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Characterization of a 15-Lipoxygenase in Human Breast Carcinoma BT-20 Cells: Stimulation of 13-HODE Formation by TGF_α/EGF

Nagi Reddy, Angela Everhart, Thomas Eling,[1] and Wayne Glasgow[2]
Laboratory of Molecular Biophysics, Eicosanoid Biochemistry Section, NIEHS, P.O. Box 12233, Research Triangle Park, North Carolina 27709

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Epidemiological and experimental data suggest a role for polyunsaturated fatty acids in the etiology of breast cancer. In this report we have studied arachidonic acid and linoleic acid metabolism in the human breast carcinoma cell line BT-20 which overexpresses both EGF receptor and the homologous *erbB-2* oncogene product. EGF and TGF_α stimulated DNA synthesis in these cells which was attenuated by the addition of a lipoxygenase inhibitor, NDGA. The addition of a prostaglandin H synthase inhibitor did not alter DNA synthesis. Analytical studies reveal little arachidonic acid metabolism while linoleic acid was metabolized to 13-hydroxyoctadecadienoic acid (13-HODE). The formation of 13-HODE was inhibited by the addition of NDGA and was dependent on EGF or TGF_α. These results suggest the metabolism of linoleic acid by a n-6 or 15-lipoxygenase regulated by EGF/TGF_α. RT-PCR was used to isolate a clone, and sequenced the cDNA for this enzyme and it was found to be identical to the human 15-lipoxygenase previously characterized from human pulmonary tissue. EGF/TGF_α did not alter the expression of this enzyme suggesting a potential post-translational regulation of activity. This study provides a link between metabolism of linoleic acid and growth factor regulation of cell proliferation in a human breast carcinoma cell line.

Specific metabolites of arachidonic and linoleic acid have been characterized as modulators of growth factor signal transduction in various cell lines. Our laboratory has reported that in BALB/c 3T3 fibroblasts, epidermal growth factor (EGF) stimulates the production of prostaglandin (PG) E_2, an arachidonate metabolite (1), and 13(S)-hydroxyoctadecadienoic acid (HODE), a linoleic acid metabolite (2). In these cells we observed that prostaglandin H synthase inhibitors were only partially effective in blocking EGF-dependent DNA synthesis, but lipoxygenase inhibitors completely blocked the mitogenic response. Treatment of quiescent cells with exogenous 13(S)-HODE resulted in a three to four-fold potentiation of EGF-dependent DNA synthesis (2). In a more extensive study using Syrian hamster embryo (SHE) cells (3), EGF enhanced the metabolism of endogenous as well as exogenous linoleic acid to 13(S)-HODE (4). Based on enzyme inhibitor studies and analytical structural characterizations, the linoleate metabolite appears to be formed by a n-6 lipoxygenase. This SHE cell enzymatic activity is most likely a part of the 15-lipoxygenase family of enzymes described in rabbit reticulocytes and human reticuloyctes, eosinophils, and tracheal and bronchial epithelial cells (5-7). The activity of this presumed 15-lipoxygenase was regulated by the EGF receptor tyrosine kinase activity (4). The addition of 13(S)-HODE or its precursor, 13(S)-hydroperoxyoctadecadienoic acid (HpODE), augmented EGF-dependent DNA synthesis in SHE cells (3). This biological activity was highly selective in that the corresponding metabolites of arachidonic acid such as 15(S)-hydroxyeicosatetraenoic acid (HETE) and 15(S)-hydroperoxyeicosatetraenoic acid (HpETE) were not effective. Moreover, the stereoisomer 13(R)-HODE was essentially inactive in modulating the EGF mitogenic response (8). The results suggest that the formation of 13(S)-HODE/13(S)-HpODE is a component of the EGF signaling pathways and these lipid metabolites can modulate communication between the cell surface EGF receptor with the nucleus.

Overexpression and amplification of EGF receptor (*erbB-1*) and the homologous *c-erbB-2* proto-oncogene product have been identified as adverse prognostic factors in human breast cancer (9,10). Furthermore, lino-

[1] To whom correspondence should be addressed. Fax: 919-541-1460. E-mail: Eling@niehs.nih.gov.
[2] Current address: Division of Basic Medical Sciences, Mercer University School of Medicine, 1550 College Street, Macon, Georgia 31207.

