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ROLE OF LIPOXYGENASES IN BREAST CANCER

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TABLE OF CONTENTS:

1. ABSTRACT

The interaction of growth factors such as epidermal growth factor (EGF) with their receptors on breast cancer cells can lead to the hydrolysis of phospholipids and release of fatty acids such as arachidonic acid which can be further metabolized by the lipoxygenase (LO) pathway. Several LO products have been shown to stimulate oncogenes and have mitogenic and chemotactic effects. The 12-LO product, 12-hydroxyeicosatetraenoic acid (12(S)HETE), has been shown to play a key role in mediating several steps of the process of hematogenous metastasis and tumor cell adhesion. 12-LO can also be activated by several growth factors and inflammatory cytokines. A growing body of evidence suggests that specific metabolites of arachidonic and/or linoleic acid serve as central elements in signal pathways necessary for cell mitogenesis as induced by growth factors or oncogenic transformation. This review examines the role of LOs in breast cancer. The growth of breast cancer cells has been shown to increased by certain LO products and, LO pathway inhibitors could block the growth of some breast cancer cells. 12-LO activity and expression was increased in breast cancer tissues relative to the uninvolved normal tissue, and also in cultured breast cancer cells relative to normal breast cells. Treatment of the breast cancer cell line, MCF-7 cells, with epidermal growth factor (EGF), led to significant increases in 12-LO activity and expression. Thus, activation of the 12-LO pathway may play a key role in basal and EGF-induced breast cancer cell growth.

2. INTRODUCTION

Human breast cancer cell proliferation involves a complex interaction between growth factors, steroid hormones and peptide hormones. However, the role of free lipids in these events is not very clear, although several several studies have suggested that fatty acids and other enzymatically oxidized lipids may play a significant role in the development and progression of breast cancer. Growth factors and hormones can activate several phospholipases leading to the release of lipids such as arachidonic acid, which in turn can be further metabolized by several pathways, including the lipoxygenase pathway. Lipoxygenase products have been shown to have potent inflammatory, growth, chemotactic and angiogenic effects in cells. They have also been implicated in the process of hematogenous metastasis. In the present manuscript, we have outlined the role of the lipoxygenase pathway and its products in breast cancer.

3. ARACHIDONIC ACID METABOLISM AND THE LIPOXYGENASES

The interaction of growth factors and hormones with their cell surface receptors stimulates a cascade of signaling events including the activation of receptor tyrosine kinases, activation of several downstream signal transducing proteins and kinases and increased transcription of multiple genes. In addition, growth factor-induced activation of phospholipases can lead to the hydrolysis of membrane phospholipids, thereby releasing lipids such as arachidonic and linoleic acids. It has been suggested that the release of arachidonic acid from the *sn*-2 position of membrane phospholipids may be one of the signals leading to cellular proliferation (1). Arachidonic acid is also the precursor for several eicosanoids with potent biological effects including inflammation and cell growth (2). Arachidonic acid or one of

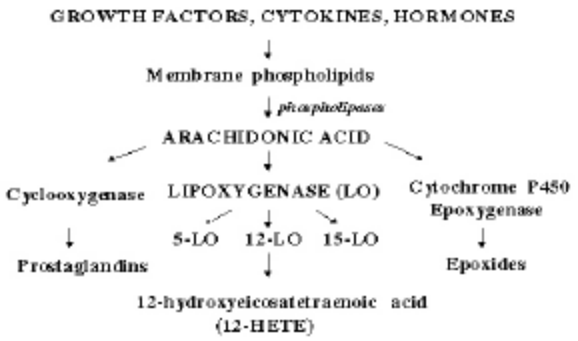

Figure 1. Arachidonic acid metabolism.

its biologically active eicosanoid metabolites may play a role in cellular growth and inflammation.

The 20-carbon arachidonic acid can be metabolized by three major pathways, the cyclooxygenase pathway which leads to the formation of prostaglandins, the lipoxygenase (LO) pathway which forms hydroperoxyeicosatetraenoic acids (HPETEs), hydroxyeicosatetraenoic acids (HETEs) and leukotrienes and thirdly, the cytochrome P-450 monooxygenase pathway which leads to the formation of epoxides such as epoxyeicosatrienoic acids as well as HETEs (3) (figure 1). The lipoxygenases, mainly called 5-, 12- and 15-lipoxygenases (LOs) are named for their ability to insert molecular oxygen at the 5, 12, or 15 carbon atom of arachidonic acid (4). The 5-lipoxygenase pathway leads to the formation of 5-HETE and leukotrienes while the 12- and 15-lipoxygenases can form 12- and 15-HETEs.

Studies show that there are at least two major isoforms of 12-LOs (5-7). The platelet-type has been cloned from human platelets and the megakaryocytic cell line, HEL (8,9). The other 12-LO, namely the leukocyte-type, has been detected in porcine leukocytes (10), pituitary (11), vascular smooth muscle cells (12) and also in human adrenal glomerulosa cells (13), human monocytes, endothelial and vascular smooth muscle cells (14). The cDNA for porcine leukocyte 12-lipoxygenase has been cloned (10). This porcine leukocyte 12-lipoxygenase is only 65% homologous to the human platelet 12-lipoxygenase (8-10) whereas it is 87% homologous to human 15-lipoxygenase (10,15). A bovine-trachea epithelial-cell 12-lipoxygenase cDNA has also been cloned which is 86% identical to the human 15-lipoxygenase and 89% identical to the porcine 12-lipoxygenase (16). The two distinct 12-lipoxygenase cDNAs, namely platelet- and leukocyte-type, have recently been cloned from the same species, namely the mouse (17). 15-lipoxygenase has been cloned from human reticulocytes (15). Platelet 12-LO differs from leukocyte 12-LO in substrate specificity. The former is much less active with C18 fatty acids such as linoleic acid in comparison with arachidonic acid while leukocyte 12-LO has broader substrate specificity reacting with C18 and C22 unsaturated fatty acids as efficiently as with arachidonic acid (5).

