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Title: BLT2, a leukotriene B4 receptor 2, as a novel prognostic biomarker of triple-negative breast cancer

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Running Title: Role of BLT2 in TNBC progression

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ABSTRACT

Triple-negative breast cancer (TNBC) is considered to be a notorious type of cancer due to its aggressive metastatic potential and poor prognosis. Recent evidence suggests that BLT2, a low-affinity LTB_4 receptor is critically associated with the phenotypes of TNBC cells, including invasion, metastasis, and survival. Furthermore, in a group of 545 breast cancer patients with metastasis, we observed that the high-BLT2 subgroup had a lower disease-free-survival rate than the low-BLT2 subgroup. Thus, we theorized that anti-BLT2 strategies could facilitate the development of new therapies used for TNBC. This review focuses on recent discoveries regarding BLT2 and its roles in as a novel prognostic biomarker in TNBC.

Keywords:

Triple-negative breast cancer, TNBC; leukotriene B4 receptor-2, BLT2

Breast cancer is the most common cancer diagnosis in women and the second leading cause of cancer-related death in women worldwide. Several different subtypes of breast cancers have been identified. Triple-negative breast cancer (TNBC), being among the most devastating types of breast cancer, is a disease with high morbidity and mortality rates. TNBC patients account for only 15-20% of all breast cancer patients (1), and patients with TNBC have an increased likelihood of distant recurrence and death compared to patients with other types of breast cancer. TNBC patients also tend to develop visceral metastases early during their disease. A recent study reported the five-year survival rate for TNBC as approximately 30% lower than other types of breast cancer (2). TNBC is very heterogeneous and lacks expression of the estrogen receptor (ER), progesterone receptor (PR), and epidermal growth factor receptor 2 (HER2). The absence of ER/PR/HER2 reduces the therapeutic effects of many targeted anti-cancer drugs, cytotoxic drugs, and radiation; as a result, patients with TNBC are only managed with standard chemotherapy (3). Therefore, the identification of new therapeutic targets for TNCB is urgently needed.

Leukotriene B_4 (LTB_4) is synthesized from arachidonic acid via the 5-lipoxygenase pathway, and it mediates intracellular functions via G protein-couple receptors (GPCRs): LTB_4 receptor 1 (BLT1) and LTB_4 receptor 2 (BLT2) (4). BLT2 is a low-affinity LTB_4 receptor that can be stimulated through various ligands, such as 12(S)-hydroxy-5(Z),8(Z),10(E),14(Z)-eicosatetraenoic acid (12(S)-HETE), and 12(S)-hydroxy-5(Z),8(E),10(E)-heptadecatrienoic acid (12-HHT), as well as LTB_4 (5). Recent studies have suggested that BLT2 is closely associated with various aspects of survival, invasion, and metastasis in TNBC (6). This review focuses on the recently discovered roles of BLT2 in TNBC progression.

Role of BLT2 in the survival and proliferation of TNBC cells

TNBC cells are known to be insensitive to most specific target treatments, including targeted small-molecule drugs (7). Therefore, conventional chemotherapeutic agents, such as Taxol and anthracyclines, are still the preferred treatments for TNBC patients. However, these drugs are not targeted and hair loss, diarrhea, nausea, and vomiting are among their many undesirable side effects. Many TNBC studies focus on survival and proliferation mechanisms in order to identify more effective therapeutic treatments (8). One of the mechanisms for TNBC progression is the generation of reactive oxygen species (ROS). ROS are tumorigenic by virtue of their ability to increase cell proliferation, survival, and DNA damage induction, leading to genetic lesions that cause tumorigenicity and sustain subsequent tumor progression (9,10). Due to their stimulating actions on TNBC proliferation, up-regulation of ROS generation plays an important role in aggressive cancer phenotypes (11). In TNBC, tumor-associated ROS are produced from nicotinamide adenine dinucleotide phosphate-oxidase (NOX) complexes. Recent studies have showed that the NOX1 cascade attenuates the transcriptional activity of tumor suppressor and tumor cell apoptosis processes (12). Our previous study showed that BLT2 is critically associated with the generation of ROS via NOX1, supporting the proliferation and survival of human TNBC MDA-MB-468 cells (13). In summary, BLT2 is suggested to play critical role in TNBC survival and proliferation (Figure 1).

