Elevated Expression of 12/15-Lipoxygenase and Cyclooxygenase-2 in a Transgenic Mouse Model of Prostate Carcinoma


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ABSTRACT

Changes in expression of arachidonic acid (AA) metabolizing enzymes are implicated in the development and progression of human prostate carcinoma (Pca). Transgenic mouse models of Pca that progress from high-grade prostatic intraepithelial neoplasia (HGPIN) to invasive and metastatic carcinoma could facilitate study of the regulation and function of these genes in Pca progression. Herein we characterize the AA-metabolizing enzymes in transgenic mice established with a prostate epithelial-specific long probasin promoter and the SV40 large T antigen (LPB-Tag mice) that develop extensive HGPIN and invasive and metastatic carcinoma with neuroendocrine (NE) differentiation. Murine 8-lipoxygenase (8-LOX), homologue of the 15-LOX-2 enzyme that is expressed in benign human prostatic epithelium and reduced in Pca, was not detected in wild-type or LPB-Tag prostates as determined by enzyme assay, reverse transcription-PCR, and immunohistochemistry. The most prominent AA metabolite in mouse prostate was 12-HETE. Wild-type prostate (dorso-lateral lobe) converted 1.6 ± 0.5% [14C]AA to 12-HETE (n = 7), and this increased to 8.0 ± 4.4% conversion in LPB-Tag mice with HGPIN (n = 13). Quantitative real-time reverse transcription-PCR and immunostaining correlated the increased 12-HETE synthesis with increased neoplastic epithelial expression of 12/15-LOX, the leukocyte-type (L) of 12-LOX and the murine homologue of human 15-LOX-1. Immunostaining showed increased L12-LOX in invasive carcinoma and approximately one-half of metastatic foci. COX-2 mRNA was detectable in neoplastic prostates with HGPIN but not in wild-type prostate. By immunostaining, COX-2 was increased in the neoplastic epithelium of HGPIN but was absent in foci of invasion and metastasis. We conclude that (a) AA metabolism in wild-type mouse prostate differs from humans in the basal expression of LOXs (15-LOX in human, absence of its 8-LOX homologue in mouse prostate); (b) increased expression of 12/15-LOX in HGPIN and invasive carcinoma of the LPB-Tag model is similar to the increased 15-LOX-1 in high-grade human Pca; and (c) the LPB-Tag model shows increased COX-2 in HGPIN, and therefore, it may allow additional definition of the role of this enzyme in the subset of human HGPINs or other precursor lesions that are COX-2 positive, as well as investigation of its contribution to neoplastic cell proliferation and tumor angiogenesis in Pca.

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INTRODUCTION

Human Pca progresses from precursor lesions such as HGPIN in the peripheral zone to invasive carcinoma, to more extensive organ confined tumor, and to tumors penetrating the prostatic capsule, which without or following definitive local treatment can progress to regional and systemic metastatic disease (1–3). In addition, advanced and metastatic Pca commonly progresses to clinically hormone refractory disease (androgen insensitivity), limiting treatment options in advanced disease (4). Determining molecular changes responsible for Pca development and progression has been difficult in human patients because it potentially evolves over decades, and it is difficult to obtain tissue for molecular studies during the prolonged evolution in any given patient. Furthermore, therapeutic trials based on demonstrated alterations in target genes are difficult to conduct, especially in earlier stages of the disease. Hence, genetically engineered mouse (GEM) models of Pca represent a potentially powerful tool in Pca research, allowing for correlation of molecular alterations with tumor progression and interventional trials at any given stage of disease progression. However, in addition to pathology and natural history, it is essential that models be characterized for molecular alterations during tumor progression and that these changes be carefully correlated to similar possible alterations in human Pca. Therapeutic interventions demonstrated effective in GEM models are more likely to be beneficial in human Pca patients if initiating and secondary molecular changes in the GEM models are similar to those implicated in human Pca.

Altered expression and activity of AA-metabolizing LOX and COX enzymes have been suggested to contribute to the development and progression of human Pca. 15-LOX-2 is expressed in the differentiated secretory cells of benign human prostate, and benign prostate synthesizes 15-HETE as the major metabolite of AA (5, 6). In contrast, 15-LOX-2 mRNA, protein, and catalytic activity are significantly reduced or absent in the majority of Pcas (5–7). The murine homologue of 15-LOX-2 appears to be an 8-LOX, which shares 78% sequence identity (8). In contrast to reduced expression of 15-LOX-2 in Pca, 15-LOX-1 immunostaining is increased in high-grade human Pca (Gleason score ≥ 8 tumors; Ref. 9). The murine homologue of this gene is a 12/15-LOX, known as L12-LOX, which similar to the human enzyme and forms a mixture of 12-HETE and 15-HETE. The murine homologue of 15-LOX-2 appears to be an 8-LOX, which shares 78% sequence identity (8). In contrast to reduced expression of 15-LOX-2 in Pca, 15-LOX-1 immunostaining is increased in high-grade human Pca (Gleason score ≥ 8 tumors; Ref. 9). The murine homologue of this gene is a 12/15-LOX, known as L12-LOX, which similar to the human enzyme and forms a mixture of 12-HETE and 15-HETE from AA (10–12). In addition, increased P12-LOX mRNA has been reported in high-grade, high-stage Pca by semiquantitative RT-PCR.

The abbreviations used are: HGPIN, high-grade prostatic intraepithelial neoplasia; Pca, prostate carcinoma; AA, arachidonic acid; COX, cyclooxygenase; LOX, lipoxygenase; L12, leukocyte type 12; P12, platelet 12; RT-PCR, reverse transcription-PCR; HETE, hydroxyeicosatetraenoic acid; BHT, hydroxyoctadecanoic acid; Tag, T antigen; LPB-Tag, long probasin promoter SV40-large T antigen; DP, dorsal prostate; LP, lateral prostate; DLP, dorsolateral prostate; VP, ventral prostate; AP, anterior prostate; PIN, prostatic intraepithelial neoplasia; PG, prostaglandin; Gem, genetically engineered mouse; NE, neuroendocrine; MMHCC, Mouse Models of Human Cancer Consortium; HPLC, high-performance liquid chromatography; RP-HPLC, reverse phase-HPLC; PPAR, peroxisome proliferator-activated receptor; IHC, immunohistochemistry; 13-HODE, 13-hydroxyoctadecadienoic acid; EGF, epidermal growth factor.

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(13), and 5-LOX has been reported in Pca cell lines (14, 15) and more recently as increased in Pca tissues by RT-PCR and IHC (16). Finally, some recent studies have reported increased COX-2 in Pca (17, 18), whereas others have challenged this general conclusion or shown that increased COX-2 may be limited to high-grade Pca (6, 19). Increased COX-2 expression has also been noted in HGPIN (6, 18).

