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15-lipoxygenase-1 and 15-lipoxygenase-2 may have different and opposing biological functions

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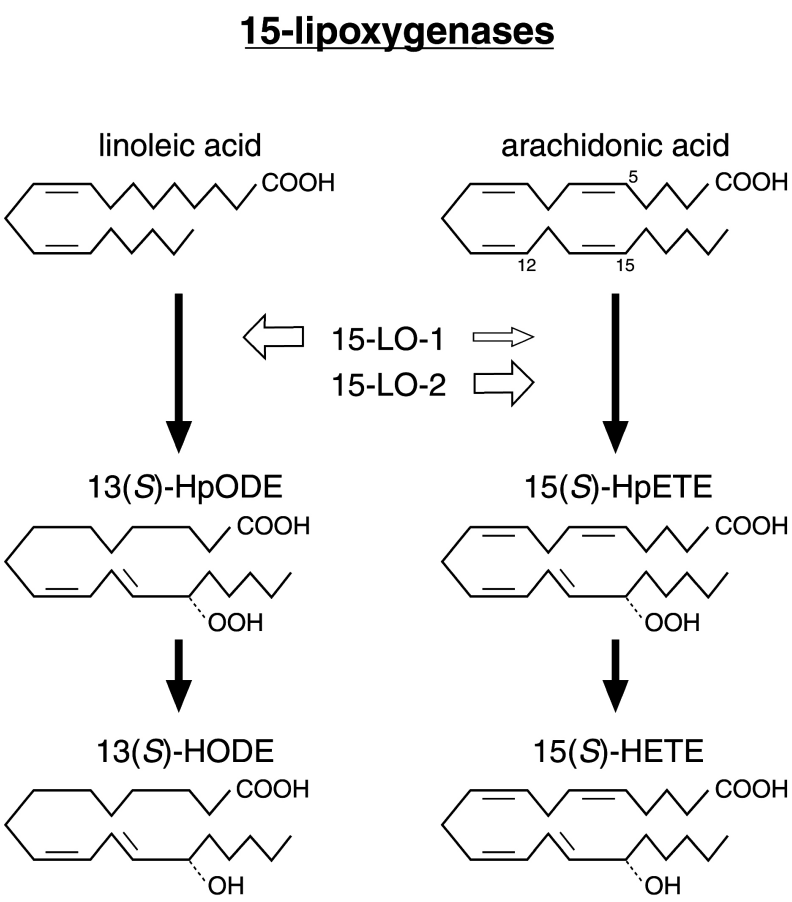
Introduction

Lipoxygenases (LOs) are a family of enzymes that oxidize unsaturated fatty acids at specific positions to form active hydroperoxy and epoxy metabolites. Three major LOs have been characterized in human tissues and are named according to the site of arachidonic acid oxygenation, 5-, 12-, and 15-LO. As shown in Figure 1, 15-LO metabolizes arachidonic acid to 15(S)-hydroperoxyeicosatetraenoic acid (HpETE) which is peroxidized to 15(S)-hydroxy-eicosatetraenoic acid (HETE). Linoleic acid is also a substrate and is metabolized to 13(S)-hydroperoxyoctadecadienoic acid (HpODE) and further converted to 13(S)-hydroxyoctadecadienoic acid (HODE). Two different 15-LOs have been identified that differ in substrate preference and in tissue distribution. 15-LO-1 preferentially metabolizes linoleic acid producing primarily 13(S)-HODE, but also metabolizes arachidonic acid to a lesser extent to 15(S)-HETE or 12(S)-HETE. 15-LO-2 primarily produces 15(S)-HETE from arachidonic acid (Fig. 1).



Expression of 15-LO-1 is observed in reticulocytes, eosinophils, monocytes/macrophages, tracheobronchial epithelial cells, prostate adenocarcinoma, and colorectal carcinoma (1-6). The mRNA for 15-LO-2 is present in prostate, lung, skin, and cornea (7). Given the differences in tissue distribution and in substrate specificity of these two enzymes, they likely serve different purposes. However, the biological functions of these lipoxygenase enzymes are unclear.

Biological Roles of 15-Lipoxygenases

There are several hypotheses for 15-LO-1 function. This enzyme may play a role in the maturation of reticulocytes to erythrocytes via the peroxidation of mitochondria and other intracellular organelles, thereby altering the membrane structure and allowing membrane proteolysis during this erythrocyte maturation process (1,8). 15-LO-1 is present in inflammatory cells. Thus, the bioactive metabolites may modulate host defense responses (9,10). In addition, 15-LO-1 may play a role in atherosclerosis (11-14) by oxidizing low density lipoprotein. This oxidation may facilitate foam cell development and, therefore, the development of atherosclerosis. In fact, some evidence suggests that the metabolites of 15-LO may serve as ligands for peroxisome proliferator-activated receptor (PPARγ). In a reporter system for PPARγ ligand binding, 13(S)-HODE, 13(S)-HpODE, and 15(S)-HETE all show binding activity but at relatively high concentrations (15). Further, PPARγ activation has been demonstrated in 15-LO-transfected macrophages (16). The function of 15-LO-1 may be

dependent on cell type as different effectors are present in different cells.