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leic acid, the major polyunsaturated fatty acid consumed by humans, is observed to stimulate tumorigenesis and metastasis in several animal and human model systems of mammary carcinoma (11-13). Many of these effects of linoleic acid in breast carcinoma cell lines can be blocked by lipoxygenase and/or prostaglandin H synthase inhibitors. This raises the possibility that the biological responses of linoleic acid may require metabolism to an active lipid mediator. Based on our findings demonstrating EGF stimulation of 13(S)-HODE production by a 15-lipoxygenase in fibroblast cell lines (2-4), we hypothesized that an analogous metabolic pathway might be operative in some breast carcinoma cell lines. For these investigations we selected the BT-20 human breast carcinoma cell line as these cells express high levels of both EGF receptor and *c-erbB-2*. Our previous work in SHE cells demonstrated the importance of EGF receptor activation in stimulating linoleic acid metabolism.

In the present study, we examined the EGF-dependent metabolism of arachidonic and linoleic acid in the BT-20 cell line. We observed little prostaglandin H synthase metabolism of arachidonic acid or linoleic acid but did observe a 15-lipoxygenase dependent formation of 13(S)-HODE. We have also investigated activation of linoleic metabolism by other ligands like TGF_α which bind to the EGFR and stimulate mitogenesis in BT-20 cells and are known to promote growth in carcinoma cell lines. Using RT-PCR, DNA hybridization and sequencing, we characterized the 15-lipoxygenase present in the human BT-20 cell line as identical to the 15-lipoxygenase previously reported in human pulmonary tissue (14).

EXPERIMENTAL PROCEDURES

Materials. Radionucleotides, enhanced chemiluminesence (ECL) detection reagents and nitrocellulose membranes (Hybond-C super) were purchased from Amersham (Arlington Heights, IL). Taq polymerase, EMEM trypsin, gentamicin, and bovine serum albumin were bought from Gibco-BRL Life Sciences Technologies (Gaithersburg, MD). Fetal calf serum was from Hyclone Laboratories (Logan, UT). Human BT-20 cell were obtained from ATCC (Rockville, MD). [1-^{14}C]Arachidonic acid and [1-^{14}C]linoleic acid (40-60 mCi/mmol) were from DuPont-New England Nuclear (Boston, MA). [*methyl*-^{3}H]-Thymidine (70 Ci/mmol) was purchased from ICN Radiochemicals (Irvine, CA). Unlabeled 13(S)-HODE was from Cayman Chemical (Ann Arbor, MI). The human 15-lipoxygenase cDNA probe was obtained from Oxford (Oxford, MI). EGF and TGF_α were obtained from Collaborative Research Associates (Bedford, MA). Indomethacin, nordihydroguaiaretic acid (NDGA), and the calcium ionophore A23187 were from Sigma (St. Louis, MO). C_{18}-PrepSep columns were from Fisher Scientific. Gene Clean-2 Kits obtained from Bio-101 (Vista, Ca). All solvents were HPLC grade and were from Baker (Phillipsburg, NJ).

EGF/TGF_α stimulation of DNA synthesis. BT-20 cells were grown at 37°C in a humidified 5% CO_2/95% air atmosphere. The culture medium was EMEM containing 10% fetal calf serum and gentamicin (10 μg/ml). After reaching 70-80% confluence in 96-well dishes, cells were washed with serum-free EMEM and then incubated in serum-free media for 16-20 hours. Following growth arrest by serum deprivation, cells were treated with 0.2 ml of serum-free EMEM containing EGF (10 ng/ml) or TGF_α (1 ng/ml) in the presence of [^{3}H]-thymidine (1 μCi/well). In experiments with inhibitors, cells were pre-treated with various concentrations of indomethacin or NDGA for 30 min prior to the addition of growth factor. As described in detail previously (3,4), DNA synthesis was assessed through incorporation of radioactive thymidine into trichloroacetic acid-insoluble material after 24 hr. Samples were processed for liquid scintillation counting in Ecolume.