4. REGULATION OF LIPOXYGENASE BY GROWTH FACTORS AND CYTOKINES

Evidence suggests that tumor cells as well as several normal cells have LO activity (18) and both arachidonic acid as well as linoleic acids are converted to LO products such as HETEs and hydroxyoctadecadienoic acids (HODEs). The 12-LO and 15-LO pathways can be activated by growth factors and cytokines. In A431 epidermoid carcinoma cells, epidermal growth factor (EGF) could induce platelet 12-LO mRNA expression (19,20). Recent studies have indicated that EGF can stimulate 13-HODE formation in BT-20 breast cancer cells by a 15-LO-dependent mechanism (21). Human vascular smooth muscle cell 12-LO expression was increased by treatment with angiotensin II (12,14), while human monocyte 15-LO was induced by interleukin-4 and interleukin-13 (22,23). Porcine 12-LO in vascular smooth muscle cells was also markedly upregulated by platelet-derived growth factor (24) and by cytokines such as interleukins-1, -4 and -8 (25). Thus, increased leukocyte-type 12-LO activity and expression may play a role in the growth-promoting effects of these factors.

5. ACTIONS OF LIPOXYGENASE PRODUCTS RELATED TO CELL GROWTH AND ADHESION.

Lipoxygenase products such as the hydroxyeicosatetraenoic acids (HETEs) have been shown to have actions highly relevant to cellular growth and migration (18). They have significant mitogenic and chemotactic properties and can also stimulate the expression of several oncogenes (26-29). The 12-LO product, 12-hydroxyeicosatetraenoic acid (12(S)-HETE) has been shown to play a role in the growth promoting effects of angiotensin II in vascular smooth muscle and adrenal cells (30,31) and the chemotactic effects of platelet-derived growth factor (24). The LO products of linoleic acid can also potentiate the mitogenic effects of epidermal growth factor (EGF) (32,33) and linoleic acid can stimulate the growth of MCF-7 breast cancer cells (34,35). Linoleic acid metabolism enhances the proliferative response in mouse mammary epithelial cells and in human breast epithelial cells (36-38). The direct growth effects of linoleic acid , however, seemed more visible with the ER negative cell line MDA-MB-231 than with the ER positive MCF-7 cells (34). LO products rather than cyclooxygenase products were found to play a major role in linoleic acid-stimulated growth of mouse mammary tumor cell line (38).

The 12-LO product 12(S)-HETE, has been shown to play an important role in mediating several major steps of the process of hematogenous metastasis of cancer cells (39,40). 12(S)-HETE could mediate the adhesion of tumor cells to the subendothelial matrix following endothelial retraction by a protein kinase C- dependent process (40,41). 12-HETE was also shown to increase tumor cell motility and invasive potential (39,40). LO products such as 12- and 15-HETE also have angiogenic and mitogenic effects on endothelial cells (26). Liu et al. demonstrated that 12-HETE was the predominant arachidonic acid metabolite produced by highly metastatic tumor cells. Furthermore, these highly metastatic cells synthesized much greater amounts of 12-HETE than the low metastatic tumor cells, suggesting that biosynthesis of 12-HETE by tumor cells is a determinant of their metastatic potential (42). Evidence shows that arachidonic acid metabolism by lipoxygenase plays a key role in the adhesion of MDA-MB-435 breast cancer cells to collagen IV (43). Furthermore, platelet 12-lipoxygenase

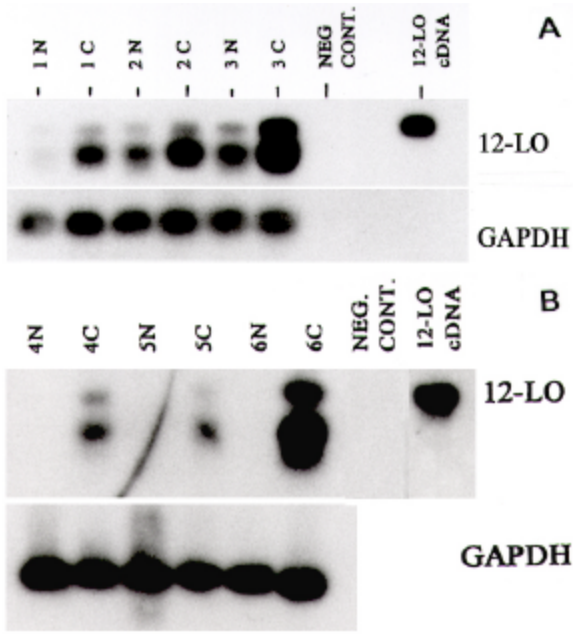

Figure 2. **A)** Leukocyte-type 12-LO mRNA expression in matched normal (N) and cancerous (C) breast tissue samples from patients 1-3. **B)** Same from patients 4-6. Results shown are Southern Blots of the RT-PCR amplified products from the RNA extracted from tissue samples. Hybridization with a leukocyte 12-LO oligonucleotide probe is seen in the upper panels (333 bp) while hybridization with a GAPDH probe to control for PCR amplification efficiency in the corresponding samples is shown in the lower panels (284 bp). The positive control, leukocyte 12-LO cDNA amplification, is seen at the far right. In the negative controls, PCR was run with no RNA. Reprinted with permission from the The Endocrine Society, 1997. *J. Clin. Endocrinol. Metabol.* 82(6)1790-1798.

