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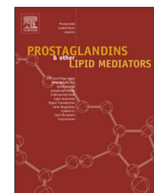
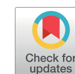


Review

Beyond leukotriene formation—The noncanonical functions of 5-lipoxygenase



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ABSTRACT

5-lipoxygenase (5-LO) is the key enzyme in the biosynthesis of leukotrienes and specialized proresolving lipid mediators (SPM). It is mainly expressed in leukocytes and is part of the innate immune system. 5-LO can shuttle between the cytosol and the nucleus. Upon cell activation the protein translocates from soluble cellular compartments to the nuclear membrane. Besides FLAP which is required for cellular leukotriene and SPM formation, 5-LO interacts with other proteins like coactosin-like protein (CLP), Dicer, β-catenin and p53. In this review, the factors involved in the regulation of 5-LO expression, the role of 5-LO in the regulation of stem cell proliferation and differentiation and its biological functions apart from leukotriene and SPM formation are summarized.

1. Canonical 5-lipoxygenase functions

The 5-lipoxygenase (5-LO) pathway was discovered several decades ago [1,2]. Subsequently, many studies have been performed to characterize the enzymes involved in the pathway, i.e. 5-LO, leukotriene (LT)A_4 hydrolase, LTC_4 synthase and other enzymes involved in the generation of 5-LO products derived from arachidonic acid like 5-HETE or LTs which include leukotriene B_4 (LTB_4) as well as the cysteinyl-containing leukotrienes LTC_4, D_4 and E_4 [3]. The early studies on the biological functions revealed that LTs are mediators of inflammatory and allergic responses [4–6]. Many subsequent studies showed that the 5-LO pathway is part of the innate immune system and plays an important role in host defense reactions [7–9]. Although arachidonic acid is by far the preferred substrate, 5-LO can also metabolize oxidized fatty acids derived from arachidonic acid, eicosapentaenoic acid or docosahexaenoic acid which leads to the formation of specialized pro-resolving mediators (SPM) [10] suggesting that 5-LO is involved in the onset and the resolution of inflammatory reactions.

Intriguingly, in resting cells 5-LO is localized in soluble compartments, i.e. either in the cytosol or in the nucleus depending on the cell type (Fig. 1) [11,12]. In resting cells, nuclear 5-LO is located in the euchromatin [13]. At present, the significance of this observation is unclear but could be related to a regulatory role of 5-LO in transcription. Upon cell activation, 5-LO translocates to the nuclear membrane where it interacts with the 5-LO-activating protein (FLAP) for catalysis (Fig. 1). Cellular localization of 5-LO (nuclear or cytosolic) is regulated by at least three nuclear localization sequences and a nuclear export sequence [14,15] which in part contain phosphorylation sites so that their function is regulated by phosphorylation (see below).

Among the enzymes of the lipoxygenase family, 5-LO has several unique properties. 5-LO activity is stimulated by diacylglycerides which bind to the C2-like domain of 5-LO (Fig. 2) [16]. 5-LO binds ATP, its activity is strongly calcium-dependent and it requires FLAP for efficient product formation in intact cells (for review see [17,18]. Furthermore, it interacts with dicer and coactosin-like protein (CLP) [19]. CLP acts as a chaperone for 5-LO and upregulates its LTA_4 production [20,21].

5-LO has been shown to be a substrate for various protein kinases in-vitro and in-vivo (for review see [22]). Phosphorylation of different residues has divergent consequences for 5-LO subcellular localization and activity. In vitro, protein kinase (PK)A was found to phosphorylate 5-LO at Ser523 [23], p38 mitogen-activated protein kinase activated protein kinase (MAPKAPK, MK)-2/3, PKA, and CaMK-II at Ser-271, and ERK1/2 at Ser663 [24,25] (Fig. 2). The tyrosine kinases Fgr, HCK and Yes have been shown to be able to phosphorylate Tyr42, Tyr53 and either Tyr94 or Tyr445 [26]. However, whether all of these phosphorylations occur in-vivo is not clear and physiological functions of most of them are unknown. In vivo, phosphorylation at Ser271 and Ser523 has been shown. It was found that phosphorylation of Ser523 inhibits 5-LO activity and leads to the cytosolic localization, obviously by blocking the function of the nuclear localization sequence which comprises Ser523 [27] (Fig. 1). Phosphorylation of 5-LO at Ser271 inhibits the nuclear export by the blockade of the nuclear export sequence

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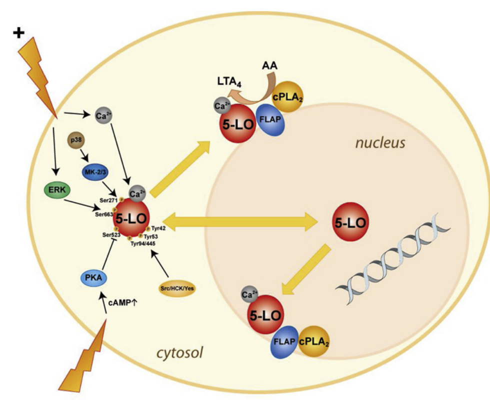

Fig. 1. Cellular localization and regulation of 5-LO activity. 5-LO can be either located in the cytosol or nucleus, depending on the cell type. Upon stimulation e.g. by calcium, it translocates to the nuclear envelope where it colocalizes with FLAP and $cPLA_2$ which releases arachidonic acid from the nuclear membrane. FLAP transfers arachidonic acid to 5-LO where it is converted to LTA_4. 5-LO can be phosphorylated by extracellular signal–regulated kinases 1/2 (ERK) at Ser663, MK-2/3 at Ser271 and PKA at Ser523.

located around Ser271 so that the nuclear localization of 5-LO is maintained [15]. Translocation of 5-LO from the cytoplasm to the nucleus was observed in neutrophils following adherence to surfaces or during cell differentiation of monocytes [11]. However, the signaling pathways that mediate the nuclear import of 5-LO in the different cell types are unknown. Nuclear localization of 5-LO has been associated with a higher synthetic capacity for leukotriene B_4 formation than the cytoplasmic localization [28] whereas enhanced SPM formation was associated with cytosolic 5-LO [29]. Consequently, it was suggested that cytosolic 5-LO drives SPM formation whereas nuclear localization promotes leukotriene formation [29,30]. However, this consideration is rather simplistic since 5-LO activation is linked with translocation to the nuclear envelope either from a cytosolic or soluble nuclear compartment [31] and because oxidized fatty acids require FLAP in order to be accepted as substrate by 5-LO [32,33]. Subsequent studies confirmed that lipoxin and resolvin formation by the 15-/5-LO pathway as well as by platelet/leukocyte interactions are FLAP-dependent [34]. The biochemical data suggest that the enzymatic formation of SPM by the 15-/5-LO pathway preferentially occurs at the nuclear membrane and not in the soluble compartment.

2. Regulation of 5-lipoxygenase expression

According to its function in immune reactions, 5-LO is mainly expressed in leukocytes. Granulocytes, monocytes/macrophages, mast cells, dendritic cells and B lymphocytes express 5-LO, whereas platelets, endothelial cells and erythrocytes do not [22] (Table 1). In the skin, Langerhans cells strongly express 5-LO [35]. 5-LO expression on T cells has been a matter of debate for many years. These cells express FLAP but show low 5-LO expression when cultured under the usual cell culture conditions [36]. However, freshly isolated T cells seem to express 5-LO which is rapidly down-regulated during cell cultivation [37]. In myeloid cells and cell lines, 5-LO is upregulated by agents that induce cell differentiation (for review see [22]) and the combination of calcitriol and TGF-β was by far the most potent combination [38]. The data suggest that 5-LO is a TGF-β and vitamin D response gene. In monocytes, 5-LO is upregulated during differentiation to M1 macrophages whereas IL-4 which triggers M2 polarization leads to downregulation of 5-LO expression [39]. In B-lymphocytes, 5-LO expression shows a strong cell cycle dependency where cell proliferation leads to a rapid down-regulation of 5-LO expression [40]. Furthermore, 5-LO expression is observed in many cancer tissues and cells suggesting that the 5-LO pathway is involved in cancer development [41]. This observation might be related to the growth stimulating effect of 5-LO and its metabolites on cancer cells and/or to the modulation of the cross talk between infiltrating immune cells and the tumour microenvironment by promoting escape mechanisms of the tumor from the immune system [42].

Human 5-LO is encoded by the ALOX5 gene that is located on chromosome 10 and comprises 71.9 kb (Fig. 3A). It is divided in 14 exons separated by 13 introns (termed introns A–M). The main 5-LO transcript, containing all 14 exons, encodes for 673 amino acids and is translated to a protein with a molecular weight of 77.9 kDa [43]. By now, several alternatively spliced 5-LO transcripts are known. While most of them contain premature termination codons, three shorter transcripts missing either exon 4, exon 13 or a part of exon 12, are not subjected to nonsense-mediated mRNA-decay and therefore lead to putative 5-LO protein isoforms [44–46].