Arachidonic and linoleic acid metabolism. Metabolism assays were conducted in duplicate. BT-20 cells were grown on 150 mm culture dishes in EMEM containing 10% fetal calf serum and gentamicin (10 μg/ml) at 37°C in a 5% CO_2/95% air atmosphere. After reaching 70-80% confluence, cells were washed twice with serum-free EMEM and then incubated for 24 hr in serum-free EMEM. The growth arrested cells were washed again with fresh serum-free media and then treated with 10 ml of serum-free EMEM containing either EGF (10 ng/ml), TGF_α (1 ng/ml) or calcium ionophore A23187 (5 μM) for 4 hr at 37°C. These treatments were done in the presence of either [^{14}C]linoleic acid or [^{14}C]arachidonic acid (3 μCi; final concentration 10 μM). In some experiments, BT-20 cells were preincubated with serum-free EMEM containing indomethacin (10μM) or NDGA (10 μM) for 30 min prior to growth factor addition. Radiolabeled fatty acid compounds were extracted from the incubation medium by acidification to pH 3.5 with glacial acetic acid and application to a C_{18}-PrepSep column preconditioned with 10 ml of methanol followed by 10 ml of water. The column was then washed with 10 ml of water and the sample eluted with 5 ml of methanol and subsequently evaporated to dryness under argon. Samples were reconstituted in 50% methanol (pH 3.5) for analysis by reverse phase-HPLC. Cellular pellets were washed twice with ice-cold PBS and used for protein analysis and total RNA isolation.

Reverse phase-HPLC analyses were conducted with a C_{18} Ultrasphere column (5 μm; 4.6 × 250 mm; Altex Scientific, Beckman Instruments) equipped with a Waters model 6000A pump and a Waters Model 717 Autosampler injector. Metabolite separation was achieved utilizing a 55-100% methanol stepwise gradient at 1.1 ml/min as described previously (3). Eluted radioactivity was monitored using a Flo-One/Beta detector (Radiomatic Instruments, Tampa, FL) linked with a Gateway 2000 3865X/16 computer for data processing.

Western Blot analysis. After treating the cells, the culture media was removed and plates were rinsed twice with ice-cold PBS. Cells were scraped into PBS medium and washed. The cell pellet was resuspended in lysis buffer (100mM Tris, pH 8.0, 3 mM EDTA, 0.5mM PMSF, 1mg/ml leupeptin, and 1mg/ml pepstatin), sonicated and then centrifuged at 14,000×g for 15 min. Both pellet and supernatant was solubilized in Laemmli's loading buffer and separated in 10% acrylamide gel. Proteins were electrotransferred to nitrocellulose membrane. The blot was blocked with 5% skim milk for 1 hr, followed by probing with human anti-15-lipoxygenase antibodies (A gift from Dr. E. Sigal) for 1 hr. The blots were visualized by an Amersham ECL system.

Total RNA isolation. Total RNA was isolated by following the method of Chomczynski and Sacchi (15). The RNA was prepared by lysing the cells in acid guanidium isothiocyanate (4N guanidium isothiocyanate, 2% sarcosine, 100mM Tris-HCl, pH6.5 and 100mM of freshly added β-mercaptoethanol). The DNA in the lysate was sheared by passage through 18-guage hypodermic needles. Total RNA was precipitated from the lysate by adding sodium acetate to a final concentration of 200 mM and then adding an equal volume of isopropanol. The pellet was resuspended in guanidium buffer and extracted with phenol-chloroform and precipitated with two volumes of ethanol. The RNA pellet was washed once with 70% ethanol, dried, resuspended in DEP-treated water and quantitated.

Northern blot analysis. Total RNA samples (20μg) were fractionated on 1%-agarose gel containing 6% formaldehyde and transferred to a nitrocellulose membrane (Schleicher & Schull, Keene, NH, USA)

by capillary blotting (16). The blots were UV cross linked, and prehybridized for 1 hr before hybridization overnight with [^{32}-P]-labeled human 15-lipoxygenase cDNA probe at 42°C. Blots were washed twice with 2× SSC containing 0.2% SDS at room temperature for 20 min, then washed twice with 1× SSC containing 0.2% SDS at 42°C for 30 min. The blots were exposed to Kodak X-OMAT % film for 2-4 days with intensifying screens at −80°C.