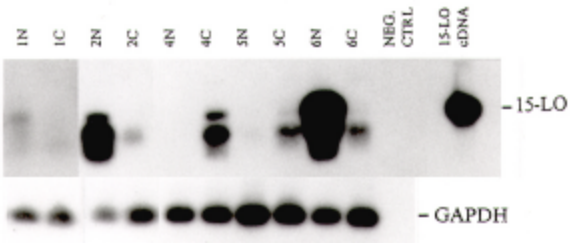

Figure 3. 15-LO mRNA expression in matched normal (N) and cancerous (C) breast tissue samples from patients 1,2,4,5 and 6. Hybridization with a 15-LO oligonucleotide probe is seen in the upper panel (333 bp) while hybridization with a GAPDH oligonucleotide probe (control for PCR) is seen in the lower panel (284 bp). The positive control (15-LO cDNA amplification) is seen on the far right. Reprinted with permission from the Endocrine Society 1997, *J. Clin. Endocrinol. Metabol.* 82(6)1790-1798.

transfected MCF-7 breast cancer cells exhibited enhanced growth in athymic nude mice (44). The involvement of arachidonate LOs, particularly 12-LO, in the inhibition of apoptosis was demonstrated by Tang et al (45). This suggests that an increased concentration of 12-HETE produced by activated platelets, the tumor cells themselves, leukocytes or by vascular cells could facilitate the proliferative, adhesive and metastatic processes. Thus, a growing body of evidence suggests that specific metabolites of arachidonic and/or linoleic acid serve as central elements in signal pathways necessary for cell mitogenesis as induced by growth factors or oncogenic transformation.

However, very few studies have examined the cellular and molecular regulation of the LO enzymes in human breast cancer tissues and cells. We recently examined whether the leukocyte-type 12-LO expression is upregulated in breast cancer cells and tissue sections and also whether EGF, a breast cell growth factor, can induce LO activity and expression in breast cancer cells (46).

6. EXPRESSION AND REGULATION OF 12-LO IN BREAST CANCER CELLS AND TISSUES.

6.1 Leukocyte-type 12-LO mRNA expression in breast tissue samples.

In order to determine whether 12-LO mRNA expression was altered in breast cancer tissues, we screened six sets of uninvolved and cancer involved breast tissue samples from 6 patients for the presence of the leukocyte-type 12-LO mRNA (46). Total RNA from these samples was subjected to a specific RT-PCR (13,46) to detect 12-LO mRNA levels owing to its the low levels in these tissues. Figures 2A and 2B show Southern blots run with the RT-PCR products from the 12 samples obtained from these 6 patients. Hybridization was performed with a [^{32}P]-labeled porcine leukocyte 12-LO oligonucleotide probe (upper panels of both Figures 2A and 2B). The size of the expected PCR product was 333 bp. It is clearly seen that in each patient, the cancerous section had a much higher level of 12-LO mRNA expression than the corresponding normal section. In fact, in patients 4-6, 12-LO mRNA was barely expressed in the normal sections. After correction for amplification of the internal control, GAPDH mRNA (PCR product 284 bp), densitometric analysis revealed 3- to 30-fold greater 12-LO mRNA expression in the cancer sections than in the corresponding uninvolved section from the same patients. These results suggest that malignant breast tissues express a much higher level of the 12-LO mRNA *in vivo* when compared to matched uninvolved tissues.

In addition to the expected 333 bp 12-LO PCR product, an additional lower band (approximately 300 bp) was observed in all the samples. The identity of this transcript was not clear but since this band was regulated in exactly the same manner as the 333bp band, it may be related to 12-LO mRNA.

6.2 Human 15-LO mRNA expression in breast tissue samples.

Since human 15-LO and leukocyte-type 12-LO are very homologous, we examined the expression of 15-LO mRNA in the same patient tissue samples as above. We used a specific RT-PCR approach which distinguishes between 15-LO and leukocyte-type 12-LO (12,13). Figure 3 is a Southern blot of RT-PCR amplified products to examine 15-LO mRNA expression in normal and cancer tissue sections from the same patients 1, 2, 4, 5 and 6. The positive control, 15-LO cDNA is

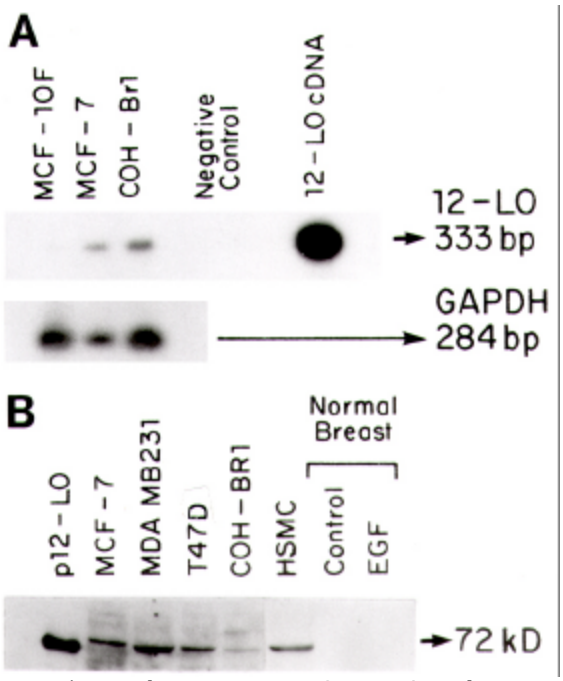

Figure 4. Leukocyte-type 12-LO mRNA and protein expression in breast cancer cell lines and normal breast cell lines. Figure 4A is a Southern blot of the RT-PCR amplified products obtained from 1 microgram each total RNA from two breast cancer cell lines (MCF-7 and COH-BR1) as well as from an immortal non-tumorigenic breast epithelial cell line (MCF-10F). Hybridization was with a leukocyte 12-LO oligonucleotide probe which resulted in the expected 333 bp amplified product. The positive control for PCR, 12-LO cDNA amplification is seen at the far right. GAPDH mRNA amplification (284 bp) is seen in the lower panel. Figure 4B shows an immunoblot to detect leukocyte 12-LO protein in the breast cancer cell lines, MCF-7, MDA-MB-231, T47D and COH-BR1 (lanes 2-5), human vascular smooth muscle cells (lane 6) as well as the primary normal human breast epithelial cell AC113 (alone or treated for 24 hr with EGF 50 ng/ml, lanes 7 and 8). Equal amounts of cell lysates (50 microgram protein) were loaded in each lane. Blots were probed with a peptide antibody to porcine leukocyte 12-LO. Authentic porcine leukocyte 12-LO is seen in lane 1.Reprinted with permission from the Endocrine Society 1997, J. Clin. Endocrinol. Metabol. 82(6)1790-1798.

seen on the far right. The size of the 15-LO PCR product is 333 bp. Strong expression of 15-LO mRNA in human breast tissue and cancer was observed. However, its expression was enhanced in the cancer- involved sections in only two of the 5 samples.

