BMB
reports

Mini Review

Cytosolic phospholipase A_2, lipoxygenase metabolites, and reactive oxygen species

*Cheolmin Kim, Joo-Young Kim & Jae-Hong Kim**
School of Life Sciences and Biotechnology, Korea University, Seoul 136-701, Korea

Reactive oxygen species (ROS) are generated in mammalian cells via both enzymatic and non-enzymatic mechanisms. Although certain ROS production pathways are required for the performance of specific physiological functions, excessive ROS generation is harmful, and has been implicated in the pathogenesis of a number of diseases. Among the ROS-producing enzymes, NADPH oxidase is widely distributed among mammalian cells, and is a crucial source of ROS for physiological and pathological processes. Reactive oxygen species are also generated by arachidonic acid (AA) metabolites, which are released from membrane phospholipids via the activity of cytosolic phospholipase A_2 ($cPLA_2$). In this study, we describe recent studies concerning the generation of ROS by AA metabolites. In particular, we have focused on the manner in which AA metabolism via lipoxygenase (LOX) and LOX metabolites contributes to ROS generation. By elucidating the signaling mechanisms that link LOX and LOX metabolites to ROS, we hope to shed light on the variety of physiological and pathological mechanisms associated with LOX metabolism. [BMB reports 2008; 41(8): 555-559]

Reactive oxygen species (ROS) are highly reactive O_2 metabolites, such as superoxide radicals ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals (•OH) (1). Although ROS have classically been regarded as cytotoxic and harmful, recent evidence suggests that superoxide radicals and H_2O_2 may also function as essential components of signal transduction pathways (2). ROS have also been implicated in cell proliferation, survival, migration, and adhesion pathways (1-4). The targets of ROS include key signaling molecules, such as transcription factor nuclear factor κB (NF-κB), mitogen-activated protein kinases, tyrosine phosphatases, and phosphatase and tensin homologue (PTEN), which hydrolyzes the 3-phosphate group of the bioactive lipid, phosphatidylinositol 3,4,5-triphosphate (1, 5-7).

*Corresponding author. Tel: 82-2-3290-3452; Fax: 82-2-923-0851; E-mail: jhongkim@korea.ac.kr

Received 8 July 2008

Keywords: Arachidonic acid, Cytosolic phospholipase A_2, Eicosanoids, Lipoxygenase, Reactive oxygen species

Until recently, phagocytic NADPH oxidase was the best-characterized example of ROS production in mammalian cells. This enzyme catalyzes the respiratory burst (1) and is comprised of a catalytic subunit (i.e., gp91phox, or NOX2), regulatory subunits (i.e., p22phox, p47phox, p40phox, and p67phox), and a small GTPase (i.e., Rac). Non-phagocytic cells also generate significant quantities of ROS, albeit in much smaller amounts than in phagocytes (i.e., only a small percentage of the ROS levels detected in activated neutrophils) (2). In non-phagocytic cells, ROS are produced via a variety of cellular oxidative metabolic processes, including NADPH oxidase, xanthine oxidase, arachidonic acid (AA) metabolism by cyclooxygenases (COX; more accurately, prostaglandin G/H synthase) and lipoxygenases (LOX), and the mitochondrial respiratory chain (2). In the 1990s, an increase in the number of sensitive assays available facilitated the detection of ever-smaller quantities of ROS, as well as enzyme generation in a variety of non-phagocytic cell types. Over the past several years, non-phagocytic NADPH oxidases (e.g., NOX1, 3, 4, 5 & DUOX1 and 2) homologous to gp91 (i.e., NOX2) have been demonstrated to catalyze ROS production in non-phagocytic cells (1).

Arachidonic acid is released from glycerophospholipids in the nuclear envelope and from the plasma membrane via the activity of cytosolic phospholipase A_2 ($cPLA_2$), and is subsequently metabolized by COX and LOX to generate a variety of bioactive eicosanoids, including prostaglandins, thromboxanes, and leukotrienes (as shown schematically in Fig. 1) (8, 9). ROS may be generated as a byproduct during the oxidation step of AA by COX and LOX (2). In addition to the oxidative metabolic processes of AA by COX and LOX, AA itself has also been reported to activate NADPH oxidase directly, thereby inducing ROS generation (10-12). Over the past few years, LOX- and COX-generated AA metabolites have been demonstrated to stimulate the generation of ROS by NOXs (11, 13), thus revealing the existence of an inter-connecting signaling system between eicosanoids and NOXs. The generation of ROS by AA metabolites may also perform pivotal roles in various cell signaling pathways, although the mechanisms by which AA metabolites mediate ROS generation remain to be clearly elucidated. In this review, we describe recent studies into the generation of ROS by eicosanoids, and in particular the generation of ROS by AA metabolic pathways via LOX.