The promoter region upstream of the 5′ end of ALOX5 contains eight GC boxes but neither TATA nor CAT boxes and thus resembles the characteristics of a housekeeping gene [43] (Fig. 3A). The transcription factors Sp1 and Egr-1 were shown to bind to these boxes in this promoter region [47,48]. Furthermore, binding of transcription factors to the promoter is regulated by DNA methylation and histone acetylation. The 5-LO promoter is silenced by DNA methylation [49] and promoter demethylation has been shown to induce 5-LO expression in non-myeloid cells [50]. Addition of the HDAC inhibitor trichostatin A leads to 5-LO promoter activation and ChIP analysis revealed an enhanced binding of Sp1/Sp3 as well as RNA polymerase II to two proximal GC boxes in the 5-LO promoter [51,52]. Enhanced 5-LO expression correlated with the trichostatin A-induced activation of the H3K4 methylase MLL (mixed lineage leukemia) which generates H3K4me3 signatures at the 5-LO promoter and upregulates transcription [53]. Interestingly, the oncogenic MLL-AF4 fusion protein leads to an up to 50-fold activation of the 5-LO promoter suggesting that 5-LO expression is deregulated in leukemias carrying chromosome translocations involving MLL.

5-LO expression in myeloid cells can be strongly induced by treatment with calcitriol and TGF-β [38]. Interestingly, the calcitriol effect is mediated by vitamin D receptor (VDR)-dependent stimulation of transcriptional elongation and not by activation of the 5-LO promoter [54]. In a subsequent study, it was found that regulation of the elongation of 5-LO transcripts by calcitriol depends on the interaction of the VDR with AF4 or the ectopic elongation activator AF4-MLL [55]. AF4 (AFF1) is a member of the transcription elongation complex, which mediates the conversion of RNA polymerase II from the initiation into the elongation form by phosphorylation of the carboxy terminal domain at Ser2. This explains the induction of the elongation form of RNA polymerase II by calcitriol at the distal part of the 5-LO gene [54]. This mechanism is supported by the identification of several vitamin D response elements located within the ALOX5 gene including a prominent VDR binding site located in intron 4 [56,57] (Fig. 3B).

In myeloid cells, TGF-β induces 5-LO expression and activity [58]. It is known that activation of TGF-β receptors leads to phosphorylation of SMAD3 and the subsequent translocation of a SMAD3/4 complex to the nucleus where it acts as a transcription factor complex in concert with other transcriptional regulators [59]. Regarding 5-LO, SMAD-dependent stimulation of transcript initiation as well as elongation have been observed [60,61]. Mutational studies revealed two functional SMAD binding elements (SBEs) close to the tandem array GC box within the 5-LO core promoter (Fig. 3A). Induction of 5-LO promoter activity by SMAD3/4 is MLL-dependent and knockdown of the MLL complex component MEN1 attenuated the SMAD effect [61]. MEN1 has been reported to interact with SMAD3 [62] so that it is most likely that SMAD3/4 recruits the transcription activator MLL to the 5-LO promoter via MEN1. Recently, it has been shown that apoptotic cells down-regulate 5-LO expression in tumor-associated macrophages by MerTK-dependent induction of c-Myb which leads to transcriptional repression of 5-LO [63].

A comprehensive survey of the available ChIP-Seq data sets of

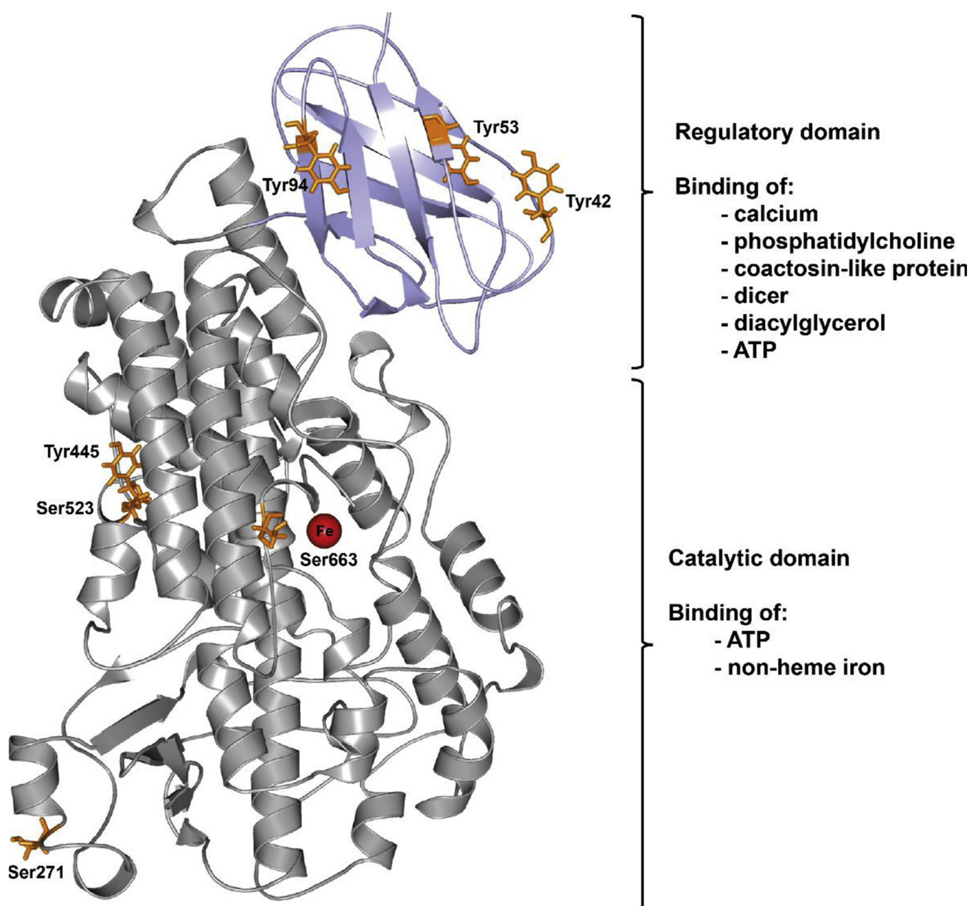

Fig. 2. Structure of 5-LO with phosphorylation sites. 5-LO can be divided in an N-terminal regulatory C2-like domain (light blue) and the catalytic domain (grey) that contains the non-heme iron (red sphere). Phosphorylation sites are shown in orange. The structure was built using the model of 5-LO wild type [105] based on the crystal structure of Stable-5LOX [106].

Table 1
Expression and enzymatic activity of 5-LO in blood cells.

Cell type	RNA	Protein	Activity	References
Granulocytes	+ +	+ +	+ +	[113]
B-lymphocytes	+ +	+ +	-/+ [a]	[36,114,115]
T-lymphocytes	+	+/- [b]	–	[36,37,116]
Dendritic cells	+	+	+	[117,118]
Mast cells	+	+	+	[119]
Monocytes/Macrophages	+ +	+ +	+ +	[120–122]
Erythrocytes	–	–	–	[123]
Platelets	–	–	–	[124]

[a] Only enzymatically active after redox stimulus in whole cells or after cell lysis.

[b] Freshly isolated primary T cells express 5-LO, but expression is rapidly down regulated under cell culture conditions.

histone markers and transcription factors reveals that the 5-LO gene contains three enhancer sites in the introns C, D and G where many transcription factors which are key regulators of stem cell function and lineage determinants bind (Fig. 3). RNA polymerase II signals also rise in these areas which might point to the fact that these sites contain polymerase II arrest sites where the enhancers can trigger transcriptional elongation. In accordance with the predominant expression of 5-LO in leukocytes and its differentiation-dependent regulation, master transcription factors for myeloid and B-cell differentiation (SMAD1, Wnt (TCF4), C/EBPα, GATA2) bind to the 5-LO gene (Fig. 3B). Interestingly, there is a co-occupancy of all four master regulators which was reported to regulate hematopoietic regeneration during stress response [64]. Prominent binding can be also observed for PU.1, a master regulator of B cell differentiation [65] and for RUNX1 and RUNX3 (Fig. 3B). RUNX1 plays a key role in the generation of hematopoietic cells from endothelial cells within the dorsal aorta, a process called the endothelial-hematopoietic transition and it is essential for hematopoiesis [66]. RUNX3 is involved in the immunoglobulin class switching in B cells [65]. Other key regulators of cell differentiation and survival which bind to the 5-LO gene are p53 (see below) and the vitamin D receptor (VDR) (Fig. 3B) which is not surprising since 5-LO is a vitamin D responsive gene (see above) and 5-LO is induced by cytostatic drugs in a p53-dependent manner [67]. We did not find ChIP-seq data sets for SMAD3 in leukocytes but found a data set where the human hepatic stellate cell line LX2 was treated with TGF-β and calcitriol and the genome wide VDR and SMAD3 distribution was investigated by ChIP-seq [68]. Binding of both transcription factors could be observed at multiple sites throughout the 5-LO gene and binding was not restricted to a few strong signals. This is in line with previous observations that the TGF-β and vitamin D effects could not be located to distinct response elements within the 5-LO gene [54,60,61]. Alternatively, SMAD1 activation by TGF-β has also been shown [69] so that in light of the prominent binding of SMAD1 to enhancers in the 5-LO gene, SMAD1 might play a key role in 5-LO regulation by TGF-β.

Taken together, 5-LO is a gene which is prominently expressed in leukocytes. It is upregulated in a cell differentiation-dependent manner. Key determinants are calcitriol and TGF-β in myeloid cells which obviously act in concert with other master regulators of stem cell regeneration and differentiation including RUNX1, SMAD1, Wnt, C/EBPα, GATA2 and p53 to drive 5-LO expression in myeloid and lymphocytic cell lineages.