RT-PCR of 15 lipoxygenase. The sequences of oligonucleotide primers used in PCR reaction are as follows:

Sense: external- AC(ATC)GG(CAG)GG(CAG)GG(CAG)GG(CAG)CA
Antisense: external- CAT(GAT)GT(AG)CA(GAT)GG(GAT)GC(GA)TT
Sense: internal- TT(CG)TG(CT)CC(TC)GA(CT)GA(CT)
Antisense: internal- CCAGGAGTACCAGTCCAACTGGCC

Ten μg of total RNA were reverse transcribed using Pharmacia first strand cDNA synthesis kit priming with oligo(dT)18 primer. Two μl of the cDNA mixture were amplified by PCR with sense and antisense degenerate primers specific for 15-lipoxygenases. PCR reactions were carried out in a Perkin-Elmer Model 480 thermal cycler, in 50 ul reaction volume. The buffer contained 67mM Tris-HCl, pH 8.8, 2mM $MgCl_2$, 17mM ammonium sulfate, and 10mM mercaptoethanol, 0.2mM primers and 0.2mM dNTP's. Typically, the amplifications were performed for 30 cycles of 94°C, 1min; 60°C, 1min; and 72°C, 1min. Reaction products were separated on 2% agarose gels and visualized by ethidium bromide and bands were confirmed by Southern blot analysis by probing with 15-lipoxygenase cDNA.

Cloning and sequencing of 15-lipoxygenase cDNA. The positive bands of PCR products from the agarose gels were cut out, the DNA isolated using a gene clean kit, and cloned into the TA vector (Invitrogene, San Diego, CA). Positive clones were selected and plasmid DNA was isolated and sequenced using the Sequenase version 2.0 DNA sequencing kit (US Biochemical, Cleveland, Ohio).

RESULTS

Our initial experimental approach was to define the response of BT-20 cells to growth factor ligands which bind to the EGF receptor. Cells were growth-arrested by incubation in serum-free media for 24 hr and treated with EGF or TGF_α over a concentration range of 0.01 to 100 ng/ml. DNA synthesis was assessed by measuring [^{3}H]thymidine incorporation over a 24 hr period. Both EGF and TGF_α stimulated a dose-dependent increase in DNA synthesis with the EGF maximal response at 10 ng/ml and the maximal TGF_α response at 0.1 ng/ml (data not shown). In addition to being more potent in BT-20 cells, TGF_α elicited a mitogenic response of greater magnitude than that of EGF. Accordingly, we used TGF_α at 0.1 ng/ml in subsequent experiments with BT-20 cells.

The effect of prostaglandin H synthase (PGHS) and lipoxygenase inhibitors on TGF_α-induced DNA synthesis was utilized to determine whether arachidonic and/or linoleic acid metabolism by prostaglandin H synthase or lipoxygenases might be involved in mitogenic signal transduction in BT-20 cells. Serum-deprived cells were pre-incubated with various concentrations of either indomethacin (PGHS inhibitor) or NDGA (lipoxygenase inhibitor) for 30 min prior to stimulation with TGF_α. The effects of these inhibitors on DNA synthesis are shown in Figure 1. Treatment of BT-20 cells with indomethacin over a broad concentration range did not alter TGF_α-stimulated [^{3}H]thymidine incorporation. Indomethacin modestly attenuated (20%) DNA synthesis only at a high concentration of 100 μM. Thus, inhibition of prostaglandin biosynthesis had little effect on the growth factor response in BT-20 cells. In contrast, treatment of cells with NDGA between 1-10 μM resulted in almost complete inhibition of TGF_α-induced DNA synthesis (Figure 1). As seen in our previous studies with SHE and BALB/c 3T3 fibroblasts (2), inhibition of lipoxygenase activity in BT-20 cells results in a profound decrease in the cellular response to growth factors which activate the EGF receptor and subsequent signaling pathways.