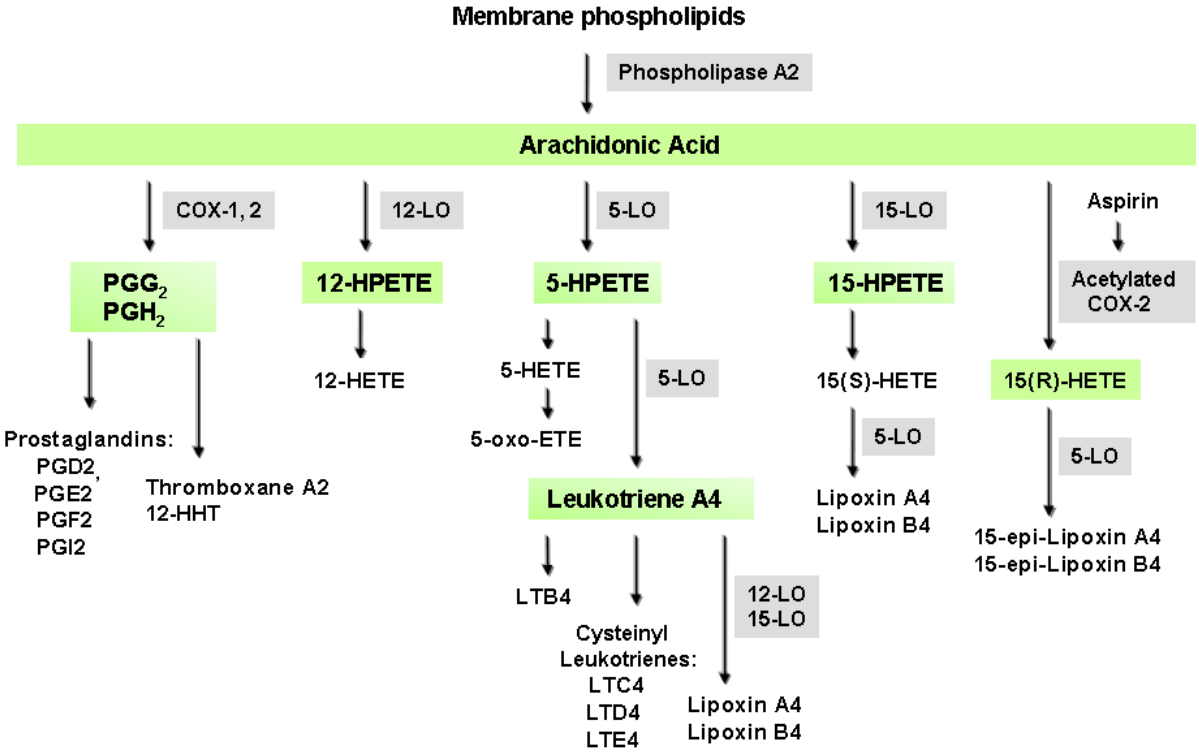

Fig. 1. Generation of various bioactive eicosanoids via the metabolism of arachidonic acid (AA) by lipoxygenases and cyclooxygenases.

Lipoxygenases and reactive oxygen species

Lipoxygenases are a group of closely related dioxygenases that catalyze the addition of oxygen to AA and polyunsaturated fatty acids (PUFAs), yielding hydroperoxyl derivatives including hydroperoxyeicosatetraenoic acids (HPETEs) (8, 14). Lipoxygenases are classified as 5-, 8-, 12-, or 15-LOX, according to the site of oxygen insertion within AA. Upon reduction, the corresponding hydroxyl derivatives hydroxyeicosatetranoic acid (HETE), leukotriene (LT), and lipoxins are generated (15) (Fig. 1). These oxidized metabolites perform crucial functions in the regulation of many biological processes, and aberrant oxidative AA metabolism has been implicated in the pathogenesis of a variety of human diseases, including cancer, atherosclerosis, rheumatoid arthritis, Alzheimer's dementia, and aging (15, 16). During LOX-catalyzed metabolism, unstable byproducts of AA hydroperoxidation can function as ROS (15, 17, 18). In addition, the metabolism of 5-, 12-, and 15-LOX produce leukotrienes, 12(S)-HETE, and 15(S)-HETE, which can induce NADPH oxides to stimulate ROS production (11, 13). For example, a 5-LOX metabolite [i.e., leukotriene B_4 (LTB_4)] has been shown to activate NADPH oxidase in non-phagocytes (19-22). In addition, a variety of cytokines and growth factors are known to generate transient ROS bursts in non-phagocytic cells, a process which can be blocked by inhibitors of either the lipoxygenase pathway or NADPH oxidase (23-28), thereby suggesting the existence of a inter-connected signaling link between 5-LOX metabolites and NOX.