6.3 12-LO mRNA and protein expression in breast cancer and normal breast cell lines.

Since tissue samples contain a variety of cell types, we also compared leukocyte-type 12-LO mRNA expression in normal versus cancer cell lines. Figure 4A shows a Southern blot of the RT-PCR amplified products from total RNA from two breast cancer cell lines MCF-7 and COH-BR1 as well as an immortal, non-tumorigenic breast epithelial cell line MCF-10F. There was very little basal expression of 12-LO mRNA (333 bp PCR product) in MCF-10F cells (figure 4A). However, distinct expression of 12-LO was seen in the two cancer cell lines MCF-7 and COH-BR1 (7- and 11-fold greater than the MCF-10F cells, corrected for GAPDH mRNA amplification (284 bp) seen in the lower panel). The positive control for PCR, 12-LO cDNA amplification, is seen in the far right. Thus, breast cancer cell lines such as MCF-7 and COH-Br1 had a much higher level of 12-LO mRNA expression relative to the control cell line MCF-10F.

In addition, we compared 12-LO protein expression in four breast cancer cell lines, MCF-7, MDA-MB-231, COH-BR1 (46) and T47D with that in a normal breast epithelial cell line, AC113, specimen 161 (from Dr. Martha R. Stampfer, University of California, Berkeley) (46). The results of the immunoblot, using a peptide antibody to porcine leukocyte 12-LO (13,24) is seen in Figure 4B. All the 4 breast cancer cell lines showed a band around 75 kD similar to the band seen with human vascular smooth muscle cells on lane 5. Authentic porcine leukocyte 12-LO is in lane 1. The human 12-LO appeared to migrate at a slightly higher molecular size than porcine 12-LO. In contrast, the normal breast epithelial cell line did not show this 12-LO band with or without 24 hr EGF treatment (lanes 6 and 7).

6.4 The effect of EGF on cell-associated 12(S)-HETE levels.

In order to evaluate whether a potent breast epithelial cell growth factor can affect the LO pathway, we examined whether EGF can increase the formation of the 12-LO product, 12(S)-HETE, in MCF-7 breast cancer cells. A 4 hr treatment of the cells with EGF did not affect the levels of released 12(S)-HETE as measured after extraction by a specific radioimmunoassay (12). In contrast, this treatment with EGF (25-100 ng/ml) led to a dose-dependent increase in the levels of cell-associated 12(S)-HETE as seen in Figure 5. EGF also increased intracellular LO enzyme activity (46).

6.5 The effect of EGF on leukocyte-type 12-LO protein expression.

Figure 6 shows that a 36 hr treatment with EGF also led to a marked increase (2-3 fold) in levels of the 12-LO protein (approximately 75 kd). Authentic porcine leukocyte 12-LO is shown in the far left lane. It is noted that the 12-LO in these human MCF-7 cells appeared at a slightly higher molecular weight than the porcine 12-LO.

No clear evidence for the presence of platelet 12-LO in the MCF-7 cells was obtained in these studies either in the basal state, or after treatment with EGF (46). However, the possibility that a platelet-type of 12-LO also contributes to 12(S)-HETE formation in breast tissue samples and in the local environment of tumor cells cannot be completely ruled out.

6.6. The effect of LO and cyclooxygenase inhibitors on the growth of MCF-7 cells.

In order to evaluate the potential functional significance of altered 12-LO expression in the breast cancer cells, we examined the effect of two specific structurally dissimilar 12-LO inhibitors, cinnamyl-3,4-dihydroxy-cyanocinnamate (CDC) and baicalein on the proliferative rates of MCF-7 cells (46). Figure 7 shows that both LO

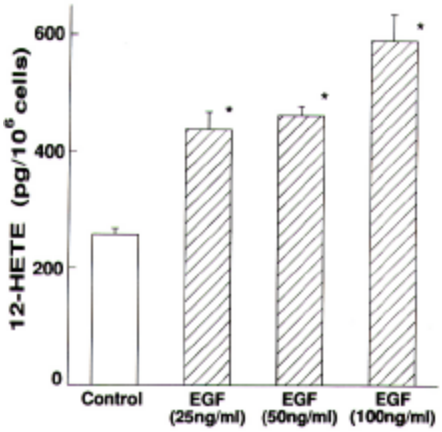

Figure 5. The effect of EGF on the levels of cell-associated immunoreactive 12-HETE in MCF-7 breast cancer cells. Serum-starved MCF-7 cells were treated for 4 hr with EGF in medium containing 0.2% BSA. 12-HETE levels in the cell pellets were quantitated by radioimmunoassay after deacylation and extraction as described (12,24). *, $p < 0.01$ vs control obtained by analysis of variance (ANOVA).Reprinted with permission from the Endocrine Society 1997, *J. Clin. Endocrinol. Metabol.* 82(6)1790-1798.