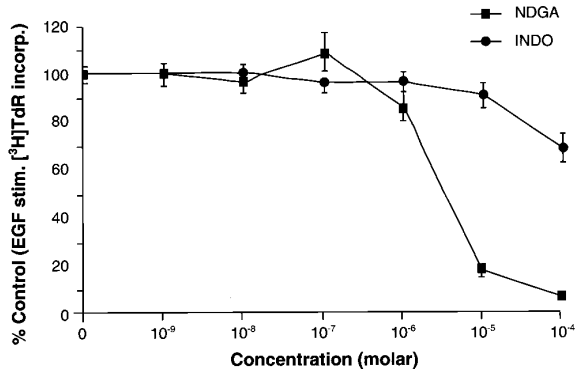

FIG. 1. Effects of NDGA and indomethacin on TGF_α-stimulated DNA synthesis in BT-20 cells. Cells were grown to near confluence in 96-well plates and then were serum-depleted for 24 hr. After preincubation with inhibitors for 30 min, TGF_α (0.1 ng/ml) was added and DNA synthesis measured by [^{3}H]thymidine incorporation after 24 hr. Data (mean ± S.E., five determinations) are expressed relative to stimulation by TGF_α alone (designated 100%=60,000 dpm). Results are representative of at least three different experiments.

The inhibitor studies suggest that lipoxygenase metabolites may modulate growth factor signal transduction in BT-20 cells. Thus, we were interested in characterizing the metabolism of both arachidonic and linoleic acid in this human breast carcinoma cell line. After over-night incubation in serum-free media, cells were activated with either A23187(5 μM) or TGF_α (0.1 ng/ml) for 4 hr in the presence of radiolabeled substrate. The calcium ionophore A23187 serves as an excellent non-physiological stimulus of arachidonic/linoleic acid metabolism in many cellular systems. Figure 2 depicts the reverse-phase HPLC radiochromatogram from the extracted incubation mixture of BT-20 cells with A23187 and [^{14}C]arachidonic acid. We observed very little metabolism of arachidonic acid in these experiments with essentially no production of prostaglandins (15-30 min range, retention time) and low levels of HETEs detected (60-70 min range, retention time) (Figure 2). The recovered amounts of HETE material did not allow for further structural characterization. In

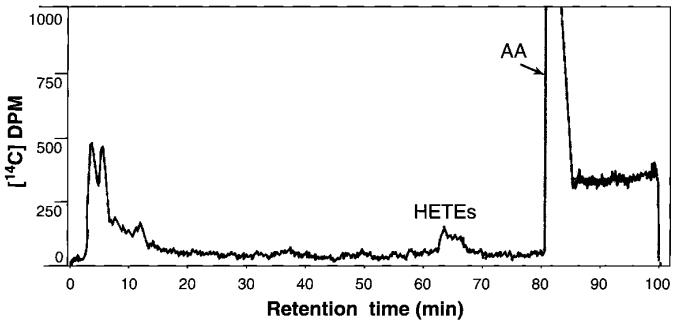

FIG. 2. Reverse phase-HPLC radiochromatogram of [^{14}C]-arachidonic acid metabolism in BT-20 cells. BT-20 cells were incubated for 4 hr at 37°C with 10 μM radiolabeled arachidonic acid and stimulated with 5 μM A23187. Acidified organic extracts were analyzed by reverse phase-HPLC as described in "Experimental Procedures".

metabolism studies with control cells which received no stimulus or in cells treated with TGF$_\alpha$, we did not observe any arachidonate metabolites (data not shown).

In contrast, when [^{14}C]linoleic acid was used as substrate, a prominent metabolite was noted which eluted at 62 min in this reverse phase-HPLC system (Figure 3). Incubation of serum-deprived BT-20 cells with linoleic acid in the absence of any additional stimulus resulted in a low production of linoleate metabolites (Figure 3A). However, when treated with TGF$_\alpha$, we found BT-20 cells to actively metabolize linoleic acid to a mono-hydroxy fatty acid (Figure 3B). This metabolite was identified as 13-HODE based on co-elution in HPLC analyses with authentic 13-HODE standard. The TGF$_\alpha$-stimulated production of 13-HODE in BT-20 cells was inhibited by NDGA (Figure 3C), but not by indomethacin (Figure 3D) indicating that the linoleate metabolite is formed by a n-6 or 15-lipoxygenase.