3. 5-LO and Dicer

In a search for 5-LO interacting proteins with the yeast two hybrid system, the K12H4 helicase which was later termed Dicer was identified

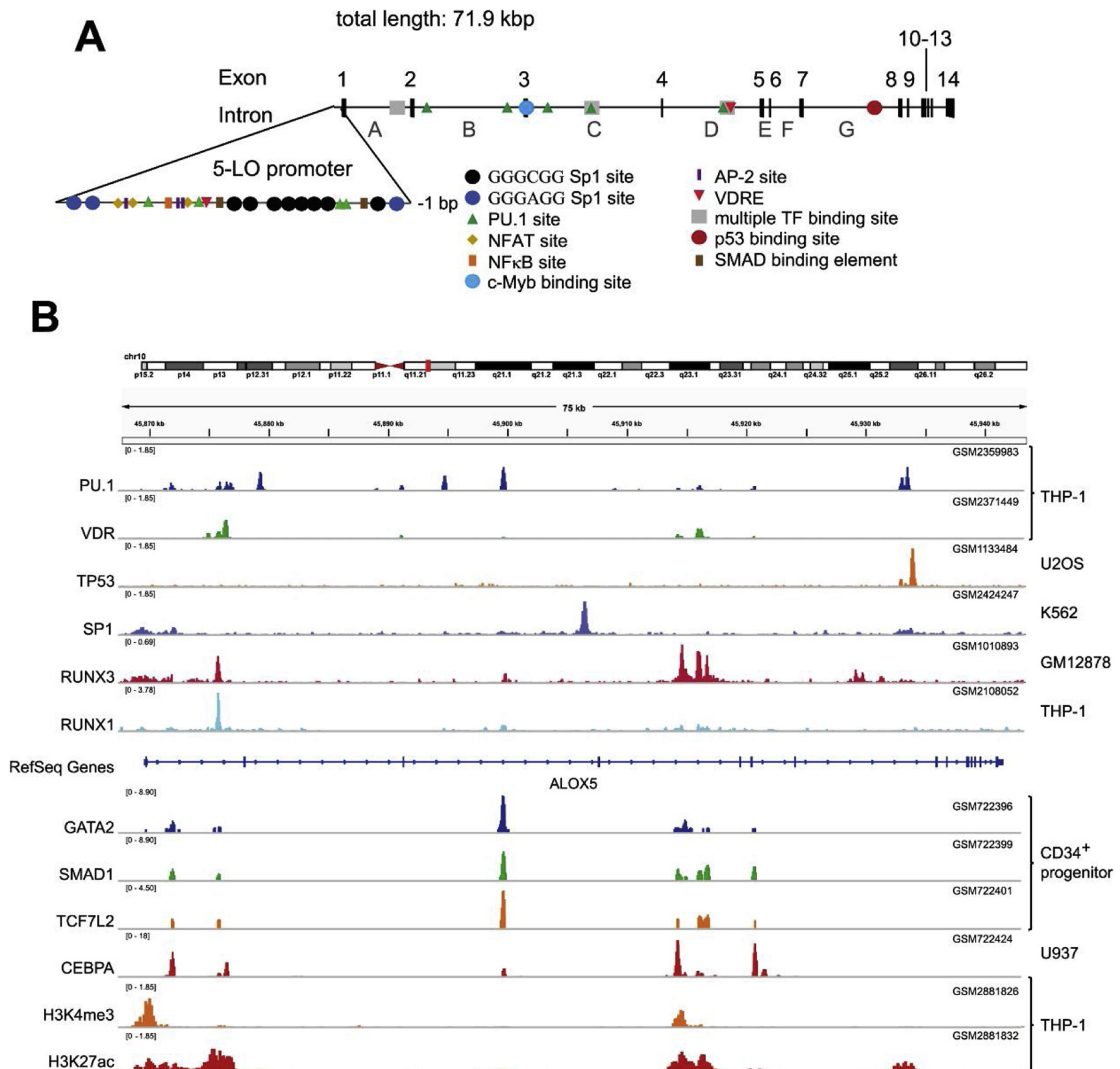

Fig. 3. A) Schematic representation of the human ALOX5 gene coding for 5-LO. Consensus binding sites for transcription factors present in the 5-LO promoter are shown on the left. Functionality has been demonstrated for SMAD, VDR and Sp1. B) ChIP-Seq data on histone markers and transcription factors which prominently bind the ALOX5 gene, displayed in the Integrative Genomics Viewer [107]. H3K4me3, H3K27Ac, PU.1 and VDR signals are shown for THP-1 cells treated for 24 h with 100 nM calcitriol [108,109], U2OS cells were treated with doxorubicin [110]. Sp1, RUNX3 [111], RUNX1 [112], GATA2, SMAD1, TCF7L2 (Wnt signalling) and C/EBPα [64] show strong binding to distinct enhancer sites of 5-LO.

as one of the 5-LO interaction partners [19] (Fig. 2). Dicer is a multi-domain RNA helicase/RNase III catalyzing final steps in the biosynthesis of microRNAs (miRNAs) and small interfering RNA (siRNA) from dsRNA substrates [70]. miRNAs are small non-coding RNAs that control gene expression. They mediate either translational repression or degradation of their target transcripts after they are directed to their binding sites in the 3′ untranslated region of their target mRNAs. Thus, a distinct miRNA can modulate the expression of a variety of mRNAs carrying appropriate recognition sites for the miRNA. It is well established that miRNA control many physiological processes such as cell type maintenance, cell proliferation, tissue differentiation, apoptosis, signal transduction, organ development and stem cell properties [71]. Distinct miRNA expression patterns in various leukocyte types point to an important function of miRNAs in the regulation of the immune system, and dysregulation can result in pathological inflammatory responses and cancer [72]. Therefore, the observed interaction of 5-LO with Dicer is of considerable interest and raised the question whether 5-LO alters Dicer activity and regulates physiological processes independent from arachidonic acid metabolism.

5-LO expression itself is also regulated by miRNAs. miR-219 which is induced by resolvin D1 was found to modulate 5-LO expression [73]. Three miRNAs, miR-216a-3p, miR-19a-3p and miR-125b-5p have been shown to directly target the 5-LO mRNA 3′UTR [37,74]. MiR-216-3p also targets cyclooxygenase (COX)-2 and it was shown to inhibit colorectal cancer proliferation and to downregulate COX-2 and 5-LO [74]. Stimulation of human T lymphocytes with phytohaemagglutinin resulted in a strong downregulation of 5-LO mRNA expression and in the induction of miR-19a-3p [37]. The inhibition of miR-19a-3p with an antagomir led to a significant increase in 5-LO mRNA expression in T lymphocytes. The data suggest that miR-19a-3p and miR-125b-5p

target 5-LO in a cell type- and stimulus-specific manner. Interestingly, miR-19a belongs to the miR-17 ~ 92 cluster, which was reported to inhibit TGF-β and Wnt signaling [75,76] and to be associated with T cell proliferation [77]. Another interesting aspect is the observation that overexpression of miR-125b-5p blocks the differentiation of the promyelocytic cell line HL-60 by DMSO [78], regulates hematopoiesis and overexpression induces myeloid leukemia in mice [79]. Of note, 5-LO expression is upregulated during HL-60 cell differentiation [80,81]. In B cell development, miR-125b-5p and miR-19a play an important role in the germinal center reaction, and it has been shown that both miRNAs are upregulated in germinal center cells where 5-LO is suppressed [82–84].

From the yeast two hybrid system data, it is known that the interaction occurs between 5-LO and the C-terminal 140 amino acids of Dicer (1912 amino acids in total) [85]. Binding between 5-LO and Dicer was confirmed with purified proteins and it was shown that the N-terminal C2-like regulatory domain of 5-LO interacts with Dicer [85] (Fig. 2). GST pull-down experiments with wildtype 5-LO and with a mutated 5-LO protein where three Trp (W13/75/102) were changed to Ala suggest that these residues are involved in binding since the 5-LO mutant is still catalytically active but lost its ability to interact with Dicer. The same mutation also interrupted coimmunoprecipitation of HA-tagged 5-LO and FLAG-tagged Dicer C-term from transfected HEK293 cells. Regarding Dicer activity, 5-LO alters the Dicer product pattern in vitro, inducing the formation of 10–12 bp small RNAs [85]. in vitro, the 5-LO interaction domain of Dicer stimulates Ca^{2+}-induced 5-LO activity, albeit not as efficiently as phosphatidylcholine or CLP. At present, it is unclear whether modulation of dicer activity requires catalytically active 5-LO or not. Furthermore, only in-vitro data are available at the moment and the functional consequences of the 5-LO/Dicer interaction at the cellular level is unclear. No data are available whether 5-LO can alter the miRNA spectrum generated by Dicer in 5-LO positive cell types and whether this is associated with certain functional consequences.

4. 5-LO as effector and regulator of p53

As already mentioned, expression of 5-LO along with other proteins of the leukotriene generating machinery is well documented in cancers from different origin such as breast, prostate, blood and GI tract tissues. This overexpression often correlates with tumour size and stage, metastasis potential, tumour microvessel density and poor patient survival in colon carcinomas. Still, the mechanism underlying 5-LO-driven tumorigenesis remains elusive. Interestingly, enzymatic activity is barely detectable in 5-LO overexpressing cells (for review see [41]). From studies employing animal models or tumour cell lines two well established tumour-promoting pathways, the wnt/β-catenin axis and the p53 network emerged which were found to interact with 5-LO on the protein as well as on the product level [67,86–88].

