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5-Lipoxygenase: Its Noncanonical Function Unravels its Inhibitors as Powerful Antileukemic Drugs

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1. Introduction

Besides its function as key enzyme in the biosynthesis of leukotrienes (***Figure 1***), there is accumulating evidence that 5-lipoxygenase (5-LO) has additional, noncanonical functions. The enzyme is mainly expressed in leukocytes. After stimulation of neutrophils, the enzyme is activated by an increase in intracellular calcium concentration and by phosphorylation by 5-LO kinases. Leukotrienes are considered as proinflammatory mediators which are involved in host defense reactions and which contribute to allergic and inflammatory reactions (1). Zileuton (***Figure 2***) is the only approved 5-LO inhibitor, which binds to the iron ion in the active cite. Cys-LT_1 receptor antagonists which block the action of LTC_4 and its metabolites in the lung are approved for the treatment of asthma and allergic rhinitis. Expression of the enzyme is regulated in a cell cycle and cell differentiation-dependent manner (2). It is a TGF-β and vitamin D response gene which seems to be controlled by transcription factors that regulate stemness, lineage-specific differentiation of myeloid and lymphocytic cells including p53, SMAD1, C/EBPα, GATA2, PU.1, RUNX1, RUNX3 and the WNT pathway (3). Aberrant 5-LO expression is observed in many cancer tissues and cells which suggested that the 5-LO pathway might be involved in cancer development (for review see (4)). A clear evidence for the role of 5-LO in cancer came from the observation that 5-LO knockout prevents the development of chronic myeloid leukemia in a murine BCR/ABL leukemia model (5). Interestingly, 5-LO knockout does not seem to lead to a defect in normal hematopoiesis. First mechanistic insights into the role of 5-LO came from the observation that 5-LO alters nuclear trafficking of p53 and leads to inhibition of apoptosis (6).

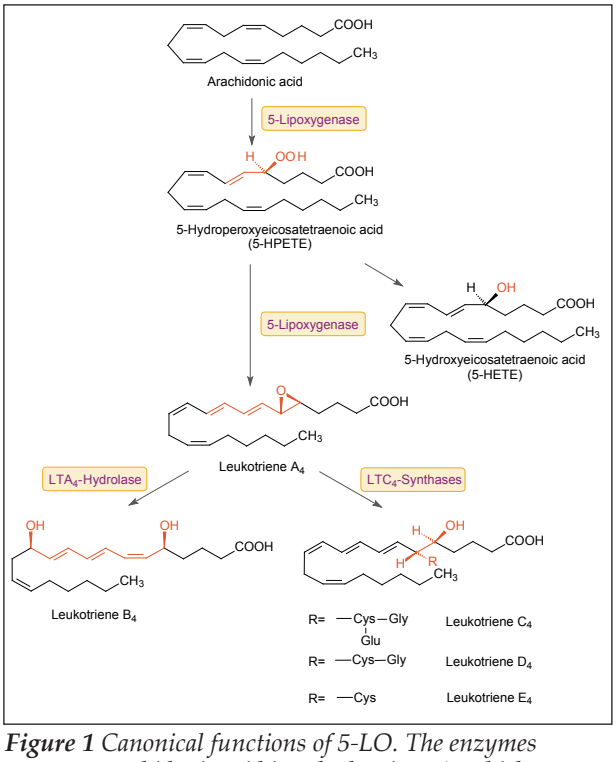

Figure 1 *Canonical functions of 5-LO. The enzymes converts arachidonic acid into leukotriene A_4 which can be subsequently converted into leukotriene B_4 by LTA_4 hydrolase or into leukotriene C_4 by LTC_4 synthase.*

2. The noncanonical 5-LO functions

A key role of 5-LO in cell proliferation and the maintenance of leukemic stem cells was also shown by us in a PML/RARα-positive stem cell model of acute myeloid leukemia (7). The noncompetitive 5-LO inhibitor CJ-13,610 (8) abolished the aberrant replating efficiency of PML/RARα-expressing hematopoietic stem and progenitor cells and inhibited the long term and short term stem cell capacity but no cytotoxic effect of CJ-13,610 was observed in the

PML/RARα-negative control cells. Mechanistically, we have shown that the 5-LO protein can in-

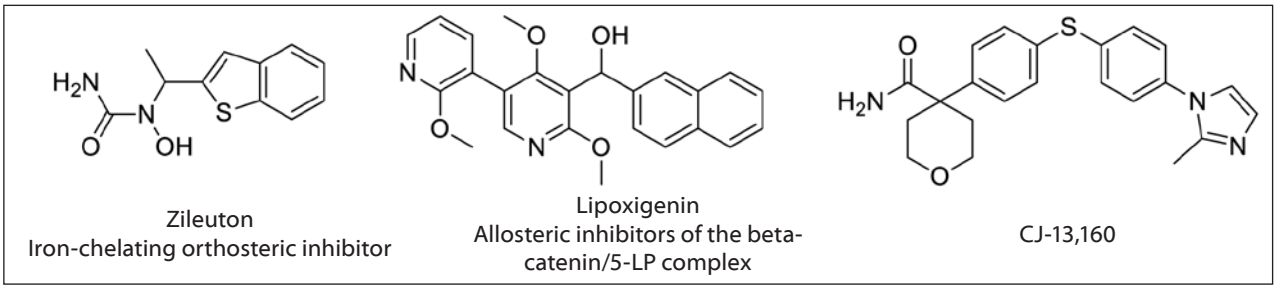

Figure 2 *5-LO inhibitors that interfere with different functions of the enzyme*

teract with p53 and β-catenin (7, 9) suggesting that 5-LO acts as STRaND (shuttling transcriptional regulator and non-DNA binding) to modulate gene transcription. Regarding WNT signalling, it seems that 5-LO acts as a kind of chaperone which regulates translocation of β-catenin between the cytosol and the nucleus. β-Catenin relies on chaperones to enter and exit the nucleus since it lacks nuclear localization (NLS) and nuclear export (NES) signals whereas 5-LO contains NLS and NES motifs. The regulation of cellular localization and stability of β-catenin by 5-LO seems to be part of a regulatory circuit for the modulation of gene transcription to regulate stem cell replication and leukocyte differentiation as part of the immune response and of tissue regeneration and resolution of inflammation.

The link between 5-LO and WNT signaling could be confirmed recently by screening for WNT inhibitors which led to identification of a 3,5-substituted-2,4-dimethoxypyridine derivative, lipoxygenin (***Figure 2***) as inhibitor of Wnt signalling (10). It turned out that the compound does not directly interfere with β-catenin but that it is a nonredox-type, noncompetitive 5-LO inhibitor which modulates the β-catenin/5-LO complex and reduces β-catenin levels in the nucleus, similar to the structurally unrelated compound CJ-13,160 (7). Subsequent studies revealed that CJ-13,160 and lipoxygenin not only inhibit Wnt signaling but also interfere with hedgehog, TGF-β, BMP and activin A signaling in the same concentration range (10). The data suggest that 5-LO inhibitors might be useful to treat certain types of leukemia and cancers.

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