As the murine homologues of these AA-metabolizing genes have been identified, GEM models may allow for elucidation of their contribution to Pca as well as their suitability as therapeutic targets. This study represents a characterization of the changes in AA-metabolizing enzymes with Pca progression in the LPB-Tag model of Pca (20). Rapidly growing lines (e.g., LPB-Tag 12T-5, 12T-7s, 12T-7f) develop marked epithelial proliferation with atypia (analogous to PIN) in all prostate lobes, accompanied by hypercellular stroma in the AP and DLP but not VP (20–22). These mice rarely progress to invasive carcinoma because the degree of prostate enlargement prevents maintaining such mice for sufficient periods of time. However, with castration and regression of the in situ prostate lesion, these mice frequently develop invasive and metastatic poorly differentiated carcinoma with NE differentiation (21–23). The slower growing 12T-10 line develops HGPIN (without hypercellular stroma), which progresses to microinvasive carcinoma, to more extensive invasive carcinoma with both glandular and NE differentiation, to larger invasive NE carcinoma, with development of lymph node, liver, and lung metastases, paralleling progression to androgen-insensitive disease (24). In the current study, expression of 8-LOX, P12-LOX, L12-LOX, and COX-2 was investigated in wild-type mice and with Pca progression in LPB-Tag mice by a combination of enzyme activity assays, mRNA analysis, and IHC and results compared with those observed in human benign prostate and Pca.

MATERIALS AND METHODS

Transgenic Mouse Lines, Tissue Procurement, and Histopathological Assessment. LPB-Tag mice were established on a CD1 background using the androgen-regulated LPB and the large Tag of the SV40 early region, with a deletion mutation to prevent expression of the small t antigen (20). The general histopathological alterations in the faster growing lines used herein, 12T-7f, 12T-5, and 12T-7s, were described in the original report of this model (20). More recent reviews have incorporated updated concepts on the histopathological alterations in the intact and castrated mice (21, 22). The histopathological alterations with tumor progression from PIN to invasive and metastatic carcinoma with progressive NE differentiation and loss of AR expression in the LPB-Tag 12T-10 line have been reported previously (24). The pathology terminology conforms to that developed in several recent mouse prostate pathology consensus conferences sponsored by the MMHCC of the National Cancer Institute, chaired by Dr. Scott Shappell (Vanderbilt University Medical Center). The most recent classification established at the Jackson Laboratory in Bar Harbor, Maine, in October 2001 is in general used herein and is to be reported in detail in an upcoming review. Although the current MMHCC classification does not specifically allow for grading of PIN, because of the marked cytological atypia of these in situ lesions in the LPB-Tag mouse, the progression to invasive carcinoma (which is a subclassification of PIN specified by the MMHCC), and in keeping with prior descriptions of the model (24), the advanced forms of the PIN lesions used herein are in general referred to as HGPIN.

All animal experiments were performed according to the guidelines of the Institutional Animal Care and Use Committee. At sacrifice, individual prostate lesion, these mice containing such mice for sufficient periods of time. However, with castration and regression of the in situ prostate lesion, these mice frequently develop invasive and metastatic poorly differentiated carcinoma with NE differentiation (21–23). The slower growing 12T-10 line develops HGPIN (without hypercellular stroma), which progresses to microinvasive carcinoma, to more extensive invasive carcinoma with both glandular and NE differentiation, to larger invasive NE carcinoma, with development of lymph node, liver, and lung metastases, paralleling progression to androgen-insensitive disease (24). In the current study, expression of 8-LOX, P12-LOX, L12-LOX, and COX-2 was investigated in wild-type mice and with Pca progression in LPB-Tag mice by a combination of enzyme activity assays, mRNA analysis, and IHC and results compared with those observed in human benign prostate and Pca.

AA Incubation, Lipid Extraction, and RP-HPLC Analysis. Portions of individual prostate lobes of wild-type and LPB-Tag mice were homogenized in 4× volume of buffer [50 mm Tris-HCl with 150 mm NaCl (pH 7.5)]. Protein concentrations were determined by the method of Bradford (Bio-Rad, Hercules, CA), and incubations were performed in 200 µl at a protein concentration of 1 µg/µl. Incubations were begun by addition of 50 µm (final concentration) [3H]AA (New England Nuclear, Boston, MA). Reactions were incubated for 45 min at 37°C with continual agitation and terminated by addition of 2.5 volume of cold methanol. Products were extracted and recovered by C18 Sep-Pak extraction and analyzed by RP-HPLC as previously described (5, 6), using a Beckman Ultrasphere 5-µm octadecyl silica column (25 × 0.46 cm) with a solvent system typically of methanol/water/glacial acetic acid at 85:15:0.01 (by volume) at a flow rate of 1.1 ml/min. This resulted in a retention time for 12-HETE of ~11 min. Unlabeled HETEs (5-HETE, 8-HETE, 9-HETE, 11-HETE, 12-HETE, and 15-HETE) were added to each sample before HPLC analysis to allow for exact determination of retention times of each HETE within individual analyses. UV spectra and profiles at
205, 220, 235, and 270 nm were recorded using a Hewlett-Packard 1040A diode array detector, and radioactivity was monitored online using a Radiomatic Instruments Flo-One detector (5, 6). 12-HETE formation (the most prominent product identified) was quantitated as percent AA substrate conversion (6). 12-HETE was carried out on the methyl ester derivative by chromatography on a Chiralpak AD column with a solvent of hexane/methanol at 100:2 (by volume) as described previously (27). In some samples, the possible identity of more polar products on RP-HPLC analysis as PGs was investigated by coinjection of cold TxB2, 6-keto-PGF1α, and corresponding to a portion of the larger cDNA standard fragment (10). The primers for L12-LOX were set on one listed above, which generate a 462-bp amplifier, spanning multiple introns and corresponding to a portion of the larger cDNA standard fragment (10). The primers for β-actin were 5′-ACGGCCAGTGTACCATATGGT-3′ (forward) and 5′-AGGGGCCGAGCTACCTC-3′ (reverse), which generate a 372-bp amplifier.