Preserving genomic integrity, p53 is often referred to as ‘guardian of the genome’. The protein is extensively regulated on different levels such as gene transcription, splicing and translation depending on the tissue [89,90]. In addition, p53 is modified post-transcriptionally by phosphorylation and acetylation. These different aspects result in a myriad of p53 variants with differing activities. P53 is a transcriptional regulator that is activated by genotoxic stress where it induces the transcription of genes that trigger growth arrest to allow DNA repair of the affected cell. If DNA damage is irreparable, p53 can directly trigger apoptosis or cell senescence and also sensitize to ferroptotic death in order to prevent tumor formation [91]. Furthermore, stem cells use p53 to control renewal and differentiation [92]. Due to its fundamental role in the integrity of the genome, it is not surprising that p53 is the by far most frequently mutated gene in human cancer where its function is attenuated or lost due to gain of function or frameshift mutations [93].

The first report pointing to an interaction of 5-LO with p53 was published by Catalano et al. [88]. It was shown that 5-LO is time-dependently upregulated by genotoxic stress induced by UV irradiation, oxidative agents (H_2O_2) or treatment with the cytostatic drugs etoposide and doxorubicin in different human cancer cell lines. Since cells carrying a p53 frameshift mutation also upregulated 5-LO upon these stimuli, the authors concluded that this upregulation was p53 independent. Overexpression of 5-LO in 5-LO negative cell lines conferred resistance to apoptosis induced by the genotoxic agents whereas a catalytically inactive 5-LO mutant showed no protective effect. Consistently, inhibition of 5-LO by the inhibitor AA-861 as well as knockdown of the enzyme via antisense technology sensitized 5-LO overexpressing cells to doxorubicin-induced apoptosis. Of note, exogenous addition of 5-HETE also inhibited p53-induced apoptosis. The authors investigated the underlying mechanism further and found that 5-LO overexpression decreases the p53-dependent upregulation of BAX and PIG3, two proteins related to apoptosis induction, in A549 cells treated with doxorubicin. Of interest, the p53-mediated upregulation of p21 and mdm2 was not affected by 5-LO. This suggests a selective inhibition of apoptosis by 5-LO while p53-triggered cell cycle arrest seems to be spared. Furthermore, expression of luciferase constructs from different p53 target gene promoters was hindered in the presence of 5-LO which was probably due to alteration of p53 nuclear trafficking by disruption of the p53/PML interaction in nuclear bodies which was dependent on 5-HETE. Cellular 5-LO co-localization with p53 was not investigated in this study [88].

p53-dependent 5-LO expression was observed in DU145 prostate cancer cells [94]. Recently, 5-LO was identified as a direct p53 target [67]. p53 binding to the ALOX5 gene was found by ChIP-seq analysis in actinomycin D treated U2OS cells, yielding a prominent p53 signal in intron G (Fig. 3). Sequence analysis revealed a DNA consensus sequence for p53 in intron G which interestingly overlaps with the histone methylation mark H3K4me1 suggesting that the p53 binding site is located in a transcriptional enhancer region. Accordingly, 5-LO was upregulated upon genotoxic stress induced by actinomycin D or etoposide along with p53 expression in different cell lines. A p53 mutant (R273 H) without DNA binding capacity did not induce 5-LO expression upon genotoxic stress. Absence or knockdown of p53 completely abrogated the genotoxic stress-induced 5-LO induction. Of interest, confocal microscopy revealed a co-localization of 5-LO and p53 upon stimulation with actinomycin D or etoposide and coimmunoprecipitations confirmed this finding. In addition, the presence of 5-LO impaired the upregulation of p53 target genes in reporter gene assays in HEK293 cells pointing to a direct influence of the 5-LO protein on p53 function (Fig. 4).

In contrast to its pro-tumorigenic role, 5-LO was also shown to regulate cell senescence by inducing growth arrest in cells via a ROS-dependent p53 activation [95]. Here, the authors showed that human fibroblasts (WI-38) arrested with an H-Ras mutant (HRasV12) strongly upregulate global 5-LO, p53 and p21 content and also show an increase in nuclear 5-LO. In addition, cytosolic phospholipase A_2 ($cPLA_2$) and

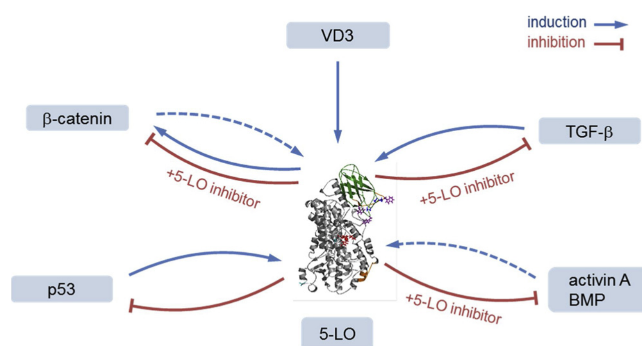

Fig. 4. Developmental signaling pathways which regulate (induce) 5-LO expression and which are modulated themselves by 5-LO. The dashed lines indicate pathways where only binding but no functional data have been reported.

COX-2 are moderately upregulated. The HRasV12 overexpression went along with elevated ROS levels that induced the growth arrest in cell culture and were dependent on 5-LO and p53/p21. In line, hypoxic cells carrying the H-Ras mutant still overexpressed 5-LO but continued to proliferate. This was due to reduced 5-LO-dependent ROS levels resulting from the minimal oxygen level present. Of note, 5-LO did not upregulate p53 in this system but rather influenced p53 activity via ROS-triggered phosphorylation.

Taken together, there is substantial evidence that 5-LO expression is regulated by p53. Furthermore, 5-LO itself, in addition to its catalytic activity, seems to regulate p53 trafficking and transcriptional activity. Further studies employing 5-LO and p53 knockout cells as well as physiologic concentrations of 5-LO-derived lipid mediators will help to decipher the exact role of the enzyme in regulation of cell death and tumorigenesis.

5. 5-LO as regulator of the Wnt and other developmental pathways

The canonical Wnt signaling pathway is a highly conserved pathway which regulates embryonic development, adult tissue homeostasis, cell polarization, stem cell biology, cell differentiation, and proliferation [96]. Additionally, the Wnt signaling is of pivotal importance in cancer development and crucially affects tumor initiation, cancer cell proliferation, cancer cell apoptosis, self-renewal of leukemic cells and metastasis [97]. In particular, dysregulation of the Wnt//β-catenin signaling pathway seems to play an important role in the self-renewal of leukemic stem cells [98]. Dysregulated 5-LO expression has been found in many types of leukemia which also show deregulated Wnt/β-catenin signaling (for review see [99]). Recently, it was found that 5-LO is strongly upregulated in AML1/ETO-positive AML [100]. In the same study, it was then shown that loss of 5-LO expression reduces the leukemic activity of RUNX1-ETO9a, MLL-AF9 and PML-RARα. Interestingly, these leukemia-associated fusion proteins are also known to induce aberrant activation of canonical Wnt signaling which in turn leads to an increased self-renewal capacity of leukemic stem cells [101].

Another link between leukemia development and the 5-LO pathway came from the observation that 5-LO is required for the aberrant self-renewal capacity of leukemic stem cells in a murine BCR/ABL chronic myeloid leukemia model [86]. Recipients of BCR/ABL transduced bone marrow cells from 5-LO negative donor mice failed to develop chronic myeloid leukemia, whereas recipients of BCR/ABL-transduced bone marrow cells from wild type donor mice developed the disease and died within 4 weeks. In the absence of 5-LO, myeloid leukemia cells gradually disappeared in the leukemic mice. Interestingly, 5-LO knockout does not seem to lead to a defect in normal hematopoiesis and did not affect BCR/ABL-induced acute lymphoid leukemia [86] indicating a lineage-specific effect. Interestingly, the 5-LO inhibitor zileuton at 300 mg/kg twice a day had similar effects as 5-LO knockout suggesting that 5-LO activity might play a role in CLL development. The impaired self-renewal of the myeloid leukemic stem cells in the absence of 5-LO correlated with a reduction of GATA-1 and β-catenin expression which suggests a link between aberrant BCR/ABL activity, 5-LO and Wnt signaling.

A key role of 5-LO for the maintenance of leukemic stem cells was also shown in a PML/RARα-positive stem cell model of acute myeloid leukemia [87]. The 5-LO inhibitor CJ-13,610 abolished the aberrant replating efficiency of PML/RARα-expressing hematopoietic stem and progenitor cells and CJ-13,610 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. CJ-13,610 even slightly stimulated the stem cell capacity in control cells. Furthermore, stem cell suppression by CJ-13,610 in PML/RARα-positive cells coincided with inhibition of Wnt signaling. Mechanistically, it was found by coimmunoprecipitation that 5-LO interacts with β-catenin and that catalytically inactive 5-LO prevents translocation of β-catenin and traps the protein at the nuclear envelope [87]. By that way, CJ-13,610 prevents the entrance of β-catenin into the nucleus and transcriptional activation of Wnt target genes. Of note, 5-LO knockout did not affect the aberrant stem cell capacity in the PML/RARα model suggesting that trapping of β-catenin by inactive 5-LO is essential for the effect or that the inhibitors interact with additional targets to prevent Wnt activity (Fig. 4).

