

# Emerging role of cysteinyl leukotrienes in cancer

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## Abstract

Cysteinyl leukotrienes (CysLTs) are inflammatory lipid mediators that play a central role in the pathophysiology of several inflammatory diseases. Recently, there has been an increased interest in determining how these lipid mediators orchestrate tumor development and metastasis through promoting a pro-tumoral microenvironment. Upregulation of CysLTs receptors and CysLTs production is found in a number of cancers and has been associated with increased tumorigenesis. Understanding the molecular mechanisms underlying the role of CysLTs and their receptors in cancer progression will help investigate the potential of targeting CysLTs signaling for anti-cancer therapy. This review gives an overview of the biological effects of CysLTs and their receptors, along with current knowledge of their regulation and expression. It also provides a recent update on the molecular mechanisms that have been postulated to explain their role in tumorigenesis and on the potential of anti-CysLTs in the treatment of cancer.

## KEYWORDS

Cysteinyl leukotrienes, inflammation, cancer, metastasis

## INTRODUCTION

Cysteinyl leukotrienes (CysLTs) are one of the major constituents of the eicosanoid family of bioactive inflammatory lipid-mediators. They are rapidly generated at the site of inflammation in response to immunological and nonimmunological stimuli following the release of arachidonic acid through the 5-lipoxygenase (5-LOX) pathway (Figure 1A). The term CysLTs includes leukotriene C<sub>4</sub>(LTC<sub>4</sub>), leukotriene D<sub>4</sub>(LTD<sub>4</sub>), and leukotriene E<sub>4</sub>(LTE<sub>4</sub>); they are structurally different from leukotriene A<sub>4</sub> (LTA<sub>4</sub>) and leukotriene B<sub>4</sub> (LTB<sub>4</sub>), which are commonly known as leukotrienes (LTs). Leukotrienes exhibit diverse biological effects such as contraction of bronchial smooth muscle, stimulation of vascular permeability, and attraction and activation of leukocytes (Hammarström, 1983).

Historically, leukotrienes were identified in 1938 as active components of slow-reacting substance of anaphylaxis (SRS-A) that causes contraction of smooth muscle in lung perfusates (Haeggström & Funk, 2011). Murphy and colleagues later identified CysLTs as lipids conjugated to glutathione (GSH). The term “leukotrienes” was introduced at that time by Samuelsson for lipids generated by “leuko”cytes that contain three conjugated double bonds, i.e. “tri-enes”.

The CysLTs are mainly biosynthesized by leukocytes from the myeloblastic lineage (in particular by eosinophils, basophils, and mast cells), and monoblastic lineages (monocytes/macrophages) as well as by dendritic cells (Peters-Golden & Henderson, 2007). CysLTs are primarily studied for their bronchoconstriction properties during allergic inflammation but are also known to increase vascular permeability and to regulate smooth muscle tone. CysLTs have a clear role in asthma and allergic rhinitis but have also been implicated in the pathogenesis of many acute and chronic inflammatory diseases including cardiovascular, gastrointestinal, immune, and neurodegenerative diseases (Haeggström & Funk, 2011).

Inflammation in the tumor microenvironment is now recognized as one of the hallmarks of cancer. Lipid mediators such as CysLTs play a central role in inflammation and tissue homeostasis, and are involved in cancer development. Given that both cancer cells and non-cancer stromal cells are potent producers of CysLTs, tumor progression involves an integrated response to CysLTs signaling pathways. In this review we will focus on recent insights into the role of CysLTs in tumorigenesis, from their involvement in triggering tumor epithelial cell proliferation, survival, and migration, to their involvement in adapting the tumor microenvironment (TME) by influencing angiogenesis and inflammation. Targeting CysLTs signaling could thus offer new strategies to block cancer-related inflammation.

## BIOLOGY OF CYSTEINYL LEUKOTRIENES

### 2.1. Intracellular cysteinyl leukotriene biosynthesis

During an inflammatory event, arachidonic acid (AA) is released from cell membrane-derived phospholipids by phospholipase A<sub>2</sub>, particularly cytosolic PLA<sub>2</sub>α (c PLA<sub>2</sub>α). Once mobilized into the cytosol, AA is metabolized by 5-lipoxygenase (5-LOX) in conjunction with 5-lipoxygenase-activating protein (FLAP) to generate the unstable epoxyde leukotriene A<sub>4</sub> (LTA<sub>4</sub>) through the sequential lipoxygenation of AA to 5-hydroperoxyeicosatetraenoic acid (5-HpETE) and dehydration to LTA<sub>4</sub> (Figure 1B). LTA<sub>4</sub> is further conjugated to reduced glutathione (GSH) by LTC<sub>4</sub> synthase (LTC<sub>4</sub>S) to form LTC<sub>4</sub>. Subsequently LTC<sub>4</sub> is exported from the cell via multidrug resistance-associated proteins 1 and 4 (MRP<sub>1/4</sub>) (Rius, Hummel-Eisenbeiss, & Keppler, 2008) where a γ-glutamyl transpeptidase (GGT) cleaves the glutamic acid moiety to form LTD<sub>4</sub>, which in turn, is transformed into LTE<sub>4</sub> by cleavage of the glycine moiety through dipeptidase enzyme (DPEP). LTC<sub>4</sub> and LTD<sub>4</sub> are rapidly metabolized in the blood circulation to LTE<sub>4</sub> with a half-life between 30s and 4 min (Keppler, Huber, Baumert, & Guhlmann, 1989). LTE<sub>4</sub>, the most stable among all CysLTs, accumulates in plasma and urine, and thus serves as a clinical marker (Mayatepek & Hoffmann, 1995).

Several studies indicate that the production of CysLTs in cells is highly regulated and compartmented. Upon cell stimulation, intracellular calcium is increased and this leads to the translocation of cPLA<sub>2</sub>α (Schievella, Regier, Smith, & Lin, 1995) and 5-LOX to the nuclear envelope where FLAP and LTC<sub>4</sub>S are embedded in the membrane ready to support the biosynthesis of CysLTs (Samuelsson, Dahlén, Lindgren, Rouzer, & Serhan, 1987). It has been shown in osteosarcoma cells that 5-LOX translocation is a FLAP-independent process (Kargman, Vickers, & Evans, 1992). On the other hand, FLAP enhances the activity of 5-LOX by binding to arachidonic acid (AA) and presenting it to 5-LOX. FLAP can also act as a scaffold for 5-LOX creating a multiprotein biosynthetic complex on both outer and inner membranes (Mandal et al., 2008). 5-LOX is rapidly inactivated in a Ca<sup>2+</sup>-dependent manner. It is not clear how AA, released by cPLA<sub>2</sub>α, reaches its binding site in FLAP but it is suggested that both cPLA<sub>2</sub>α and 5-LOX are in direct contact within the nuclear membrane and that all biosynthetic components of the CysLTs pathway are spatially interconnected (Haeggström & Funk, 2011). The compartmentalization of CysLTs biosynthesis suggests that this pathway may have additional intracellular/intranuclear functions due to the presence of cysLTs receptors inside the nuclei of colorectal adenocarcinoma and human mast cells (Y. Jiang, Borrelli, Kanaoka, Bacskai, & Boyce, 2007a; Nielsen, Campbell, et al., 2005).

Furthermore, lipid bodies of eosinophils and neutrophils have also been identified as a source of leukotriene synthesis (Murphy, 2001). All the enzymes required for LTC<sub>4</sub> synthesis are present within eosinophil lipid bodies and several stimuli, such as eotaxin and RANTES has been shown to elicit the formation of lipid bodies and promote LTC<sub>4</sub> production (Bandeira-Melo, Phoofolo, & Weller, 2001).

#### 1. Enzymes in cysteinyl leukotriene biosynthesis

##### 2. 5-LOX

As described above, 5-LOX catalyzes the first two steps in CysLTs biosynthesis in which AA is converted into the unstable epoxide LTA<sub>4</sub> through 5-HpETE. The human 5-lipoxygenase (5-LOX) gene encodes the mature 78-kDa protein consisting of 675 amino acids. 5-LOX is essentially expressed by bone marrow-derived cells of myeloid origin such as granulocytes (i.e. neutrophils, eosinophils, basophils), monocytes/macrophages,

mast cells, as well as dendritic cells and B lymphocytes (Haeggström, 2018). The location of 5-LOX in resting cells is cell-type specific, residing in either the cytosol or the euchromatin region of the nucleus. For example, 5-LOX is cytoplasmic in peripheral blood neutrophils and eosinophils, but resides in the nucleus of alveolar macrophages and mast cells (Thomas G. Brock, 2005). The distribution and localization of 5-LOX in human neutrophils may also vary depending on the gender of the individual. While 5-LOX has been localized in the cytoplasm of resting cells and is supposed to be translocated to nucleus upon stimulation in female neutrophils, 5-LOX has been detected in both the cytosolic and nuclear compartments of resting male neutrophils (Rakesh Kumar Singh, 2020). Although detected in the cytosolic and nucleosolic fraction of resting cells, cellular activation leads to 5-LOX translocation, in a calcium-dependent manner, to the nuclear envelope where colocalization with FLAP is a prerequisite for LT synthesis (Evans, Ferguson, Mosley, & Hutchinson, 2008). In all cell types, upon increased intracellular calcium and p38 MAPK-activated protein kinase 2 (MK2)-mediated phosphorylation and activation, 5-LOX is translocated to the nuclear membrane (Moore & Pidgeon, 2017). These observations indicate that both cytoplasmic and nuclear 5-LOX can be activated to produce leukotrienes, although in eosinophils nuclear import of 5-LOX from cytosol is associated with a reduced capacity to synthesize LTC<sub>4</sub> (T. G. Brock, Anderson, Fries, Peters-Golden, & Sporn, 1999).

The expression of 5-LOX is tightly regulated (Figure 1C) and is increased during differentiation processes. Hence, human monocytes lose 5-LOX expression when kept in culture for 7 days - probably due to the lack in required growth factors (Rådmark, Werz, Steinhilber, & Samuelsson, 2007). 5-LOX expression is increased by different cytokines and growth factors such as TGF- $\beta$  and vitamin D3 in HL-60 cells and Mono Mac 6 cells (Steinhilber, Rådmark, & Samuelsson, 1993), IL-3 in mouse mast cells (Murakami et al., 1995), and GM-CSF in mature neutrophils (Pouliot, McDonald, Khamzina, Borgeat, & McColl, 1994). On the other hand, IL-4 reduces 5-LOX expression in monocytes and maturing dendritic cells (Spanbroek et al., 2001). 5-LOX activity can potentially be modulated through the action of different kinases as it has been shown - at least *in vitro* - that 5-LOX is phosphorylated at several sites including: Ser271 by MK2, Ser663 by ERK, Ser523 by protein kinase A (PKA), and Tyr42, Tyr53 and Tyr94 by Src kinase (Rådmark, Werz, Steinhilber, & Samuelsson, 2015). Phosphorylation of 5-LOX by activation of MK2 and ERK enhances leukotriene formation by inducing nuclear translocation whereas phosphorylation by PKA directly inhibits leukotriene synthesis and interferes with 5-LOX nuclear translocation (Luo et al., 2004; Werz, Bürkert, et al., 2002; Werz, Szellas, Steinhilber, & Rådmark, 2002). In addition, 5-LOX activity is inhibited by nitric oxide and glutathione peroxidase (Thomas G. Brock, 2005). Intriguingly, neutrophils and monocytes from males have a lower capacity to produce leukotrienes, as compared to these cells from females - which paradoxically is due to androgen-induced ERK activation (Haeggström & Funk, 2011). However, such studies need further investigation to establish a possible link between gender-specific CysLTs synthesis and the occurrence of hormone-dependent cancers.

