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15-LOX-2: Tissue distribution, enzymatic properties, possible biological function and role of reduced expression in prostate cancer

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Introduction

15-LOX-2 was originally cloned from human hair rootlets by Brash et al. during investigations of lipoxygenases expressed in human skin (1). The cDNA derived amino acid sequence showed only 35-40 % sequence identity to the previously characterized 5-LOX, 12-LOX, and the reticulocyte-type of 15-LOX (15-LOX-1). Within the expanding LOX family, human 15-LOX-2 appears to be most homologous to the murine 8-LOX (78 % identical at the cDNA and protein levels) (2, 3). Mouse 8-LOX is expressed in the skin, increased with topical phorbol esters (4-6), and may contribute to keratinocyte differentiation (7). Human 15-LOX-2 maps to the short arm of chromosome 17, in a similar locus as several other human LOXs (unpublished results).

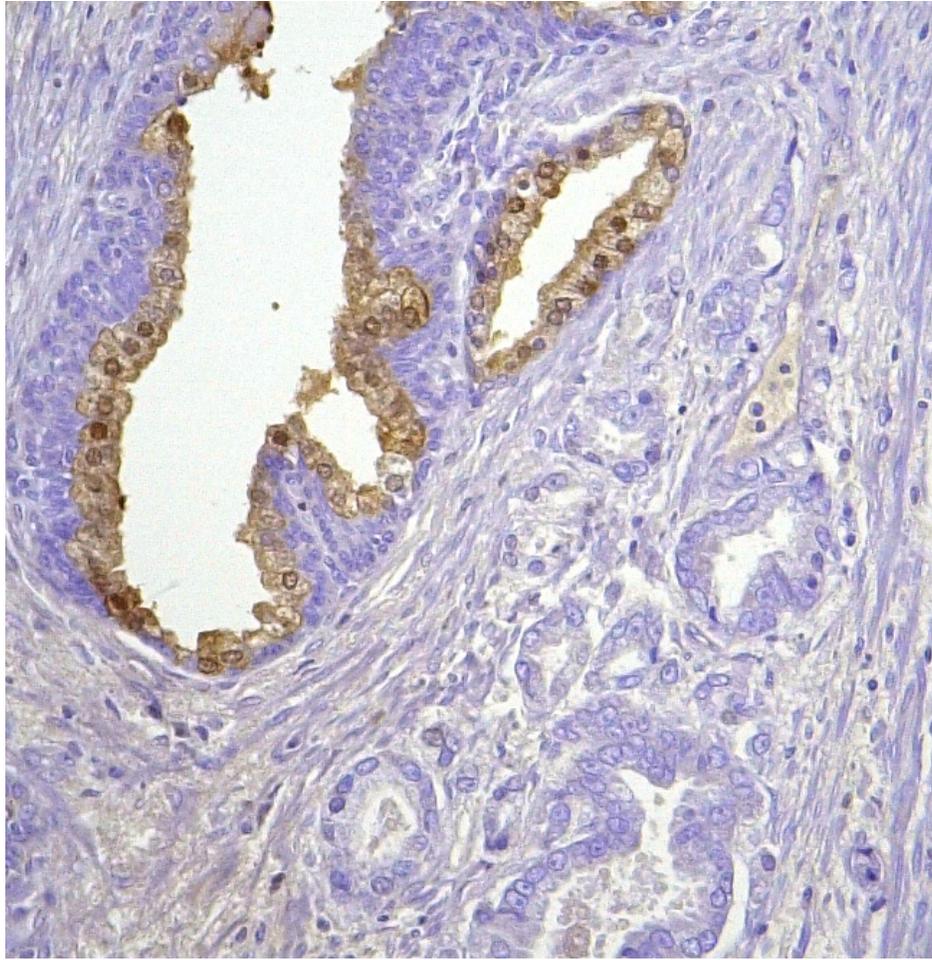
Enzymology of 15-Lox-2

The enzymatic properties of 15-LOX-2 have been investigated using protein expressed in HEK 293 cells (1) and baculovirus (8). Compared to the reticulocyte lipoxygenase (15-LOX-1) (9), 15-LOX-2 has distinct enzymatic properties, including exclusive incorporation of molecular oxygen at the C-15 position of arachidonic acid with formation only of 15S-hydroperoxy-eicosatetraenoic acid (15S-HPETE). In addition, 15-LOX-2 forms product steadily over time (up to one hour or greater) without undergoing significant suicide inactivation (1). Finally, while both arachidonic acid (AA) and linoleic acid are substrates for the two types of 15-lipoxygenase, linoleate is the preferred substrate for 15-LOX-1, whereas arachidonate is preferred by 15-LOX-2. When Kilty et al. reported the cloning of 15-LOX-2 cDNA (which they

referred to as 15-LOXb) from a prostate library, they also reported cloning of an alternately spliced form, corresponding to elimination of a single exon (8). This splice variant has different enzymatic properties, which may have biologic significance in tissues expressing this form, including reduced substrate affinity, more evidence of suicide inactivation, and a lower susceptibility to LOX inhibitors (8). Similar to some other LOX enzymes, translocation of 15-LOX-2 to cell membranes upon activation and augmentation of activity by Ca²⁺ has been reported (8).

Expression & Tissue Distribution

15-LOX-2 appears to have a unique tissue distribution, being expressed in epithelial cells, which often have secretory differentiation. 15-LOX-2 mRNA was detected in skin, cornea, lung, and prostate by Northern analysis (1). We have used immuno-histochemistry and in situ hybridization to characterize the sites of expression of 15-LOX-2 in several tissues. In benign prostate, 15-LOX-2 is uniformly expressed in the differentiated secretory (luminal) cells in the peripheral zone (where most prostate cancers arise) and to slightly less extent in the transition zone (where benign prostatic hyperplasia occurs) and central zone (surrounding the ejaculatory ducts) (10). 