The link between 5-LO and Wnt was confirmed recently by another study where a screening for Wnt inhibitors was performed in which a 3,5-substituted-2,4-dimethoxypyridine derivative, lipoxygenin, was identified as inhibitor of Wnt signalling [102]. It turned out that the compound does not directly interfere with β-catenin but that it is a nonredox-type 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 [87]. Subsequent studies revealed that CJ-13,160 and lipoxygenin not only inhibit Wnt signalling but also interfere with hedgehog, TGF-β, BMP and activin A signaling in the same concentration range [102] (Fig. 4). Lipoxygenin and CJ-13,610 promote cardiac differentiation of human induced pluripotent stem cells which is in accordance with the expected pharmacological profile. Of note, there is a good correlation between the 5-LO inhibitory potency and the capacity to interfere with developmental pathways with both compounds.

The mechanism how both compounds interfere with hedgehog, TGF-β, BMP and activin A signalling is unknown at the moment. 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 [103]. 5-LO contains functional NLS and NES motifs (see above) so that 5-LO might act as STRaND (shuttling transcriptional regulator and non-DNA binding) protein in the Wnt pathway by regulating β-catenin localization [104].

6. Conclusion

Besides its function as key enzyme in the biosynthesis of leukotrienes and SPM, there is accumulating evidence that 5-LO has additional, noncanonical functions. It interacts with Dicer and might be a modulator of miRNA formation under certain physiological conditions. The enzyme is mainly expressed in leukocytes. It is regulated in a cell cycle and cell differentiation-dependent manner. 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 and the Wnt pathway (Fig. 4). Recently, it has become evident that the 5-LO protein can interact with p53 and β-catenin suggesting a function of 5-LO as modulator of gene transcription which might be part of a regulatory circuit for fine tuning of gene transcription to adapt immune functions to certain physiological and pathophysiological conditions and to regulate stem cell replication and leukocyte differentiation as part of the immune response and of tissue regeneration and resolution of inflammation. An interesting aspect is that 5-LO can serve as drug target and that certain small molecule 5-LO inhibitors have been identified that inhibit aberrant stem cell activity of certain leukemic cells by interference with the Wnt signalling pathway. One interesting aspect will be to optimize these inhibitors for the modulation of developmental pathways such as Wnt.

Declarations of interest

None.