### 2.2.2. FLAP

Unlike other enzymes in the leukotriene pathway, FLAP does not present any enzymatic activity. However, studies have demonstrated that FLAP is necessary for 5-LOX activity and thus CysLTs biosynthesis by acting as a substrate carrier that presents AA to 5-LOX (Gomes, Felipe da Costa, & Colquhoun, 2018). FLAP is a member of the membrane-associated protein involved in eicosanoid and GSH metabolism or MAPEG family along with LTC4S with whom it shares 33% sequence identity. However, FLAP lacks a GSH binding site and GSH-conjugating activity (Lam & Austen, 2002). It has been localized to the nuclear envelope of resting and activated neutrophils and is distributed between the inner and outer nuclear membranes and peripheral ER (Christmas, Weber, McKee, Brown, & Soberman, 2002). Similarly to 5-LOX, GM-CSF upregulates FLAP in human neutrophils, suggesting a coordinated regulation of these two proteins (Pouliot et al., 1994) (Figure 1C). In addition, FLAP is upregulated by IL-5 in human eosinophils (Cowburn, Holgate, & Sampson, 1999) and by TNF- $\alpha$  and lipopolysaccharide (LPS) in THP-1 human monocytic cells (Reddy, Serio, Hodulik, & Bigby, 2003).

### 2.2.3. LTC4S

LTC4S catalyzes the first committed step in the biosynthesis of CysLTs through conjugation of GSH to

LTA<sub>4</sub>. Unlike conventional GSH-transferases, LTC<sub>4</sub>S does not conjugate xenobiotics and is selective for LTA<sub>4</sub> (Lam & Austen, 2002). LTC<sub>4</sub>S is expressed in cells of myeloid origin such as basophils, eosinophils, mast cells and monocytes/macrophages (Peters-Golden & Henderson, 2007). LTC<sub>4</sub>S is also detected in several cells that lack 5-LOX - and therefore cannot produce the LTA<sub>4</sub> substrate - such as platelets, vascular smooth cells and endothelial cells but allows these cells to implement transcellular biosynthesis of CysLT when they interact with LTA<sub>4</sub>-producing cells (Capra et al., 2015). This topic will be further discussed in paragraph 1.3.

Some biophysical evidence has been presented indicating that FLAP and LTC<sub>4</sub>S form functional dimers and trimers within the membrane and that together with 5-LOX, they form an interconnected machinery necessary for CysLTs biosynthesis (Haeggström & Funk, 2011). LTC<sub>4</sub>S is an 18-kDa membrane protein that is present in the outer nuclear membrane and peripheral endoplasmic reticulum but is excluded from the inner nuclear membrane. The active site of LTC<sub>4</sub>S is localized in the lumen indicating that LTC<sub>4</sub> must be returned to the cytoplasmic side of the membrane for its export (Christmas et al., 2002). Induction of genes encoding MAPEGs such as FLAP and LTC<sub>4</sub>S is mediated by a series of transcription factors such as C/EBP $\alpha$ , C/EBP $\delta$ , and C/EBP $\epsilon$ , NF- $\kappa$ B and Early growth response protein 1 (EGR-1) (Higgins & Hayes, 2011). Upregulation of LTC<sub>4</sub>S is mediated by TGF $\beta$  in THP-1 cells (Riddick et al., 1999), IL-3 and IL-5 in human eosinophils (Boyce et al., 1996) and IL-4 in human cord blood-derived mast cells (Hsieh, Lam, Penrose, Austen, & Boyce, 2001) (Figure 1C). On the other hand, TNF- $\alpha$  downregulates LTC<sub>4</sub>S expression in THP-1 cells via a transcriptional mechanism involving activation of p50 and p65 (Serio, Luo, Luo, & Mao, 2007).

#### 2.2.4. Other enzymes with LTC<sub>4</sub>S activity

As mentioned above LTC<sub>4</sub>S is a member of the MAPEG superfamily. This family further comprises FLAP, MGST1, MGST2, MGST3, and microsomal prostaglandin E synthase (mPGES) according to sequence identity, structural properties, and enzymatic activities (Jakobsson, Morgenstern, Mancini, Ford-Hutchinson, & Persson, 1999). Among this family, MGST2 and MGST3 have been shown to catalyze conjugation of LTA<sub>4</sub> with glutathione (Jakobsson, Mancini, & Ford-Hutchinson, 1996; Jakobsson, Mancini, Riendeau, & Ford-Hutchinson, 1997). MGST2 demonstrates a 44% identity with LTC<sub>4</sub>S and accounts for LTC<sub>4</sub> synthesis in nonhematopoietic cells such as in the endothelium and testis (Scoggan, Jakobsson, & Ford-Hutchinson, 1997; Sjöström, Jakobsson, Heimbürger, Palmblad, & Haeggström, 2001). MGST2 is predominantly detected in human endothelial cells and liver microsomes where it potentially is the principal enzyme responsible for LTC<sub>4</sub> production (Scoggan et al., 1997). MGST3 presents 27% amino acid identity with LTC<sub>4</sub>S and is widely expressed being found in human heart, skeletal muscle, and adrenal cortex, as well as in brain, placenta, liver, and kidney tissues. MGST3 is able to catalyze *in vitro* both the reduction of 5-HPETE to 5-HETE and the production of LTC<sub>4</sub> from LTA<sub>4</sub> and GSH that may occur under pathophysiological situations (Jakobsson et al., 1997).

#### 2.2.5. $\gamma$ -Glutamyl transpeptidases (GGT1, GGT5)

GGT1 is the principal  $\gamma$ -glutamyl transpeptidase that cleaves glutamic acid from LTC<sub>4</sub> to form LTD<sub>4</sub> at the plasma membrane. For many years, GGT1 was thought to be the only enzyme that could cleave this gamma-glutamyl bond until Heisterkamp and colleagues reported in 1991 the discovery of a human enzyme, later named GGT5, with 39.5% homology to human GGT1 that could also metabolize LTC<sub>4</sub> to LTD<sub>4</sub> although with less catalytic activity for LTC<sub>4</sub> than GGT1 (Wickham, West, Cook, & Hanigan, 2011). GGT1 and GGT5 are both extracellular enzymes, type II membrane glycoproteins, and the only catalytic activity members of the GGT family (Wickham et al., 2011). GGT1 is up-regulated by IL-1 $\beta$  in A549 epithelial lung cancer cells (Lukic et al., 2016), and GGT5 by IFN $\gamma$  and IL-4 in human macrophages (Hanigan, Gillies, Wickham, Wakeham, & Wirsig-Wiechmann, 2015) (Figure 1C). In many human tissues, GGT1 and GGT5 are expressed by different cell types or localized differently on polarized cells, which may result in unique roles for GGT1 and GGT5 (Hanigan et al., 2015).

#### 2.2.6. Dipeptidases (DPEP1, DPEP2)

DPEP1 (also known as renal dipeptidase) is a 55kDa membrane-bound dipeptidase which hydrolyzes a wide range of substrates, including the conversion of LTD<sub>4</sub> to LTE<sub>4</sub>. It was long believed to be the only enzyme able to cleave glycine from LTD<sub>4</sub> to form LTE<sub>4</sub> until the identification of DPEP2 (Habib, Shi, Cuevas, & Lieberman, 2003). DPEP1 is a zinc-dependent metalloproteinase that is expressed aberrantly in several cancers although its role remains controversial. DPEP1 expression is increased in colorectal cancer (CRC) and has been identified as a marker of High-Grade Intraepithelial Neoplasia (IEN) and CRC (Eisenach et al., 2013).

### Transcellular CysLT biosynthesis

CysLTs are primarily produced by stimulated leukocytes that express all the enzymes required for their synthesis (Figure 1A). However, many cells lacking expression of 5-LOX were found to contain the enzymes responsible for the biochemical conversion of the unstable LTA<sub>4</sub> into the final bioactive CysLTs. It is now clear that the production of CysLTs within a tissue, in particular in tumor tissues and sites of inflammation, takes place in a more complex manner through the cooperation of multiple cell types in which transformation of AA occurs in one cell type and then LTA<sub>4</sub> is passed on to a second cell to complete the transformation into CysLTs. This phenomenon defined as “transcellular biosynthesis,” has been well observed *in vitro* when neutrophils or monocytes expressing 5-LOX are incubated with endothelial cells, platelets, or vascular smooth cells, which express LTC<sub>4</sub>S (Capra et al., 2015). This transcellular process was later demonstrated *in vivo* in a mouse model of peritoneal inflammation (Zarini, Gijón, Ransome, Murphy, & Sala, 2009). LTA<sub>4</sub>H is a highly unstable molecule that has to be shielded from hydrolytic attack by water. Albumin, liposomes and fatty acid binding protein have been shown to act as chaperones and can facilitate transcellular trafficking of LTA<sub>4</sub> during cell to cell interactions (Dickinson Zimmer, Voelker, Bernlohr, & Murphy, 2004; Fiore & Serhan, 1989).

Transcellular metabolism could represent an important contributor to the production of CysLTs in patho-physiologic situations such as cancer. It has been shown that more than 50% of the LTA<sub>4</sub> produced by bovine polymorphonuclear leukocytes (PMNL) was released into the extracellular environment instead of being metabolized by the cells that synthesized it. On the other hand, when washed platelets were added to PMNL, LTC<sub>4</sub> was the main metabolite excreted (Sala, Testa, & Folco, 1996). This phenomenon could be largely underestimated because it is well known that immune cells, platelets, endothelial cells, and cancer cells interact with each other under inflammatory conditions potentially involved in adapting the tumor microenvironment by influencing angiogenesis, inflammation, immunosuppression or direct tumor growth.

### CYSTEINYL LEUKOTRIENE RECEPTORS

Cysteinyl leukotrienes signal through two sets of G protein-coupled receptors (GPCRs): CysLT<sub>1</sub>R and CysLT<sub>2</sub>R for LTC<sub>4</sub> and LTD<sub>4</sub>, and CysLT<sub>3</sub>R, also known as GPR99, for LTE<sub>4</sub>. These receptors are located on the outer plasma membrane of inflammatory cells and interact with G proteins in the cytoplasm, thereby eliciting increases in intracellular calcium and reduction in cAMP (Peters-Golden & Henderson, 2007). The classification of CysLT<sub>1</sub>R and CysLT<sub>2</sub>R was initially based on the differential profiles of guinea pig trachea contractions induced by either LTC<sub>4</sub> or LTD<sub>4</sub> and whether the response was being sensitive or resistant to a class of CysLTRs antagonists (Labat et al., 1992). This nomenclature was later introduced based on the observation that CysLT<sub>1</sub> receptor was sensitive to inhibition by classic antagonists, whereas the CysLT<sub>2</sub> receptor was not. Later, the cloning of these receptors in 1999 and 2000 largely confirmed these pharmacological characteristics of the CysLT<sub>1</sub> and CysLT<sub>2</sub> receptors (Heise et al., 2000; Lynch et al., 1999).

