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Inverse Relationship between 15-Lipoxygenase-2 and PPAR-γ Gene Expression in Normal Epithelia Compared with Tumor Epithelia[1]

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Abstract

15-Lipoxygenase-2 (15-LOX-2) synthesizes 15-*S*-hydroxyeicosatetraenoic acid (15-*S*-HETE), an endogenous ligand for the nuclear receptor, peroxisome proliferator–activated receptor-γ (PPAR-γ). Several studies have described an inverse relationship between 15-LOX-2 and PPAR-γ expression in normal *versus* tumor samples. To systematically determine if this is a ubiquitous phenomenon, we used a variety of epithelial and nonepithelial cells and some tissues to further evaluate the extent of this inverse relationship. The levels of mRNA or protein were measured by reverse transcriptase polymerase chain reaction or Western gray level intensity, whereas distribution was determined by *in situ* hybridization or immunofluorescence. 15-*S*-HETE was measured by liquid chromatography/tandem mass spectrometry. Normal epithelial cells/samples generally expressed high levels of 15-LOX-2 along with the enzyme product 15-*S*-HETE, but both levels were reduced in cancer cells/samples. In contrast, most cancer cells expressed high levels of PPAR-γ mRNA and protein, which were absent from normal epithelial cells. Overall, the inverse relationship between these two genes was primarily restricted to epithelial samples. Forced expression of PPAR-γ reduced 15-LOX-2 protein levels in normal cells, whereas forced expression of 15-LOX-2 in tumor cells suppressed PPAR-γ protein levels. These results suggest that feedback mechanisms may contribute to the loss of 15-LOX-2 pathway components, which coincide with an increase in PPAR-γ in many epithelial cancers.

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Introduction

Lipoxygenase (LOX) enzymes catalyze the conversion of polyunsaturated fatty acids to hydroxy fatty acids [1,2]. LOX enzymes contain a nonheme iron (Fe^{3+}) that promotes hydrogen abstraction and dioxygenation of 1,4-diene moieties, followed by protonation of the peroxyl radical to form monohydroperoxy fatty acids predominantly from mobilized lipid pools [2–4]. A wide variety of LOXs are found in nature, which are described based on the carbon atom that each enzyme peroxygenates on a particular substrate [2–4]. The primary lipid targets of LOX activity are arachidonic acid (AA) and linoleic acid (LA). Two forms of 15-LOX occur in humans: 15-lipoxygenase-1 (15-LOX-1), originally isolated from reticulocytes [5], and 15-lipoxygenase-2 (15-LOX-2), first described in skin [6]. 15-LOXs are distinguished by their catalytic properties. 15-LOX-2 preferentially peroxidates AA on carbon 15 to generate 15-*S*-hydroperoxyeicosatetraenoic acid, which is hydrolyzed to 15-*S*-hydroxyeicosatetraenoic acid (15-*S*-HETE) [3,6]. In contrast, 15-LOX-1 preferentially peroxidates LA on carbon 13 to form 13-*S*-hydroperoxyoctadecadienoic acid, which is hydrolyzed to 13-*S*-hydroxyoctadecadienoic acid (13-*S*-HODE) [7]. 15-LOX-1 also generates 15-*S*-hydroperoxyeicosatetraenoic acid from AA (thus the nomenclature), but less efficiently than 15-LOX-2 [7]. A variety of oxidized lipids, including 15-*S*-HETE, can activate the nuclear receptor peroxisome proliferator–activated receptor γ (PPAR-γ) during inflammation and prostate cancer [8–11].

15-LOX-2 expression is most studied in human prostate, a tissue initially reported to have one of the highest expression levels of this gene [6]. Shappell et al. [12] reported that 15-LOX-2 expression was high in benign prostatic epithelium, but was low in malignant prostatic adenocarcinoma. Tang et al. [13] reported that 15-LOX-2 levels were high in normal

Abbreviations: AA, arachidonic acid; LA, linoleic acid; 15-LOX-1, 15-lipoxygenase-1; 15-LOX-2, 15-lipoxygenase-2; 15-*S*-HETE, 15-*S*-hydroxyeicosatetraenoic acid; PPAR, peroxisome proliferator–activated receptor; 13-*S*-HODE, 13-S-hydroxyeicosatetraenoic acid; IgG, immunoglobulin G; RT-PCR, reverse transcriptase polymerase chain reaction; BSA, bovine serum albumin; PBS, phosphate-buffered saline; DAPI, 4′,6-diamidino-2-phenylindole dilactate; LC/MS/MS, liquid chromatography/tandem mass spectrometry; ISH, *in situ* hybridization

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prostatic epithelial cells and, when expressed, also acted as negative cell cycle regulator. Further work by Bhatia et al. [14] suggested that tumor-suppressive functions of 15-LOX-2 may not necessarily depend solely on AA-metabolizing activity or localization to the nucleus. Jack et al. [15] also correlated 15-LOX-2 levels with tumor grade (> 5 Gleason score); expression was reduced in higher-grade tumors. Moreover, 15-LOX-2 expression was significantly reduced in high-grade prostatic intraepithelial neoplasia compared with benign tissue [15]. In addition, 15-*S*-HETE production was lower in prostatic adenocarcinoma compared with benign prostatic hyperplasia, which produced both 15-LOX-2 and 15-*S*-HETE [8,12]. These studies suggest that the loss of the 15-LOX-2 pathway may contribute to increased proliferation and reduced differentiation in prostatic adenocarcinoma.