Eicosanoids generated via the 5-LOX pathway are known to harbor at least six receptors. These include OXE, which recognizes 5-HETE and 5-oxo-6,8,11,14-eicosatetraenoic acid (5-oxo-ETE); the FOG7 receptor, which is believed to recognize the 5-oxo-ETE metabolite, 5-oxo-7-glutathionyl-8,11,14-eicosatetraenoic acid (FOG7); BLT1 and BLT2, which recognize LTB4 and other eicosanoids such as 12-HETE; and CysLT1 and CysLT2, which recognize the cysteinyl leukotrienes LTC4, LTD4, and LTE4. The results of recent studies have demonstrated that these receptors perform unique functions in cell signaling and pathophysiology (29). Cyclooxgenase (COX) transforms AA into prostaglandin H2 (PGH2), the immediate precursor of a variety of prostanoids including prostaglandin (PG) and thromboxane A2. Thromboxane A2 and 12-HHT are generated from PGH2 in an equimolar ratio, in a reaction catalyzed by thromboxane synthase (Fig. 1). COX metabolites (e.g., PGF2α are generated in a COX-dependent manner, and are capable of stimulating NADPH oxidase. However, delayed PGF2α-dependent activation of the NADPH oxidase system requires the transcriptional upregulation of NOX-1 (30). Therefore, short-term delays may occur in eicosanoid-medi-

ated NOX activation, although the details of this mechanism remain to be clearly elucidated (11).

5-LOX metabolites and reactive oxygen species

The 5-lipoxygenase enzyme, which was initially described 1976 by Borgeat *et al*. (31), is a 78 kDa protein which catalyzes the biosynthesis of potent bioactive eicosanoids, such as LTs and HETEs. The biosynthesis of LT begins with the metabolism of AA by 5-LOX. These soluble dioxygenases incorporate oxygen molecules at position C5 of the fatty acid, yielding 5(S)-hydroperoxyeicosatetraenoic acid (5-HPETE), which is subsequently metabolized by 5-LOX to generate the unstable epoxide, leukotriene A4 (LTA4). LTA4 can be converted to LTB4 via LTA4 hydrolase, or to LTC4 via LTC4 synthase (16). The most relevant pathophysiological function performed by LTs involves the regulation of inflammatory immune responses. Leukotriene B4 (LTB4) is a potent activator of neutrophil chemotaxis, whereas the cysteinyl leukotrienes (CysLTs) (i.e., LTC4, LTD4, and LTE4) are key mediators of allergic inflammation (16, 32). LTB4, which was the first leukotriene to be isolated, elicits a variety of inflammatory responses, including leukocyte activation, chemotaxis, and degranulation (8, 33, 34). Over the past few years, it has been reported that LTB4 treatment of fibroblasts and neutrophils results in ROS generation, and that LTB4-induced chemotaxis is mediated by a NADPH oxidase-dependent cascade. These observations suggest that LTB4-induced ROS generation occurring via NOX is crucial to cell chemotaxis (34, 35). In addition, Giembycz has reported that LTB4 promotes the robust, receptor-mediated activation of NADPH oxidase in guinea pig eosinophils (21, 36). LTB4 has also been previously shown to promote the phosphorylation and translocation of p47phox, thereby stimulating NADPH oxidase (22). Collectively, these results suggest that the 5-LOX metabolite, LTB4, appears to stimulate NOX, thereby generating ROS that mediate a variety of signaling pathways in non-phagocytic cells.