15-Lipoxygenases and Cancer

In cell culture, our laboratory has demonstrated an association between 15-LO-1 expression and cell differentiation. Hill (4) demonstrated that the addition of retinoic acid to normal human tracheobronchial epithelial (NHTBE) cells caused differentiation into mucociliary epithelium concomitant with increased 15-LO-1 expression and decreased prostaglandin-H synthase-2 (COX-2) expression. COX-2 metabolizes arachidonic acid to prostaglandins. With the differentiation of these cells, there was a shift in fatty acid metabolism from prostaglandins to 15-LO-1 metabolites. This shift in metabolism may play a role in the differentiation process. Likewise, Kamitani (17) obtained similar results in the colorectal carcinoma cell line, Caco-2 cells. Treatment of Caco-2 cells with the histone deacetylase inhibitor, sodium butyrate, caused the cells to undergo differentiation and apoptosis. These cells normally express COX-2, but not 15-LO-1. During treatment with sodium butyrate, 15-LO-1 was up-regulated, while COX-2 was down-regulated. Likewise, a shift in metabolism from prostaglandins to 15-LO

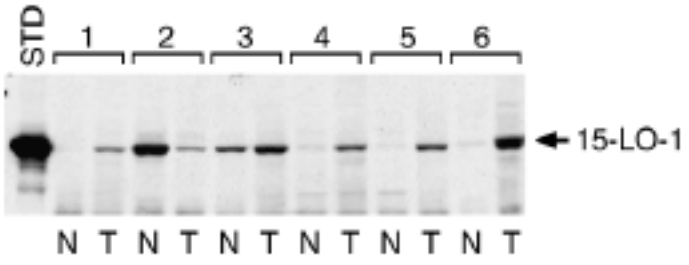
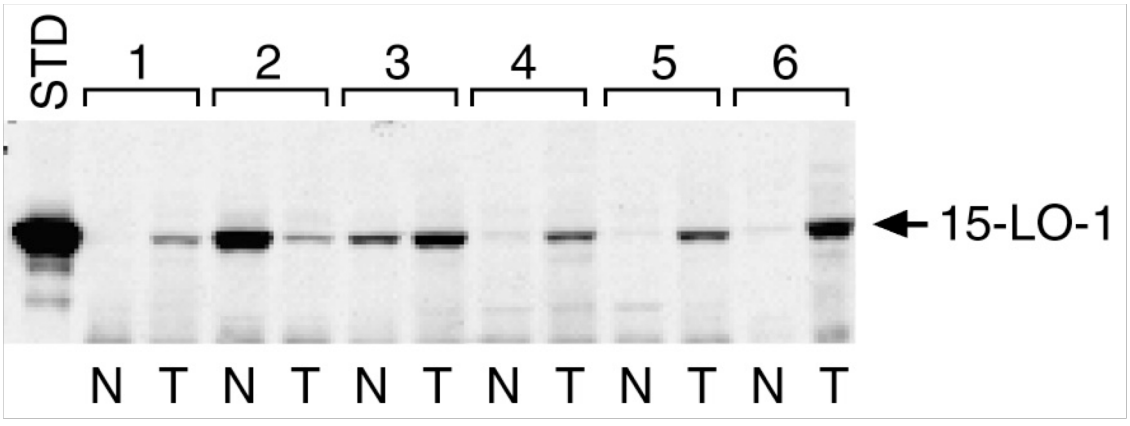


Figure 2. The expression of 15-LO-1 in paired colorectal tumor tissues and normal adjacent tissues. Western blot analysis was performed using an anti-human-15-LO-1 polyclonal antibody. T represents tumor tissue and N represents matched normal adjacent tissue. STD, 15-LO-1 standard. (ref. 6)

metabolites was observed. These data suggest that the balance of fatty acid metabolism may play an important role in cell growth/differentiation properties, particularly in colon cancer.