These receptors show differential affinity toward CysLTs (Table 1). While CysLT<sub>1</sub> receptor has a specific affinity to CysLTs in the following order : LTD<sub>4</sub>> LTC<sub>4</sub>>>LTE<sub>4</sub>(Lynch et al., 1999), CysLT<sub>2</sub> receptor responds equally to LTC<sub>4</sub> and LTD<sub>4</sub> with a low affinity : LTD<sub>4</sub>= LTC<sub>4</sub>>>LTE<sub>4</sub>(Heise et al., 2000). CysLT<sub>3</sub>R only exhibits substantial affinity for LTE<sub>4</sub>(Kanaoka, Maekawa, & Austen, 2013). CysLTRs are phylogenetically related to P2Y nucleotide receptors, and both CysLT<sub>1</sub>R and CysLT<sub>2</sub>R have been reported to signal in response to nucleotides in mast cells (Mellor et al., 2003) although it was later demonstrated that LTD<sub>4</sub> and uridine diphosphate signal through separate receptors in monocytes (Capra et al., 2005).

### CysLT<sub>1</sub>R

CysLT<sub>1</sub>R encodes a 337-amino acid protein and is expressed in many human tissues (Table 1) including lung, spleen, small intestine, placenta, and heart, as well as in several cell types such as peripheral blood leukocytes other myeloid lineage hematopoietic cells, and smooth muscle cells (Bäck et al., 2014; Lynch et al., 1999). CysLT<sub>1</sub>R is generally associated with the classical physiological functions elicited by CysLTs, such as bronchoconstriction, increased vascular permeability, leukocyte activation, activation of transcription factors and release of inflammatory mediators and cytokines (Bäck et al., 2014). CysLT<sub>1</sub>R is known to signal through elevation of intracellular calcium. Activation of the CysLT<sub>1</sub>R triggers signaling through either or both the Gq- and the Gi-protein depending on the cell type, most commonly through Gq (Nielsen, Campbell, et al., 2005). LTD<sub>4</sub>-initiated desensitization of CysLT<sub>1</sub>R is associated with its internalization which is dependent on a protein kinase C-dependent mechanism (Capra et al., 2005).

This receptor is targeted by antiasthmatic drugs such as Montelukast, Pranlukast, and Zafirlukast (Table 2). Some physiological functions in cells expressing both CysLT<sub>1</sub>R and CysLT<sub>2</sub>R receptors have been attributed to CysLT<sub>1</sub>R based on antagonist effects. However, most leukotriene receptor antagonists, except Montelukast and MK-571, are also active on CysLT<sub>2</sub>R. In fact, CysLT<sub>1</sub>R and CysLT<sub>2</sub>R exhibit redundant activities as they both mediate cysteinyl-leukotriene-induced modulation of smooth muscle cell contraction, regulation of vascular permeability, and leukocyte activation (Bäck et al., 2014).

### CysLT<sub>2</sub>R

CysLT<sub>2</sub>R encodes a 345-amino acid protein with 40% sequence identity to CysLT<sub>1</sub>R. (Heise et al., 2000). CysLT<sub>2</sub>R is mainly expressed in leukocytes, including eosinophils, basophils, mast cells, and macrophages/monocytes (Bäck et al., 2011; Peters-Golden & Henderson, 2007), but also in airway muscle cells, vascular endothelial cells, vascular smooth muscle cells, and platelets (Table 1). Studies using CysLT<sub>2</sub>R-deficient mice or mice overexpressing CysLT<sub>2</sub>R in the lungs indicate that CysLT<sub>2</sub>R does not mediate bronchoconstriction, but rather contributes to inflammation, vascular permeability, and tissue fibrosis (Beller, Maekawa, Friend, Austen, & Kanaoka, 2004; Hui et al., 2004). CysLT<sub>2</sub>R has been shown to mediate the synthesis and release of IL-33 from alveolar epithelial cells (Liu et al., 2018) and enhances platelet-mediated airway eosinophilic inflammation (Liu et al., 2019). There is currently no selective inhibitor of CysLT<sub>2</sub>R that has entered into clinical practice, but HAMI3379, BayCysLT2, and compound 15b reportedly inhibit CysLT<sub>2</sub>R with high specificity (Table 2).

### CysLT<sub>3</sub>R

Recently, GPR99 or P2Y<sub>15</sub>, previously described as an oxoglutarate receptor, has been identified as CysLT<sub>3</sub>R with high affinity to LTE<sub>4</sub> (Kanaoka et al., 2013) (Table 2). CysLT<sub>3</sub>R mRNA expression is mainly detected in the tissue of the respiratory tract and kidneys (Inbe, Watanabe, Miyawaki, Tanabe, & Encinas, 2004) (Table 1). Little is known about the pathophysiological role of CysLT<sub>3</sub>R. Signaling through CysLT<sub>3</sub>R increased the number of IL-25-producing airway brush cells having modified cellular functions (Bankova et al., 2018) and caused vascular leakage during inflammatory responses (Kanaoka et al., 2013) suggesting a role in allergic and respiratory disease associated with type 2 inflammation.

### Other identified CysLTs-related receptors

In addition to the CysLT<sub>1</sub>, CysLT<sub>2</sub>, and CysLT<sub>3</sub> receptors, additional receptors have recently been reported to be involved in CysLTs signaling. These additional receptors include GPR17 (Ciana et al., 2006), P2Y<sub>6</sub>, PPAR $\gamma$  (Sailaja Paruchuri et al., 2008), and P2Y<sub>12</sub> (Sailaja Paruchuri et al., 2009). GPR17 and P2Y<sub>6</sub> receptors have shown substantial affinity to LTD<sub>4</sub>, whereas the latter two receptors, PPAR $\gamma$ , and P2Y<sub>12</sub> have shown a preferential binding to LTE<sub>4</sub> (R.K. Singh, Gupta, Dastidar, & Ray, 2010). GPR17 is a dual uracil nucleotide-CysLTs receptor highly expressed in organs that can typically undergo ischemic injury (brain, heart, and kidney) (Ciana et al., 2006). GPR17 is activated by endogenous CysLTs, including LTC<sub>4</sub> and LTD<sub>4</sub>, and inhibited by Montelukast. GPR17 has also been shown to form a dimer with CysLT<sub>1</sub>R thus preventing LTD<sub>4</sub>-mediated activation of CysLT<sub>1</sub>R (Maekawa, Balestrieri, Austen, & Kanaoka, 2009). However, its role as a CysLTR

has been disputed (Bened-Jensen & Rosenkilde, 2010). P2Y<sub>6</sub>, a purinergic subfamily of receptors, has been identified as a promoter of cell survival that acts as a supporting receptor for CysLT<sub>1</sub>R. P2Y<sub>6</sub> does not bind directly with CysLTs but mediates the activation of CysLT receptors. CysLT<sub>1</sub>R antagonists have also been shown to inhibit the P2Y<sub>6</sub> receptor (Y. Jiang, Borrelli, Bacskai, Kanaoka, & Boyce, 2009; Lau, Chow, Au, & Ko, 2011). P2Y<sub>12</sub>, is a canonic type of purinergic receptor that is activated by ADP. The P2Y<sub>12</sub> receptor inhibition with the selective antagonist Clopidogrel is clinically used to prevent platelet aggregation and thrombosis (Cattaneo, 2015). P2Y<sub>12</sub> receptor was initially identified *in silico* as a receptor for LTE<sub>4</sub>. It was later shown that P2Y<sub>12</sub> receptor is required for LTE<sub>4</sub>-mediated pulmonary inflammation and can induce activation of Erk in transfected CHO cells, an effect of LTE<sub>4</sub> inhibited by Clopidogrel (R. K. Singh et al., 2010). However, it is still unclear whether LTE<sub>4</sub> binds directly to P2Y<sub>12</sub> and whether its downstream signaling interacts with CysLT<sub>3</sub>R-mediated activation.

### 3. CYSTEINYL LEUKOTRIENES IN CANCER

#### 3.1. Altered metabolism of 5-LOX pathway

Emerging evidence indicates that lipoxygenase pathways are involved in carcinogenesis and 5-LOX is generally considered as being pro-carcinogenic. Altered metabolism of 5-LOX is found in several cancer types and has been shown to be associated with increased tumorigenesis (Wang & Dubois, 2010). Although most attention has been focused on the cyclooxygenase pathway, a few reports indicate that 5-LOX expression is absent in normal epithelia but upregulated by pro-inflammatory stimuli and constitutively expressed in a number of cancer types including colon, oesophagus, lung, renal, pancreatic, prostate and breast cancer (Moore & Pidgeon, 2017). 5-LOX expression has been shown to be present during the early neoplastic changes of pancreatic cancer, well before progression to invasive disease (Knab, Grippo, & Bentrem, 2014). 5-LOX overexpression positively correlates with high risk factors for malignant transformation to colorectal adenocarcinoma (Wasilewicz et al., 2010). These results support the role of 5-LOX in early stages of carcinogenesis. Furthermore, 5-LOX correlates with tumor stage and lymph node metastasis in colorectal adenocarcinomas (Barresi, Grosso, Vitarelli, Tuccari, & Barresi, 2007). As mentioned before, FLAP is a protein necessary for the activity of 5-LOX. FLAP mRNA is also aberrantly expressed in breast cancers compared to normal tissues and this expression correlates with a poor prognosis (W. G. Jiang, Douglas-Jones, & Mansel, 2006).

The mechanisms by which 5-LOX contributes to tumorigenesis have not been fully elucidated. Several studies suggest a role for 5-LOX in apoptosis. 5-LOX inhibition has been shown to induce apoptosis in pancreatic cancer cells (Tong, Ding, Witt, & Adrian, 2002), and prostate cancer cells (Ghosh & Myers, 1998). These inhibitors were later shown to decrease Bcl-2 protein and Mcl-1, and to increase Bax expression in human pancreatic cancer cells (Tong et al., 2002).

#### 3.2. Pathophysiological functions of cysteinyl leukotrienes

CysLTs receptors have been implicated in the pathogenesis of many acute and chronic inflammatory diseases. The role of CysLT<sub>1</sub>R is not only well described in respiratory diseases such as asthma, but also in chronic vascular and immune diseases whereas CysLT<sub>2</sub>R has been particularly involved in cardiovascular diseases. However, several studies also indicate a role for CysLTRs in various cancers (Bäck et al., 2014).