It has been previously established that LTB_4 functions by binding to the BLT1 and BLT2 receptors (33, 37, 38). Most studies of LTB4 receptors have focused on the high-affinity receptor, BLT1, which is expressed exclusively in inflammatory cells (e.g., leukocytes) and performs a function in inflammatory processes (33). By way of contrast, BLT2 evidences a low affinity for LTB4 and is expressed in a broad variety of tissues, with the highest levels observed in the spleen, leukocytes, and ovaries (37, 38). Although the physiological functions of BLT2 have yet to be thoroughly explained, the results of recent studies appear to suggest that BLT2 plays a pivotal role in ROS generation (19). For example, the presence of BLT2 is known to be critical for ROS generation via NOX1, thereby mediating Ras transformation (19). Furthermore, increased ROS levels in Ras-transformed cells are believed to be elicited via either the autocrine or paracrine effects of high levels of LTB4, which amplifies the LTB4-dependent cascade via BLT2. Recently, Okuno *et al*. reported that 12(S)-hydroxyheptadeca-5Z, 8E, 10E-trienoic acid (12-HHT) is a natural *in vivo* lipid agonist of BLT2, along with LTB4 and 12(S)-HETE (39). In fact, 12-HHT can be abundantly detected in tissues and bodily fluids, and has long been regarded as a byproduct with no specific activity (39). Previous studies have demonstrated that 12-HHT and thromboxane A2 are produced from PGH2 in an equimolar ratio, and that this reaction is catalyzed by thromboxane synthase in activated blood platelets and macrophages. However, it remains to be determined whether 12-HHT is capable of generating ROS via the stimulation of BLT2.

12-LOX metabolites and ROS

12-lipoxygenase (12-LOX), a member of the lipoxygenase superfamily, catalyzes the stereospecific oxygenation of AA to 12(S)-hydroperoxyeicosatetraenoic acid (HPETE) and 12(S)-hydroxyeicosatetraenoic acid (HETE) (40). At least three types of 12-LOX have been characterized–platelet-type, leukocyte-type, and epidermal 12-LOX. Platelet-type 12-LOX synthesizes 12(S)-HPETE and 12(S)-HETE from AA, whereas leukocyte-type 12-LOX synthesizes 12(S)- and 15(S)-HETE. Each 12-LOX isozyme has been detected in various cell types, including smooth muscle cells, keratinocytes, endothelial cells, and tumor cells. In addition, elevated 12-LOX activity has been implicated in hypertension, inflammation, thrombosis, and the development of skin tumors (41). Although the physiological relevance of the 12-LOX and NADPH oxidase pathways has yet to be firmly established, several researchers have suggested that 12-LOX acts upstream of the NADPH oxidase pathways. It was recently reported that 12(S)-HETE, a product of 12-LOX, activates the NADPH oxidase pathway to generate ROS during HIV-induced thrombocytopenia and platelet fragmentation (23). De Carvalho *et al*. identified a signaling connection between 12-LOX and NADPH oxidase, using a mouse knockout model. For example, in the pathogenesis of colon cancer, 12-LOX regulates Nox1-dependent ROS production to enhance the spread and proliferation of colon epithelial cells (13). However, the exact role of 12-LOX in the activation of Nox1 remains to be clarified in further studies (40).

15-LOX metabolites and reactive oxygen species

Previous studies have established that 15-LOX catalyzes the addition of oxygen molecules onto carbons 13 or 15 of its substrates (18). This enzyme was initially discovered in rabbit reticulocytes and immature red blood cells (42, 43). Later, the reticulocyte-type of 15-LOX was designated 15-LOX-1. In 1997, Brash *et al*. cloned a second type of 15-LOX from human hair roots. This epidermal-type 15-LOX was designated 15-LOX-2, and is expressed in the skin, prostate gland, lungs, and the cornea (44). It is well known that 15-LOX-1 converts linoleic acid and AA to 13-(S)-HPODE and 13-(S)-HODE, respectively. Additionally, 15-LOX-2 can convert AA and linoleic acid to

15-(S)-HPETE and 15-(S)-HETE, respectively. As is the case with other LOX families, 15-LOXs (i.e., 15-LOX-1 and 15-LOX-2) are believed to regulate ROS generation. It has also been reported that the anti-carcinogenic properties of 15-LOX-2 and its metabolites arise from ROS generation and the subsequent activation of p38 (15). Recently, it has been suggested that 15-LOX may play a role in NOX activation. For example, Suraneni *et al*. previously reported that Nox may be an important mediator of ROS generation via 15-LOX (14). However, further studies will be necessary to characterize the relationship between 15-LOX and NOX, with regard to cell signaling.