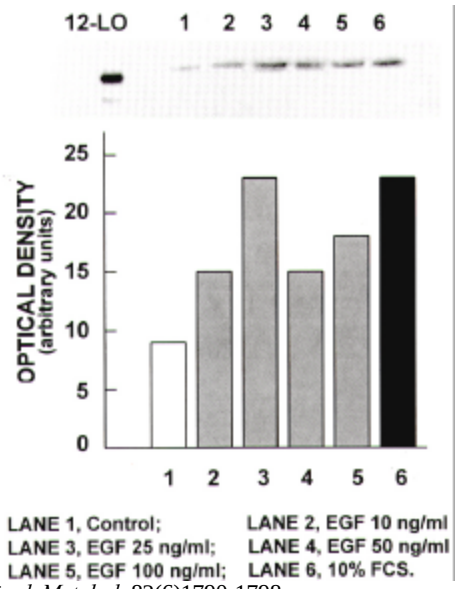

Figure 6. The effect of EGF on leukocyte-type 12-LO protein expression in MCF-7 cells. Serum-starved cells were treated for 36 hr with EGF. Equal amounts of protein lysates were electorphoresed and subjected to immunoblotting with an antibody raised against to a peptide derived from the sequence of the porcine leukocyte 12-LO. Authentic porcine leukocyte 12-LO protein (Oxford Biomedical Research Co., Oxford, MI) was loaded in the lane on the extreme left. Densitometric representation of the blot is seen in the bar graph below in arbitrary optical density units. EGF (50 and 25 ng/ml) led to significant increases in 12-LO levels (1.8 ± 0.2 fold and 2.3 ± 0.4 fold respectively, both $p < 0.001$). Reprinted with permission from the Endocrine Society 1997, *J. Clin. Endocrinol. Metabol.* 82(6)1790-1798.

inhibitors led to a marked inhibition of the serum-induced growth of these cells. Since both CDC and baicalein may also block the 5-LO pathway, we also checked the effect of a highly specific 5-LO inhibitor, AA-861. Figure 7 shows that, while this 5-LO inhibitor also had inhibitory effects on the proliferation of the MCF-7 cells, it was not as potent as CDC or baicalein. A cyclooxygenase inhibitor, ibuprofen, at the same concentration as CDC and baicalein, had no significant effect on cell proliferation (figure 7). These results indicate that the LO pathway may mediate, at least in part, the growth of breast cancer cells. However, 12-HETE may not be the only LO product involved in breast cancer and other LO products generated by the 5-LO or other pathways may also play a role.

7. MECHANISMS INVOLVED IN LIPOXYGENASE PRODUCT MEDIATED BREAST CANCER DEVELOPMENT.

There are several mechanisms by which lipoxygenase products may mediate cellular growth. They have been shown to lead to the activation of oncogenes such as c-*fos* and *ras* (29,47). HETEs can also activate protein kinase C directly (39,48,49) or indirectly by incorporating into membrane phospholipids which then generate HETE-containing diacylglycerol species to activate protein kinase C (50). 12-HETE can also mimic the protein kinase C activator and tumor promoter, phorbol myristate acetate, in enhancing tumor cell integrin expression and adhesion (51). Evidence also suggests that 12-HETE can lead to G-protein mediated activation of phospholipase C (52). Studies have also shown that LO products can modulate intracellular calcium levels (53). Furthermore, a receptor-mediated mechanism of action with high affinity binding sites for 12-HETE has also been demonstrated in certain cells (52,54,55). Activation of the leukocyte-type of 12-lipoxygenase may cause structural modification of membrane phospholipids since it can not only oxidize free fatty acids but also those esterified to phospholipids, unlike the platelet form of 12-lipoxygenase (56).

LO products may also mediate cellular effects by the activation of key growth and stress related kinases which are signaling components of gene transcription (57). In vascular smooth muscle cells, lipoxygenase products of arachidonic acid (HETEs) and linoleic acid (HODEs) could directly activate mitogen activated protein kinases (MAPKs) (58,59). Furthermore, in the CHO-AT1a cells, LO inhibitors could attenuate AII-induced MAPK (ERK1/2) activity, and LO products could directly activate ERK1/2 (60). Very recent evidence in the CHO-AT1a cells suggests that LO products, such as 12-HETE, also directly activate another member of the MAPK family, the c-Jun aminoterminal kinase (JNK) and furthermore, LO inhibitors blocked AII-induced JNK activation. (61). LO metabolites have also been shown to mediate the growth promoting effects of EGF, and the mechanism appeared to involve regulation of the tyrosine kinase activity of the EGF receptor (62). Thus, arachidonic acid and its LO metabolites may mediate growth factor-induced activation of specific protein kinases and hence play a key role in the aberrant behavior of cancer cells.

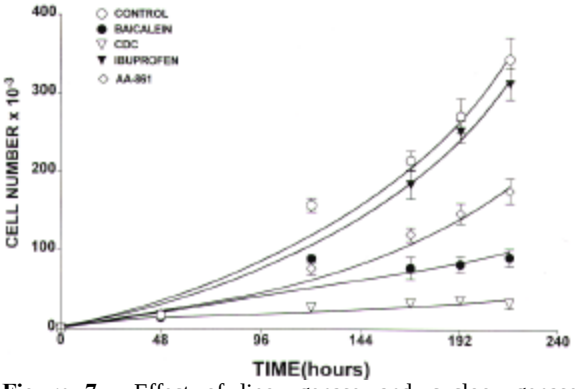

Figure 7. Effect of lipoxygenase and cyclooxygenase inhibitors on the growth of MCF-7 cells. The inhibitors (10 micromolar each) were added every 48 hr to MCF-7 cells growing in DME medium containing 5% FCS and cell counts obtained on a Coulter counter. Reprinted with permission from the Endocrine Society 1997, *J. Clin. Endocrinol. Metabol.* 82(6)1790-1798

8. PERSPECTIVE

Increased activity and expression of LO enzymes in tumor cells can lead to excess accummulation of products with potent growth promoting, chemotactic, inflammatory and angiogenic effects, which therefore implicate them in the pathogenesis of breast cancer. There are currently no clinically available safe and selective inhibitors of 12-LO enzymes. One approach could be to use gene therapy to block these enzymes. We have developed a novel ribozyme or catalytic RNA directed against the porcine leukocyte 12-LO (63). We showed that this ribozyme can efficiently cleave 12-LO mRNA *in vitro* and can also dose-dependently decrease 12-lipoxygenase mRNA and protein expression when transfected into porcine vascular smooth muscle cells (63,64). Hence, therapeutic modalities and novel ribozyme technology approaches to effectively block these pathways may provide new anti cancer therapies.