Many studies suggest a relationship between patient survival and the expression of CysLTRs. Clinically, CysLT<sub>1</sub>R is overexpressed in gastric cancer, renal cell carcinoma, transitional cell carcinoma, prostate cancer, colon cancer and testicular cancer (Magnusson, Ehrnström, Olsen, & Sjölander, 2007; Matsuyama & Yoshimura, 2010; Venerito et al., 2011). Furthermore, strong expression of CysLT<sub>1</sub>R correlates with poor prognosis in patients with prostate, breast, and colon cancers (Bäck et al., 2014; Wang & Dubois, 2010). Some studies identified a hotspot mutation in CysLT<sub>2</sub>R as a driver oncogene in uveal melanoma and in blue

nevi although in other cancer types CysLT<sub>2</sub>R seems to exert anti-cancer properties (Slater et al., 2018). CysLT<sub>1</sub>R has been shown to signal survival and proliferation in colon cancer (Table 3), suggesting an important role for this receptor in tumor development, whereas reduced expression of CysLT<sub>2</sub>R in colon cancer is associated with poor prognosis and CysLT<sub>2</sub>R signaling leads to terminal differentiation of colon carcinoma cells and growth inhibition (Magnusson et al., 2007). Interestingly, LTD<sub>4</sub> is able to upregulate CysLT<sub>2</sub>R in both non-transformed intestinal cells and colon cancer cells but upregulates CysLT<sub>1</sub>R expression in non-transformed cells only, possibly due to the already high expression of CysLT<sub>1</sub>R in cancer cells (Yudina, Parhamifar, Bengtsson, Juhas, & Sjölander, 2008). It has also been demonstrated that CysLT<sub>2</sub>R negatively regulates CysLT<sub>1</sub>R signalling in human mast cells (Y. Jiang, Borrelli, Kanaoka, Bacskai, & Boyce, 2007b). Lack of CysLT<sub>2</sub>R may facilitate the formation of CysLT<sub>1</sub>R homodimers, leading to increased LTD<sub>4</sub> signaling, which may promote tumorigenesis. These data thus suggest that the balance between these two receptors is important for tumor progression and disease outcome.

### 3.3. Role of cysteinyl leukotrienes in carcinogenesis

As aforementioned, pro-inflammatory CysLTs are produced by various normal and cancer cell types. These bioactive lipids can modulate tumor progression through several mechanisms (Table 3, Figure 2), such as the activation of CysLTs receptors on cancer cells, which in turn regulate cell proliferation, apoptosis and migration, or the induction of epithelial and immune cells to secrete growth factors, pro-inflammatory mediators and angiogenic factors that switch a normal microenvironment to a tumor microenvironment (TME).

#### 3.3.1. Proliferation and survival

CysLTs induce cell proliferation in various cell types including bone marrow cells, smooth muscle cells, endothelial cells, and intestinal cells (Bäck et al., 2014). The activation of LTD<sub>4</sub>-CysLT<sub>1</sub>R signaling has been shown to promote cell proliferation and survival through multiple pathways (Table 3). LTD<sub>4</sub> induces cell proliferation in non-tumoral intestinal Int407 cells via a Ras-independent, PKC $\epsilon$ -dependent activation of ERK-1/2, and via a parallel Ras-dependent pathway (Sailaja Paruchuri, Hallberg, Juhas, Larsson, & Sjölander, 2002). LTD<sub>4</sub>-induced activation of CysLT<sub>1</sub>R also promotes intestinal cell proliferation and survival by activation of  $\beta$ -catenin signaling via a PI3K-dependent phosphorylation of GSK3 $\beta$  (Maryna Mezhybovska, Wikström, Öhd, & Sjölander, 2006). In colon cancer cells, LTD<sub>4</sub>-CysLT<sub>1</sub>R signaling induces nuclear translocation of  $\beta$ -catenin and subsequent MYC and CCND1 transcription in HT116 cells, but not in HT29 cells, and reduces the GSK-3 $\beta$ -induced degradation of  $\beta$ -catenin (Salim, Sand-Dejmek, & Sjölander, 2014). LTD<sub>4</sub> not only induces  $\beta$ -catenin translocation to the nucleus but also to the mitochondria where it is suggested to physically interact with Bcl-2 to promote survival (Maryna Mezhybovska et al., 2006).

Hence, LTD<sub>4</sub> promotes cell survival by increasing expression of the anti-apoptotic protein Bcl-2 in intestinal Int407 cells (Maryna Mezhybovska et al., 2006; Öhd et al., 2003), and in colon cancer Caco-2 and HT-116 cells (Bellamkonda, Sime, & Sjölander, 2015; Wikström, Öhd, et al., 2003). LTD<sub>4</sub> also prevents apoptosis induced by COX-2 inhibition in intestinal cells by interfering with caspase 8 or caspase 3 activation and by activating MEK/Erk signaling (Öhd et al., 2003; Wikström, Juhas, & Sjölander, 2003; Wikström, Öhd, et al., 2003). LTD<sub>4</sub> - but not LTC<sub>4</sub> - has indeed been shown to increase cell survival by up-regulating COX-2 expression and prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) production in intestinal cells (Öhd et al., 2003) and colon cancer cells (Bellamkonda et al., 2015; Ramin Massoumi, Nielsen, Azemovic, & Sjölander, 2003), suggesting a crosstalk between CysLTs and the prostaglandins pathways. LTD<sub>4</sub>-induced increase of COX-2 is mediated by MAPK/Erk1-2 signaling in Int407 and Caco-2 cells (Wikström, Öhd, et al., 2003). Some results further suggest that LTD<sub>4</sub> signals a switch from cell death to cell survival. LTD<sub>4</sub> is known to increase MAPK protein expression in monocytic leukemia cells via a PKC $\alpha$  and Raf1 signaling pathway (Hoshino, Izumi, & Shimizu, 1998). MAPK potentially plays a role in protecting cells against apoptosis by counteracting cell death induced by loss of matrix contact, cytoskeletal integrity or extracellular mitogenic factors and LTD<sub>4</sub> promotes intestinal cells survival by causing a PKC-dependent upregulation of active  $\beta$ 1-integrins and

enhancing  $\beta 1$ -integrin-dependent adhesion to collagen IV (R. Massoumi & Sjölander, 2001).

The presence of at least two distinct intracellular signalling pathways could explain the versatile activities of CysLTs in cells under inflammatory conditions (Hoshino et al., 1998; Sailaja Paruchuri & Sjölander, 2003). It has been shown that LTD<sub>4</sub> can induce cell survival and proliferation by activating two parallel signaling pathways in Int407 cells: a PKC $\alpha$ /CREB pathway promoting cell survival and a PKC $\epsilon$ /ERK-1/2/p90<sup>RSK</sup> pathway promoting proliferation (Sailaja Paruchuri & Sjölander, 2003). CysLT<sub>1</sub>R inhibition significantly reduces proliferation of both non-tumoral intestinal cell lines and colon cancer cells but inhibits survival only in non-tumoral intestinal cells. Colon cancer cells exhibit nuclear and cell surface CysLT<sub>1</sub>R and blockade of cell surface CysLT<sub>1</sub>R impairs proliferation signaling, but not cell survival. However, an apoptotic response could be induced by cell-permeable 5-LOX inhibitor that impairs endogenous production of CysLTs suggesting that nuclear CysLT<sub>1</sub>R has a role in CysLT survival signalling (Nielsen, Massoumi, Sonnerlind, & Sjölander, 2005; S. Paruchuri, Mezhybovska, Juhas, & Sjölander, 2006).

The proliferative effects of CysLTs also depend on the receptor subtype. While CysLT<sub>1</sub>R signaling induces proliferation and survival in intestinal epithelial cells and colon cancer cells, CysLT<sub>2</sub>R plays a role in terminal differentiation and growth inhibition in colon carcinoma cell lines (Magnusson et al., 2007; Mehdawi et al., 2017; Satapathy & Sjolander, 2018). CysLT<sub>2</sub>R signaling also appears to have no effect on the level of apoptosis in MCF-7 breast cancer cells (Magnusson, Bengtsson, et al., 2011). However, LTC<sub>4</sub> through CysLT<sub>2</sub>R signaling upregulates tumor-suppressor enzyme 15-PGDH in colon cancer cells via JNK/AP-1 and TNF $\alpha$  signaling, downregulates COX-2 expression, and increases expression of the differentiation markers sucrase-isomaltase and mucin-2 (Mehdawi et al., 2017; Satapathy & Sjolander, 2018).

### 3.3.2. Migration, Invasion

Cell migration is an essential step for tumor invasion and metastasis. LTD<sub>4</sub> induces non-transformed intestinal cell migration through the activation of the PI3K-Rac cascade (Sailaja Paruchuri, Broom, Dib, & Sjölander, 2005). In epidermal carcinoma A431 cells, both LTD<sub>4</sub> and LTC<sub>4</sub>-induced CysLT<sub>1</sub>R signaling regulated the EGF-induced second wave of Rac1 activation, a small GTPase which plays an important role in cell migration, by increasing Tiam1 expression. (Magi et al., 2014). LTD<sub>4</sub> has been involved in the regulation of cell-matrix adhesion and migration of colon cancer cells by increasing adhesion to collagen I. LTD<sub>4</sub> regulates the adhesive properties and migration of Caco-2 cells by up-regulating COX-2 and stimulating PGE<sub>2</sub> production resulting in enhanced activation and expression of  $\alpha 2\beta 1$ -integrins (Ramin Massoumi et al., 2003). Epithelial-mesenchymal transition (EMT) is an essential step to cancer cell invasion and metastasis. LTD<sub>4</sub> increases the migration of HT-116 colon cancer cells and decreases E-cadherin expression, a key mediator of the EMT process (Salim et al., 2014). LTD<sub>4</sub> further promotes cell invasion and EMT in SW480 colon cancer cells, and increases expression of MMP-9, a metalloproteinase known to be involved in the induction of EMT in breast cancers (Vinnakota et al., 2017). Conversely CysLT<sub>2</sub>R signaling was assessed to reduce cell migration in MCF-7 breast cancer cells (Magnusson et al., 2007), and to induce cell differentiation markers and decrease MMP-2 and MMP-9 in colon cancer cells (Mehdawi et al., 2017; Satapathy & Sjolander, 2018).

### 3.3.3. Cancer-initiating cells

The role of inflammatory lipid mediators in tumor progression is well recognized. However, whether this includes promotion of cancer-initiating cells (CICs) remains unclear. Some studies suggest that leukotrienes are involved in the regulation of stem cell homeostasis indicating their potential capacity to regulate cancer stem cell growth.

LTD<sub>4</sub> - but not LTC<sub>4</sub> or LTE<sub>4</sub> - via CysLT<sub>1</sub>R signaling supports proliferation of CD34+ hematopoietic progenitor cells (HPCs) induced by cytokines such as IL-3, and activates the MAPK/Erk pathway leading to increase  $\alpha 4\beta 1$  and  $\alpha 5\beta 1$  integrin-dependent adhesion suggesting that CysLTs may contribute to HPCs retention and proliferation during inflammation (Boehmler et al., 2009). LTD<sub>4</sub> and PGE<sub>2</sub> increase the ALDH<sup>+</sup> population, a marker of cancer-initiating cells in CRC, and their resistance to radiation and 5-FU. Also, LTD<sub>4</sub> promoted expression of self-renewal genes in ALDH<sup>+</sup> CICs, the colony formation capacity, and

tumor growth in a xenograft model of colon cancer further supporting a role for LTD<sub>4</sub> in the promotion of cancer stemcell-like characteristics and thus tumor progression (Bellamkonda et al., 2015).