The one-step real-time RT-PCR reactions consisted of the following steps: reverse transcription at 55°C for 15 min; denaturation at 94°C (P- and L12-LOX) or 95°C (β-actin) for 1 min; amplification for 45 cycles; and melting curve analysis from 65°C to 95°C at a rate of 0.1°C under continuous fluorescence monitoring. The amplification programs consisted of heating at 20°C/s to 94°C (P- and L12-LOX) or 95°C (β-actin), cooling at 20°C/s to 58°C (P- and L12-LOX) or 55°C (β-actin), annealing at 58°C or 55°C for 10 s, heating at 20°C/s to 72°C, elongation at 72°C for either 14 s (for P12-LOX), 18 s (for L12-LOX), or 15 s (for β-actin), and heating at 5°C/s to either 85°C (P12-LOX, β-actin) or 88°C (L12-LOX) for fluorescence acquisition. The specificity of the amplifier in each reaction was confirmed by the melting curve analysis, with internal gel confirmation that this large peak corresponded to the expected amplifier (28). The contribution to fluorescence signal of any nonspecific products and/or primer dimers was eliminated by increasing the temperature to 2°C below the melting temperature of the specific product, which eliminated any other minor cDNAs (which have lower melting temperatures; Ref. 28). Copy numbers of mRNA were calculated from serially diluted standard curves generated from purified cDNA template (28). 1:10 serial dilutions over three to six orders of magnitude range were used to generate the standard curves (10^11–10^1 copies for β-actin, 10^7–10^8 copies for P12-LOX, 10^2–10^6 copies for L12-LOX). The serially diluted standards were simultaneously amplified with the unknown samples to generate a linear standard curve using the fit points method of analysis with four points. Standard curves for P12-LOX, L12-LOX, and β-actin all had correlation coefficients of 1.00. Control samples run in triplicate had a variance of ±10%. Wild-type and neoplastic prostate samples were always run in the same assay for each gene product. When mRNA was detected in biological samples, the amplification curves fell within the range of the standard curves, and copy numbers of the unknown samples were calculated using the Lightcycler software (version 3).

IHC. All IHC was performed on formalin-fixed, paraffin-embedded sections using standard techniques using an avidin-biotin method on a Ventana Benchmark system (Ventana Medical Systems, Tucson, AZ). Refs. 5–7). The antibody for 8-LOX was a polyclonal antibody specific for human 15-LOX-2 and murine 8-LOX (8, 29), without cross-reactivity for 15-LOX-1 (murine L12-LOX), 5-LOX, or P12-LOX (8). The antibody was typically used at a 1:1000 dilution, without antigen retrieval. Sections of philbob esterase treated mouse skin, which results in epidermal thickening and increased 8-LOX expression in a thickened granular layer (8), were used as a positive control. The antibody for P12-LOX was a rabbit polyclonal antibody purchased from Oxford Biomedical Research, Inc. specific for P12S-LOX and cross-reacting with both the human and mouse enzyme. The usual dilution was 1:50, and Trilogy pressure cooker (Cell Marque, Hot Springs, AR) antigen retrieval was used. Sections of mouse spleen were used for megakaryocytes as a positive control, with expected granular cytoplasmic staining of these platelet precursors. The rabbit polyclonal antibody for murine L12-LOX was prepared, expressed, and characterized (30) and was used at a 1:1000 dilution with citrate steam antigen retrieval. Known expression in bronchial epithelium was used in mouse lung sections as a positive control. In addition, as immunostaining was detected in benign and especially neoplastic murine prostate, specificity of the immunostaining was additionally confirmed by performing immunostaining on lung sections of a 12/15-LOX (L12-LOX) knockout mouse (30). COX-2 immunostaining was performed with a polyclonal antibody purchased from Cayman Chemical specific for COX-2 and cross-reacting with both the human and mouse enzyme (6). The antibody was used at a 1:100 dilution with citrate antigen retrieval. Known strong expression in terminal vas deferens of the rodent was used as a positive control (31).
Again, as strong immunostaining was detected in the neoplastic mouse prostate, the specificity of the IHC assay was confirmed by negative immunostaining on similar terminal vas deferens sections from a COX-2 knockout mouse (32), generously provided by Dr. Matthew Breyer (Vanderbilt University Medical Center). Extent of immunostaining in benign prostate glands in wild-type mice or HGPIN in LPB-Tag mice was assessed semiquantitatively as to4 wild-type mice or HGPIN in LPB-Tag mice was assessed semiquantitatively as 0 to + as follows: 0, negative; 1+, rare immunostaining, <5% of cells in the section; 2+, focal, with immunostaining in 10–25% of cells or up to 50% of epithelial cells within 10–25% of gland profiles; 3+, immunostaining in 25–50% of cells or extensive immunostaining in 25–50% of gland profiles; and +, diffuse, with immunostaining in >50% of cells or extensive immunostaining in >50% of gland profiles. Immunostaining in smaller foci of invasive carcinoma and metastatic carcinoma foci was assessed as either positive or negative because immunostaining was typically either absent or diffusely present.

RESULTS

AA Metabolism in Wild-Type Mouse Prostate. Of the three lobes of the mouse prostate, AP, VP, and DLP, the latter is the most prominently involved site of neoplastic transformation in the fast-growing LPB-Tag lines and thus was used for the majority of the AA metabolism studies. Incubation of wild-type DLP tissue from adult mice with [14C]AA resulted in the formation of [14C]12-HETE as the major metabolite (Fig. 1A). Identification of this product as 12-HETE was based on coelution with the authentic standard on both reverse phase (Fig. 1A) and normal-phase HPLC (data not shown), as well as the characteristic UV absorption spectrum (5, 6). 12-HETE formation was detectable in four of seven DP, LP, or DLP samples from 7 different adult wild-type mice. In the four positive wild-type samples, the amount of 12-HETE formed was 1.59 ± 0.49% AA conversion (mean ± SD; range, 1.04–2.20%). 12-HETE formation was also detected in four of five VP specimens, with 1.46 ± 1.13% AA conversion in the positive samples (mean ± SD; range, 0.76–3.15%). In samples from 3 mice in which both DLP and VP were examined, 12-LOX catalytic activity was slightly higher in the DLP than VP in all 3 (1.41 versus 1.07, 2.20 versus 0.86, 1.70 versus 0.76% AA conversion for DLP versus VP, respectively). 12-HETE formation was also noted in 2 of 2 incubated wild-type AP specimens.

Because normal human prostate expresses 15-LOX-2 (5, 6) and the mouse homologue of this enzyme is an 8-LOX (8), representative samples of wild-type mouse prostate were examined carefully for the formation of 8-HETE. This metabolite runs close to 12-HETE on RP-HPLC and can be distinguished only by initial RP-HPLC to isolate the peak of 12-HETE/8-HETE followed by the easily accomplished chromatographic resolution of 12-HETE and 8-HETE on normal-phase HPLC. None of the wild-type DLP or VP specimens showed detectable formation of 8-HETE. 5-HETE was also clearly absent as determined by RP-HPLC analysis (Fig. 1A).