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10. REFERENCES

1. Millar JBA, E. Rozengurt: Arachidonic acid release by bombesin. A novel postreceptor target for heterologous mitogenic desensitization. *J Biol Chem.* 265, 19973-19979 (1990).

2. DiMarzo V: Arachidonic acid and eicosanoids as targets and effectors in second messenger interactions. *Prost. Leuk. Essent. Fatty Acids.* 53, 239-254 (1995).

3. Smith WL: The eicosanoids and their biochemical mechanisms of action. *Biochem. J.* 259, 315-324 (1989).

4. Yamamoto S: "Enzymatic" lipid peroxidation: Reactions of mammalian lipoxygenases. *Free Radical Biology and Medicine* 10, 149-159 (1991).

5. Yamamoto S: Mammalian lipoxygenases: molecular structures and functions. *Biochim Biophys Acta.* 1128, 117-131 (1992).

6. Funk CD: The molecular biology of mammalian lipoxygenases and the quest for eicosanoid functions using lipoxygenase deficient mice. *Biochim. et. Biophys. Acta* 1304, 65-84 (1996).

7. Sigal E: The molecular Biology of mammalian arachidonic acid metabolism. *Am J Physiol.* 260 (Lung Cell. Mol. Physiol.), L13-L28 (1991).

8. Funk CD, L. Furci & G.A. FitzGerald: Molecular cloning, primary structure and expression of the human platelet/erythroleukemia cell 12-lipoxygenase. *Proc Natl Acad Sci USA* 87, 5638-5642 (1990).

9. Izumi T, S. Hoshiko, O. Radmark & S. Samuelsson: Cloning of the cDNA for human 12-lipoxygenase. *Proc Natl Acad Sci USA* 87, 7477-7481 (1990).

10. Yoshimoto T, H. Suzuki, S. Yamamoto, T. Takai, C. Yokoyama & T. Tanabe: Cloning and sequence analysis of the cDNA for arachidonate 12-lipoxygenase of porcine leukocytes. *Proc Natl Acad Sci USA* 87, 2142-2146 (1990).

11. Ueda N, A. Hiroshima, K. Natsui, F. Shinjo, T. Yoshimoto, S. Yamamoto, K. Ii, K. Gerozissis & F. Dray: Localization of arachidonate 12-lipoxygenase in parenchymal cells of porcine anterior pituitary. *J Biol Chem.* 265, 2311-2316 (1990).

12. Natarajan R, J.L. Gu , J. Rossi, N. Gonzales, L. Lanting, L. Xu & J.L. Nadler: Elevated glucose and angiotensin II increase 12-lipoxygenase activity and expression in porcine aortic smooth muscle cells. *Proc Natl Acad Sci USA* 90, 4947-4951 (1993).

13. Gu JL, R. Natarajan, J. Ben-Ezra, G. Valente, S. Scott, T. Yoshimoto, S. Yamamoto, J.J. Rossi, J.L & Nadler L: Evidence that a leukocyte-type of 12-lipoxygenase is expressed and regulated by angiotensin II in human adrenal glomerulosa cell. *Endocrinology* 134, 70-77 (1994).

14. Kim JA, J.L. Gu, R. Natarajan, J. Esteban, J.A. Berliner & J.L. Nadler: A leukocyte-type of 12-lipoxygenase is expressed in human vascular and mononuclear cells: evidence for upregulation by angiotensin II. *Arterioscler Throm Vasc Biol.* 15, 942-948 (1995).

15. Sigal E, C.S. Craik, E. Highland, D. Grunberger, L.L. Costelo, R.A.F. Dixon & J.A. Nadel: Molecular cloning and primary structure of human 15-lipoxygenase. *Biochem Biophys Res Commun.* 157, 457-464 (1988).

16. DeMarzo N, D.L. Sloane, L. Dicharry, E. Highland & E. Sigal: Molecular cloning and expression of an airway 12-lipoxygenase. *Am. J. Physiol.* 262, L198-207 (1992).

17. Chen X, U. Kurre, N.A. Jenkins, N.G. Copeland & C.D. Funk: cDNA cloning, expression, mutagenesis c-terminal iso-

leucine, genomic structure, and chromosomal localizations of murine 12-lipoxygenases. *J Biol Chem.* 269, 13979-13987 (1994).

18. Spector AA, J.A. Gordon & S.A. Moore: Hydroxyeicosatetraenoic acids (HETEs). *Prog Lipid Res.* 27:271-329 (1988).

19. Chang W-C, Y. Liu, C. Ning, H. Suzuk, T. Yoshomoto & S. Yamamoto: Induction of arachidonate 12-lipoxygenase by epidermal growth factor in A431 cells. *J Biol Chem*. 268, 18734-18739 (1993).

20. Hagmann W, X. Gao, J. Timar, Y.Q. Chen, A. Strohmaier, C. Fahrenkoph, D. Kagawa, M. Lee, A. Zacharek & K.V. Honn: 12-lipoxygenase in A431 cells: Genetic identity, modulation of expression, and intracellular localization. *Exp. Cell. Res.* 228, 197-205 (1996).

21. Reddy N, A. Everhart, T. Eling & W. Glasgow: Characterization of a 15-lipoxygenase in human breast carcinoma BT-20 cells: Stimulation of 13-HODE formation by TGF /EGF. *Biochem. Biophys. Res. Commun.* 231, 111-116 (1997).

22. Conrad DJ, H. Kuhn, M. Mulkins, E. Highland & E. Sigal: Specific inflammatory cytokines regulate the expression of human monocyte 15-lipoxygenase. *Proc Natl Acad Sci USA* 89, 217-222 (1992).