Conclusions and future perspectives

There is mounting evidence to suggest that eicosanoids and ROS are involved in a variety of pathological processes, as well as the inflammatory response. However, the mechanisms by which eicosanoids mediate the specific signaling pathways involved in ROS generation have yet to be clearly demonstrated. Future studies of the eicosanoid receptors that mediate ROS generation, in addition to their relationships to NOX, should be expected to further our understanding of the signaling pathways involved in ROS generation.

Acknowledgments

This work was supported by a grant from the Diseases Network Research Program of the Korean Ministry of Education, Science, and Technology.

REFERENCES

1. Lambeth, J. D. (2004) NOX enzymes and the biology of reactive oxygen. *Nat. Rev. Immunol.* **4**, 181-189.
2. Edderkaoui, M., Hong, P., Vaquero, E. C., Lee, J. K., Fischer, L., Friess, H., Buchler, M. W., Lerch, M. M., Pandol, S. J. and Gukovskaya, A. S. (2005) Extracellular matrix stimulates reactive oxygen species production and increases pancreatic cancer cell survival through 5-lipoxygenase and NADPH oxidase. *Am. J. Physiol. Gastrointest. Liver. Physiol.* **289**, G1137-47.
3. Suh, Y. A., Arnold, R. S., Lassegue, B., Shi, J., Xu, X., Sorescu, D., Chung, A. B., Griendling, K. K. and Lambeth, J. D. (1999) Cell transformation by the superoxide-generating oxidase *Mox1*. *Nature*. **401**, 79-82.
4. Simon, H. U., Haj-Yehia, A. and Levi-Schaffer, F. (2000) Role of reactive oxygen species (ROS) in apoptosis induction. *Apoptosis*. **5**, 415-418.
5. Lee, S. R., Kwon, K. S., Kim, S. R. and Rhee, S. G. (1998) Reversible inactivation of protein-tyrosine phosphatase 1B in A431 cells stimulated with epidermal growth factor. *J. Biol. Chem.* **273**, 15366-15372.
6. Lee, S. R., Yang, K. S., Kwon, J., Lee, C., Jeong, W. and Rhee, S. G. (2002) Reversible inactivation of the tumor suppressor PTEN by H2O2. *J. Biol. Chem.* **277**, 20336-20342.
7. Torres, M. and Forman, H. J. (2003) Redox signaling and the MAP kinase pathways. *Biofactors*. **17**, 287-296.
8. Samuelsson, B., Dahlen, S. E., Lindgren, J. A., Rouzer, C. A. and Serhan, C. N. (1987) Leukotrienes and lipoxins: structures, biosynthesis, and biological effects. *Science*. **237**, 1171-1176.
9. Funk, C. D. (2001) Prostaglandins and leukotrienes: advances in eicosanoid biology. *Science*. **294**, 1871-1875.
10. Kim, C. and Dinauer, M. C. (2006) Impaired NADPH oxidase activity in Rac2-deficient murine neutrophils does not result from defective translocation of p47phox and p67phox and can be rescued by exogenous arachidonic acid. *J. Biol. Chem.* **79**, 223-234.
11. Luchtefeld, M., Drexler, H. and Schieffer, B. (2003) 5-Lipoxygenase is involved in the angiotensin II-induced NAD(P)H-oxidase activation. *Biochem. Biophys. Res. Commun.* **308**, 668-672.
12. Shiose, A. and Sumimoto, H. (2000) Arachidonic acid and phosphorylation synergistically induce a conformational change of p47phox to activate the phagocyte NADPH oxidase. *J. Biol. Chem.* **275**, 13793-13801.
13. de Carvalho, D. D., Sadok, A., Bourgarel-Rey, V., Gattacceca, F., Penel, C., Lehmann, M. and Kovacic, H. (2008) Nox1 downstream of 12-lipoxygenase controls cell proliferation but not cell spreading of colon cancer cells. *Int. J. Cancer*. **122**, 1757-1764.
14. Mahipal, S. V., Subhashini, J., Reddy, M. C., Reddy, M. M., Anilkumar, K., Roy, K. R., Reddy, G. V. and Reddanna, P. (2007) Effect of 15-lipoxygenase metabolites, 15-(S)-HPETE and 15-(S)-HETE on chronic myelogenous leukemia cell line K-562: reactive oxygen species (ROS) mediate caspase-dependent apoptosis. *Biochem. Pharmacol.* **74**, 202-214.
15. Schweiger, D., Furstenberger, G. and Krieg, P. (2007) Inducible expression of 15-lipoxygenase-2 and 8-lipoxygenase inhibits cell growth via common signaling pathways. *J. Lipid Res.* **48**, 553-564.
16. Werz, O. and Steinhilber, D. (2006) Therapeutic options for 5-lipoxygenase inhibitors. *Pharmacol. Ther.* **112**, 701-718.
17. Uchida, K. (2003) 4-Hydroxy-2-nonenal: a product and mediator of oxidative stress. *Prog. Lipid Res.* **42**, 318-343.
18. Brash, A. R. (1999) Lipoxygenases: occurrence, functions, catalysis, and acquisition of substrate. *J. Biol. Chem.* 274, 23679-23682.
19. Choi, J. A., Kim, E. Y., Song, H., Kim, C. and Kim, J. H. (2008) Reactive oxygen species are generated through a BLT2-linked cascade in Ras-transformed cells. Free. *Radic. Biol. Med.* **44**, 624-634.
20. Woo, C. H., Lee, Z. W., Kim, B. C., Ha, K. S. and Kim, J. H. (2000) Involvement of cytosolic phospholipase A2, and the subsequent release of arachidonic acid, in signalling by rac for the generation of intracellular reactive oxygen species in rat-2 fibroblasts. *Biochem. J.* **348** (Pt 3), 525-530.
21. Lindsay, M. A., Perkins, R. S., Barnes, P. J. and Giembycz, M. A. (1998) Leukotriene B4 activates the NADPH oxidase in eosinophils by a pertussis toxin-sensitive mechanism that is largely independent of arachidonic acid mobilization. *J. Immunol.* **160**, 4526-4534.
22. Serezani, C. H., Aronoff, D. M., Jancar, S. and Peters-Golden, M. (2005) Leukotriene B4 mediates p47 phox phosphorylation and membrane translocation in pol-