To further characterize the presence of a 15-lipoxygenase gene at the molecular level, we did Northern analysis of total RNA isolated from either control or BT-20 cells incubated with TGF$_\alpha$ (10ng/ml) for 4 hours. The RNA was probed with a cDNA for the human 15-lipoxygenase coding region (Figure 4). The results shown in Figure 4 indicate that 15-lipoxygenase is present in the BT-20 cell line but we did not observe any differences in the expression of this gene in control and TGF$_\alpha$-treated cells. The levels of 15-lipoxygenase protein was examined by Western analysis using specific antibodies to human 15-lipoxygenase obtained from human lung. We did observe faint bands with the human anti-15-lipoxygenase antibody that migrated at approximately 70kDa, but the results were not conclusive (data not shown).

We used RT-PCR and Southern blot analysis to characterize the 15-lipoxygenase in these cells. Total RNA was isolated and used for RT-PCR with 15-lipoxygenase specific primers. We designed several sets of primers and used PCR to enrich the 15-lipoxygenase message. RT-PCR was performed without template as a negative control with each set of primers to check for any carry-over contamination. Due to the low amount of 15-lipoxygenase in BT-20 cells, we used nested PCR technique to amplify this gene. The outermost set of primers was used for the first round amplification, and a second round PCR was performed using the innermost set of primers and first round product. The second round PCR product gave a prominent 330 bp product (Figure 5) and it was confirmed as 15-lipoxygenase by Southern Blot analysis probed with human 15-lipoxygenase (data not shown). To further identify this prod-

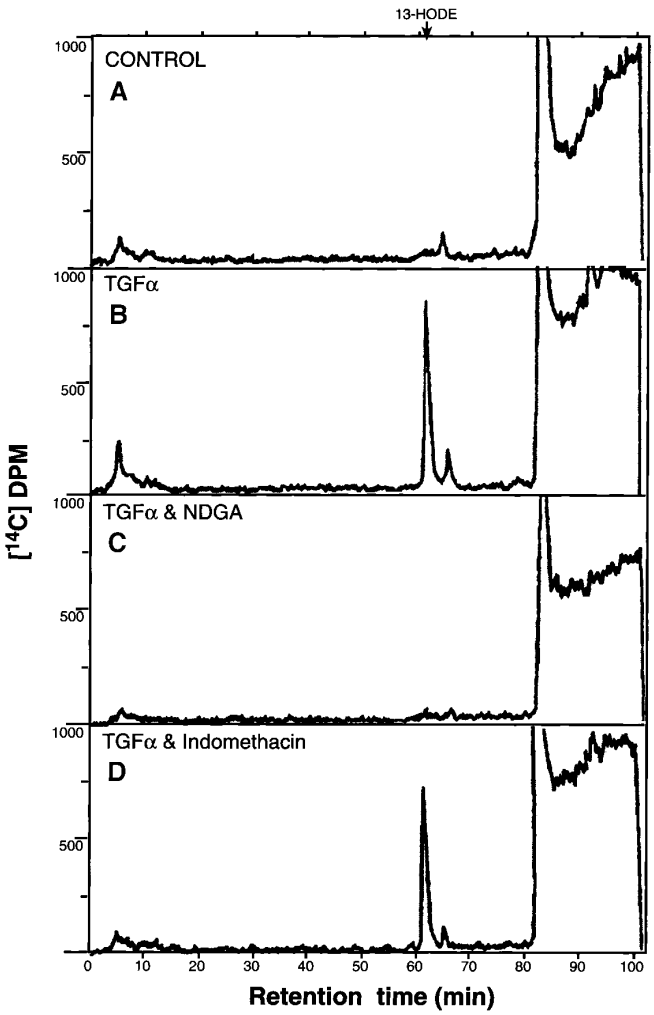

FIG. 3. Reverse phase HPLC radiochromatograms of [^{14}C]linoleic acid metabolism in BT-20 cells. Serum-deprived, growth arrested BT-20 cells were incubated with 10 μM radiolabeled linoleic acid for 4 hr at 37°C and treated with **A,** vehicle control; **B,** 0.1 ng /ml TGF$_\alpha$; **C,** 0.1 ng/ml TGF$_\alpha$ in the presence of 10 μM NDGA; or **D,** 0.1 ng/ml TGF$_\alpha$ in the presence of 10 μM indomethacin. Extractions and HPLC analyses were performed as described under Experimental Procedures. The retention time of authentic 13-HODE standard is indicated on the chromatogram.