23. Nasser GM, J.D. Morrow, L.J. Robert II, F.J. Lakkis & K.F. Badr: Induction of 15-lipoxygenase by IL-13 in human blood monocytes. *J Biol Chem.* 269, 27631-27634 (1994).

24. Natarajan R, W. Bai, J.L. Gu, V. Rangarajan & J.L. Nadler: Platelet-derived growth factor BB mediated regulation of 12-lipoxygenase in porcine aortic smooth muscle cells. *J. Cell. Physiol.* 169, 391-400 (1996).

25. Natarajan R, J. Rosdahl, N. Gonzales & W. Bai: Regulation of 12-lipoxygenase by cytokines in vascular smooth muscle cells. *Hypertension* 30, 873-879 (1997).

26. Setty BNY, J.E. Graeber & M.J. Stuart: The mitogenic effects of 15- and 12-hydroxyeicosatetraenoic acids on endothelial cells may be mediated via diacylglycerol kinase inhibition. *J Biol Chem.* 262, 17613-17622 (1987).

27. Nakao J, T. Ooyama, H. Ito, W.C. Chang & S. Murota: Comparative effects of lipoxygenase products of arachidonic acid on rat aortic smooth muscle cell migration. *Atherosclerosis* 44, 339-342 (1982).

28. Nakao J, H. Ito, T. Kanayasu & S. Murota: Stimulatory effect of insulin on aortic smooth muscle cell migration induced by 12-hydroxy-5,8,10,14-eicosatetraenoic acid and its modulation by elevated glucose levels. *Diabetes* 34, 185-191 (1985).

29. Haliday E, C. Ramesha & G. Ringold: TNF induces c-fos via a novel pathway requiring the conversion of arachidonic acid to a lipoxygenase metabolite. *Embo J.* 10, 109-115 (1991).

30. Natarajan R, N. Gonzales, L. Lanting, J.L. Nadler: Role of the lipoxygenase pathway in angiotensin II-induced vascular smooth muscle cell hypertrophy. *Hypertension* 23[suppl I], I-142-I-147 (1994).

31. Natarajan R, N. Gonzales, P.J. Hornsby & J.L. Nadler: Mechanism of angiotensin II-induced proliferation in bovine adrenal cortical cells. *Endocrinology* 131, 1174-1180 (1992).

32. Glasgow WC & T.E. Eling: Epidermal growth factor stimulates linoleic acid metabolism in BALB/c3T3 fibroblasts. *Mol Pharmacol.* 38, 503-510 (1990).

33. Glasgow WC, C.A. Afshari, J. Carl Barret & T.E. Eling: Modulation of the epidermal growth factor mitogenic response by metabolites of linoleic and arachidonic acid in Syrian hamster embryo fibroblasts. *J Biol Chem.* 267, 10771-10779 (1992).

34. Rose DP & J.M. Connolly: Stimulation of growth of human breast cancer cell lines in culture by linoleic acid. *Biochem Biophys Res Commun.* 164, 277-283 (1989).

35. Rose DP & J.M. Connolly: Effects of fatty acids and inhibitors of eicosanoid synthesis on the growth of a human breast cancer cell line in culture. *Cancer Res.* 50, 7139-7144 (1990).

36. Bandyopadhyay GK, W. Imagawa, D.R. Wallace & S. Nandi: Proliferative effects of insulin and epidermal growth factor on mouse mammary epithelial cells in primary culture. *J Biol Chem.* 263, 7567-7573 (1988).

37. Balakrishnan A, S. Cramer, G.K. Badyopadhyay, W. Imagawa, J. Yang, J. Elias, C.W. Beattie, T.K. Das Gupta & S. Nandi: Differential proliferative response to linoleate in cultures of epithelial cells from normal human breast and fibroadenomas. *Cancer Res*. 49, 857-862 (1989).

38. Buckman DK, N.E. Hubbard & K.L. Erickson: Eicosanoids and linoleate-enhanced growth of mouse mammary tumor cells. *Prost Leuk Essen Fatty acids* 44, 177-184 (1991).

39. KV Honn, IM Grossi, J Timar, H Chopra & JD Taylor: Platelets and Cancer metastasis. In: Microcirculation and cancer metastasis. Eds: Weiss L, Buchanan M and Orr FW, CRC Press, London-Oxford (1991).

40. Honn KV, D.G. Tang, X. Gao, I.A. Butovich, B. Liu, J. Timar & W. Hagmann: 12-Lipoxygenases and 12(S)-HETE: role in cancer metastasis. *Cancer and Metastasis Reviews* 13, 365-396 (1994).

41. Liu B, J. Timar, J. Howlet, C.A. Diglio & K.V. Honn: Lipoxygenase metabolites of arachidonic and linoleic acids modulate the adhesion of tumor cells to endothelium via regulation of protein kinase C. *Cell Regulation* 2, 1045-1055 (1991).

42. Liu BN, L.J. Marnett, A. Chaudhary, J. Chuan, L.A. Blair, C.R. Johnson, C.A. Diglio & K.V. Honn: Biosynthesis of 12(S)-hydroxyeicosatetraenoic acid by B16 amelanotic melanoma cells is a determinant of their metastatic potential. *Lab Invest.* 70, 314-323 (1994).

43. Palmantier R, J.D. Roberts, W.C. Glasgow, T.E. Eling & K. Olden: Regulation of the adhesion of a human breast carcinoma cell line to type IV collagen and vitronectin: Roles for lipoxygenase and protein kinase C. *Cancer Res.* 56, 2206-2212 (1996).

44. Liu X, J.M. Connolly & D.P. Rose: The 12-lipoxygenase gene-transfected MCF-7 human breast cancer cell line exhibits estrogen-independent, but estrogen and omega-6-fatty acid-stimulated proliferation in vitro, and enhanced growth in athymic nude mice. *Cancer Lett*. 109, 223-230 (1996).