yunsaturated fatty acid-stimulated neutrophils. *J. Biol. Chem.* **78**, 976-984.
23. Nardi, M., Feinmark, S. J., Hu, L., Li, Z. and Karpatkin, S. (2004) Complement-independent Ab-induced peroxide lysis of platelets requires 12-lipoxygenase and a platelet NADPH oxidase pathway. *J. Clin. Invest.* **113**, 973-980.
24. Woo, C. H., Eom, Y. W., Yoo, M. H., You, H. J., Han, H. J., Song, W. K., Yoo, Y. J., Chun, J. S. and Kim, J. H. (2000) Tumor necrosis factor-alpha generates reactive oxygen species via a cytosolic phospholipase A2-linked cascade. *J. Biol. Chem.* **275**, 32357-32362.
25. Lo, Y. Y. and Cruz, T. F. (1995) Involvement of reactive oxygen species in cytokine and growth factor induction of c-fos expression in chondrocytes. *J. Biol. Chem.* **270**, 11727-11730.
26. Bae, Y. S., Kang, S. W., Seo, M. S., Baines, I. C., Tekle, E., Chock, P. B. and Rhee, S. G. (1997) Epidermal growth factor (EGF)-induced generation of hydrogen peroxide. Role in EGF receptor-mediated tyrosine phosphorylation. *J. Biol. Chem.* **272**, 217-221.
27. Sundaresan, M., Yu, Z. X., Ferrans, V. J., Irani, K. and Finkel, T. (1995) Requirement for generation of H2O2 for platelet-derived growth factor signal transduction. *Science.* **270**, 296-299.
28. Colavitti, R., Pani, G., Bedogni, B., Anzevino, R., Borrello, S., Waltenberger, J. and Galeotti, T. (2002) Reactive oxygen species as downstream mediators of angiogenic signaling by vascular endothelial growth factor receptor- 2/KDR. *J. Biol. Chem.* **277**, 3101-3108.
29. Rubin, P. and Mollison, K. W. (2007) Pharmacotherapy of diseases mediated by 5-lipoxygenase pathway eicosanoids. *Prostaglandins Other Lipid Mediat.* **83**, 188-197.
30. Katsuyama, M., Fan, C. and Yabe-Nishimura, C. (2002) NADPH oxidase is involved in prostaglandin F2alpha-induced hypertrophy of vascular smooth muscle cells: induction of NOX1 by PGF2alpha. *J. Biol. Chem.* **277**, 13438-13442.
31. Borgeat, P., Hamberg, M. and Samuelsson, B. (1976) Transformation of arachidonic acid and homo-gamma-linolenic acid by rabbit polymorphonuclear leukocytes. Monohydroxy acids from novel lipoxygenases. *J. Biol. Chem.* **251**, 7816-7820.
32. Claria, J. and Arroyo, V. (2003) Prostaglandins and other cyclooxygenase-dependent arachidonic acid metabolites and the kidney in liver disease. *Prostaglandins Other Lipid Mediat.* **72**, 19-33.
33. Yokomizo, T., Izumi, T., Chang, K., Takuwa, Y. and Shimizu, T. (1997) A G-protein-coupled receptor for leukotriene B4 that mediates chemotaxis. *Nature.* **387**, 620-624.