Role of BLT2 in the interaction between TNBC cells and immune cells

The interactions between cancer and immune cells have been suggested to be important components of tumor and TNBC progression (14,15). Cell adhesion molecules are involved in cell to cell interaction, which is an especially important component in the cancer-immune system (16,17). Intercellular adhesion molecule 1 (ICAM-1) is a member of the immunoglobulin superfamily and is expressed on many cell types, such as leukocytes,

endothelial cells, and cancer cells (18,19). In these cell types, ICAM-1 can be up-regulated in response to pro-inflammatory cytokines or stimuli (20). Moreover, studies have shown that ICAM-1 promotes TNBC progression, aggressiveness, and shorter recurrence-free survival (21-24). Our recent study demonstrated that BLT2 regulates LPS-induced ICAM-1 expression in MDA-MB-231 cells via BLT2-ERK-NF-kB-linked cascades (Figure 1). Therefore, up-regulation of BLT2 in TNBC potentially increases TNBC adhesion to monocytes (25). Our findings suggest that BLT2 can influence the microenvironment of TNBC, suggesting another critical role for BLT2 in TNBC progression.

Role of BLT2 in the invasion and metastasis of TNBC cells

The metastatic phenotype is a major cause of death in cancer patients. Various mechanisms are involved in the metastasis of TNBC, including the acquisition of invasive characteristics in the tumor microenvironment (26,27). Moreover, this particular cancer type shows enhanced metastatic potential. Patients with TNBC are known to have an increased risk of metastasis and distant recurrence compared to patients with other types of breast cancer (28). Given that metastasis can significantly contribute to cancer severity, finding new therapeutic targets for these types of metastatic, aggressive breast cancer is critical for treating patients.

Recently, pro-inflammatory cytokine interleukins (IL)-6 and IL-8 have been suggested to play roles in the invasion and metastasis of breast cancer (29). IL-6 has reportedly been correlated with TNBC progression, and clinical studies have shown increased IL-8 levels in aggressive breast tumor tissue when compared with normal breast tissue (30,31). Due to the importance of pro-inflammatory cytokines in TNBC invasion, in-depth study is needed to validate a better target for inflammatory signaling. Our recent studies have shown that IL-6 and IL-8 are produced through a BLT2-dependent pathway (6,32) (Figure 1). Kim et al. found that

BLT2 expression was increased in human TNBC cell lines, human TNBC MDA-MB-231, and MDA-MB-435 cells when compared with its expression in a human breast cancer cell line, MCF-10A. Therefore, we examined whether BLT2 can reduce the invasiveness and secretion of IL-8. MDA-MB-231 and MDA-MB-435 cells were treated with a specific BLT2 antagonist LY255283 or transfected with BLT2 small interfering RNA (siRNA). In these experiments, we demonstrated that IL-8 secretion, invasiveness in TNBC cells, and lung invasion in a TNBC xenograft mouse model are regulated in a BLT2-dependent manner. Park et al. also demonstrated that IL-6/IL-8 is downstream of the BLT2-dependent pathway in LPS-treated TNBC cell lines and an MDA-MB-231 xenograft mouse model. The activity of NF-κB is up-regulated in TNBC, and the NF-κB signaling pathway plays a crucial role in regulating the invasiveness of TNBC. In TNBC, NF-κB signaling cascades, which are related to invasion, are regulated by the BLT2-NOX-1 pathway, leading to the production of IL-6 and IL-8 in TNBC (Figure 1). Together, these observations suggest that BLT2 plays a critical role in TNBC cell invasion and metastasis via IL-6/IL-8 up-regulation.

Significance of BLT2 in lymph node-positive breast cancer patients and HER2-negative breast cancer patients

We analyzed BLT2 with *Breastmark* by dichotomizing gene expression data around a media into ‘high’ and ‘low’ expression, and with 50% of patient samples with lymph node metastasis in each group, in order to investigate whether BLT2 contributes to breast cancer patient survival (33,34). Figure 2 shows Kaplan-Meier curves for the high- and low-BLT2 subgroups; the high-BLT2 subgroup exhibited a lower disease-free-survival (DFS) rate than the low-BLT2 subgroup. Additionally, we fit a univariate COX proportional hazards model in which those with higher BLT2 expression are 1.34-fold more likely to have disease progression