### 3.3.4. Oxidative DNA damage

LTC<sub>4</sub> has been shown to promote oxidative DNA damage, a primary driver of carcinogenesis, contributing to genomic instabilities and increased mutations. ER-stress triggers LTC<sub>4</sub> synthesis by MGST2-based biosynthetic machinery and CysLTR nuclear translocation. Activation of this pathway elicits nuclear translocation of NADPH oxidase 4 (NOX4) and generation of ROS. The use of LTC<sub>4</sub> antagonists attenuates ER stress-triggered cell death, correlating with reduced cleavage of caspase 3, whereas inhibition of the LTC<sub>4</sub> transporter MRP<sub>1</sub> by reversan does not reduce cleaved caspase-3 levels, thereby further demonstrating the intracrine nature of LTC<sub>4</sub> action on cell death (Dvash, Har-Tal, Barak, Meir, & Rubinstein, 2015).

### 3.3.5. Deregulation of cell energetics

Changes in glycolysis - also known as the Warburg effect - are implicated in the transformation and regulation of cancer cell growth, and are mediated by the “master regulator” c-Myc oncogene. LTD<sub>4</sub>- CysLT<sub>1</sub>R signaling activates transcription of c-Myc via induction of β-catenin nuclear translocation in intestinal Int407 cells (Salim et al., 2014). LTD<sub>4</sub> induces the PI3K-Akt signaling pathway in Int407 cells, which has been shown to stimulate aerobic glycolysis in cancer cells (Elstrom et al., 2004). LTD<sub>4</sub> also triggers metabolic activity in both non-transformed Int407 cells and colon cancer Caco-2 cells by increasing NADPH activity, the ATP/ADP ratio, and the transcription of mitochondrial DNA genes (M. Mezhybovska, Yudina, Abhyankar, & Sjölander, 2009).

### 3.3.6. Tumor-promoting inflammation

During chronic inflammation, pro-inflammatory mediators such as cytokines, chemokines and eicosanoids such as CysLTs, contribute to the shift from a normal tissue microenvironment to a TME capable of promoting tumor progression. This TME is associated with every step of tumorigenesis, from initiation, through tumor promotion, to metastatic progression. A strong association between chronic inflammation and cancer occurs in CRC that arises in patients with inflammatory bowel disease (IBD) (Wang & Dubois, 2010). Inflammatory mediators such as CysLTs are increased at sites of inflammation and urinary LTE<sub>4</sub> can serve as a biomarker for IBD (Stanke-Labesque, Pofelski, Moreau-Gaudry, Bessard, & Bonaz, 2008). Several studies have shown that nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin reduce the incidence of colon, breast, and prostate cancer (Grivennikov, Greten, & Karin, 2010). The mechanism by which inflammatory mediators contribute to tumor cell transformation and progression has not however been completely resolved.

In response to tumor-associated or chronic inflammation, the recruitment of immune cells that are phenotypically different to normal immune cells can maintain inflammation and induce angiogenesis (Wang & Dubois, 2010). Many studies have focused on the role of 5-LOX and leukotriene LTB<sub>4</sub> on immune regulation but CysLTs are also potent leukocyte chemoattractants. LTD<sub>4</sub> has been shown to induce a chemotactic response in human monocytic leukaemia THP-1 cells (Hoshino et al., 1998) and in Th17 lymphocytes, which play a role in tumorigenesis (Kim & Lee, 2015). LTD<sub>4</sub>, via activation of transcriptional regulator EGR-1, induces production of cytokines, such as IL-8, IL-6, and TNFα by Hodgkin lymphoma cells which may contribute to chemotaxis of eosinophils into the tumor site and promote the synthesis of other pro-inflammatory compounds by immune cells contributing to a TME (Han et al., 2015; Schain et al., 2008). Tumor-associated macrophages (TAMs), such as M2 macrophages, play an important role in the progression of cancer. M2 macrophages secrete various cytokines including TNFα which is known to promote proliferation, invasion and metastasis of tumor cells, as well as LTD<sub>4</sub> which may further promote immune cell infiltration. M2-like macrophage medium enriched in TNFα and LTD<sub>4</sub> promotes MMP-9 expression and induction of the EMT in colon cancer cells (Vinnakota et al., 2017). TNF-α and LTD<sub>4</sub> also increase COX-2 expression in non-transformed, and 5-LOX and LTC<sub>4</sub>S in both non-transformed and cancer cell lines. TNF-α and LTD<sub>4</sub>, to a

lesser extent, upregulate CysLT<sub>1</sub>R levels, These results suggest that inflammatory mediators can upregulate the eicosanoid pathway, including the CysLTs and their receptors thus providing important mechanisms for both maintaining inflammation and for tumor progression (Yudina et al., 2008).

### 3.3.7. Angiogenesis

Angiogenesis is a hallmark of cancer as tumor growth and invasion are heavily dependent on inner-tumor angiogenesis and are influenced by vascular stability. Several cells in the TME secrete various pro-angiogenic factors that stimulate endothelial cell recruitment, proliferation, migration and tubule formation. In general, endothelial cells do not have the capacity to produce leukotrienes *de novo* due to low or negligible enzyme expression; however they express CysLT receptors suggesting the possibility of interactions between CysLT produced by cancer cells and non-cancer cells in the microenvironment. CysLT<sub>1</sub>R and CysLT<sub>2</sub>R are both detected in vascular endothelial cells (ECs) and vascular smooth muscle cells. In particular, CysLT<sub>2</sub> is the main CysLTR expressed by these cells and is associated with vascular permeability. LTD<sub>4</sub>-CysLT<sub>2</sub>R signaling has been shown to regulate angiogenesis in isolated murine ECs and Matrigel implants in WT mice, and to enhance ECs contraction and permeability via the Rho-dependent myosin light chain 2 and vascular endothelial (VE)-cadherin axis (Duah et al., 2019). CysLTs have been consistently shown to induce endothelial cell proliferation *in vitro* (M a r y n a M e z h y b o v s k a e t a l., 2006; Sailaja Paruchuri & Sjölander, 2003; Wikström, Öhd, et al., 2003). Both LTC<sub>4</sub> and LTD<sub>4</sub> promote angiogenesis in an *in vivo* chick chorioallantoic membrane system (Tsopanoglou, Pipili-Synetos, & Maragoudakis, 1994). LTC<sub>4</sub> - but not LTD<sub>4</sub> - stimulated *in vitro* tube formation and migration of vascular endothelial cells and endothelial cell migration suggesting a potential synergistic action between LTC<sub>4</sub>, and LTD<sub>4</sub> on angiogenesis (Kanayasu et al., 1989). On the other hand, restoration of 15-PGDH expression through LTC<sub>4</sub>-CysLT<sub>2</sub>R signaling promotes anti-angiogenic action against colon cancer cells, decreases the VEGFR-1 protein level, and disrupts tube formation in HUVEC (Satapathy & Sjölander, 2018). The CysLT<sub>1</sub>R antagonist Montelukast, but not the CysLT<sub>2</sub>R antagonist BayCysLT<sub>2</sub>, inhibits basal microvessel outgrowth from rat thoracic aortic rings, and both drugs inhibit an LTD<sub>4</sub>-induced model of aortic ring angiogenesis (Xu et al., 2010). CysLT<sub>1</sub> receptor antagonists also reduce tumor angiogenesis in a mouse xenograft model of colorectal cancer, and reduce tumor VEGF levels (Savari, Liu, Zhang, Sime, & Sjölander, 2013). Interestingly, the recognized anti-asthmatic effect of Montelukast is modulated by VEGF polymorphisms, and Montelukast has been postulated to attenuate airway inflammation by reducing VEGF expression (Lee, Kim, Park, Jin, & Lee, 2004).

## 4. TARGETING CYSTEINYL LEUKOTRIENE SIGNALING IN CANCER MODELS

Given the effects of CysLTs on the diverse oncogenic pathways described above, the therapeutic potential of targeting CysLT signaling for anti-cancer therapy has been investigated.

### 4.1. Anti-leukotriene drugs

The 5-LOX pathway and leukotrienes have long been linked to cancer. Several studies have aimed at targeting 5-LOX or FLAP to inhibit endogenous production of leukotrienes in *in vivo* cancer models. Among the 5-LOX inhibitors that have been developed, Zileuton has shown the most beneficial effects in many inflammatory diseases, and is today marketed in the United States for treatment of asthma (Haeggström, 2018). While Zileuton has had promising effects for airway disease, this drug has not yet been tested in patients with cancer. Zileuton has been investigated in animal studies and has shown promising results for multiple cancers including those of colon, lung, and pancreas (Knab et al., 2014). Zileuton reduced polyp formation in an APC<sup>Δ468</sup> mouse model of polyposis and decreased tumor burden in LoVo and HT29 colon cancer murine xenograft models (Gounaris et al., 2015; Melstrom et al., 2008). Inhibition of 5-LOX not only has a direct effect on cancer cells but has also been demonstrated to be efficient in targeting the TME. Genetic or pharmacological inhibition of 5-LOX inhibited neutrophils pro-metastatic activity and reduced subsequent lung metastasis in a mouse breast cancer model (Wculek & Malanchi, 2015). Similar to Zileuton, MK-886, a FLAP inhibitor, has shown promising results *in vitro* and *in vivo*. MK-886 and Zileuton prevents lung tumors in mice (Gunning, Kramer, Steele, & Pereira, 2002). MK-886 has been shown to reduce pancreatic

cancer growth in a hamster model (Knab et al., 2014). Several studies have shown that a combination of a cyclooxygenase inhibitor and a 5-LOX or FLAP inhibitor exhibits an additive effect on reducing tumor growth in mouse model xenografts of human colon, oesophageal, breast and skin cancers (Barry et al., 2009; Chen et al., 2004; Fegn & Wang, 2009; Rioux & Castonguay, 1998). Few studies have evaluated the effect of specifically targeting the CysLTs pathway. Indeed, despite its central role in inflammatory diseases, very few specific inhibitors of LTC<sub>4</sub>S have been developed so far (Kleinschmidt et al., 2015; Munck Af Rosenschöld et al., 2019). TK05 is one of the most potent LTC<sub>4</sub>S inhibitors reported and significantly reduces vascular permeability *in vivo* (Kleinschmidt et al., 2015) but has not as yet been tested in cancer models.