More polar products (eluting earlier on RP-HPLC) were also consistently noted (Fig. 1A). Although PGs would be expected to elute in this region of the chromatogram, the polar radioactivity was mainly not PGs because the radiolabeled products did not coelute with PG standards on a more highly resolving RP-HPLC system. Also, formation of the main polar products was not inhibited by the nonselective COX inhibitor indomethacin (data not shown). It is likely this polar radioactivity mainly represents trihydroxy products derived originally from 12-HPTEA via epoxyalcohols, although their identity was not additionally investigated here.

AA Metabolism in LPB-Tag 12T-7f Mice. Metabolism of [14C]AA in the neoplastic LPB-Tag prostate was examined in DLP of 13–19-week-old 12T-7f mice. At these time points, the neoplastic prostate is characterized by PIN, with uniform lobular expansion by proliferating atypical epithelium accompanied by a hypercellular stroma (20–22). Occasional microscopic foci of invasive adenocarcinoma and NE carcinoma are noted in a minority of samples (20–22). 12-HETE formation from exogenous AA was markedly increased in the HGPIN-containing DLP of LPB-Tag 12T-7f mice compared with wild-type mouse prostates (Figs. 1B and C), with 8.0 ± 4.4% AA conversion (mean ± SD; range, 2.8–18.6%; n = 13; P < 0.05 LPB-Tag versus wild-type control). Again, as 12-HETE and 8-HETE coelute on RP-HPLC, the nature of this product as 12-HETE was confirmed by normal phase HPLC (data not shown). The product was 12-HETE of 12S stereochemistry as determined by chiral column analysis (Fig. 1D).

As in wild-type prostate, there was no detectable formation of 5-HETE or 8-HETE in LPB-Tag 12T-7f mouse prostate. Comparison of AA metabolism in DLP and VP lobes showed in 2 LPB-Tag 12T-7f mice, distinctively higher formation of 12-HETE in the DLP compared with VP (18.6 versus 3.7 and 7.7 versus 0% AA conversion to 12-HETE, respectively).

More polar products on RP-HPLC analysis were also noted as more prominent than similar eluting peaks in wild-type mouse prostate incubations (Fig. 1B). As for wild-type mouse prostate, these peaks did not comigrate with PG standards, and their formation was not inhibited by indomethacin. A prominent peak eluting in the middle of
the chromatographic run was increased in DLP incubations of LPB-Tag mouse prostates compared with wild type (data not shown). Although this peak migrates on RP-HPLC close to the COX-derived metabolite HHT, it did not cochromatograph with exogenously added HHT (n = 3). Furthermore, its formation was not significantly modified in LPB-Tag prostate incubations by indomethacin, the COX-1 inhibitor SC560, or the COX-2 inhibitors SC58125 or celecoxib (data not shown). The product may be an epoxyalcohol derivative of 12-HPETE, although its identity was not additionally investigated in the current study.

Absence of 8-LOX mRNA and Immunostaining in Wild-Type and LPB-Tag Mouse Prostates. Compatible with the absence of detectable 8-LOX catalytic activity, 8-LOX mRNA was not detected by RT-PCR in any of two wild-type DLP samples nor in 8 of 9 13–19-week 12T7f samples, with a faint band detected in 1 of 2 19-week samples (data not shown). The same antibody that recognizes human 15-LOX-2 in the human prostate (Fig. 2A) cross-reacts with the murine homologue 8-LOX (8). With good positive control immunostaining in phorbol-ester-treated mouse skin (Fig. 2B), 8-LOX immunostaining was not detected in wild-type prostates or in HGPIN, invasive, or metastatic carcinoma in intact or castrated LPB-Tag mice (Table 1). Similar negative immunostaining was observed in PIN sections of DLP and VP of two LPB-Tag 12T-5 and two LPB-Tag 12T-11 mice (data not shown).

**Table 1 Immunostaining for LOX and COX-2 enzymes in normal and neoplastic prostate epithelium of wild-type and LPB-Tag transgenic mice**

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<th>Enzyme</th>
<th>Wild Type</th>
<th>Transgenic</th>
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<td>Epithelium, mean ± SD*</td>
<td>Epithelium, n with focal or greater/total a</td>
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<td>8-LOX</td>
<td>0.0 ± 0.0</td>
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<td>P12-LOX</td>
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* Mean ± SD of immunoscore (see “Materials and Methods”).

** Number of mice with immunoscore ≥2/total number assessed.

d NA, not applicable (i.e., invasion and metastases not frequent in intact 12T7f or in younger 12T10 mice); ND, not determined.

f Includes castrated (cast) fast growing lines 12T5, 12T7s, 12T7f, and 12T11 mice.

g Two positive animals had 4/4+ in multiple metastatic sites each; the section with negative lung metastasis in another animal failed to show expected positive control staining of normal bronchial epithelium raising concern over tissue quality/fixation.

h 12T10 mice 6 and 7 months old.

i 12T10 mice 6 and 7 months old.
increase in LPB-Tag mice, the P12-LOX and L12-LOX or 12/15-LOX. By conventional RT-PCR, mRNA for both enzymes was detectable in wild-type and 13–19-week 12T-7f DLP samples (data not shown). By quantitative real-time RT-PCR, signals for P12-LOX (with amplification curves falling within the range of the standard curve) were consistently detected in adult wild-type mouse DLPs, 6-week 12T-7f DLPs, and 13–19-week 12T-7f DLPs, but there was no difference in P12-LOX mRNA copy number in neoplastic LPB-Tag prostate versus wild-type prostate (copy number 5.07 ± 3.14 × 10⁻⁷/β-actin copy number, mean ± SD, n = 6, for wild-type mice versus 3.49 ± 4.41 × 10⁻⁵/β-actin copy number, mean ± SD, n = 12, for LPB-Tag 12T-7f mice). The real-time RT-PCR assay for L12-LOX mRNA was not as sensitive as for the housekeeping gene β-actin was readily quantifiable by real-time RT-PCR in these samples, with similar ranges of values as in the neoplastic prostate samples. In contrast to the wild-type samples, L12-LOX mRNA was detected in 6 of 14 13–19-week 12T7f DLPs, including 3 of 9 13–17-week samples (copy number 2.32 ± 4.01 × 10⁻³/β-actin copy number, mean ± SD) and 3 of 5 18–19-week samples (copy number 1.74 ± 2.59 × 10⁻⁶/β-actin copy number, mean ± SD).