45. Tang DG, Y.Q. Chen & K.V. Honn. Arachidonate lipoxygenases as essential regulators of cell survival and apoptosis. *Proc. Natl. Acad. Sci. USA.* 93, 5241-5246 (1996).

46. Natarajan R, R. Esworthy, W. Bai, J.L. Gu, S. Wilczynski & J.L. Nadler: Increased 12-lipoxygenase expression in breast cancer cells and tissues. Regulation by epidermal growth factor. *J. Clin. Endocrinol. Metabol.* 82, 1790-1798 (1997).

47. Yu CL, M. Tsai & D.W. Stacey: Serum stimulation of NIH 3T3 cells induces the production of lipids able to inhibit GTPase-activating protein activity. *Molr. Cell. Biol.* 10, 6683-6689 (1990).

48. Shearman MS, Z.Naor, K. Segikuchi, A. Kishimoto & Y. Nishizuka: Selective activation of the gamma-subspecies of protein kinase C from bovine cerebellum by arachidonic acid and its lipoxygenase metabolites. *FEBS Lett.* 243, 177-182 (1989).

49. Natarajan R, L. Lanting,, L Xu & J.L. Nadler: Role of specific isoforms of protein kinase C in angiotensin II and lipoxygenase action in rat adrenal glomerulosa cells. *Mol. Cell. Endocrinol.* 101, 59-66 (1994).

50. Legrand AB, J.A. Lawson, B.O. Meyrick, L.A. Blair & J.A. Oates: Substitution of 15-hydroxyeicosatetraenoic acid in the phosphoinositide signaling pathway. *J. Biol. Chem.* 266, 7570-7577 (1991).

51. Grossi IM, L.A. Fitzgerald, L.A.Unbarger, K.D. Nelson, C.D. Diglio, J.D. Taylor & K.V. Honn: Bi-directional control of membrane expression and/or activation of the tumor cell IRGpIIB/IIIa receptor and tumor cell adhesion by lipoxygenase products of arachidonic and linoleic acid. *Cancer Res.* 49, 1029-1035 (1989).

52. Liu B, W.A. Khan, Y.A. Hannun, J. Timar, J.D. Taylor, S. Lundy, I. Butovich & K.V. Honn: 12(S)-Hydroxyeicosatetraenoic acid and 13(S)-hydroxyoctadecadienoic acid regulation of protein kinase C- in melanoma cells: Role of receptor-mediated hydrolysis of inositol phospholipids. *Proc. Natl. Acad. Sci. USA* 92, 9323-9327 (1995).

53. Saito F, M.T. Hori, Y. Ideguchi, M. Berger, M. Golub, N. Stern & M.L. Tuck: 12-lipoxygenase products modulate calcium signals in vascular smooth muscle cells. *Hypertension* 20, 138-143 (1992).

54. Gross E, T. Ruzicka, B.V. Restoroff, W. Stolz & K.N. Klotz: High affinity binding and lack of growth-promoting activity of 12(S)hydroxyeicosatetraenoic acid, 12(S)HETE, in a human epidermal cell line. *J. Invest. Dermatol*. 94, 446-450 (1990).

55. Herbertsson H & S. Hammarstrom: High affinity binding sites for 12(S)hydroxy-eicosatetraenoic acid, 12(S)HETE in carcinoma cells. *FEBS Lett*. 298, 249-252 (1992).

56. Takahashi Y, W.C. Glasgow, H. Suzuki, Y. Taketani, S. Yamamoto, M. Anton, H. Kuhn & A. Brash: Investigation of the oxygenation of phopspholipids by the porcine leukocyte and human platelet arachidonate 12-lipoxygenases. *Eur. J. Biochem.* 218, 165-171 (1993).

57. Davis RJ: The mitogen-activated protein kinase signal transduction pathway. *J. Biol. Chem.* 268, 14553-14556 (1993).

58. Rao G, A.S. Baas, W.C. Glasgow, T.E. Eling, M.D. Runge & R.W. Alexander: Activation of MAP kinases by arachidonic acid and its metabolites in vascular smooth muscle cells. *J. Biol. Chem.* 269, 32586-32591 (1994).

59. Rao GN, R.W. Alexander & M.S. Runge: Linoleic acid and its metabolites, hydroperoxyocatadecadienoic acids, stimulate c-*fos*, c-*jun*, and c-*myc* mRNA expression, mitogen-activated protein kinase activation, and growth in rat aortic smooth muscle cells. *J. Clin. Invest.* 96, 842-847 (1995).

60. Wen Y, J.L. Nadler, N. Gonzales, S. Scott, E. Clauser & R. Natarajan: Mechanisms of angiotensin II-induced mitogenic responses: Key role of 12-lipoxygenase and biphasic mitogen activated protein kinase activity. *Am. J. Physiol* 271: (*Cell Physiol*)40, C1212-C1220 (1996).

61. Wen Y, S. Scott, Y. Liu, N. Gonzales & J.L. Nadler: Evidence that angiotensin II and lipoxygenase products activate the c-Jun NH2-terminal kinase. Circ. Res. 81, 651-655 (1997).

62. Eling TE & W.C. Glasgow: Cellular proliferation and lipid metabolism: importance of lipoxygenases in modulating epidermal growth factor-dependent mitogenesis. *Cancer Metastasis Rev.* 13, 397-410 (1994).

63. Gu JL, D. Veerapanane, J. Rossi, R. Natarajan, L. Thomas & J. L Nadler: Ribozyme-mediated inhibition of expression of leukocyte-type 12-lipoxygenase in porcine aortic vascular smooth muscle cells. *Circ. Res.* 77, 14-20 (1995).

64. Gu JL, J.L. Nadler & J.J. Rossi: Use of a hammerhead ribozyme with cationic liposomes to reduce leukocyte-type 12-lipoxygenase expression in vascular smooth muscle. *Mol. Cell Biochem.* 172, 47-57 (1997).

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