15-LOX-2 was not expressed in the prostate gland basal cells, stroma, vessels, ejaculatory ducts, urothelium, or seminal vesicles. In incubations with exogenous AA analyzed by reverse phase HPLC, benign prostate uniformly synthesized 15-HETE as the major AA metabolite (10). By immunohistochemistry, 15-LOX-2 was reduced (>25 % 15-LOX-2 immunonegative) or completely absent in 10 of 18 prostate carcinomas (Figure 1) (10).



15-Lox-2 and Prostate Cancer

In 3 pairs of benign and tumor from the same patients' radical prostatectomy specimens, 15-HETE formation from AA was reduced or absent in tumor compared to benign (10). More recently, we have utilized snap frozen tissues obtained intraoperatively in 60 patients undergoing prostatectomy, with rigid correlation to histology of tissues processed for RNA and enzyme assays. 15-LOX-2 mRNA was detected in 21 of 25 benign prostate samples and 23 of 29 benign samples formed 15-HETE from exogenous AA. In contrast, 15-LOX-2 mRNA and

activity were significantly reduced in tumor versus benign tissue (11). 15-HETE formation was detected in 6 of 18 pure tumor specimens overall. Whether this is from 15-LOX-2 or 15-LOX-1 was not established. Elevated expression of 15-LOX-1 in prostate cancers has been reported (12), and more details are given in the accompanying review on 15-LOX-1 by Nixon and Eling.

Better treatment strategies for prostate cancer are predicated on understanding factors that control the proliferation and differentiation of benign and malignant cells. This applies especially in the most susceptible peripheral zone of the prostate, where most prostate cancers arise and appear to develop from in situ precursor lesions designated high grade prostatic intraepithelial neo-plasia (HGPIN) (13-15). In this context we are interested in the potential role 15-LOX-2 might play in prostate cell differentiation and how its reduced expression may relate to different grades or stages of the disease. Recently we found that reduced expression of 15-LOX-2 in prostate cancer correlates with tumor differentiation (grade) and may occur early in prostate carcinogenesis. In a series of 70 radical prostatectomy specimens, immunohistochemistry demonstrated that in contrast to essentially uniform expression in differentiated benign glands, 15-LOX-2 was completely absent in 23 cases and substantially (> 50 %) reduced in 45 others (16). Reduced expression correlated with grade, being retained particularly in Gleason score 5 tumors (which include generally transition zone well differentiated or Gleason pattern 2 components) versus a statistically significant reduction in Gleason score 6, 7, and > 8 tumors. In a subset of patients with multifocal tumors or different regions of tumor with different grades, 15-LOX-2 was significantly reduced in higher vs. lower grade foci (16). Compared to benign glands, 15-LOX-