IHC for the two 12-LOX enzymes in mouse prostate tissues strongly supported the L12-LOX over the P12-LOX as the source for enzyme activity in wild-type prostate and its increase in LPB-Tag prostates. P12-LOX immunostaining gave generally negative results in prostates, with no sign of increased expression in the neoplastic prostate tissues. With good positive control immunostaining in mouse spleen megakaryocytes (Fig. 2C), only rare focal (<5% or 1+) cytoplasmic granular epithelial P12-LOX immunostaining was seen in 1 of 5 wild-type or LPB-CAT control DLP sections. No appreciable increase in P12-L0X immunostaining was noted in HGPIN of 12T7f mice 6–21 weeks of age (Table 1), paralleling those tissues incubated with AA. P12-LOX immunostaining was also not appreciable in HGPIN of 12T-10 mice or in atrophic PIN lesions, invasive carcinoma, and metastatic carcinoma in castrated fast-growing lines (Table 1). With good positive control immunostaining for L12-LOX and specificity of the immunohistoassay confirmed using sections from the L12-LOX knockout mouse (Fig. 3, A and B), L12-LOX immunostaining was detected in the epithelium of wild-type prostates (Fig. 3, Table 1). Moreover, the extent and particularly the intensity of immunostaining was increased in the epithelium of HGPIN in both 12T-7f and 12T-10 lines (Fig. 3, Table 1), clearly supporting this enzyme as the source of increased 12-LOX catalytic activity in LPB-Tag mice. L12-LOX immunostaining was also noted in invasive NE carcinomas in 10 of 11 mice (Fig. 3, Table 1). L12-LOX immunostaining was heterogeneous in metastatic foci, noted in metastases of 3 of 8 separate mice (Fig. 3, Table 1). In 2 mice examined with multiple metastases, both liver and lung metastases were strongly positive.

**COX-2 Was Increased in HGPIN but not Invasive and Metastatic Carcinoma in LPB-Tag Mice.** COX-2 mRNA was not detected by RT-PCR in 2 wild-type DLP samples. Of a group of 9 13–19-week LPB-Tag 12T-7f animals with HGPIN in the DLP, 2 18–19-week samples gave a positive RT-PCR result for COX-2 expression. Immunostaining for COX-2 protein additionally suggested an increase in expression in PIN of LPB-Tag mice (Fig. 4, Table 1). Whereas COX-2 immunostaining was not detected in most prostates of wild-type mice, increased COX-2 immunostaining was noted in HGPIN of intact fast-growing Tag lines. Approximately one-third of 12T-7f mice showed focal or greater COX-2 immunopositivity in the HGPIN lesions, with weaker immunostaining noted in the remainder. Focal COX-2 immunopositivity was also seen in partially atrophic HGPIN lesions in castrated fast-growing lines, including in foci of stratified epithelium occasionally showing features compatible with transitional or urothelial metaplasia (Refs. 21–23; Fig. 4, Table 1). Increased COX-2 immunostaining was more pronounced and uniform in the HGPIN lesions in 12T-10 mice (Fig. 4, Table 1). This was particularly true in the VP, and the immunostaining was also evident in the younger age ranges (Table 1). In the older range of 12T-10 mice in which invasive and metastatic lesions are more routinely encountered (24), the HGPIN lesions of both DLP and VP typically showed extensive COX-2 immunostaining (Fig. 4, Table 1). In striking contrast to L12-LOX expression in both HGPIN and invasive carcinoma, COX-2 immunostaining was absent in invasive carcinoma (n = 15) and metastatic carcinoma (n = 9) in both castrated fast growing lines and intact 12T-10 mice (Fig. 4, Table 1).

**DISCUSSION**

GEM models potentially offer an important advance in the study of causes of Pca and the testing of possible treatments. Models that progress from PIN to invasive and metastatic carcinoma may allow for elucidation of molecular changes accompanying defined stages of tumor development and progression. The use of these models will depend upon careful validation of their relevance to human Pca (21, 22, 33). Criteria for characterization of GEM models include histopathological and genetic or molecular (22), with careful comparison to features of human tumors at different stages of progression. Individual models well characterized for specific relevance to particular aspects of human Pca will be useful in determining the contribution of specific pathways to cancer progression and response to treatments based on manipulating those pathways.

The SV40 early region or large Tag has been used with several promoters to produce GEM models that vary in PIN to invasive and metastatic carcinoma (20, 24, 34–37). The prostate epithelial expression of Tag sequesters p53 and pRB, which have been implicated in human Pca, including in early, low stage Pca and in subsets of HGPIN, as well as advanced disease, such that the genetic manipulations used in the LPB-Tag mouse have relevance to human Pca (3, 22). The current study investigated possible secondary changes in expression of AA-metabolizing enzymes accompanying tumor progression in the LPB-Tag model. There is growing interest in the possible role of altered AA metabolism in human Pca development and progression (5, 6, 9, 13, 14, 17–19, 38–40), with the need for better animal models to additionally define the etiologic role of these genes and their suitability as targets for therapeutic intervention. The results of the current study indicate that there are both some similarities and some differences in changes of AA metabolizing genes in the mouse model and those described thus far in human Pca (Table 2).

In the benign human prostate, the major AA metabolite is 15S-HETE (5). 15-LOX-2 mRNA is essentially uniformly detected in benign prostate, and by IHC, 15-LOX-2 is reduced or absent in prostate tumors (5–7). We have previously demonstrated that the 15-LOX-2 product, 15S-HETE, activates transcription by the nuclear receptor PPARγ and inhibits proliferation of Pca cells (28). Hence, in the human prostate, 15-LOX-2-derived 15S-HETE may constitute an endogenous ligand for PPARγ and reduced 15-LOX-2 in Pca may contribute to tumor development or progression by altered expression of PPARγ-regulated genes.
Fig. 3. Leukocyte (L-) 12-LOX immunostaining in wild-type and LPB-Tag mouse prostate. A, positive control showing strong bronchial epithelial immunostaining in mouse lung (arrowheads). B, confirmation of specificity of immunostaining, with absence of similar bronchial epithelial immunostaining in section of L12-LOX (12/15-LOX) knockout mouse (arrowheads). C, weak focal cytoplasmic immunostaining in secretory epithelial cells in DLP of 15-week-old wild-type mouse (top right). D, focal cytoplasmic immunostaining in...
The metabolism of AA in the normal mouse prostate has not been previously characterized. At the cDNA and protein level, the murine 8-LOX has 78% sequence identity to human 15-LOX-2 (8) and is considered to represent the murine homologue of 15-LOX-2 (12). However, in the current study, we observed that the murine 8-LOX is not similarly expressed in the normal mouse prostate. By a combination of RT-PCR, IHC, and enzyme activity assays, we did not detect 8-LOX expression in wild-type or LPB-Tag mouse prostates. The reasons or functional significance of this potentially evolutionary difference in enzyme expression between mouse and human prostate remain to be elucidated.