In view of what is known about 15-LOX-2 expression in prostate and the limited information available for other tissues, we examined the expression of 15-LOX-2 and its relationship with PPAR-γ expression in normal or cancer samples from various epithelial and nonepithelial sources. We hypothesized that an inverse relationship exists between 15-LOX-2 and PPAR-γ expression in normal samples compared to tumor samples, which may be a generalized phenomenon. As an extension of this hypothesis, we also hypothesized that complex mechanisms limit the coexpression of 15-LOX-2 pathway components and PPAR-γ in cells, particularly in the presence of substrate or ligand. We set out to determine if an inverse relationship between these two genes was common to epithelial tissues. We also determined whether forced expression of either 15-LOX-2 or PPAR-γ would affect the endogenous expression of its counterpart, or be altered by AA or 15-*S*-HETE. Based on these outcomes, we might expect that disrupting the counterregulation of 15-LOX-2 or PPAR-γ would profoundly influence epithelial carcinogenesis.

Materials and Methods

The primary rabbit antibody against 15-LOX-2 was purchased from Oxford Biomedical Research, Inc. (Oxford, MI). Primary antibodies against PPAR-γ [rabbit polyclonal immunoglobulin G (IgG) and mouse monoclonal IgG_1] were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). The secondary antibodies to rabbit, or mouse IgG that were conjugated to horseradish peroxidase, were obtained from Pierce Chemical Co. (Rockford, IL), or those conjugated to Alexa-488 were purchased from Molecular Probes (Eugene, OR). Deuterated liquid chromatography/tandem mass spectrometry (LC/MS/MS) standard, 15-*S*-HETE-d_8, and purified 15-*S*-HETE were purchased from Cayman Chemical Co. (Ann Arbor, MI). AA and butylated hydroxytolulene (BHT) were obtained from Sigma Chemical Co. (St. Louis, MO).

Cell Culture

Tumor cell lines derived from different epithelial and nonepithelial origins were grown in tissue culture. The cells of normal epithelial origin included normal prostate epithelial cells (PrEC), normal or immortalized normal keratinocytes (NHEK and NK), human bronchial epithelial cells (NHBE), human mammary epithelial cells (HMEC), and immortalized normal bladder cells (SVHUC), which were purchased from Cambrex BioScience (Walkersville, MD) or American Tissue Type Culture Collection (ATCC; Manassas, VA). Normal esophageal epithelial cells (EEC) were obtained from the Department of Surgery and Surgical Basic Science, Graduate School of Medicine, Kyoto University (Kyoto, Japan). Esophageal cancer cell lines TE-3 and TE-12 were obtained from the First Department of Pathology, Hiroshima University School of Medicine (Hiroshima, Japan). Skin squamous cell carcinomas (HaCaT, SCC-M7, and SCC-P9) were provided by Dr. John Clifford (Lousiana State University, Shreveport, LA). UM-UC-9 and UM-UC-14 bladder cells, referred to hereafter as U-9 and U-14 were obtained from Dr. Barton Grossman at The University of Texas M.D. Anderson Cancer Center. Other cancer cells were obtained from ATCC, including breast carcinoma cells (MCF-7, SK-BR-3, and MDA-MB-453), pancreatic carcinoma cells (AsPC-1, MIA PaCa, and Panc 28), colon carcinoma cells (HCT-115, SW-480, and SW-620), and prostate carcinoma cells (PC-3, LnCaP, and DU 145). Hepatic carcinoma cells (Hep 3B and Hep G2) cells were purchased from ATCC. The cells from nonepithelial origin including prostate stromal cells (PrSt) and prostate smooth muscle cells (PrSM) were purchased from Cambrex BioScience. NIH-3T3 cells were also purchased from ATCC. Also, cells from neuroepithelial or neural crest origins, melanocytes Cambrex BioScience, and melanoma cells (MeWo, 3S5, and 70W) were obtained from Dr. Robert Kerbel, Sunnybrook and Women's College Health Sciences Centre in Toronto, Ontario, Canada. Primary cell cultures were maintained in defined culture medium according to the manufacturer's instructions as described previously [16]. Cancer cell lines were maintained in Dulbecco's modified Eagle's medium/F-12 low-glucose medium (Invitrogen-GIBCO, Carlsbad, CA) supplemented with 5% to 10% fetal bovine serum.

Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

Total RNA was extracted using RNA STAT-60 reagent (Tel-Test, Inc., Friendswood, TX) and treated with DNase I before use in RT-PCR. One microgram of RNA was reverse-transcribed with mouse mammary tumor virus reverse transcriptase (Life Technologies, Inc., Rockville, MD). 15-LOX-2 (351 bp) was amplified by the primer set S 5′-AACTCACCCCCACCACCATACACA-3′ and AS 5′-TTCCCGCCTCCATCTCCCAAAGT-3′ as previously described [6]. Primer pairs were used in RT-PCR analysis to detect PPAR-γ (γS 5′-TCTCTCCGTAATGGAAGACC-3′; γAS 5′-GCATTATGAGCATCCCCAC-3′; γ2S 5′-GCGATTCCTTCACTGCTAC-3′) [17] and 36B4 (S, 5′-CAGCTCTGGAGAACTGCTG-3′; AS 5′-GTGTACTCAGTCTCCACAGA-3′) [18]. The PCR products were electrophoretically separated, transferred onto a nylon membrane, and probed with 15-LOX-2 (5′-TCTACCAAGAACAGAGTCTC-3′), total γ (5′-GAGTACCAAAGTGCAATCAA-3′),