A role for COX-2 in colon cancer is well established, as elevated expression of this enzyme is prevalent in human colorectal tumors. Nonsteroidal anti-inflammatory drugs (NSAIDs), which inhibit COX-2, reduce the incidence of colorectal cancer (18,19). NSAIDs can also potentiate the sodium butyrate induction of 15-LO-1 in human colorectal cells in culture, further shifting the metabolism from COX-2 metabolites to 15-LO-1 metabolites (17). The mechanisms of how these drugs and COX-2 alter colon cancer are not clear. These observations combined with the results in model systems where expression of COX-2 and 15-LO-1 counterbalance each other led Ikawa (6) to investigate the expression of 15-LO-1 in human colon tumors compared to normal adjacent tissues. Surprisingly, these studies revealed elevated levels of 15-LO-1 in the tumor tissues compared to the normal adjacent tissues (Fig. 2) similar to the pattern observed for COX-2 (Fig.3) (6).



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The expression of 15-LO-1 was localized to the epithelium in the colorectal tumor tissues. These data demonstrate that differences in the expression occur in model systems and in tumor tissues and suggest that 15-LO-1 may play a role in the development of colorectal cancer.

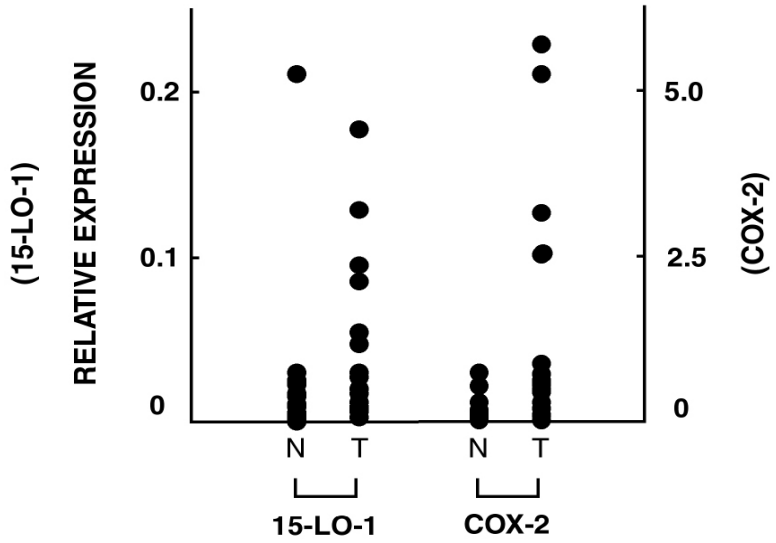

Figure 3. Comparison of the distribution of the relative expression of 15-LO-1 and COX-2. Western blots of the colorectal tumor tissue and normal adjacent tissues were scanned. Densitometry was performed to assess relative expression. N represents normal tissue and T represents tumor

In addition to the expression of 15-LO-1 in human colorectal tumors, in collaboration with this laboratory, Kelavkar (5) demonstrated the expression of 15- LO-1 in prostate adenocarcinoma tissues. 15-LO-1 was localized primarily in the epithelium of these tissues. These studies revealed that 15-LO-1 is expressed at significantly higher levels in prostate adenocarcinoma compared to normal tissue. Likewise, 15-LO-1 metabolites were detected at higher levels in the adenocarcinoma samples.

The level of 15-LO-1 expression strong ly correlated with the degree of malignancy, as assessed by Gleason staging. Interestingly, the level of mutant p53 also correlated with the levels of 15-LO-1. The p53 protein is a tumor suppressor and mutations are often associated with the development of cancer. In addition, Kelavkar (20) demonstrated that mutant p53 can activate the 15-LO-1 promoter. These data suggest a strong correlation between 15-LO-1 and cancer progression. In contrast, Shappell (21) analyzed normal prostate tissues and prostate adenocarcinoma tissues for 15-LO-2 mRNA expression and found that 15-LO-2 was preferentially expressed in the normal prostate tissues. In agreement with these findings, arachidonic acid was converted to 15(S)-HETE in the normal prostate tissues. At first assessment, the data for 15-LO-1 and 15-LO-2 appear to be in opposition. However, since 15-LO-1 is expressed preferentially in prostate adenocarcinomas and 15-LO-2 is primarily expressed in normal prostate tissue, these two enzymes may play opposing roles. These two enzymes also prefer different substrates and the primary metabolites are different.

Our laboratory has demonstrated that 15-LO-1 expression may alter cell growth. The products, 13(S)-HpODE and 13(S)-HODE, enhance epidermal growth factor (EGF)-induced mitogenesis in Syrian hamster embryo (SHE) cells (22). In this system, EGF stimulated the metabolism of exogenous or endogenous linoleic acid to 13(S)-HpODE/13(S)-HODE, dependent on tyrosine kinase activity. The addition of tyrosine kinase inhibitors not only inhibited EGF-induced mitogenesis, but also the formation of 15-LO-1 metabolites. Further, the exogenous addition of 13(S)-HpODE or 13(S)-HODE, but not 15(S)-HETE, in combination

with EGF to SHE cells inhibited the dephosphorylation of the EGF receptor, thereby up-regulating the EGF cascade and potentiating the mitogenic response (23). Therefore, the 15-LO-1 linoleic acid metabolites, 13(S)-HpODE and 13(S)-HODE, can up-regulate EGF dependent cell proliferation and enhance MAP kinase activity, but the 15-LO-2 arachidonic acid metabolite, 15(S)-HETE, is not active. Taken together, these investigations suggest different roles for 15-LO-1 and 15-LO-2 and their respective metabolites, 13(S)-HODE and 15(S)-HETE.