In the current study, the major AA-metabolizing activity detected in epithelial cells of VP of adult wild-type mouse, including at apical cell membrane (arrowhead). A, increased immunostaining in neoplastic epithelium of HGPIN in DLP of 17-week-old LPB-Tag 12Tf mouse, similar to tissues incubated in Fig. 1. Note absence of immunostaining in neoplastic stroma. B, diffuse strong L12-LOX immunostaining in epithelium of partially regressed HGPIN, including in larger more central dilated ducts (arrowhead, top right) and connecting smaller acinar profiles (arrowheads toward bottom) in DLP of LPB-Tag 12T-11 mouse castrated at 21 weeks and sacrificed at 47 weeks. Note absence of staining in residual hypercellular stroma (asterisk). C, diffuse COX-2 immunostaining in HGPIN containing residual glands of DLP of 11-month-old 12T-10 mouse (arrowheads), with complete absence of COX-2 expression in admixed invasive NE carcinoma (arrowheads). D, negative COX-2 immunostaining in neoplastic epithelium of HGPIN in 12T-10 mouse prostate (arrowhead), with complete absence of immunostaining in immediately adjacent invasive poorly differentiated carcinoma with NE differentiation (asterisk). E, focal COX-2 immunostaining in HGPIN of proximal prostatic duct in LP of 9-month-old 12T-10 mouse (arrowheads). Note strong immunostaining in included portion of vas deferens (asterisk, bottom left). F, diffuse COX-2 immunostaining in HGPIN of 8-month-old 12T-10 mouse. G, COX-2 immunostaining in HGPIN containing residual glands of DLP of 11-month-old 12T-10 mouse (arrowheads), with complete absence of COX-2 expression in admixed invasive NE carcinoma (asterisk). H, COX-2 immunostaining in histologically identical NE carcinoma metastatic to liver in 11-month-old 12T-10 mouse (same mouse as H); normal liver hepatocytes seen at bottom. J, focal COX-2 immunostaining in neoplastic epithelial cells of HGPIN (in situ lesion with diffuse lobular expansion) in DLP of 22-week-old LPB-Tag 12Tf mouse. Cytoplasmic granular and occasional perinuclear immunostaining was noted focally in both the more central located duct lumens and the connecting smaller acinar profiles, but the extent of immunostaining was typically less than in 12T-10 mice. K, focal COX-2 immunostaining in neoplastic epithelium (partially regressed, focally stratified with transitional metaplasia) of HGPIN (arrowheads) in the DLP of LPB-Tag 12T-11 mouse castrated at 21 weeks and sacrificed at 47 weeks. Note absence of immunostaining in residual hypercellular stroma (asterisks). L, prominent diffuse COX-2 immunostaining in foamy macrophages in stroma (top right) coursing through epithelium (asterisk) and in gland lumen (bottom left) in DLP of a 12Tf mouse castrated at 16 weeks and sacrificed at 22 weeks. M, absence of COX-2 immunostaining in invasive carcinoma with NE differentiation (asterisks) in contrast to focal residual immunostaining in partially regressed PIN (arrowheads) of VP of LPB-Tag 12T-5 mouse castrated at 22 weeks and sacrificed at 29 weeks.

Fig. 4. COX-2 immunostaining in wild type and LPB-Tag mouse prostate. A, positive control, with strong immunostaining in epithelium of the mouse vas deferens. B, confirmation of specificity of immunostaining, with absence of similar vas deferens epithelial immunostaining in similar section from COX-2 knockout mouse. C, absence of appreciable immunostaining in VP of wild-type adult mouse. D, focal increased immunostaining in HGPIN in VP from LPB-Tag 12T-10 mouse. E, extensive COX-2 immunostaining in neoplastic epithelium of HGPIN in 12T-10 mouse prostate (arrowhead), with complete absence of immunostaining in immediately adjacent invasive poorly differentiated carcinoma with NE differentiation (asterisk). F, focal COX-2 immunostaining in HGPIN of proximal prostatic duct in LP of 9-month-old 12T-10 mouse (arrowheads). Note strong immunostaining in included portion of vas deferens (asterisk, bottom left). G, diffuse COX-2 immunostaining in HGPIN of 8-month-old 12T-10 mouse. H, COX-2 immunostaining in HGPIN containing residual glands of DLP of 11-month-old 12T-10 mouse (arrowheads), with complete absence of COX-2 expression in admixed invasive NE carcinoma (asterisk). I, negative COX-2 immunostaining in neoplastic epithelium of HGPIN (arrowheads) in the DLP of LPB-Tag 12T-11 mouse castrated at 21 weeks and sacrificed at 47 weeks. Note absence of immunostaining in residual hypercellular stroma (asterisks). J, prominent diffuse COX-2 immunostaining in foamy macrophages in stroma (top right) coursing through epithelium (asterisk) and in gland lumen (bottom left) in DLP of a 12Tf mouse castrated at 16 weeks and sacrificed at 22 weeks. M, absence of COX-2 immunostaining in invasive carcinoma with NE differentiation (asterisks) in contrast to focal residual immunostaining in partially regressed PIN (arrowheads) of VP of LPB-Tag 12T-5 mouse castrated at 22 weeks and sacrificed at 29 weeks.

The metabolism of AA in the normal mouse prostate has not been previously characterized. At the cDNA and protein level, the murine 8-LOX has 78% sequence identity to human 15-LOX-2 (8) and is considered to represent the murine homologue of 15-LOX-2 (12). However, in the current study, we observed that the murine 8-LOX is not similarly expressed in the normal mouse prostate. By a combination of RT-PCR, IHC, and enzyme activity assays, we did not detect 8-LOX expression in wild-type or LPB-Tag mouse prostates. The reasons or functional significance of this potentially evolutionary difference in enzyme expression between mouse and human prostate remain to be elucidated.

