Review Article

BLT2 is a pro-tumorigenic mediator during cancer progression and a therapeutic target for anti-cancer drug development

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Abstract: Cancer is a leading cause of death worldwide and has been linked to inflammation. Leukotriene B\(_4\) (LTB\(_4\)) is synthesized from arachidonic acid via the 5-lipoxygenase pathway and is a potent chemoattractant for inflammatory cells. LTB\(_4\) was recently shown to be associated with the pathogenesis of inflammatory diseases, including cancer. Of the two known LTB\(_4\) receptors, BLT1 and BLT2, the biological roles of the low-affinity LTB\(_4\) receptor 2, BLT2, have only recently been elucidated. This review focuses on recent discoveries regarding BLT2 and its roles in cancer progression and the downstream signaling mechanisms of the BLT2-linked signaling cascade in cancer cells. We believe that these findings will facilitate the development of new cancer treatments.

Keywords: Leukotriene B\(_4\) receptor 2 (BLT2), leukotriene B\(_4\), NADPH oxidase, reactive oxygen species, nuclear factor-kB, cancer progression

Introduction

Cancer caused the deaths of 8 million people worldwide in 2010, a 38% increase from 1990 according to the Global Burden of Disease Study 2010 [1]. The development of cancer requires a series of distinct but interconnected properties that allow cancer cells to survive, proliferate, and spread. These characteristics were described as the “Hallmarks of Cancer” by Hanahan and Weinberg [2]. A newly recognized characteristic of cancer is tumor-promoting inflammation [2]. The inflammatory microenvironment creates a local environment suitable for tumor initiation and growth and is hypothesized to be involved in the majority of tumors, even tumors in which a causal relationship with inflammation has not yet been confirmed [3-6].

A pro-inflammatory lipid mediator, LTB\(_4\), plays a particularly important role in the tumor microenvironment because it is a very potent chemoattractant involved in neutrophil migration [7-9]. In addition, LTB\(_4\) helps attract and activate other leukocytes at the inflammation site, including macrophages [10], eosinophils [11], immature mast cells [12], dendritic cells [13], and T cells [14]. LTB\(_4\) is synthesized from arachidonic acid (AA), which is released from membrane phospholipids via the activity of cytosolic phospholipase A\(_2\) (cPLA\(_2\)) (Figure 1) [15]. Free AA is subsequently metabolized to LTA\(_4\) by 5-lipoxygenase (5-LOX) in conjunction with 5-LOX-activating protein (FLAP). LTA\(_4\) is then hydrolyzed by LTA\(_4\) hydrolase (LTA\(_4\)H) to LTB\(_4\) [16, 17]. Many studies have demonstrated that LTB\(_4\) is involved in numerous inflammatory diseases [18, 19], including asthma [20], atherosclerosis [21], arthritis [22], chronic obstructive pulmonary disease [23], and inflammatory bowel disease [24]. More recently, LTB\(_4\) has been shown to be involved in numerous aspects of cancer. Treatment with 5-LOX, FLAP, and LTA\(_4\)H inhibitors decreases cancer cell survival and reduces both the number and volume of tumors in mouse models [10, 19].

LTB\(_4\) mediates biological functions via the G-protein-coupled receptors (GPCRs) LTB\(_4\) receptor-1 (BLT1) and LTB\(_4\) receptor-2 (BLT2) [25]. Until recently, the majority of studies on LTB\(_4\) receptors focused on BLT1, a high-affinity LTB\(_4\) receptor first discovered in 1996 as a
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The role of BLT2 in cancer progression was first recognized when we demonstrated that BLT2 is upregulated in a variety of human cancers and that BLT2 blockade significantly attenuates Ras-induced transformation [31]. Because Ras signaling pathways are associated with various aspects of cancer progression [32], the proposed link between BLT2 and Ras signaling suggested new roles for BLT2 in human cancer. Increasing evidence indicates that BLT2 plays a critical role in mediating different aspects of cancer development, such as proliferation, survival, angiogenesis, invasion, and metastasis (Table 1) [33-41]. Consequently, elucidating the function of BLT2 may be relevant to cancer therapy.

For example, treatment with the BLT2-specific antagonist LY255283 or transfection with BLT2 siRNA induces cell cycle arrest and apoptosis in androgen receptor (AR)-positive prostate cancer cells [37], bladder cancer cells [38], and estrogen receptor (ER)-negative breast cancer cells [35], suggesting that BLT2 plays a role in mediating cancer cell survival. In addition, BLT2 may be important in angiogenesis because it is involved in the signaling pathways of angiogenic factors such as vascular endothelial growth factor (VEGF) and interleukin-8 (IL-8) [42, 43]. VEGF is a very potent angiogenic factor that is involved in tumor growth. VEGF/VEGFR signaling results in vascular permeability and endothelial cell survival, mitogenesis, migration, and differentiation [44]. Activation of BLT2 by its ligands significantly enhances VEGF-mediated angiogenesis in vitro as well as in vivo [42]. Furthermore, in mast cells, IL-8 synthesis by IL-1β appears to be mediated by BLT2-induced activation of nuclear factor-κB (NF-κB) [43]. In these studies, inhibition of BLT2 by LY255283 or siRNA markedly attenuated angiogenic activity.