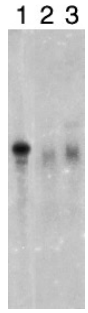

FIG. 4. Northern analysis of 15-lipoxygenase in BT20 cell line. Total RNA (20μg) was separated in 1.0%-agarose gel containing 6% formaldehyde, transferred to nylon membrane and probed with 15-lipoxygenase cDNA. Lane 1; Rabbit reticulocyte total RNA as a positive control, Lane 2; Control cells grown in EMEM medium and Lane 3; Cells were treated with TGF_α (10 ng/ml).

uct, this band was isolated and cloned into a TA-vector and sequenced in both directions. The sequence of this product was found to be identical to the published human 15-lipoxygenase sequence (data not shown). This indicates that the BT-20 cell line contains the identical 15- lipoxygenase gene present in human pulmonary tissue (14) and is consistent with the HPLC data showing the formation of the linoleic acid metabolite 13-HODE.

DISCUSSION

In this report we have examined the importance of lipid metabolism in EGF and TGF_α dependent cell proliferation of a human breast cell line, BT-20. EGF and TGF_α stimulated cell growth, dependent on the concentration of the growth factor, and increased the metabolism of the essential dietary fatty acid, linoleic acid. We observed a EGF/TGF_α dependent increase in the formation of 13-HODE, a metabolite formed by a n-6 lipoxygenase and not by prostaglandin H synthase. 13-HODE was the major linoleate metabolite formed in BT-20 cells. We detected only low levels of arachidonic acid products. Inhibition of the lipoxygenase activity attenuated the TGF_α-dependent formation of 13-HODE and TGF_α-stimulated DNA synthesis, while inhibition of prostaglandin H synthase activity did not alter TGF_α-stimulated mitogenesis or linoleic acid metabolism. These findings suggest that metabolism of linoleic acid to 13-HpODE or 13-HODE by a lipoxygenase is necessary for transmission of the EGF/TGF_α mitogenic signal from the cell surface to the nucleus in this human breast cell line.

These findings with BT-20 breast cells are supported by our previous results (3,4,8,17) which indicate that the formation of the linoleic acid metabolite, 13(S)-HODE is a component of the signaling pathway that leads to EGF-dependent proliferation of Syrian hamster embryo fibroblasts and other cell lines. Inhibition of the EGF receptor tyrosine kinase activity attenuates the EGF-dependent mitogenic response and produces a corresponding decrease in the formation of 13(S)-HODE (4). Likewise, inhibition of tyrosine phosphatases enhances EGF-dependent mitogenesis and stimulates the formation of 13(S)-HODE (4,17). Observations in the hamster cells suggest that the EGF receptor regulates the 15-lipoxygenase that metabolizes linoleic acid to 13(S)-HODE. Furthermore, the addition of 13(S)-HODE and its precursor 13(S)-HpODE, but not other linoleic acid or arachidonic acid metabolites, selectively enhances EGF-dependent mitogenesis in the Syrian hamster cells (8). Other growth factors, such as fibroblast growth factor and platelet derived growth factor, do not stimulate linoleic acid metabolism and the addition of exogenous linoleic acid metabolites do not alter the mitogenic signal transduction pathways of these specific growth factors (17,18). These results indicate a specific interaction of the lipid metabolites with the EGF signaling pathway. The findings of this current report with BT-20 cells suggest a similar role for 13-HODE formation in the EGF receptor/*erbB-2* signal transduction system present in human breast carcinoma cells (9,10).