In the current study, the major AA-metabolizing activity detected in...
Table 2: Correlation of reported alterations in AA metabolizing enzymes in human prostate carcinoma and in LPB-Tag-transgenic mouse model

| Human enzyme | Benign human prostate | Cancer IHC | Cancer mRNA | Cancer enzyme activity | HGPIN (IHC) | Pca cell lines/postulated mechanism(s) | Murine homologue | Wild-type and Tag mouse result
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<tr>
<td>15-LOX-2</td>
<td>Positive mRNA (Northern); positive activity; secretory cells by IHC (5, 6)</td>
<td>Reduced (in Gleason score ≥ 6) (5, 7)</td>
<td>Reduced (Northern) (6)</td>
<td>Reduced (5, 6)</td>
<td>Reduced (7)</td>
<td>Absent/15-HETE activates PPARγ, inhibits proliferation (28, 41)</td>
<td>mRNA (RT-PCR)/possible modulation of growth factors, down-regulation of PPARγ (48, 51, 58)</td>
<td>8-LOX (8)</td>
</tr>
<tr>
<td>15-LOX-1</td>
<td>Focal by IHC (9)</td>
<td>Increased (in Gleason score ≥ 8) (9)</td>
<td>Increased (9)</td>
<td>ND (5, 6)</td>
<td>ND</td>
<td>mRNA (RT-PCR)</td>
<td>L12-LOX (12/15-LOX) (10, 30)</td>
<td>Not present by IHC or activity in wild-type or Tag prostate; mRNA by RT-PCR</td>
</tr>
<tr>
<td>P12-LOX</td>
<td>Focal basal cells by ISH; mRNA (RT-PCR) (13); no activity detected (5, 6)</td>
<td>Possibly increased in subset and correlating with grade (59)</td>
<td>Increased (in Gleason score ≥ 8) (RT-PCR) (13)</td>
<td>Not increased (5, 6)</td>
<td>ND</td>
<td>mRNA (RT-PCR)</td>
<td>P12-LOX (10, 30)</td>
<td>Activity not present</td>
</tr>
<tr>
<td>5-LOX</td>
<td>mRNA variably by RT-PCR; protein variably by Western and IHC; 5-HETE variably by immunohistochemistry (16); activity not detected by RP-HPLC (5, 6)</td>
<td>Increased; supported by Western blots (16)</td>
<td>Possibly increased (nonquantitative RT-PCR) (16)</td>
<td>Not increased in tissue AA incubation and RP-HPLC (5, 6); possibly increased 5-HETE by immunohistochemistry, without prior separation or analysis of other HETEs to control for autooxidation (16)</td>
<td>ND</td>
<td>mRNA (RT-PCR)</td>
<td>5-LOX (30)</td>
<td>Activity not present</td>
</tr>
<tr>
<td>COX-2</td>
<td>Focal by IHC (6, 18, 19); mRNA by RT-PCR (17); mRNA in subset by RNase protection assay and ISH (6)</td>
<td>Increased in some studies (possibly correlates with grade) (6, 18, 19)</td>
<td>Possibly increased by RT-PCR (17); not commonly detected by RNase protection assay and ISH (6)</td>
<td>Not prominent by assays employed to date (6)</td>
<td>Little data; possibly increased in a subset of HGPIN (6, 18)</td>
<td>mRNA (RT-PCR)/inhibitors induce apoptosis, reduce angiogenesis (40, 55, 60)</td>
<td>COX-2 (32)</td>
<td>mRNA, IHC increased in HGPIN</td>
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Compiled information from references cited within table, indicated in parentheses.  
Results of current study.  
Increased by real-time RT-PCR analysis in tumor versus benign in the majority of snap-frozen benign-tumor pairs from radical prostatectomies with predominantly Gleason score 7.  
ND, not determined (not reported in literature to best of our knowledge).
wild-type mouse prostate was a 12S-LOX activity. The mouse has three known different 12(S)-lipoxygenases, an epidermal type (E12-LOX), a platelet type (P12-LOX), homologous to human platelet type 12-LOX, and a leukocyte type (L12-LOX or 12/15-LOX), homologous to human 15-LOX-1 (12, 30). The possible expression of E12-LOX in the murine prostate was not investigated in the current study. P12-LOX mRNA was detected in all wild-type prostates. Hence, our studies do not exclude a contribution of P12-LOX (or E12-LOX) to the 12-LOX catalytic activity in wild-type prostate or a minor contribution to the activity in the neoplastic LPB-Tag prostates. However, 12-LOX catalytic activity was increased in the neoplastic LPB-Tag prostate, and the constellation of data presented herein indicates that this increased 12-LOX activity is that of the L12-LOX (12/15-LOX). Human 15-LOX-1 and the murine 12/15-LOX (L12-LOX) are fundamentally different enzymes than 15-LOX-2, not only in terms of structure and tissue distribution but also regarding enzymatic properties (12). Similar to the human 15-LOX-1, the mouse L12-LOX forms a combination of 12-HETE and 15-HETE from AA and, in common with other animal L12-LOX isozymes, the mouse L12-LOX is also capable of generating more polar additional peroxidation products from bis-dioxygenations (12). The appearance of more polar radiolabeled products was very evident in our incubations of [14C]AA with mouse prostate tissues; formation of these products was not sensitive to COX inhibitors, and the compounds most likely represent derivatives of 12-HPETE and/or 15-HPETE. Hence, the enzymatic activity in wild-type prostate and increased in LPB-Tag prostate is quite compatible with that of the L12-LOX.

The amount of 12-HETE formation appeared to be greater in the DP (DLP) than VP in wild-type mice. The mechanisms responsible for differentially regulating growth and function of the individual mouse prostate lobes are still being characterized such that the factors regulating possible different lobar levels of L12-LOX are not known. As IHC indicates that it is the luminal epithelial cells that express L12-LOX in the wild-type mouse prostate, one possible explanation for the greater catalytic activity in the DP (DLP) is simply the increased amount of epithelium (relative to stroma), with increased luminal folding and some stratification of the luminal epithelium, present in the normal mouse DP versus VP. Greater formation of 12-HETE in the DLP versus VP was also noted for the increased activity in the LPB-Tag 12T-7f prostate. Again, as immunostaining showed that the proliferating atypical epithelium is the source of the (12/15-LOX)-derived 12-HETE, one possibility for greater 12-HETE in the DLP versus VP in Tag mice is simply the greater amount of neoplastic epithelium in the former. The in situ lesion in the DLP is characterized by a much more robust proliferation of epithelium, with a fairly uniform lobular expansion by new gland spaces but still regarded as PIN and which can progress to obvious focal invasion (20–22). Despite the differences in catalytic activity, however, immunostaining showed that a L12-LOX increase occurs in both the VP and DLP, suggesting that it may indeed contribute to tumor progression because progression to invasion occurs in both these lobes in this model, and the invasive foci are also L12-LOX immunopositive. However, as the stromal morphology is so different in these lobes, it is also tempting to speculate on a possible contribution of the epithelial derived LOX activity to the stromal cellularity increase via a paracrine effect.