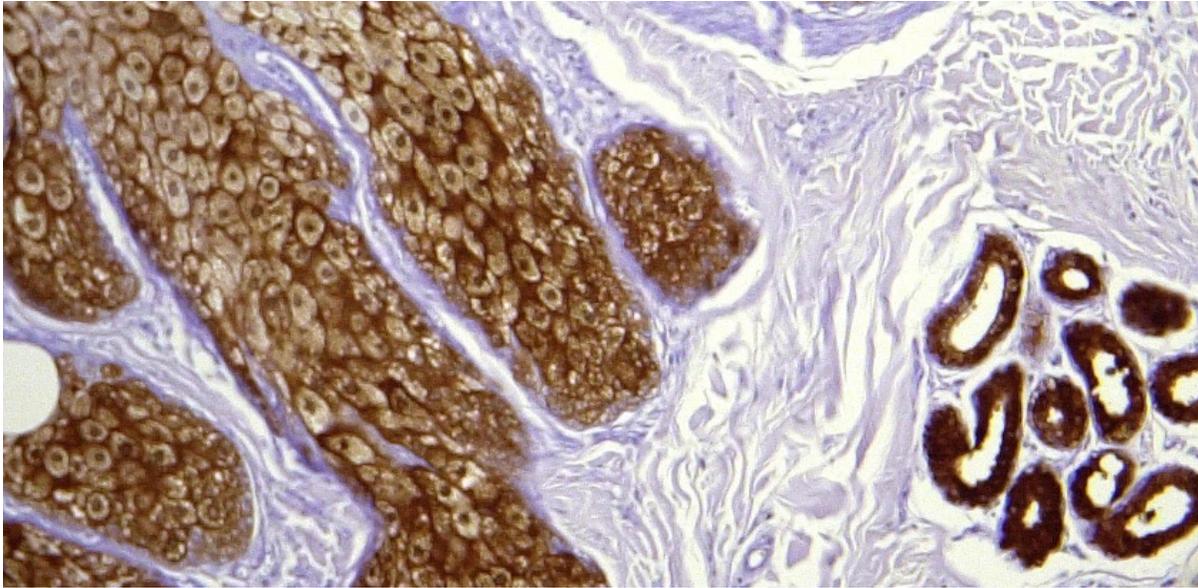
2 was significantly reduced in HGPIN glands, both away from and within areas of invasive tumor (16). Hence, reduced expression of 15-LOX-2 may be an early alteration in the development of prostate cancer, at least in a significant subset of patients.

The mechanisms whereby 15-LOX-2 derived 15-HETE contributes to cell function in tissues expressing the gene or whereby reduced 15-LOX-2 contributes to prostate cancer development or progression are still being elucidated. However, recent data suggests that 15-HETE may be able to activate gene transcription as a ligand for the nuclear receptor peroxisome proliferator activated receptor gamma (PPAR γ) (17, 18). In studies of the mechanisms of oxidized low density lipoprotein and foam cell formation, oxidized lipids including 9-HODE, 13-HODE, and 15-HETE were reported to activate PPAR γ -dependent transcription (19). We have recently made related observations in prostate cells. The prostate tumor cell line PC3 is negative for 15-LOX-2 mRNA and enzyme activity as is predictable based on the reduced expression or absence of 15-LOX-2 in prostate cancer tissues (as opposed to benign prostate). By contrast, benign and malignant prostate tissues and prostate cancer cell lines expressed PPAR γ . Exogenous 15-HETE, similar to synthetic PPAR γ agonists, caused a dose-dependent inhibition of PC3 proliferation. Furthermore, 15-HETE activated PPAR γ -dependent transcription with PPAR-response element (PPRE)-containing reporter constructs and upregulated expression of a known PPRE-containing gene (20). Hence, 15-LOX-2 derived 15-HETE may constitute an endogenous ligand for PPAR γ in prostate. A caveat here is that concentrations of 15-HETE in the order of 10mM are required to elicit these effects. Whether this requirement relates to the limited ability of exogenous 15-HETE to penetrate to the cell nucleus, or to missing synergistic factors in the in vitro PC3 cell system,

or to this being a pharmacological effect is yet to be clarified.