34. Woo, C. H., You, H. J., Cho, S. H., Eom, Y. W., Chun, J. S., Yoo, Y. J. and Kim, J. H. (2002) Leukotriene B(4) stimulates Rac-ERK cascade to generate reactive oxygen species that mediates chemotaxis. *J. Biol. Chem.* **277**, 8572-8578.
35. Woo, C. H., Yoo, M. H., You, H. J., Cho, S. H., Mun, Y. C., Seong, C. M. and Kim, J. H. (2003) Transepithelial migration of neutrophils in response to leukotriene B4 is mediated by a reactive oxygen species-extracellular signal-regulated kinase-linked cascade. *J. Immunol.* **170**, 6273-6279.
36. Perkins, R. S., Lindsay, M. A., Barnes, P. J. and Giembycz, M. A. (1995) Early signalling events implicated in leukotriene B4-induced activation of the NADPH oxidase in eosinophils: role of Ca2+, protein kinase C and phospholipases C and D. *Biochem. J.* **310** (Pt 3), 795-806.
37. Yokomizo, T., Kato, K., Terawaki, K., Izumi, T. and Shimizu, T. (2000) A second leukotriene B(4) receptor, BLT2. A new therapeutic target in inflammation and immunological disorders. *J. Exp. Med.* **192**, 421-432.
38. Kamohara, M., Takasaki, J., Matsumoto, M., Saito, T., Ohishi, T., Ishii, H. and Furuichi, K. (2000) Molecular cloning and characterization of another leukotriene B4 receptor. *J. Biol. Chem.* **275**, 27000-27004.
39. Okuno, T., Iizuka, Y., Okazaki, H., Yokomizo, T., Taguchi, R. and Shimizu, T. (2008) 12(S)-Hydroxyheptadeca-5Z, 8E, 10E-trienoic acid is a natural ligand for leukotriene B4 receptor 2. *J. Exp. Med.* **205**, 759-766.
40. Sadok, A., Bourgarel-Rey, V., Gattacceca, F., Penel, C., Lehmann, M. and Kovacic, H. (2008) Nox1-dependent superoxide production controls colon adenocarcinoma cell migration. *Biochim. Biophys. Acta.* **1783**, 23-33.
41. Nie, D., Tang, K., Diglio, C. and Honn, K. V. (2000) Eicosanoid regulation of angiogenesis: role of endothelial arachidonate 12-lipoxygenase. *Blood.* **95**, 2304-2311.
42. Schewe, T., Halangk, W., Hiebsch, C. and Rapoport, S. M. (1975) A lipoxygenase in rabbit reticulocytes which attacks phospholipids and intact mitochondria. *FEBS Lett.* **60**, 149-152.
43. Rapoport, S. M., Schewe, T., Wiesner, R., Halangk, W., Ludwig, P., Janicke-Hohne, M., Tannert, C., Hiebsch, C. and Klatt, D. (1979) The lipoxygenase of reticulocytes. Purification, characterization and biological dynamics of the lipoxygenase; its identity with the respiratory inhibitors of the reticulocyte. *Eur. J. Biochem.* **96**, 545-561.
44. Brash, A. R., Boeglin, W. E. and Chang, M. S. (1997) Discovery of a second 15S-lipoxygenase in humans. *Proc. Natl. Acad. Sci. U.S.A.* **94**, 6148-6152.