Recent studies have suggested a possible role for altered 15-LOX-1 expression in multiple human cancers, including colon, breast, and prostate (42, 43). Some controversy exists regarding altered expression of 15-LOX-1 in human colon cancer, with both increased expression (44) and reduced expression (45) reported compared with benign colon. Similarly, promitogenic effects as well as antiproliferative, prodifferentiating, and proapoptotic effects have been observed for the 15-LOX-1 metabolite 13-HODE in cell lines (45–47). The emerging story in Pca appears to be one of up-regulation of this gene compared with benign prostate, similar to the observations we report here in the GEM model of Pca. Kelavkar et al. (9) observed by IHC an increase in 15-LOX-1 in human Pca, correlating with increased grade and p53 mutations, and we have observed increased 15-LOX-1 mRNA in Pca versus benign peripheral zone prostate snap frozen during radical prostatectomy (unpublished observations). Multiple possible mechanisms have been proposed for the contribution of 15-LOX-1 and 13-HODE to tumor progression, including promotion of tumor growth and spread via induction of angiogenesis through increased vascular endothelial growth factor (48). Interestingly, both human 15-LOX-1 and the murine 12/15-LOX have been implicated in the generation of PPARγ agonists in macrophages (49, 50). However, recent studies have suggested a possible proepithelial effect of 15-LOX-1-derived 13-HODE (but not the 15-LOX-2 derived AA metabolite 15-HETE) through down-regulation of PPARγ via interaction with EGF and phosphorylation of PPARγ mediated by activated mitogen-activated protein kinase (51). PPARγ is expressed in wild-type prostate and in all lobes of the LPB-Tag mouse prostate (unpublished observations). However, a possible role of increased 12/15-LOX in reduced PPARγ activation versus other proepithelial mechanisms with tumor progression in LPB-Tag mice remains to be investigated. Interactions of the L12-LOX with growth factor signaling pathways are particularly intriguing because the invasive and metastatic tumor in the LPB-Tag mouse model is accompanied by loss of hormone dependence (24), and signaling through EGF and related pathways has been implicated as potentially important in hormone refractory human Pca (4). The LPB-Tag model may prove useful in elucidating the role of 12/15-LOX, EGF, and mitogen-activated protein kinase activation in progression to advanced, hormone refractory, and metastatic tumor.

In addition to increased expression of L12-LOX, we observed increased COX-2 immunostaining in HGPIN in LPB-Tag mouse lines. This was especially prominent in the HGPIN in the 12T-10 mouse. PG production was not conspicuous in AA incubations of PIN-containing LB 12T-7f DLPs. However, further supporting increased COX-2 activity in this model, we have recently readily detected PG_E2 in extracts of these PIN-containing DLPs by gas chromatography mass spectrometry, the levels of which are reduced by prior oral treatment of the mice with the selective COX-2 inhibitor celecoxib. However, in the current study, in contrast to COX-2 immunostaining in HGPIN, invasive and metastatic foci were consistently negative for COX-2 in both the intact 12T-10 mice and castrated fast-growing lines. This was an unexpected result, arguing against the necessity for COX-2 expression or up-regulation in tumor cells for the development of invasive and especially metastatic carcinoma in this mouse model, as has been suggested recently by some studies in human Pca. Interestingly, Gupta et al. (52) recently observed an inhibition of tumor progression with COX-2 inhibition in the TRAMP GEM model of Pca, wherein the selective COX-2 inhibitor celecoxib reduced metastases over a time course in which these mice progress from HGPIN to invasive and metastatic carcinoma. The patterns of expression of COX-2 with tumor progression in this model have not yet been reported. If COX-2 were also not expressed in these later stages of invasion, it would suggest that the beneficial effect could be exerted by retarding growth or progression of COX-2-positive HGPIN lesions, by effects on en-
dothelial cells in tumor neovasculature, or by nonspecific effects unrelated to COX-2 inhibition.

The possible increased expression of COX-2 in human Pca is controversial. Some studies have reported increased COX-2 in human Pca and possibly HGPIN, especially by IHC (17, 18). However, more recent studies have challenged this observation, suggesting that COX-2 may not be increased in Pca or that its increased expression may be limited to high-grade (Gleason score ≥ 8) tumors (6, 19). Smaller volume high-grade (Gleason score, 8–10) Pcas are increasingly being detected in the prostate-specific antigen era (53), wherein such tumors may arise (directly) from HGPIN (53). In addition, increased COX-2 expression was noted in postinflammatory atrophy in the human prostate, a condition increasingly recognized as a possible precursor for invasive Pca (19). GEM models with increased COX-2 in precursor lesions may be useful to establish chemopreventive efficacy for COX-2 inhibitors, especially for those patients with precursor lesions giving rise to high-grade tumors, who have a narrow window of opportunity for current surgical intervention for cure (53).

COX-2 expression may be regulated by wild-type p53, with increased expression in some tumors associated with p53 mutation (54). As the large Tag likely promotes carcinogenesis in LTB-PgT mice by binding to and inactivating p53 and pRb (20), this may, in part, explain the up-regulation of COX-2 as well as of 12/15-LOX in this model because up-regulation of the human homologue 15-LOX-1 has been correlated with p53 mutation/accumulation in human Pca (9). Regardless of the precise relationship between COX-2 expression in HGPIN and/or invasive Pca in human Pca and the mouse model, the increased expression of COX-2 in this model will allow for additional elucidation of the mechanisms whereby this enzyme and its metabolites may contribute to tumor development and progression. A recent study using human Pca cell lines in nude mice suggested a contribution of increased COX-2 activity to tumor angiogenesis (55). Angiogenesis involves a complex interplay of tumor, stroma, and new vessels and increased COX-2 activity to tumor angiogenesis (55). Angiogenesis using human Pca cell lines in nude mice suggested a contribution of GEM models of human Pca in that it is unlikely any single model will involve a complex interplay of tumor, stroma, and new vessels and critical role of 5-lipoxygenase. Biochem. Biophys. Res. Commun., 237: 6367–6368, 1999.

In summary, our initial studies characterizing AA metabolism in the LTB-PgT mice have confirmed our expectations regarding early GEM models of human Pca in that it is unlikely any single model will perfectly parallel all aspects of the human disease. We observed that some changes in AA metabolism occur in the LTB-PgT mouse that have also been described in human Pca (Table 2). This is similar to previous studies in other GEM models of Pca in which some secondary mutations or alterations in other genes occurred that have also been observed in human Pca (56, 57). In particular, we observed a marked increase in L12-LOX, homologue of human 15-LOX-1, which has also been described in human Pca (Table 2). This is similar to previous studies in other GEM models of Pca in which some secondary mutations or alterations in other genes occurred that have also been observed in human Pca (56, 57). In particular, we observed a marked increase in L12-LOX, homologue of human 15-LOX-1, and of 15-lipoxygenase-2 immunostaining in prostate adenocarcinoma. Am. J. Pathol., 155: 235–245, 1999.


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Elevated Expression of 12/15-Lipoxygenase and Cyclooxygenase-2 in a Transgenic Mouse Model of Prostate Carcinoma

Scott B. Shappell, Sandra J. Olson, S. Erin Hannah, et al.


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