## 4.2. Cysteinyl leukotriene receptor antagonists (LTRAs)

Cysteinyl leukotriene receptor antagonists (LTRAs) are certainly the most promising option to target CysLTs signaling as they have been on the market for more than two decades. This class of compounds, also known as “lukasts”, comprises molecules such as Montelukast, Zafirlukast, and Pranlukast, that are widely prescribed drugs for the management of bronchial asthma and allergic rhinitis (Yokomizo, Nakamura, & Shimizu, s. d.). Because of the emerging role of CysLTs in carcinogenesis and the tumor-promoting microenvironment, some studies have investigated the chemopreventive effect of LTRAs. A recent large epidemiological study on asthmatic patients showed that LTRAs decreased the risk of cancer development, especially lung, breast, colorectal, and liver cancers, in a dose-dependent manner (Tsai et al., 2016). Several *in vitro* studies have also demonstrated the potential of targeting CysLT<sub>1</sub>R pathways for anti-cancer therapy due to its effects on the oncogenic pathways described above. CysLT<sub>1</sub>R antagonist treatment has been shown to inhibit growth and/or induce apoptosis of a series of human cancer cell lines including urological (Matsuyama & Yoshimura, 2010), prostate (Matsuyama et al., 2007), lung (Tsai et al., 2017), breast (Suknuntha et al., 2018), and colon cancer cells (S. Paruchuri et al., 2006; Savari et al., 2013), as well as neuroblastomas (Sveinbjörnsson et al., 2008), glioblastomas (Piromkraipak et al., 2018), and chronic myeloid leukemia (CML) (Zovko et al., 2018). However, Montelukast failed to prevent growth and colony formation of bone marrow CD34<sup>+</sup>CD38<sup>+</sup>leukemic stem cells from CML patients (Dolinska et al., 2017). Although LTRAs have common pharmacological properties in inhibiting CysLT<sub>1</sub>R signaling, some studies indicate that Montelukast mainly induces apoptosis, while Zafirlukast exerts its action on the cell cycle (Piromkraipak et al., 2018; Suknuntha et al., 2018). Similarly, MK-5571, a selective CysLT<sub>1</sub>R antagonist that blocks the binding of LTD<sub>4</sub> but not LTC<sub>4</sub>, has been shown to inhibit colorectal cancer cell proliferation but not survival (S. Paruchuri et al., 2006) suggesting that CysLT could regulate apoptosis and cell proliferation through different pathways. LTRAs also suppress cell migration and invasion signaling of glioblastoma cells (Piromkraipak et al., 2018), colon cancer cells (Vinnakota et al., 2017) and skin cancer cells (Magi et al., 2014).

A few *in vivo* studies have reported the chemopreventive effect of CysLT<sub>1</sub>R antagonists in human colon cancer xenografts (Savari et al., 2013), in N-nitroso N-methyl urea-induced mammary carcinogenesis in rats (Jose, Amathi, Sathyamurthy, & Kumar, 2013) in a Lewis lung carcinoma metastasis model in mice (Nozaki et al., 2010), and in vinyl carbamate-induced lung tumors in mice (Gunning et al., 2002). CysLT<sub>1</sub>R antagonists Pranlukast and Montelukast reduce vascular permeability by regulating VEGF expression in the lungs of mice with allergen-induced asthma (Lee et al., 2004). These antagonists prevented tumor metastasis in a mouse model of Lewis lung carcinoma metastasis by inhibiting the permeability of peripheral capillaries. Interestingly, Pranlukast can inhibit tumor cell migration through both the brain and peripheral capillaries, whereas Montelukast inhibits cell migration only in peripheral capillaries (Nozaki et al., 2010). Quinini, a novel antiangiogenic and antagonist of CysLT<sub>1</sub>R and CysLT<sub>2</sub>R has also shown anti-tumor efficacy using *in vitro*, *ex vivo* and *in vivo* models of colorectal cancer without targeting VEGF receptors (Butler et al., 2019).

There is evidence that some of these anti-tumoral properties of LTRAs might be of a secondary nature and therefore unrelated to the CysLT<sub>1</sub>R antagonism. In particular, Montelukast, Pranlukast, and Zafirlukast inhibit PGE<sub>2</sub> formation by inhibiting mPGES-1 activity in cytokine-stimulated HeLa and A549 carcinoma cells and in LPS-stimulated human leukocyte preparations (Kahnt et al., 2013). Although Montelukast also

antagonizes the effect of GPR17 it is unknown if this receptor plays a role in the drug's anti-tumoral action (Ciana et al., 2006). Interest of CysLT<sub>2</sub>R agonist to exert anti-cancer properties has been suggested in the case of colorectal cancer and breast cancer (Burke et al., 2016; Magnusson, Liu, et al., 2011). Additionally, it has been revealed that both Pranlukast and BayCysLT2 could attenuate chemotherapy-triggered ROS production and DNA damage via an intracrine MGST2-LTC<sub>4</sub> pathway, suggesting that LTC<sub>4</sub> inhibitors may have a therapeutic potential in combination with chemotherapy to reduce the risk of secondary malignancies (Dvash et al., 2015). Moreover, *CysLT2R* oncogenic mutation has been identified as a potential therapeutic target in uveal melanoma (Slater et al., 2018) and CysLT<sub>2</sub>R antagonist BayCysLT2 reduces Lewis lung carcinoma tumor volume and metastasis in mice (Duah et al., 2019).

## 5. CONCLUSIONS

In conclusion, CysLTs as well as their receptors are not only important mediators of acute and chronic inflammation, but have been recognized to be essential regulators of cancer development in several cancer types. Targeting CysLT signaling has emerged as a promising pharmacological strategy considering the role of CysLTs in many hallmarks of cancer (Figure 2). Commercially available anti-asthmatic drugs which target the CysLT<sub>1</sub>R, have demonstrated notable anti-tumor efficacy in *in vitro* and *in vivo* cancer models. Most CysLTs inhibitory studies largely center on targeting the tumor-promoting effects of this pathway in cancer cells. However, as evidenced in this review, CysLTs also play an important role in the cross-talk between cancer cells and stromal cells and in the orchestration of a pro-tumoral inflammatory microenvironment. A major challenge for the future will be to clarify the dual role of CysLTRs and the different CysLTs in pro- and anti-tumorigenic inflammation. Further investigation is needed to understand the role of CysLTs in the different stages of cancer and the potential for modulating CysLTs signaling in combination with other antitumor therapies in order to improve responses to treatment for a range of cancer types.

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TABLES:

**Table 1. Tissue distribution of cysteinyl leukotrienes receptors**

| Receptor                                     | Tissue distribution       | Cells expressing CysLT <sub>1</sub> R  | Reference   |
|--|---------------------------|--|---|
| <b>CysLT<sub>1</sub>R</b>                    | Airway mucosa             | bronchial mucosal eosinophils, neutrophils, mast cells, macrophages, B-lymphocytes, and plasma cells   | (Zhu et al., 2005)  |
|  | Blood and bone marrow     | Peripheral blood leukocytes (eosinophils, neutrophils, monocytes/macrophages, B lymphocytes), mast cells, mononuclear cells, monocyte-derived dendritic cells, platelets | (Zhu et al., 2005), (Lynch et al., 1999), (Figuroa et al., 2003), (Hasegawa et al., 2010), (Dannull et al., 2012) |
|  | Blood vessels             | coronary artery smooth muscle cells, saphenous veins, human umbilical vein endothelial cells (HUVECs)  | (Mechiche et al., 2004), (Eaton et al., 2012), (Lötzer et al., 2003)  |
|  | Conjunctiva               | conjunctival goblet cells  | (Dartt et al., 2011)  |
|  | Heart                     | aortic valvular myofibroblasts   | (Nagy et al., 2011)   |
|  | Lung                      | bronchial smooth muscle cells, alveolar macrophages  | (Zhu et al., 2005), (Lynch et al., 1999)  |
|  | Nasal polyps              | -  | (Pérez-Novo et al., 2006)   |
|  | Pancreas                  | -  | (Lynch et al., 1999), (Sarau et al., 1999)  |
|  | Placenta                  | -  |   |
|  | Small intestine and colon | -  |   |
| <b>CysLT<sub>2</sub>R CysLT<sub>2</sub>R</b> | Spleen                    | -  |   |
|  | Adrenal gland             | medullary pheochromocytes, ganglion cells  | (Heise et al., 2000)  |
|  | Blood and bone marrow     | Peripheral blood leukocytes (eosinophils, basophils, monocytes), mast cells, mononuclear cells, monocyte-derived dendritic cells, platelets                              | (Figuroa et al., 2003), (Hasegawa et al., 2010), (Heise et al., 2000), (Gauvreau et al., 2005)                    |

| Receptor                                   | Tissue distribution              | Cells expressing CysLT1R  | Reference   |
|--|----------------------------------|---|---|
| CysLT <sub>3</sub> R                       | Blood vessels                    | vascular, and coronary artery smooth muscle cells, saphenous veins, human umbilical vein endothelial cells (HUVECs) | (Hu et al., 2005), (Sjöström et al., 2003), (Mechiche et al., 2004) |
|  | Brain and central nervous system | pituitary and spinal cord, smooth muscle cells of cerebral arteries and veins                                       | (Heise et al., 2000), (Hu et al., 2005)                             |
|  | Conjunctiva                      | conjunctival goblet cells   | (Dartt et al., 2011)  |
|  | Heart                            | cardiac Purkinje cells, myocytes, fibroblasts   | (Heise et al., 2000), (Kamohara et al., 2001)                       |
|  | Lung                             | airway smooth muscle cells, interstitial macrophages  | (Heise et al., 2000)  |
|  | Lymph node                       | -   | (Heise et al., 2000)  |
|  | Nasal mucosa                     | epithelial and glandular cells  | (Corrigan et al., 2005)   |
|  | Nasal polyps                     | -   | (Adamusiak et al., 2012)  |
|  | Placenta                         | -   | (Takasaki et al., 2000)   |
|  | Spleen                           | -   | (Heise et al., 2000)  |
|  | fetal brain                      | -   | (Inbe et al., 2004)   |
|  | kidney                           | -   | (Inbe et al., 2004), (Wittenberger et al., 2002)                    |
|  | placenta                         | -   | (Wittenberger et al., 2002)   |
| Respiratory tract (trachea and lung)       | mast cells, basophils            | (Inbe et al., 2004)   |   |
| salivary glands                            | -                                | (Inbe et al., 2004)   |   |
| Kidney, testis and smooth muscle (in mice) | -                                | (He et al., 2004)   |   |

**Table 2. Ligands of cysteinyl leukotriene receptors**

| Category | Category | Ligand           | CysLT <sub>1</sub> R Value | CysLT <sub>1</sub> R Parameter | CysLT <sub>1</sub> R Reference | CysLT <sub>2</sub> R Value | CysLT <sub>2</sub> R Parameter | CysLT <sub>2</sub> R Reference | CysLT <sub>3</sub> R Value | CysLT <sub>3</sub> R Parameter |
|----------|----------|------------------|----------------------------|--------------------------------|--------------------------------|----------------------------|--------------------------------|--------------------------------|----------------------------|--------------------------------|
| Agonists | Agonists | LTC <sub>4</sub> | 7.4-7.7                    | pEC <sub>50</sub> <sup>a</sup> | (Bäck et al., 2019)            | 7.0-8.6                    | pEC <sub>50</sub> <sup>a</sup> | (Bäck et al., 2019)            | -                          | -                              |
|          |          | LTD <sub>4</sub> | 7.3-9.4                    |                                |                                | 6.8-8.6                    |                                |                                | -                          | -                              |