We have also demonstrated the significance of BLT2 in mediating cancer invasiveness and metastasis. For example, in aggressive bladder cancer cells, BLT2 markedly enhances invasiveness by upregulating matrix metalloproteinase-9 (MMP-9) [36]. BLT2 also increases the invasiveness of ovarian cancer cells via a different pathway, with MMP-2 as the effector [39]. In aggressive breast cancer cells, BLT2 confers invasiveness via IL-8 regulation [40]. In these studies, metastatic nodule formation in mice was induced by the injection of cancer cells and was significantly attenuated by BLT2 inhibition with either an antagonist or by siRNA transfection. In addition, we recently demonstrated that BLT2-ROS signaling through MMP-9 is involved in Ras-induced invasiveness because inhibition of BLT2 function results in a significant decrease in Ras-induced invasiveness [33].

Figure 1. Schematic of LTB4 Biosynthesis. When a cell is activated, the increase in the intracellular Ca2+ concentration triggers the translocation of cPLA2 and 5-LOX to the nuclear membrane. Free arachidonic acid released by cPLA2 is metabolized to LTA4 and LTB4 via 5-LOX and LTA4H, respectively.
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Another group recently reported that BLT2 might be involved in the metastasis of pancreatic cells [45]. These studies demonstrate the importance of BLT2 in mediating invasion and metastasis in cancer, thereby presenting new opportunities for the treatment of malignant tumors.

Downstream signaling mechanisms of BLT2 in cancer cells

**NF-κB downstream of BLT2**

NF-κB is a pleiotropic transcription factor that is activated by a broad range of stimuli and is an integral protein in a number of cell signaling pathways. It induces the expression of genes involved in the regulation of biological responses such as immune responses, inflammation, and cell survival [46, 47]. NF-κB plays a critical role in the progression of the majority of cancers [48-50]. In normal resting cells, NF-κB is usually inactive. In this state, NF-κB is bound to inhibitors of kappa B (IκB) in the cytosol until it is activated by a wide array of stimuli. However, in cancer cells, NF-κB is often constitutively active, not as a result of either an IκB loss-of-function mutation or an IκB kinase (IKK) gain-of-function mutation but as a result of persistent “normal” activation in most cases [50, 51]. Our recent studies have indicated that BLT2 stimulates NF-κB activity in prostate, breast, and bladder cancer cells (Table 1 and Figure 2). BLT2 appears to mediate the activation of MMP-9 by stimulating NF-κB activity through ROS production via NOXs. This appears to promote an aggressive cancer phenotype, suggesting a critical role for BLT2 in mediating invasion and metastasis. For example, in Ras-transformed cells, NF-κB activation by the BLT2-NOX1-ROS-linked signaling cascade is involved in the upregulation of MMP-9, which leads to invasion and metastasis [33]. In aggressive 253J-BV bladder cancer cells, the BLT2-NOX1/4-ROS-NF-κB-linked signaling cascade also appears to play a role in invasion and metastasis by controlling MMP-9 [36]. Another downstream component of NF-κB is IL-8, which is a key factor in cancer invasion and angiogenesis [52, 53]. IL-8 binds to its receptors CXCR1 and CXCR2 and activates signaling pathways involving phosphatidylinositol-3-kinase (PI3K), phospholipase C, Akt, and mitogen-activated protein kinase (MAPK), resulting in the activation of various transcription factors that promote cell survival, angiogenesis, and invasion [54]. In the highly aggressive human breast cancer cell lines MDA-MB-231 and MDA-MB-435 and the mast cell line HMC-1, the activation of BLT2 markedly upregulates NOX1-dependent ROS generation, which in turn induces NF-κB-dependent IL-8 synthesis [40, 43]. In aggressive breast cancer cells, IL-8 synthesis via the BLT2-NOX1-ROS-NF-κB-linked signaling cascade contributes to invasiveness and metastasis [40]. For mast cells, the pro-inflammatory cytokine IL-1β mediates IL-8 synthesis in part through the upregulation of BLT2 expression and LTB₄ synthesis, which leads to NF-κB activation and significantly contributes to angiogenic activities [43]. The androgen receptor (AR) was previously reported to be upregulated by NF-κB in prostate cancer [55]. AR is a nuclear transcription factor that belongs to the steroid receptor superfamily and is activated by androgen to
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mediate normal development and survival of the prostate [56, 57]. However, in androgen-responsive and more progressed castration-resistant prostate cancer, AR mediates the aberrant survival and proliferation of cancer cells [57, 58]. We observed that ROS produced by BLT2-mediated NOX4 activation stimulated NF-κB-mediated AR expression. Moreover, treatment with the inhibitor 12-LOX decreased AR expression and the survival of AR-positive LNCaP prostate cancer cells, indicating that 12(S)-HETE is the ligand that activates BLT2. In conclusion, the 12(S)-HETE-BLT2-NOX4-ROS-NF-κB-linked signaling cascade upregulates AR expression to positively affect the survival of AR-positive prostate cancer cells [37].

