 2-6) for 3 h. Total cellular RNA was isolated. Each lane contained 6 μg of RNA. The Northern blot was probed sequentially with probes that recognized Cox-2 mRNA and 18S rRNA. Results of densitometry in arbitrary units were as follows: Lane 1, 33; Lane 2, 128; Lane 3, 531; Lane 4, 33; Lane 5, 100; and Lane 6, 105.](image2)

![Figure 8. All-trans-RA inhibits EGF-mediated induction of Cox-2. 1483 cells were treated with vehicle (0.01% DMSO; Lanes 1 and 2) or a range of concentrations of all-trans-RA (0.01, 0.1, 1, or 10 μM; Lanes 3-6) for 24 h. The medium was replaced with DMEM/F-12 (Lane 1) or DMEM/F-12 and EGF (1 ng/ml; Lanes 2-6) for 3 h. Total cellular RNA was isolated. Each lane contained 6 μg of RNA. The Northern blot was probed sequentially with probes that recognized Cox-2 mRNA and 18S rRNA. Results of densitometry in arbitrary units were as follows: Lane 1, 33; Lane 2, 128; Lane 3, 531; Lane 4, 400; Lane 5, 100; and Lane 6, 105.](image3)
from this result, retinoids failed to decrease levels of EGFR in these same cells. In this context, it is important to point out that the inhibitory effects of retinoids on EGF-mediated induction of Cox-2 occur within 3 h of treatment, whereas down-regulation of EGFR generally requires prolonged treatment (43). An important next step will be to determine the transcription factor(s) and promoter element(s) that mediate Cox-2 induction by EGF. One possible mechanism is that EGF induces Cox-2 expression by modulating AP-1 activity. This idea fits with the previous observation that v-src upregulates Cox-2 expression via activation of AP-1 (49). The finding that EGF caused more than a 10-fold increase in amounts of Cox-2 mRNA but only a doubling of Cox-2 promoter activity raises the possibility that EGF increased the stability of Cox-2 mRNA in addition to activating the transcription of Cox-2.

Retinoids elicit their biological response by binding to two classes of nuclear receptors, RA receptors and retinoid X receptors, both of which are expressed in 1483 cells (50). Upon ligand binding, these nuclear receptors regulate the transcription of genes containing RA-responsive elements or AP-1 sites. Although the Cox-2 promoter does not contain nucleotide sequences resembling RA-responsive elements, it does contain a cyclic AMP response element that is activated by AP-1 factors (49). Retinoids antagonize AP-1-mediated transcription of other genes (51, 52); therefore, it is possible that this is the mechanism by which retinoids inhibit EGF-mediated induction of Cox-2. In support of this idea, RA decreases AP-1 binding activity and inhibits phorbol ester-mediated induction of Cox-2 in head and neck squamous carcinoma cells (53, 54). Experiments with AP-1-selective retinoids (55) should provide further insight into the mechanism by which retinoids suppress Cox-2 expression.

REFERENCES


Retinoids Suppress Epidermal Growth Factor-induced Transcription of Cyclooxygenase-2 in Human Oral Squamous Carcinoma Cells

Juan R. Mestre, Kotha Subbaramaiah, Peter G. Sacks, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/57/14/2890

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.