|                         |  | CysLT <sub>1</sub> R   | CysLT <sub>1</sub> R   | CysLT <sub>1</sub> R   | CysLT <sub>2</sub> R   | CysLT <sub>2</sub> R           | CysLT <sub>2</sub> R      | CysLT <sub>3</sub> R | CysLT <sub>3</sub> R |
|-------------------------|--|--|--|--|--|--------------------------------|---------------------------|----------------------|----------------------|
|                         | LTE <sub>4</sub>                             | 6.4-7.2  |  |  | 5.6-7.1  |                                |                           | 8,6                  | pK <sup>b</sup>      |
| <b>Antagonist</b>       | <b>Antagonist</b>                            | BayCysLT <sub>2</sub> 6,4  | pA <sub>2</sub> <sup>d</sup>                                 |  | 8.3-8.4  | pA <sub>2</sub> <sup>d</sup>   |                           | -                    | -                    |
| <b>Antag-<br/>onist</b> | <b>Antag-<br/>onist</b>                      | BAY-<br>u<br>9773<br>compound<br>13e<br>[PMID:31811124]<br>compound<br>15b<br>[PMID:<br>31811124]<br>CR-<br>3465 | 5,3-<br>6,4<br>>9<br>6,9<br>5,3                              | pIC <sub>50</sub> <sup>d</sup><br>pIC <sub>50</sub> <sup>e</sup><br>pIC <sub>50</sub> <sup>e</sup><br>pK <sub>i</sub> <sup>c</sup> | (Ferrari<br>et al.,<br>2004)<br>(Itadani<br>et al.,<br>2015) | -<br>-<br>-                    | -<br>-                    | -<br>-               | -<br>-               |
|                         | Gemilukast<br>(ONO-<br>2570366)<br>HAMI3379- | 5,8  | pIC <sub>50</sub> <sup>c</sup>                               |  | 4,6  | pIC <sub>50</sub> <sup>c</sup> | 5                         |                      |                      |
|                         | [ <sup>3</sup> H]ICI-<br>198615              | 10,6   | pK <sub>d</sub> <sup>f</sup>                                 | (Bäck<br>et al.,<br>2019)  | 7,4-<br>8,4  | pIC <sub>50</sub> <sup>d</sup> | (Bäck<br>et al.,<br>2019) |                      |                      |
|                         | Iralukast<br>KP-<br>496                      | 7,8<br>8,64  | pK <sub>i</sub> <sup>c</sup><br>pA <sub>2</sub> <sup>c</sup> | (Ishimura<br>et al.,<br>2006)  | 6,8  | pA <sub>2</sub> <sup>d</sup>   |                           |                      |                      |
|                         | Masilukast<br>(MCC-<br>847)                  | -  | -  | (Cuthbert,<br>1996)  | -  | -                              | -                         | -                    | -                    |
|                         | MEN-<br>91507                                | 6,2-<br>6,3  | pK <sub>i</sub> <sup>c</sup>                                 | (Ohashi<br>et al.,<br>1991)  | -  | -                              | -                         | -                    | -                    |
|                         | MK-<br>571                                   | 8  | pIC <sub>50</sub> <sup>c</sup>                               | (Bäck<br>et al.,<br>2019)  | -  | -                              | -                         | -                    | -                    |
|                         | MN-<br>002                                   | -  | -  | (Cabré<br>et al.,<br>2002)   | -  | -                              | -                         | -                    | -                    |
|                         | Montelukas                                   | 8,3-<br>8,6  | pIC <sub>50</sub> <sup>c</sup>                               | (Bäck<br>et al.,<br>2019)  | -  | -                              | -                         | -                    | -                    |
|                         | Pobilukast                                   | 7,5-<br>8,3  | pIC <sub>50</sub> <sup>c,d</sup>                             |  | 6,2  | pA <sub>2</sub> <sup>d</sup>   | (Bäck<br>et al.,<br>2019) |                      |                      |

|    |  | CysLT <sub>1</sub> R   | CysLT <sub>1</sub> R   | CysLT <sub>1</sub> R   | CysLT <sub>2</sub> R   | CysLT <sub>2</sub> R   | CysLT <sub>2</sub> R             | CysLT <sub>3</sub> R | CysLT <sub>3</sub> R |
|----|--|--|--|--|--|--|----------------------------------|----------------------|----------------------|
|    |  | Pranlukast   | 8,1-10   | pIC <sub>50</sub> <sup>c</sup>   |  | 5,4  | pIC <sub>50</sub> <sup>c</sup>   | -                    | -                    |
|    |  | Quininiib  | 5,9  | pIC <sub>50</sub> <sup>d</sup>   | (Reynolds et al., 2016)  | 5,9  | pIC <sub>50</sub> <sup>d</sup>   | 3                    | -                    |
|    |  | SR2640   | 8,7  | pK <sub>i</sub> <sup>c</sup>   | (Bäck et al., 2019)  | -  | -                                | -                    | -                    |
|    |  | Sulukast   | 8,3  | pK <sub>i</sub> <sup>c</sup>   |  | -  | -                                | -                    | -                    |
|    |  | triple modulator   | 8,7  | pA <sub>2</sub> <sup>c</sup>   |  | -  | -                                | -                    | -                    |
|    |  | 10 [PMID: 29878767]  |  |  |  |  |                                  |                      |                      |
|    |  | Verlukast (MK-679)   | 8,0-8,1  | pIC <sub>50</sub> <sup>c</sup>   |  | -  | -                                | -                    | -                    |
|    |  | Zafirlukast  | 7,7-9,6  | pIC <sub>50</sub> <sup>c,d</sup>   |  | 5,1-5,2  | pIC <sub>50</sub> <sup>c,d</sup> | (Bäck et al., 2019)  | -                    |
| a. | <i>Ca<sup>2+</sup> mobilization assay</i>  | <i>Ca<sup>2+</sup> mobilization assay</i>  | <i>Ca<sup>2+</sup> mobilization assay</i>  |  |  |  |                                  |                      |                      |
| b. | <i>[<sup>3</sup>H]LTE<sub>4</sub> binding assay in CHO cells</i>   |  |  |                                  |                      |                      |
| c. | <i>against [<sup>3</sup>H]LTD<sub>4</sub> or LTD<sub>4</sub></i>   | <i>against [<sup>3</sup>H]LTD<sub>4</sub> or LTD<sub>4</sub></i>   | <i>against [<sup>3</sup>H]LTD<sub>4</sub> or LTD<sub>4</sub></i>   |  |  |  |                                  |                      |                      |
| d. | <i>against LTC<sub>4</sub> or LTD<sub>4</sub> Ca<sup>2+</sup> mobilization assay or β-arrestin assay</i> | <i>against LTC<sub>4</sub> or LTD<sub>4</sub> Ca<sup>2+</sup> mobilization assay or β-arrestin assay</i> | <i>against LTC<sub>4</sub> or LTD<sub>4</sub> Ca<sup>2+</sup> mobilization assay or β-arrestin assay</i> | <i>against LTC<sub>4</sub> or LTD<sub>4</sub> Ca<sup>2+</sup> mobilization assay or β-arrestin assay</i> | <i>against LTC<sub>4</sub> or LTD<sub>4</sub> Ca<sup>2+</sup> mobilization assay or β-arrestin assay</i> | <i>against LTC<sub>4</sub> or LTD<sub>4</sub> Ca<sup>2+</sup> mobilization assay or β-arrestin assay</i> |                                  |                      |                      |

|    | CysLT <sub>1</sub> R  | CysLT <sub>1</sub> R  | CysLT <sub>1</sub> R  | CysLT <sub>2</sub> R  | CysLT <sub>2</sub> R  | CysLT <sub>2</sub> R  | CysLT <sub>3</sub> R | CysLT <sub>3</sub> R |
|----|---|---|---|---|---|---|----------------------|----------------------|
| e. | <i>against LTD<sub>4</sub>-induced IP1 production by the antagonist</i> |                      |                      |
| f. | <i>antagonist binding assay in human lung parenchyma</i>                |                      |                      |

**Table 3. Effects of cysteinyl leukotriene receptors on cancer cells**

| CysLT            | Receptor             | Function               | Pathways   | Comments  |
|------------------|----------------------|------------------------|--|---|
| LTD <sub>4</sub> | CysLT <sub>1</sub> R | chemotaxis             | PKC $\alpha$ /Raf-1 + PTX-sensitive G-protein    | - increases MAPkinase protein expression  |
| LTD <sub>4</sub> | -                    | survival               | COX2, $\beta$ -catenin, and Bcl-2                | - increases expression of COX-2, $\beta$ -catenin   |
| LTD <sub>4</sub> | -                    | survival               | PKC  | - induced relocalisation of vinculin accom  |
| LTD <sub>4</sub> | -                    | proliferation          | 1. PKC $\epsilon$ -Raf-1-MEK1/2-ERK1/2<br>2. Ras | - activates ERK-1/2 via a PKC $\epsilon$ /Raf-1/M   |
| LTD <sub>4</sub> | -                    | proliferation survival | MAPK/Erk-1/2<br>COX2                             | - increases COX-2 expression via activation of the MAPK/Erk-1/2 pathway - increases Bcl-2 protein levels<br>(Wikström et al., 2003) |
|                  |                      |                        |  | Intestinal<br>Colorectal cancer   |
|                  |                      |                        |  | Int407<br>Caco-2  |

|                  |   |                        |                              |   |            |        |                                 |
|------------------|---|------------------------|------------------------------|---|------------|--------|---------------------------------|
| LTD <sub>4</sub> | - | proliferation survival | MAPK/Erk-1/2<br>COX2         | - increases COX-2 expression via activation of the MAPK/Erk-1/2 pathway - increases Bcl-2 protein levels    | Intestinal | Int407 | (Wikström et al., 2003)         |
| LTD <sub>4</sub> | - | proliferation survival | PKC /Raf-1/ ERK1/2/p90RSK    | - increases proliferation by activating p90 <sup>RSK</sup> protein in a PKC /Raf-1/ERK1/2-dependent process | Intestinal | Int407 | (Paruchuri and Sjölander, 2003) |
| LTD <sub>4</sub> | - | survival               | PKC $\alpha$ /CREB           | - increases survival by activating CREB in a PKC $\alpha$ -dependent process                                | Intestinal | Int407 | (Wikström et al., 2003)         |
| LTD <sub>4</sub> | - | survival               | Caspase 8 and Bid<br>MEK/Erk | - prevents apoptosis induced by COX2 inhibition by lowering caspase 8 activation and Bid truncation         | Intestinal | Int407 | (Wikström et al., 2003)         |

|                  |                      |                        |                                |   |                   |        |                            |
|------------------|----------------------|------------------------|--------------------------------|---|-------------------|--------|----------------------------|
| LTD <sub>4</sub> | -                    | proliferation survival | MAPK/Erk-1/2<br>COX2           | - increases COX-2 expression via activation of the MAPK/Erk-1/2 pathway - increases Bcl-2 protein levels  | Intestinal        | Int407 | (Wikström et al., 2003)    |
| LTD <sub>4</sub> | -                    | proliferation survival | PI3K/Akt/GSK-3β<br>PKC/Erk-1/2 | - induces β-catenin translocation to the nucleus and association with cell survival protein Bcl2 mediated by a PI3K-dependent phosphorylation of GSK-3β | Intestinal        | Int407 | (Mezhybovska et al., 2006) |
| LTD <sub>4</sub> | CysLT <sub>1</sub> R | proliferation          | -                              | - increases cell proliferation  | Colorectal cancer | Caco-2 | (Magnusson et al., 2007)   |