Studies on the formation of 13(S)-HODE in SHE cells, the characterization of the responsible enzyme, as well as the determination of the mechanism for the EGF-dependent stimulation are hampered by the lack of suitable biochemical and molecular tools. Attempts to develop these tools by the isolation and cloning of the hamster enzyme have not been successful. The results

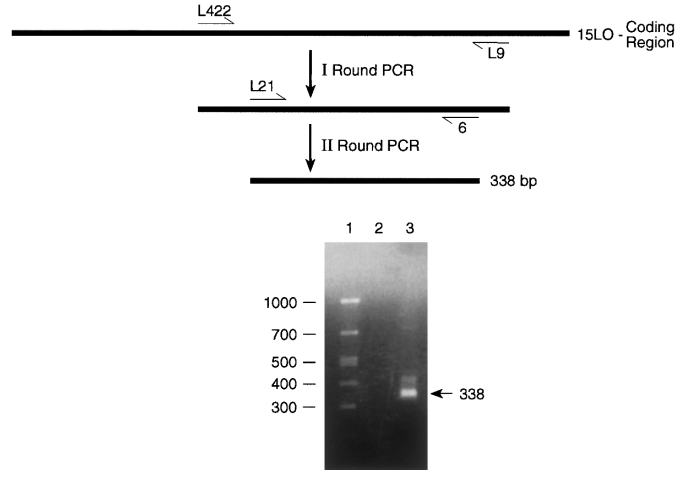

FIG. 5. RT-PCR analysis of 15 lipoxygenase from BT20 cell line. A. Map of 15-lipoxygenase coding region, primer locations and expected size PCR products were drawn. B. Total RNA from BT20 cell line reverse transcribed using MMLV-reverse transcriptase and random primers. Single strand cDNA was amplified by PCR in the presence of 15-LO specific primers. PCR product was separated on 2.0.% agarose gel and visualized by ethidium bromide staining. Lane 1; marker, lane 2; Negative control (no DNA), lane 3; RNA from BT20 cells.

presented in this study with BT-20 cells clearly indicate the presence of a n-6 lipoxygenase that appears identical to the 15-lipoxygenase previously cloned and characterized from human leukocytes and highly expressed in human airway epithelium (5-7,14). In the BT-20 cells, linoleic acid appears to be the primary physiological substrate, certainly in preference to arachidonic acid. In studies with human pulmonary cells, the 15-lipoxygenase catalyzed the metabolism of arachidonic acid to 15-HETE, but at concentration greater than 100μM (19). These studies did not report metabolism of linoleic acid in the human pulmonary system.

The incubation of the BT-20 cells with EGF or TGF$_\alpha$ did not increase the expression of the 15-lipoxygenase as measured by northern and RT-PCR analysis, despite the observation that incubation of the breast cells with EGF or TGF$_\alpha$ increased the metabolism of linoleic acid to 13-HODE. These observations suggest a potential post-translational regulation of the 15-lipoxygenase activity by EGF and TGF$_\alpha$ in the BT-20 cells. In human monocytes, interleukin-4 and interleukin-13 are reported to stimulate the transcription and translation of 15-lipoxygenase (20,21), with this effect antagonized by interferon-γ (20). Regulation of 15-lipoxygenase activity in other cellular systems is not clearly understood. Measurement of the 15-lipoxygenase in BT-20 cells at the protein level using anti-human 15-lipoxygenase antibody did not identify a strong immunoreactive band at 70 kDa as expected for a lipoxygenase. This may be due to low protein expression, but this conclusion does not agree with the observed level of metabolism. A possible explanation for this observation is a post-translational modification of the enzyme such that the antibody will not recognize the modified protein.

The role of dietary fat in breast cancer development continues to provoke controversy. Reports have associated dietary fat consumption, including linoleic acid, with enhancement of tumorigenesis and metastasis in human breast cancer (11-13). Our studies indicating metabolism of linoleic acid to a potent, biologically active compound in a breast carcinoma cell line may provide an important avenue for future research aimed towards understanding the role of lipids in breast cancer. The BT-20 cell system serves as an experimental model which overexpresses both EGF receptor and the *erbB-2* oncogene product, factors which have been clearly identified as adverse prognostic indicators in the development and progression of breast cancer (reviewed in 9). We believe our data support a role for 13-HODE as a lipid mediator which up-regulates the EGF receptor signaling pathway. Increased formation of 13-HODE as a consequence of high dietary linoleic acid could result in amplification of the EGF receptor signaling pathway leading to enhanced cellular proliferation in breast carcinoma tissue.

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