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References

[1] P. Borgeat, M. Hamberg, B. Samuelsson, Transformation of arachidonic acid and homo-γ-linolenic acid by rabbit polymorphonuclear leukocytes. Monohydroxy acids from novel lipoxygenases, J. Biol. Chem. 251 (1976) 7816–7820.
[2] P. Borgeat, B. Samuelsson, Arachidonic acid metabolism in polymorphonuclear leukocytes: unstable intermediate in formation of dihydroxy acids, Proc. Natl. Acad. Sci. U. S. A. 76 (1979) 3213–3217.
[3] B. Samuelsson, S.-E. Dahlén, J.-Å. Lindgren, C.A. Rouzer, C.N. Serhan, Leukotrienes and lipoxins: structures, biosynthesis, and biological effects, Science 237 (1987) 1171–1176.
[4] S.E. Dahlén, P. Hedqvist, S. Hammarström, B. Samuelsson, Leukotrienes are potent constrictors of human bronchi, Nature 288 (1980) 484–486.
[5] S.E. Dahlén, J. Björk, P. Hedqvist, K.-E. Arfors, S. Hammarström, J.Å. Lindgren, B. Samuelsson, Leukotrienes promote plasma leakage and leukocyte adhesion in postcapillary venules: in vivo effects with relevance to the acute inflammatory response, Proc. Natl. Acad. Sci. U. S. A. 78 (1981) 3887–3891.
[6] M. Peters-Golden, W.R. Henderson Jr., Leukotrienes, N. Engl. J. Med. 357 (2007) 1841–1854.
[7] M. Peters-Golden, C. Canetti, P. Mancuso, M.J. Coffey, Leukotrienes: underappreciated mediators of innate immune responses, J. Immunol. 174 (2005) 589–594.
[8] N. Flamand, P. Mancuso, C.H. Serezani, T.G. Brock, Leukotrienes: mediators that have been typecast as villains, Cell. Mol. Life Sci. 64 (2007) 2657–2670.
[9] M. Le Bel, A. Brunet, J. Gosselin, Leukotriene B4, an endogenous stimulator of the innate immune response against pathogens, J. Innate Immun. 6 (2014) 159–168.
[10] C.N. Serhan, N. Chiang, J. Dalli, B.D. Levy, Lipid mediators in the resolution of inflammation, Cold Spring Harb. Perspect. Biol. 7 (2014) a016311.
[11] T.G. Brock, Regulating leukotriene synthesis: the role of nuclear 5-lipoxygenase, J. Cell. Biochem. 96 (2005) 1203–1211.
[12] T.G. Brock, M. Peters-Golden, Activation and regulation of cellular eicosanoid biosynthesis, Sci. World J. 7 (2007) 1273–1284.
[13] J.W. Woods, M.J. Coffey, T.G. Brock, I.I. Singer, M. Peters-Golden, 5-lipoxygenase is located in the euchromatin of the nucleus in resting human alveolar macrophages and translocates to the nuclear envelope upon cell activation, J. Clin. Invest. 95 (1995) 2035–2046.
[14] M. Luo, C.W. Pang, A.E. Gerken, T.G. Brock, Multiple nuclear localization sequences allow modulation of 5-lipoxygenase nuclear import, Traffic 5 (2004) 847–854.
[15] N. Flamand, M. Luo, M. Peters-Golden, T.G. Brock, Phosphorylation of serine 271 on 5-lipoxygenase and its role in nuclear export, J. Biol. Chem. 284 (2009) 306–313.
[16] C. Hörnig, D. Albert, L. Fischer, M. Hörnig, O. Rådmark, D. Steinhilber, O. Werz, 1-oleoyl-2-acetylglycerol stimulates 5-lipoxygenase activity via a putative (phospho) lipid-binding site within the N-terminal C2-like domain, J. Biol. Chem. 280 (2005) 26913–26921.
[17] O. Rådmark, O. Werz, D. Steinhilber, B. Samuelsson, 5-Lipoxygenase, a key enzyme for leukotriene biosynthesis in health and disease, Biochim. Biophys. Acta 1851 (2014) 331–339.
[18] J.Z. Haeggström, C.D. Funk, Lipoxygenase and leukotriene pathways: biochemistry, biology, and roles in disease, Chem. Rev. 111 (2011) 5866–5898.
[19] P. Provost, B. Samuelsson, O. Rådmark, Interaction of 5-lipoxygenase with cellular proteins, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 1881–1885.
[20] J. Esser, M. Rakonjac, B. Hofmann, L. Fischer, P. Provost, G. Schneider, D. Steinhilber, B. Samuelsson, O. Radmark, Coactosin-like protein functions as a stabilizing chaperone for 5-lipoxygenase: role of tryptophan 102, Biochem. J. 425 (2009) 265–274.
[21] M. Rakonjac, L. Fischer, P. Provost, O. Werz, D. Steinhilber, B. Samuelsson, O. Rådmark, Coactosin-like protein supports 5-lipoxygenase enzyme activity and up-regulates leukotriene A4 production, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 13150–13155.
[22] O. Rådmark, O. Werz, D. Steinhilber, B. Samuelsson, 5-Lipoxygenase: regulation of expression and enzyme activity, Trends Biochem. Sci. 32 (2007) 332–341.
[23] M. Luo, S.M. Jones, S.M. Phare, M.J. Coffey, M. Peters-Golden, T.G. Brock, Protein kinase A inhibits leukotriene synthesis by phosphorylation of 5-lipoxygenase on serine 523, J. Biol. Chem. 279 (2004) 41512–41520.
[24] O. Werz, J. Klemm, B. Samuelsson, O. Rådmark, 5-lipoxygenase is phosphorylated by p38 kinase-dependent MAPKAP kinases, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 5261–5266.
[25] O. Werz, E. Bürkert, L. Fischer, D. Szellas, D. Dishart, B. Samuelsson, O. Rådmark, D. Steinhilber, Extracellular signal-regulated kinases phosphorylate 5-lipoxygenase and stimulate 5-lipoxygenase product formation in leukocytes, FASEB J. 16 (2002) 1441–1443.
[26] S. Markoutsa, D. Sürun, M. Karas, B. Hofmann, D. Steinhilber, B.L. Sorg, Analysis of 5-lipoxygenase phosphorylation on molecular level by MALDI-MS, FEBS J. 281 (2014) 1931–1947.
[27] M. Luo, S.M. Jones, N. Flamand, D.M. Aronoff, M. Peters-Golden, T.G. Brock, Phosphorylation by protein kinase A inhibits nuclear import of 5-lipoxygenase, J. Biol. Chem. 280 (2005) 40609–40616.
[28] M. Luo, S.M. Jones, M. Peters-Golden, T.G. Brock, Nuclear localization of 5-lipoxygenase as a determinant of leukotriene B_4 synthetic capacity, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 12165–12170.
[29] B. Cai, E.B. Thorp, A.C. Doran, M. Subramanian, B.E. Sansbury, C.S. Lin, M. Spite, G. Fredman, I. Tabas, MerTK cleavage limits proresolving mediator biosynthesis and exacerbates tissue inflammation, Proc. Natl. Acad. Sci. U. S. A. 113 (2016) 6526–6531.
[30] G. Fredman, L. Ozcan, S. Spolitu, J. Hellmann, M. Spite, J. Backs, I. Tabas, Resolvin D1 limits 5-lipoxygenase nuclear localization and leukotriene B4 synthesis by inhibiting a calcium-activated kinase pathway, Proc. Natl. Acad. Sci. U. S. A. 111 (2014) 14530–14535.
[31] A.K. Mandal, P.B. Jones, A.M. Bair, P. Christmas, D. Miller, T.T. Yamin, D. Wisniewski, J. Menke, J.F. Evans, B.T. Hyman, B. Bacskai, M. Chen, D.M. Lee, B. Nikolic, R.J. Soberman, The nuclear membrane organization of leukotriene synthesis, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 20434–20439.
[32] E. Hill, J. Maclouf, R.C. Murphy, P.M. Henson, Reversible membrane association of neutrophil 5-lipoxygenase is accompanied by retention of activity and a change in substrate specificity, J. Biol. Chem. 267 (1992) 22048–22053.
[33] J.A. Mancini, H. Waterman, D. Riendeau, Cellular oxygenation of 12-hydroxyeicosatetraenoic acid and 15-hydroxyeicosatetraenoic acid by 5-lipoxygenase is stimulated by 5-lipoxygenase-activating protein, J. Biol. Chem. 273 (1998) 32842–32847.
[34] C. Lehmann, J. Homann, A.K. Ball, R. Blöcher, T.K. Kleinschmidt, D. Basavarajappa, C. Angioni, N. Ferreiros, A.K. Häfner, O. Rådmark, E. Proschak, J.Z. Haeggström, G. Geisslinger, M.J. Parnham, D. Steinhilber, A.S. Kahnt, Lipoxin and resolvin biosynthesis is dependent on 5-lipoxygenase activating protein, FASEB J. 29 (2015) 5029–5043.
[35] R. Spanbroek, H.J. Stark, U. Janßen-Timmen, S. Kraft, M. Hildner, T. Andl, F.X. Bosch, N.E. Fusenig, T. Bieber, O. Rådmark, B. Samuelsson, A.J.R. Habenicht, 5-Lipoxygenase expression in Langerhans cells of normal human epidermis, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 663–668.
[36] P.J. Jakobsson, D. Steinhilber, B. Odlander, O. Rådmark, H.E. Claesson, B. Samuelsson, On the expression and regulation of 5-lipoxygenase in human lymphocytes, Proc. Natl. Acad. Sci. U. S. A. 89 (1992) 3521–3525.
[37] S. Busch, E. Auth, F. Scholl, S. Huenecke, U. Koehl, B. Suess, D. Steinhilber, 5-lipoxygenase is a direct target of miR-19a-3p and miR-125b-5p, J. Immunol. 194 (2015) 1646–1653.
[38] M. Brungs, O. Rådmark, B. Samuelsson, D. Steinhilber, Sequential induction of 5-lipoxygenase gene expression and activity in Mono Mac 6 cells by transforming growth factor-beta and 1,25-dihydroxyvitamin D3, Proc. Natl. Acad. Sci. U. S. A. 92 (1995) 107–111.
[39] R. Spanbroek, M. Hildner, A. Kohler, A. Müller, F. Zintl, H. Kühn, O. Radmark, B. Samuelsson, A.J. Habenicht, IL-4 determines eicosanoid formation in dendritic cells by down-regulation of 5-lipoxygenase and up-regulation of 15-lipoxygenase 1 expression, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 5152–5157.
[40] O. Werz, I. Tretiakova, A. Michel, A. Ulke-Lemee, M. Hörnig, L. Franke, G. Schneider, B. Samuelsson, O. Rådmark, D. Steinhilber, Caspase-mediated degradation of human 5-lipoxygenase in B lymphocytic cells, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 13164–13169.
[41] D. Steinhilber, A.S. Fischer, J. Metzner, S.D. Steinbrink, J. Roos, M. Ruthardt, T.J. Maier, 5-lipoxygenase: underappreciated role of a pro-inflammatory enzyme in tumorigenesis, Front. Pharmacol. 1 (2010) 143.
[42] G.Y. Moore, G.P. Pidgeon, Cross-talk between cancer cells and the tumour microenvironment: the role of the 5-lipoxygenase pathway, Int. J. Mol. Sci. 18 (2017) 236.
[43] C.D. Funk, S. Hoshiko, T. Matsumoto, O. Rådmark, B. Samuelsson, Characterization of the human 5-lipoxygenase gene, Proc. Natl. Acad. Sci. U. S. A. 86 (1989) 2587–2591.
[44] L.H. Boudreau, J. Bertin, P.P. Robichaud, M. Laflamme, R.J. Ouellette, N. Flamand, M.E. Surette, Novel 5-lipoxygenase isoforms affect the biosynthesis of 5-lipoxygenase products, FASEB J. 25 (2010) 1097–1105.
[45] A.K. Häfner, K. Beilstein, P. Graab, A.K. Ball, M.J. Saul, B. Hofmann, D. Steinhilber, Identification and characterization of a new protein isoform of human 5-Lipoxygenase, PLoS One 11 (2016) e0166591.
[46] M.J. Ochs, B. Suess, D. Steinhilber, 5-lipoxygenase mRNA and protein isoforms, Basic Clin. Pharmacol. Toxicol. 114 (2014) 78–82.
[47] S. Hoshiko, O. Rådmark, B. Samuelsson, Characterization of the human 5-lipoxygenase gene promoter, Proc. Natl. Acad. Sci. U. S. A. 87 (1990) 9073–9077.
[48] K.H. In, K. Asano, D. Beier, J. Grobholz, P.W. Finn, E.K. Silverman, E.S. Silverman, T. Collins, A.R. Fischer, T.P. Keith, K. Serino, S.W. Kim, G.T. De Sanctis, C. Yandava, A. Pillari, P. Rubin, J. Kemp, E. Israel, W. Busse, D. Ledford, J.J. Murray, A. Segal, D. Tinkleman, J.M. Drazen, Naturally occurring mutations in the human 5-lipoxygenase gene promoter that modify transcription factor binding and reporter gene transcription, J. Clin. Invest. 99 (1997) 1130–1137.
[49] J. Uhl, N. Klan, M. Rose, K.D. Entian, O. Werz, D. Steinhilber, The 5-lipoxygenase promoter is regulated by DNA methylation, J. Biol. Chem. 277 (2002) 4374–4379.
[50] E. Nagy, M. Bäck, Epigenetic regulation of 5-lipoxygenase in the phenotypic plasticity of valvular interstitial cells associated with aortic valve stenosis, FEBS Lett. 586 (2012) 1325–1329.
[51] N. Klan, S. Seuter, N. Schnur, M. Jung, D. Steinhilber, Trichostatin A and structurally related histone deacetylase inhibitors induce 5-lipoxygenase promoter activity, Biol. Chem. 384 (2003) 777–785.
[52] N. Schnur, S. Seuter, C. Katryniok, O. Radmark, D. Steinhilber, The histone deacetylase inhibitor trichostatin A mediates upregulation of 5-lipoxygenase promoter activity by recruitment of Sp1 to distinct GC-boxes, Biochim. Biophys. Acta 1771 (2007) 1271–1882.
[53] K. Ahmad, C. Katryniok, B. Scholz, J. Merkens, D. Löscher, R. Marschalek, D. Steinhilber, Inhibition of class I HDACs abrogates the dominant effect of MLL-AF4 by activation of wild-type MLL, Oncogenesis 3 (2014) e127.
[54] K.L. Stoffers, B.L. Sorg, S. Seuter, O. Rau, O. Radmark, D. Steinhilber, Calcitriol upregulates open chromatin and elongation markers at functional vitamin D response elements in the distal part of the 5-lipoxygenase gene, J. Mol. Biol. 395