|                  |                      |                          |   |   |                   |                                      |                          |
|------------------|----------------------|--------------------------|---|---|-------------------|--------------------------------------|--------------------------|
| LTD <sub>4</sub> | -                    | proliferation survival   | MAPK/Erk-1/2 COX2                         | - increases COX-2 expression via activation of the MAPK/Erk-1/2 pathway - increases Bcl-2 protein levels  | Intestinal        | Int407                               | (Wikström et al., 2003)  |
| LTC <sub>4</sub> | CysLT <sub>2</sub> R | differentiation survival | alkaline phosphatase and aminopeptidase N | - induces cell differentiation markers: increases alkaline phosphatase activity and enhances NaBT-induced increase of aminopeptidase N activity - increases caspase 3 activity and enhances sodium butyrate-induced apoptosis | Colorectal cancer | Caco-2                               | (Magnusson et al., 2007) |
| LTD <sub>4</sub> | CysLT <sub>1</sub> R | adhesion proliferation   | p44/42 ERK/MAPK Pyk 2                     | - upregulates α4β1 and α5β1 integrin-dependent adhesion - enhances cytokine-induced proliferation   | Stem cells        | CD34+ hematopoietic progenitor cells | (Boehmler et al., 2009)  |

|                  |                      |                        |                   |  |                     |        |                         |
|------------------|----------------------|------------------------|-------------------|--|---------------------|--------|-------------------------|
| LTD <sub>4</sub> | -                    | proliferation survival | MAPK/Erk-1/2 COX2 | - increases COX-2 expression via activation of the MAPK/Erk-1/2 pathway - increases Bcl-2 protein levels                   | Intestinal          | Int407 | (Wikström et al., 2003) |
|                  | CysLT <sub>1</sub> R | migration metastasis   | EGF/Rac1/Tiam1    | LTC <sub>4</sub> /LTD <sub>4</sub> regulates the second EGF-induced wave of Rac1 activation by increasing Tiam1 expression | Epidermal carcinoma | A431   | (Magi et al., 2014)     |

|                  |                      |                         |                   |   |                   |         |                         |
|------------------|----------------------|-------------------------|-------------------|---|-------------------|---------|-------------------------|
| LTD <sub>4</sub> | -                    | proliferation survival  | MAPK/Erk-1/2 COX2 | - increases COX-2 expression via activation of the MAPK/Erk-1/2 pathway - increases Bcl-2 protein levels  | Intestinal        | Int407  | (Wikström et al., 2003) |
| LTD <sub>4</sub> | CysLT <sub>1</sub> R | Proliferation migration | GSK-3β/β-catenin  | - increases β-catenin expression - induces β-catenin nuclear translocation and the subsequent transcription of MYC and CCND1-induced EMT : reduces expression of E-cadherin and β-catenin at the plasma membrane-increases migration and proliferation-inhibits GSK-3β activity (increases its phosphorylation) | Colorectal cancer | HCT-116 | (Salim et al., 2014)    |

|                  |                      |                                 |                   |  |                   |                 |                            |
|------------------|----------------------|---------------------------------|-------------------|--|-------------------|-----------------|----------------------------|
| LTD <sub>4</sub> | -                    | proliferation survival          | MAPK/Erk-1/2 COX2 | - increases COX-2 expression via activation of the MAPK/Erk-1/2 pathway - increases Bcl-2 protein levels   | Intestinal        | Int407          | (Wikström et al., 2003)    |
| LTD <sub>4</sub> | CysLT <sub>1</sub> R | stemness survival proliferation | Bcl-2 Bcl-3 COX-2 | - increases ALDH+ CICs population - increases gene expression of Bcl-2 and Bcl-3 - increases expression of COX-2 - increases colony formation capacity -increases tumor growth in a xenograft model of colon cancer - increases resistance to radiation and 5-FU | Colorectal cancer | HCT-116, Caco-2 | (Bellamkonda et al., 2015) |

|                  |                      |                        |                      |   |                   |        |                          |
|------------------|----------------------|------------------------|----------------------|---|-------------------|--------|--------------------------|
| LTD <sub>4</sub> | -                    | proliferation survival | MAPK/Erk-1/2<br>COX2 | - increases COX-2 expression via activation of the MAPK/Erk-1/2 pathway - increases Bcl-2 protein levels  | Intestinal        | Int407 | (Wikström et al., 2003)  |
| LTD <sub>4</sub> | CysLT <sub>1</sub> R | invasion migration     | β-catenin<br>MMP-9   | - increases expression of MMP-9<br>- induces EMT markers (stabilization/nuclear translocation of β-catenin)<br>- increases invasion   | Colorectal cancer | SW480  | (Vinnakota et al., 2017) |
| LTC <sub>4</sub> | CysLT <sub>2</sub> R | Differentiation        | JNK/AP-1             | - upregulates tumor suppressor 15-PGDH via JNK/AP-1 phosphorylation - increases expression of differentiation markers sucrase-isomaltase and mucin-2 - downregulates COX-2 expression | Colorectal cancer | HT-29  | (Mehdawi et al., 2017)   |

|                  |                      |                        |   |  |                   |                 |                                 |
|------------------|----------------------|------------------------|---|--|-------------------|-----------------|---------------------------------|
| LTD <sub>4</sub> | -                    | proliferation survival | MAPK/Erk-1/2<br>COX2                                | - increases COX-2 expression via activation of the MAPK/Erk-1/2 pathway - increases Bcl-2 protein levels   | Intestinal        | Int407          | (Wikström et al., 2003)         |
| LTC <sub>4</sub> | CysLT <sub>2</sub> R | survival migration     | β-catenin<br>Bcl-2<br>Caspase 3<br>MMP-2/9<br>VEGFR | - phosphorylates β-catenin - downregulates Bcl-2 and activates Caspase-3 - induces 15-PGDH in a TNFα dependent manner - decreases MMP-2 and -9, and VEGFR-1 levels | Colorectal cancer | HT-29<br>Caco-2 | (Satapathy and Sjolander, 2018) |
| LTD <sub>4</sub> | CysLT <sub>1</sub> R | metabolism             | -   | - upregulates COX-2 expression - increases CysLT <sub>1</sub> R and CysLT <sub>2</sub> R expression  | Intestinal        | Int407          | (Yudina et al., 2008)           |



|                  |               |                        |                   |  |            |        |                            |
|------------------|---------------|------------------------|-------------------|--|------------|--------|----------------------------|
| LTD <sub>4</sub> | -             | proliferation survival | MAPK/Erk-1/2 COX2 | - increases COX-2 expression via activation of the MAPK/Erk-1/2 pathway - increases Bcl-2 protein levels                                 | Intestinal | Int407 | (Wikström et al., 2003)    |
| LTD <sub>4</sub> | not specified | metabolism             | β-catenin         | - increases NADPH, ATP/ADP ratio and transcription of mtDNA genes via β-catenin accumulation - increases ROS levels and subsequent NF-κB | Intestinal | Int407 | (Mezhybovska et al., 2009) |

## FIGURE LEGENDS

**Figure 1. Leukotrienes, structures and biosynthesis. A. Key enzymes and metabolites of the leukotriene cascade** AA is converted by 5-LOX in conjunction with FLAP into LTA<sub>4</sub> through 5-HPETE. The leukotrienes are divided into two class, LTA<sub>4</sub>H metabolites such as LTB<sub>4</sub> and LTC<sub>4</sub>S metabolites, cysteinyl leukotrienes. **B. Biosynthesis pathway of cysteinyl leukotrienes.** Upon cellular stimulation, intracellular Ca<sup>2+</sup> is increased leading to translocation of cPLA<sub>2</sub>α and 5-LOX to the nuclear membrane. cPLA<sub>2</sub>α releases membrane phospholipids to produce free AA which is presented to 5-LOX by FLAP and further converted to LTA<sub>4</sub>. LTA<sub>4</sub> is conjugated to reduced glutathione by LTC<sub>4</sub>S to form LTC<sub>4</sub>, which is exported by MRP 1 and 4. In the extracellular space, LTC<sub>4</sub> is converted to LTD<sub>4</sub> by GGT and LTD<sub>4</sub> to LTE<sub>4</sub> by DPEP. Cysteinyl leukotrienes LTC<sub>4</sub> and LTD<sub>4</sub> can bind to two major receptors CysLT<sub>1</sub>R and CysLT<sub>2</sub>R whereas GPR99, or CysLT<sub>3</sub>R, is a receptor with high affinity to LTE<sub>4</sub>. The thickness of the arrows represents the receptor/ligand binding affinity. **C. Regulation of the expression of the enzymes of the cysteinyl leukotriene pathway.** Various cytokines and molecules have been shown to upregulate (in green) or downregulate (in red) the expression of 5-LOX, LTC<sub>4</sub>S or GGT. Abbreviations: 5-HPETE, 5-hydroperoxyeicosatetraenoic acid; 5-LOX, 5-lipoxygenase; AA, arachidonic acid; cPLA<sub>2</sub>α, cytosolic phospholipase A2; DPEP, dipeptidase; FLAP, 5-lipoxygenase-activating protein; GGT, γ-glutamyl transpeptidases; MRP, multidrug resistance-associated protein.

**Figure 2. Role of cysteinyl leukotrienes in carcinogenesis.**

Figure 1

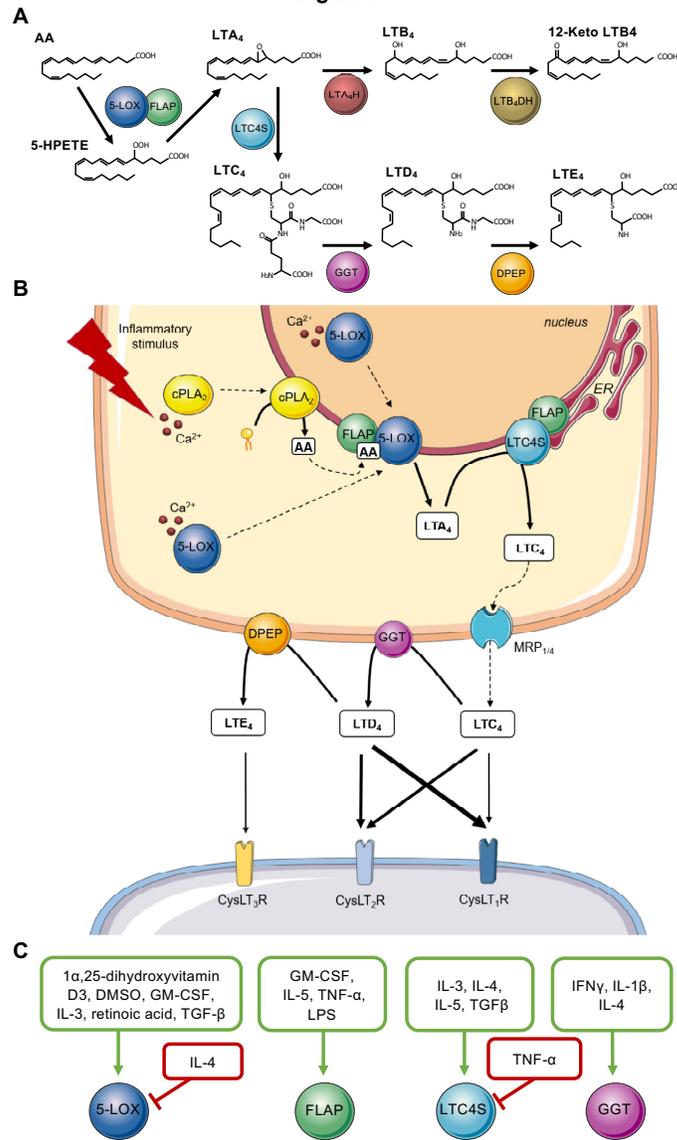


Figure 2