JAK/STAT3 downstream of BLT2

The downstream pathways of BLT2 also involve other downstream components in addition to NF-κB (Table 1 and Figure 2). For example, we have established that BLT2 confers MMP-2-mediated invasiveness by regulating Tyr705 phosphorylation-induced activation of signal transducer and activator of transcription-3 (STAT3) in ovarian cancer [39]. Janus kinase 2 (JAK2) is phosphorylated when a membrane receptor is dimerized by a ligand and phosphorylates STAT3 at Tyr705 for activation [59, 60]. Constitutive activation of STAT3 has been reported to mediate cancer development in different types of ovarian cancer [62, 63]. Previous studies have also established a link between STAT3 and MMP-2 in promoting cancer cell invasiveness [64, 65]. Therefore, BLT2 and NOX4 inhibition were used to demonstrate that the STAT3-MMP-2 pathway lies downstream of the BLT2-NOX4-ROS pathway. There is also evidence suggesting that the ROS produced by NOXs activate the signaling pathway leading from JAK by inhibiting tyrosine phosphatases (PTPs) that dephosphorylate JAK [66]. We propose that the BLT2-NOX4-ROS pathway activates the JAK2-STAT3-MMP-2 pathway in ovarian cancer cells to promote invasiveness and
metastasis by inhibiting PTPs [39]. However, the details of this mechanism must be further elucidated.

**ERK/AKT downstream of BLT2**

In bladder cancer cells, the BLT2-NOX1/4-ROS signaling cascade-induced survival of cancer cells appears to involve ERK and Akt phosphorylation [38]. ERK, which is a MAPK, and the PI3K/Akt signaling pathway have been implicated in the promotion of cell survival under mild oxidative stress conditions [67, 68]. BLT2 may mediate ERK phosphorylation in pancreatic cancer [45], and ERK appears to function downstream of the BLT2-ROS cascade in keratinocytes [69]. There are also reports indicating that Akt and BLT2 phosphorylate each other [38, 70], further demonstrating the relationship between BLT2, ERK, and Akt. BLT2 and NOX1/4 knockdown significantly reduce ERK and Akt phosphorylation [39], indicating that ERK and Akt are critically involved in the BLT2 signaling pathway that promotes cell survival in bladder cancer. The detailed signaling mechanisms, however, remain to be characterized.

Cross-talk exists between signaling pathways, such as between the RAS/RAF/ERK and RAS/PI3K/AKT pathways or between the PI3K/AKT and stress-activated protein kinase (SAPK) pathways [67, 68, 71]. BLT2 may be involved in this cross-talk because it is involved in Ras-mediated activities, Akt-mediated cell survival, and SAPK pathway-mediated apoptosis. There is also evidence that NF-kB and STAT-3 cooperate during tumorigenesis [50]. Studies are needed to determine if and the mechanism by which these signaling pathways are interconnected through BLT2; these signaling pathways could behave differently depending on the cell type and stress type.

**Conclusions and future perspectives**

In this review, we have elucidated the effects of the low-affinity LTB₄ receptor BLT2 on cancer progression. In the process of evolving into a neoplastic state and becoming malignant, cells acquire hallmark characteristics of cancer, including sustainment of proliferative signals, activation of invasion and metastasis, induction of angiogenesis, and evasion of cell death [2]. The studies reviewed here demonstrate that these characteristics are likely to be mediated, at least in part, by BLT2 activity. As shown in Figure 2, activation of BLT2 by its ligands activates a number of signaling pathways that lead to cancer phenotypes for a variety of cancers. By controlling ROS production through NOXs, BLT2 mediates the actions of critical components of intracellular signaling pathways such as Akt, JAK2, STAT3, ERK, and NF-kB, which help confer cancer phenotypes to normal cells. The exact mechanisms by which BLT2-induced ROS controls those proteins remain to be determined. Because studies of the role of BLT2 as an integral part of cancer progression have only recently begun, the mechanism by which BLT2 interacts with other signaling pathways must be further elucidated in future studies. Nonetheless, BLT2-mediated ROS production and subsequent cross-talk among the different signaling pathways may hold enormous potential for the treatment of cancer.

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