(2010) 884–896.
[55] K. Ahmad, B. Scholz, R. Capelo, I. Schweighöfer, A.S. Kahnt, R. Marschalek, D. Steinhilber, AF4 and AF4-MLL mediate transcriptional elongation of 5-lipoxygenase mRNA by 1, 25-dihydroxyvitamin D3, Oncotarget 6 (2015) 25784–25800.
[56] S. Seuter, S. Vaisanen, O. Rådmark, C. Carlberg, D. Steinhilber, Functional characterization of vitamin D responding regions in the human 5-Lipoxygenase gene, Biochim. Biophys. Acta 1771 (2007) 864–872.
[57] S.V. Ramagopalan, A. Heger, A.J. Berlanga, N.J. Maugeri, M.R. Lincoln, A. Burrell, L. Handunnetthi, A.E. Handel, G. Disanto, S.M. Orton, C.T. Watson, J.M. Morahan, G. Giovannoni, C.P. Ponting, G.C. Ebers, J.C. Knight, A ChIP-seq defined genome-wide map of vitamin D receptor binding: associations with disease and evolution, Genome Res. 20 (2010) 1352–1360.
[58] D. Steinhilber, O. Rådmark, B. Samuelsson, Transforming growth factor beta upregulates 5-lipoxygenase activity during myeloid cell maturation, Proc. Natl. Acad. Sci. U. S. A. 90 (1993) 5984–5988.
[59] C.J. David, J. Massague, Contextual determinants of TGFbeta action in development, immunity and cancer, Nat. Rev. Mol. Cell Biol. 19 (2018) 419–435.
[60] S. Seuter, B.L. Sorg, D. Steinhilber, The coding sequence mediates induction of 5-lipoxygenase expression by Smads3/4, Biochem. Biophys. Res. Commun. 348 (2006) 1403–1410.
[61] M.J. Saul, F. Groher, A.B. Hegewald, M. Müller-McNicoll, R. Marschalek, B. Suess, D. Steinhilber, TGFbeta/SMAD signalling modulates MLL and MLL-AF4 mediated 5-lipoxygenase promoter activation, Prostaglandins Other Lipid Mediat. 133 (2017) 60–67.
[62] H. Kaji, L. Canaff, J.J. Lebrun, D. Goltzman, G.N. Hendy, Inactivation of menin, a Smad3-interacting protein, blocks transforming growth factor type beta signaling, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 3837–3842.
[63] J. Ringleb, E. Strack, C. Angioni, G. Geisslinger, D. Steinhilber, A. Weigert, B. Brüne, Apoptotic cancer cells suppress 5-Lipoxygenase in tumor-associated macrophages, J. Immunol. 200 (2018) 857–868.
[64] E. Trompouki, T.V. Bowman, L.N. Lawton, Z.P. Fan, D.C. Wu, A. DiBiase, C.S. Martin, J.N. Cech, A.K. Sessa, J.L. Leblanc, P. Li, E.M. Durand, C. Mosimann, G.C. Heffner, G.Q. Daley, R.F. Paulson, R.A. Young, L.I. Zon, Lineage regulators direct BMP and Wnt pathways to cell-specific programs during differentiation and regeneration, Cell 147 (2011) 577–589.
[65] C.J. David, J. Massague, Contextual determinants of TGFbeta action in development, immunity and cancer, Nat. Rev. Mol. Cell Biol. 19 (2018) 419–435.
[66] D. Hong, A.J. Fritz, J.A. Gordon, C.E. Tye, J.R. Boyd, K.M. Tracy, S.E. Frietze, F.E. Carr, J.A. Nickerson, A.J. Van Wijnen, A.N. Imbalzano, S.K. Zaidi, J.B. Lian, J.L. Stein, G.S. Stein, RUNX1-dependent mechanisms in biological control and dysregulation in cancer, J. Cell. Physiol. 234 (2019) 8597–8609, https://doi.org/10.1002/jcp.27841.
[67] B. Gilbert, K. Ahmad, J. Roos, C. Lehmann, T. Chiba, S. Ulrich-Rückert, L. Smeenk, S. van Heeringen, T.J. Maier, B. Groner, D. Steinhilber, 5-Lipoxygenase is a direct p53 target gene in humans, Biochim. Biophys. Acta 1849 (2015) 1003–1016.
[68] N. Ding, R.T. Yu, N. Subramaniam, M.H. Sherman, C. Wilson, R. Rao, M. Leblanc, S. Coulter, M. He, C. Scott, S.L. Lau, A.R. Atkins, G.D. Barish, J.E. Gunton, C. Liddle, M. Downes, R.M. Evans, A vitamin D receptor/SMAD genomic circuit gates hepatic fibrotic response, Cell 153 (2013) 601–613.
[69] A. Ramachandran, P. Vizan, D. Das, P. Chakravarty, J. Vogt, K.W. Rogers, P. Muller, A.P. Hinck, G.P. Sapkota, C.S. Hill, TGF-beta uses a novel mode of receptor activation to phosphorylate SMAD1/5 and induce epithelial-to-mesenchymal transition, Elife 7 (2018) e31756.
[70] M.P. Perron, P. Landry, I. Plante, P. Provost, Detection of human Dicer and Argonaute 2 catalytic activity, Methods Mol. Biol. 725 (2011) 121–141.
[71] C. Carissimi, V. Fulci, G. Macino, MicroRNAs: novel regulators of immunity, Autoimmun. Rev. 8 (2009) 520–524.
[72] D. Baltimore, M.P. Boldin, R.M. O'Connell, D.S. Rao, K.D. Taganov, MicroRNAs: new regulators of immune cell development and function, Nat. Immunol. 9 (2008) 839–845.
[73] A. Recchiuti, S. Krishnamoorthy, G. Fredman, N. Chiang, C.N. Serhan, MicroRNAs in resolution of acute inflammation: identification of novel resolvin D1-miRNA circuits, FASEB J. 25 (2011) 544–560.
[74] D. Wang, Y. Li, C. Zhang, X. Li, J. Yu, MiR-216a-3p inhibits colorectal cancer cell proliferation through direct targeting COX-2 and ALOX5, J. Cell. Biochem. 119 (2018) 1755–1766.
[75] M. Dews, J.L. Fox, S. Hultine, P. Sundaram, W. Wang, Y.Y. Liu, E. Furth, G.H. Enders, W. El-Deiry, J.M. Schelter, M.A. Cleary, A. Thomas-Tikhonenko, The myc-miR-17˜92 axis blunts TGF{beta} signaling and production of multiple TGF{beta}-dependent antiangiogenic factors, Cancer Res. 70 (2010) 8233–8246.
[76] S. Landskroner-Eiger, C. Qiu, P. Perrotta, M. Siragusa, M.Y. Lee, V. Ulrich, A.K. Luciano, Z.W. Zhuang, F. Corti, M. Simons, R.L. Montgomery, D. Wu, J. Yu, W.C. Sessa, Endothelial miR-17 approximately 92 cluster negatively regulates arteriogenesis via miRNA-19 repression of WNT signaling, Proc. Natl. Acad. Sci. U. S. A. 112 (2015) 12812–12817.
[77] A.A. Khan, L.A. Penny, Y. Yuzefpolskiy, S. Sarkar, V. Kalia, MicroRNA-17˜92 regulates effector and memory CD8 T-cell fates by modulating proliferation in response to infections, Blood 121 (2013) 4473–4483.
[78] M. Bousquet, C. Quelen, R. Rosati, V. Mansat-De Mas, R. La Starza, C. Bastard, E. Lippert, P. Talmant, M. Lafage-Pochitaloff, D. Leroux, C. Gervais, F. Viguie, J.L. Lai, C. Terre, B. Beverlo, C. Sambani, A. Hagemeijer, P. Marynen, G. Delsol, N. Dastugue, C. Mecucci, P. Brousset, Myeloid cell differentiation arrest by miR-125b-1 in myelodysplastic syndrome and acute myeloid leukemia with the t(2;11)(p21;q23) translocation, J. Exp. Med. 205 (2008) 2499–2506.
[79] A.A. Chaudhuri, A.Y. So, A. Mehta, A. Minisandram, N. Sinha, V.D. Jonsson, D.S. Rao, R.M. O'Connell, D. Baltimore, Oncomir miR-125b regulates hematopoiesis by targeting the gene Lin28A, Proc. Natl. Acad. Sci. U. S. A. 109 (2012) 4233–4238.
[80] S. Kargman, C.A. Rouzer, Studies on the regulation, biosynthesis, and activation of 5-lipoxygenase in differentiated HL60 cells, J. Biol. Chem. 264 (1989) 13313–13320.
[81] M. Brungs, O. Rådmark, B. Samuelsson, D. Steinhilber, On the induction of 5-lipoxygenase expression and activity in HL-60 cells: effects of vitamin D3, retinoic acid, DMSO and TGFß, Biochem. Biophys. Res. Commun. 205 (1994) 1572–1580.
[82] M. Gururajan, C.L. Haga, S. Das, C.M. Leu, D. Hodson, S. Josson, M. Turner, M.D. Cooper, MicroRNA 125b inhibition of B cell differentiation in germinal centers, Int. Immunol. 22 (2010) 583–592.
[83] R. Malumbres, K.A. Sarosiek, E. Cubedo, J.W. Ruiz, X. Jiang, R.D. Gascoyne, R. Tibshirani, I.S. Lossos, Differentiation stage-specific expression of microRNAs in B lymphocytes and diffuse large B-cell lymphomas, Blood 113 (2009) 3754–3764.
[84] Y. Mahshid, M.R. Lisy, X. Wang, R. Spanbroek, J. Flygare, B. Christensson, M. Bjorkholm, B. Sander, A.J. Habenicht, H.E. Claesson, High expression of 5-lipoxygenase in normal and malignant mantle zone B lymphocytes, BMC Immunol. 10 (2009) 2.
[85] V. Dincbas-Renqvist, G. Pepin, M. Rakonjac, I. Plante, D.L. Ouellet, A. Hermansson, I. Goulet, J. Doucet, B. Samuelsson, O. Radmark, P. Provost, Human Dicer C-terminus functions as a 5-lipoxygenase binding domain, Biochim. Biophys. Acta 1789 (2009) 99–108.
[86] Y. Chen, Y. Hu, H. Zhang, C. Peng, S. Li, Loss of the Alox5 gene impairs leukemia stem cells and prevents chronic myeloid leukemia, Nat. Genet. 41 (2009) 783–792.
[87] J. Roos, C. Oancea, M. Heinssmann, D. Khan, H. Held, A.S. Kahnt, R. Capelo, E. la Buscato, E. Proschak, E. Puccetti, D. Steinhilber, I. Fleming, T.J. Maier, M. Ruthardt, 5-Lipoxygenase is a candidate target for therapeutic management of stem cell-like cells in acute myeloid leukemia, Cancer Res. 74 (2014) 5244–5255.
[88] A. Catalano, P. Caprari, S. Soddu, A. Procopio, M. Romano, 5-lipoxygenase antagonizes genotoxic stress-induced apoptosis by altering p53 nuclear trafficking, FASEB J. 18 (2004) 1740–1742.
[89] D.W. Meek, Regulation of the p53 response and its relationship to cancer, Biochem. J. 469 (2015) 325–346.
[90] S. Kim, S.S. An, Role of p53 isoforms and aggregations in cancer, Medicine (Baltimore) 95 (2016) e3993.
[91] M.R. Junttila, G.I. Evan, p53–a Jack of all trades but master of none, Nat. Rev. Cancer 9 (2009) 821–829.
[92] A.K. Jain, M.C. Barton, p53: emerging roles in stem cells, development and beyond, Development 145 (2018), https://doi.org/10.1242/dev.158360.
[93] V.J.N. Bykov, S.E. Eriksson, J. Bianchi, K.G. Wiman, Targeting mutant p53 for efficient cancer therapy, Nat. Rev. Cancer 18 (2018) 89–102.
[94] Y. Torosyan, A. Dobi, S. Naga, K. Mezhevaya, M. Glasman, C. Norris, G. Jiang, G. Mueller, H. Pollard, M. Srivastava, Distinct effects of annexin A7 and p53 on arachidonate lipoxygenation in prostate cancer cells involve 5-lipoxygenase transcription, Cancer Res. 66 (2006) 9609–9616.
[95] A. Catalano, S. Rodilossi, P. Caprari, V. Coppola, A. Procopio, 5-Lipoxygenase regulates senescence-like growth arrest by promoting ROS-dependent p53 activation, EMBO J. 24 (2005) 170–179.
[96] H. Clevers, Wnt/beta-catenin signaling in development and disease, Cell 127 (2006) 469–480.
[97] H. Clevers, R. Nusse, Wnt/beta-catenin signaling and disease, Cell 149 (2012) 1192–1205.
[98] E.K. Siapati, M. Papadaki, Z. Kozaou, E. Rouka, E. Michali, I. Savvidou, D. Gogos, D. Kyriakou, N.I. Anagnostopoulos, G. Vassilopoulos, Proliferation and bone marrow engraftment of AML blasts is dependent on beta-catenin signalling, Br. J. Haematol. 152 (2011) 164–174.
[99] J. Roos, S. Grösch, O. Werz, P. Schröder, S. Ziegler, S. Fulda, P. Paulus, A. Urbschat, B. Kühn, I. Maucher, J. Fettel, T. Vorup-Jensen, M. Piesche, C. Matrone, D. Steinhilber, M.J. Parnham, T.J. Maier, Regulation of tumorigenic Wnt signaling by cyclooxygenase-2,5-lipoxygenase and their pharmacological inhibitors: a basis for novel drugs targeting cancer cells? Pharmacol. Ther. 157 (2015) 43–64.
[100] R.C. DeKelver, B. Lewin, K. Lam, Y. Komeno, M. Yan, C. Rundle, M.C. Lo, D.E. Zhang, Cooperation between RUNX1-ETO9a and novel transcriptional partner KLF6 in upregulation of Alox5 in acute myeloid leukemia, PLoS Genet. 9 (2013) e1003765.
[101] C. Muller-Tidow, B. Steffen, T. Cauvet, L. Tickenbrock, P. Ji, S. Diederichs, B. Sargin, G. Kohler, M. Stelljes, E. Puccetti, M. Ruthardt, S. deVos, S.W. Hiebert, H.P. Koeffler, W.E. Berdel, H. Serve, Translocation products in acute myeloid leukemia activate the Wnt signaling pathway in hematopoietic cells, Mol. Cell. Biol. 24 (2004) 2890–2904.
[102] S. Brand, S. Roy, P. Schroder, B. Rathmer, J. Roos, S. Kapoor, S. Patil, C. Pommerenke, T. Maier, P. Janning, S. Eberth, D. Steinhilber, D. Schade, G. Schneider, K. Kumar, S. Ziegler, H. Waldmann, Combined proteomic and in silico target identification reveal a role for 5-lipoxygenase in developmental signaling pathways, Cell Chem. Biol. 25 (2018) 1095–1106 e1023.
[103] R.G. Morgan, J. Ridsdale, A. Tonks, R.L. Darley, Factors affecting the nuclear localization of beta-catenin in normal and malignant tissue, J. Cell. Biochem. 115 (2014) 1351–1361.
[104] M. Lu, M.R. Muers, X. Lu, Introducing STRaNDs: shuttling transcriptional regulators that are non-DNA binding, Nat. Rev. Mol. Cell Biol. 17 (2016) 523–532.
[105] A.K. Häfner, M. Cernescu, B. Hofmann, M. Ermisch, M. Hörnig, J. Metzner, G. Schneider, B. Brutschy, D. Steinhilber, Dimerization of human 5-lipoxygenase, Biol. Chem. 392 (2011) 1097–1111.
[106] N.C. Gilbert, S.G. Bartlett, M.T. Waight, D.B. Neau, W.E. Boeglin, A.R. Brash, M.E. Newcomer, The structure of human 5-lipoxygenase, Science 331 (2011)