Synthetic PPAR γ agonists have been shown to inhibit proliferation and possibly induce differentiation in tumor cell lines from multiple organs, including breast (21), colon (22), and urinary bladder (23), in which endogenous ligands have not been identified. Furthermore, these agents are widely available and utilized as anti-diabetic agents in patients with type II diabetes (24) and are in clinical trials for human malignancies, including prostate cancer (25). In a recent study, loss of function mutations in PPAR γ , previously reported in colon cancer (26), were not observed in a large number of prostate cancers (27). Although loss of heterozygosity at chromosome regions including PPAR γ were observed in 40 % of prostate carcinomas, PPAR γ appears to be expressed in most or all of prostate carcinomas (27). Hence, we believe that reduced 15-LOX-2 in prostate carcinoma may contribute to the malignant phenotype by reduced transcription of PPAR γ -regulated genes, the specific identify of which still remain to be defined. In a brief clinical trial with the synthetic PPAR γ agonist troglitazone, reduction in serum PSA was observed in a significant subset of patients with both androgen sensitive and androgen insensitive prostate cancer (27). Hence, identifying prostate cancer patients with reduced 15-LOX-2 and understanding the relationship between androgen receptor (AR) signaling and 15-LOX-2 expression (see below) may help identify patients who could be more likely to benefit from this promising therapy.

By immunohistochemistry and in situ hybridization, we have also characterized the distribution of 15-LOX-2 in skin. 15-LOX-2 is uniformly and strongly expressed in differentiated secretory cells of sebaceous glands and also in apocrine and eccrine glands (Figure 2) .



Interestingly, these secretory cutaneous adnexal glands, like the prostate, are androgen regulated. Sebocytes like prostate epithelial cells express 5 α -reductase, which converts circulating testosterone to dihydrotestosterone (DHT), a strong agonist for AR (28-31). Whereas induction of sebocyte differentiation in vitro has been difficult to achieve with DHT alone, synthetic agonists for PPAR γ can induce sebocyte differentiation and act additively with AR (32). These results suggest that expression of 15-LOX-2 may in part be AR regulated and that a 15-LOX-2/PPAR γ pathway may contribute to secretory differentiation in 15-LOX-2 expressing AR regulated tissues. Supporting AR modulation of 15-LOX-2, we have observed that 15-LOX-2 is not expressed in prepubertal (vs. postpubertal) prostates and expression is reduced in benign prostate epithelium in adult patients who have received prior anti-androgen therapy (manuscript in preparation). Interestingly, in contrast to cutaneous sebaceous glands, however, eyelid Meibomian glands, modified sebaceous glands - which are also AR regulated (33, 34), are uniformly negative for 15-LOX-2. Sebaceous carcinomas, mostly derived from Meibomian glands, are also negative for 15-LOX-2 (Shappell et al.)

Conclusion

Expression of other tissue specific genes may modulate 15-LOX-2 expression in androgen sensitive tissues. In contrast to extensive studies on the regulation of 15-LOX-1 expression (35), data regarding regulation of 15-LOX-2 expression have not been reported. Current studies in our laboratory are addressing possible direct or indirect mechanisms whereby AR may modulate 15-LOX-2 expression and studying other factors which may modify 15-LOX-2 expression, primarily in prostate cells. As the distribution of 15-LOX-2 appears quite unique amongst mammalian LOX enzymes, ongoing and future efforts to clarify the function of 15-LOX-2 derived 15-HETE will shed important insight into normal function and diseases of these organs.

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