Conclusion

Further research is needed to delineate the specific functions of 15-LO-1 and 15-LO-2. These functions may be dependent on cell type and on the system under investigation. The endogenous metabolism needs to be measured in model systems to determine how the levels of 15(S)-HETE and 13(S)-HODE change as the expression of 15-LO-2 decreases and 15-LO-1 increases during neoplasic progression. These types of experiments will give a better view on the potential roles of the 15-LO enzymes. It will be necessary to identify the intracellular targets for 13(S)-HpODE and 13(S)-HODE in comparison with the target for 15(S)-HETE, the primary metabolite of 15-LO-2, to gain the necessary insight into 15-LO function. B theme is emerging that implicates opposing roles for 15-LO-1 and 15-LO-2 in the regulation of cell proliferation and tumor progression.

References

1. Rapoport, S. M., Schewe, T., Wiesner, R., Halangk, W., Ludwig, P., Janicke-Hohne, M., Tannert, C., Hiebsch, C., and Klatt, D. (1979) Eur J Biochem 96(3), 545-61

2. Nadel, J. A., Conrad, D. J., Ueki, I. F., Schuster, A., and Sigal, E. (1991) J Clin Invest 87(4), 1139-45

3. Nassar, G. M., Morrow, J. D., Roberts, L. J. D., Lakkis, F. G., and Badr, K. F. (1994) J Biol Chem 269(44), 27631-4

4. Hill, E. M., Bader, T., Nettesheim, P., and Eling, T. E. (1996) Am J Physiol 270 (5 Pt 1), L854-62

5. Kelavkar, U. P., Cohen, C., Kamitani, H., Eling, T. E., and Badr, K. F. (2000) Carcinogenesis 21, in press

6. Ikawa, H., Kamitani, H., Calvo, B. F., Foley, J. F., and Eling, T. E. (1999) Cancer Res 59(2), 360-6

7. Brash, A. R., Boeglin, W. E., and Chang, M. S. (1997) Proc Natl Acad Sci U S A 94(12), 6148-52

8. Kuhn, H., and Brash, A. R. (1990) J Biol Chem 265(3), 1454-8

9. Ford-Hutchinson, A. W. (1991) Eicosanoids 4(2), 65-74

10. Samuelsson, B., Dahlen, S. E., Lindgren, J. A., Rouzer, C. A., and Serhan, C. N. (1987) Science 237(4819), 1171-6

11. Feinmark, S. J., and Cornicelli, J. A. (1997) Biochem Pharmacol 54(9), 953-9

12. Funk, C. D. (1996) Biochim Biophys Acta 1304(1), 65-84

13. Kuhn, H., and Chan, L. (1997) Curr Opin Lipidol 8(2), 111-7

14. Kuhn, H., Heydeck, D., Hugou, I., and Gniwotta, C. (1997) J Clin Invest 99(5), 888-93

15. Nagy, L., Tontonoz, P., Alvarez, J. G., Chen, H., and Evans, R. M. (1998) Cell 93(2), 229-40

16. Huang, J. T., Welch, J. S., Ricote, M., Binder, C. J., Willson, T. M., Kelly, C., Witztum, J. L., Funk, C. D., Conrad, D., and Glass, C. K. (1999) Nature 400(6742), 378-82

17. Kamitani, H., Geller, M., and Eling, T. (1998) J Biol Chem 273(34), 21569-77

18. Marnett, L. J. (1992) Cancer Res 52(20), 5575-89

19. DuBois, R. N., and Smalley, W. E. (1996) J Gastroenterol 31(6), 898-906

20. Kelavkar, U. P., and Badr, K. F. (1999) Proc Natl Acad Sci U S A 96(8), 4378-83

21. Shappell, S. B., Boeglin, W. E., Olson, S. J., Kasper, S., and Brash, A. R. (1999) Am J Pathol 155(1), 235-45

22. Glasgow, W. C., Afshari, C. A., Barrett, J. C., and Eling, T. E. (1992) J Biol Chem 267(15),10771-9

23. Glasgow, W. C., Hui, R., Everhart, A. L., Jayawickreme, S. P., Angerman-Stewart, J., Han, B. B., and Eling, T. E. (1997)