217–219.
[107] H. Thorvaldsdottir, J.T. Robinson, J.P. Mesirov, Integrative Genomics Viewer (IGV): high-performance genomics data visualization and exploration, Brief. Bioinform. 14 (2013) 178–192.
[108] V. Nurminen, A. Neme, S. Seuter, C. Carlberg, The impact of the vitamin D-modulated epigenome on VDR target gene regulation, Biochim. Biophys. Acta Gene Regul. Mech. 1861 (2018) 697–705.
[109] S. Seuter, A. Neme, C. Carlberg, P.U. Epigenomic, 1-VDR crosstalk modulates vitamin D signaling, Biochim. Biophys. Acta Gene Regul. Mech 1860 (2017) 405–415.
[110] D. Menendez, T.A. Nguyen, J.M. Freudenberg, V.J. Mathew, C.W. Anderson, R. Jothi, M.A. Resnick, Diverse stresses dramatically alter genome-wide p53 binding and transactivation landscape in human cancer cells, Nucleic Acids Res. 41 (2013) 7286–7301.
[111] E.P. Consortium, An integrated encyclopedia of DNA elements in the human genome, Nature 489 (2012) 57–74.
[112] K.H.M. Prange, A. Mandoli, T. Kuznetsova, S.Y. Wang, A.M. Sotoca, A.E. Marneth, B.A. van der Reijden, H.G. Stunnenberg, J.H.A. Martens, MLL-AF9 and MLL-AF4 oncofusion proteins bind a distinct enhancer repertoire and target the RUNX1 program in 11q23 acute myeloid leukemia, Oncogene 36 (2017) 3346–3356.
[113] P. Borgeat, B. Samuelsson, Metabolism of arachidonic acid in polymorphnuclear leukocytes. Structural analysis of novel hydroxylated compounds, J. Biol. Chem. 254 (1979) 7865–7869.
[114] P.G. Schulam, W.T. Shearer, Evidence for 5-lipoxygenase activity in human B cell lines. A possible role for arachidonic acid metabolites during B cell signal transduction, J. Immunol. 144 (1990) 2696–2701.
[115] P.J. Jakobsson, P. Shaskin, P. Larsson, S. Feltenmark, B. Odlander, M. Aguilar-Santelises, M. Jondal, P. Biberfeld, H.E. Claesson, Studies on the regulation and localization of 5-lipoxygenase in human B-lymphocytes, Eur. J. Biochem. 232 (1995) 37–46.
[116] P.E. Poubelle, P. Borgeat, M. Rola-Pleszczynski, Assessment of leukotriene B4 synthesis in human lymphocytes using high performance liquid chromatography and radioimmunoassay methods, J. Immunol. 139 (1987) 1273–1277.
[117] R. Spanbroek, M. Hildner, D. Steinhilber, N. Fusenig, K. Yoneda, O.R. Samuelsson, A.J. Habenicht, 5-lipoxygenase expression in dendritic cells generated from CD34+ hematopoietic progenitors and in lymphoid organs, Blood 96 (2000) 3857–3865.
[118] H. Harizi, N. Gualde, Dendritic cells produce eicosanoids, which modulate generation and functions of antigen-presenting cells, Prostaglandins Leukot. Essent. Fatty Acids 66 (2002) 459–466.
[119] T. Shimizu, T. Izumi, Y. Seyama, K. Tadokoro, O. Radmark, B. Samuelsson, Characterization of leukotriene A4 synthase from murine mast cells: evidence for its identity to arachidonate 5-lipoxygenase, Proc. Natl. Acad. Sci. U. S. A. 83 (1986) 4175–4179.
[120] A.O.S. Fels, N.A. Pawlowski, E.B. Cramer, T.K.C. King, Z.A. Cohn, W.A. Scott, Human alveolar macrophages produce leukotriene B4, Proc. Natl. Acad. Sci. U.S.A. 79 (1982) 7866–7870.
[121] C.F. Bennett, M.-Y. Chiang, B.P. Monia, S.T. Crooke, Regulation of 5-lipoxygenase and 5-lipoxygenase-activating protein expression in HL-60 cells, Biochem. J. 289 (1993) 33–39.
[122] M.E. Goldyne, G.F. Burrish, P. Poubelle, P. Borgeat, Arachidonic acid metabolism among human mononuclear leukocytes: lipoxygenase-related pathways, J. Biol. Chem. 259 (1984) 8815–8819.
[123] F.A. Fitzpatrick, W. Ligget, J. McGee, S. Bunting, D. Morton, B. Samuelsson, Metabolism of leukotriene A4 by human erythrocytes, J. Biol. Chem. 259 (1984) 11403–11407.
[124] M. Hamberg, B. Samuelsson, Prostaglandin endoperoxides. Novel transformations of arachidonic acid in human platelets, Proc. Natl. Acad. Sci. U. S. A. 71 (1974) 3400–3404.