than those with lower BLT2 expression (Hazard ratio (HR) = 1.34; 95% CI = (1.036, 1.735); p = 0.025584). Patients with a basal subtype in the high-BLT2 expression group also had significantly reduced DFS (data not shown). These results suggest that BLT2 plays a significant role in the lymph node metastasis of breast cancer patients. Another dataset showed the effect of BLT2 up-regulation, at the mRNA level, on overall survival rates (Figure 3). In the Metabric dataset (StudyID brca_metabric) from the cBioportal for Cancer Genomics (35), the overall survival of 56 cases with up-regulated BLT2, at mRNA level, was significantly lower than in the remaining whole sequenced cases (Figure 3) ($p = 0.041$). Furthermore, 52 of 56 cases with up-regulated BLT2 were identified as HER2-negative (Figure 3). In general, most patients with metastatic breast cancer have HER2-negative breast cancer (Reference PMID: 28942029 DOI: 10.1016/j.ctrv.2017.09.001), implying that BLT2 plays a role in the breast cancer malignancy of a subset of patients with metastasis and HER2 negativity. These results suggest that BLT2 is associated with malignancy in breast cancer, such as in TNBC.

CONCLUSION AND FUTURE PERSPECTIVES

In this review, we discussed the role of BLT2 in TNBC progression. TNBC is commonly associated with a poor prognosis, which is unpredictable and measured by considering standard clinicopathological values, such as lymph node status or size at initial presentation (36). The studies reviewed in this article demonstrate that these characteristics are likely to be mediated, at least in part, by BLT2. As shown in Figure 1, BLT2 activation through its ligands activates a number of signaling pathways that support the malignant phenotypes of TNBC cells. Our findings reveal a previously unrecognized role BLT2 plays in the progression of TNBC. Our study enhances the understanding of the molecular mechanism of TNBC and provides potential targets for developing new therapies to be used in the treatment of TNBC patients.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest regarding the contents of this article.

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FIGURE LEGENDS

Figure 1. Comprehensive overview of the BLT2 signaling pathways. See text for details.

Figure 2. The Kaplan-Meier curves of the overall survival rates of patients with lymph node metastasis stratified by BLT2 expression (n = 545; HR = 1.341; 95% CI = (1.036, 1.735), p = 0.03).

Figure 3. Overall survival of breast cancer patients with and without up-regulated BLT2 expression at the mRNA level and a pie chart of the patients' HER2 status. Absolute patient numbers for each subgroup are indicated.

Figure 1

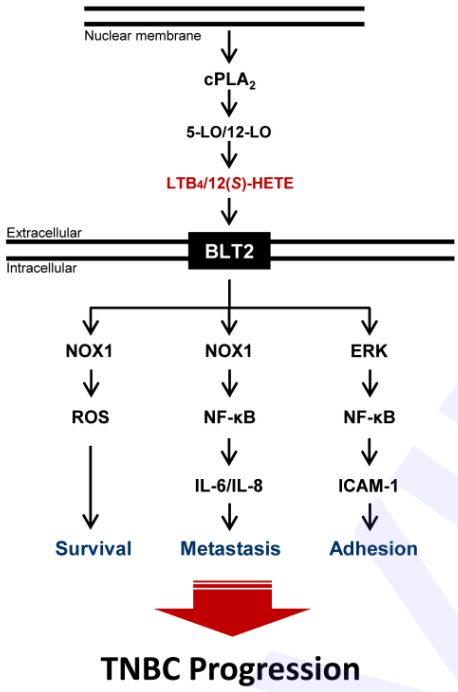


Fig. 1.

Figure 2

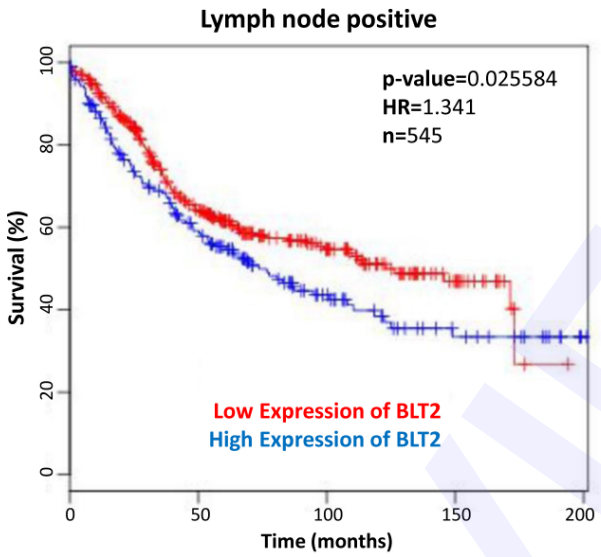
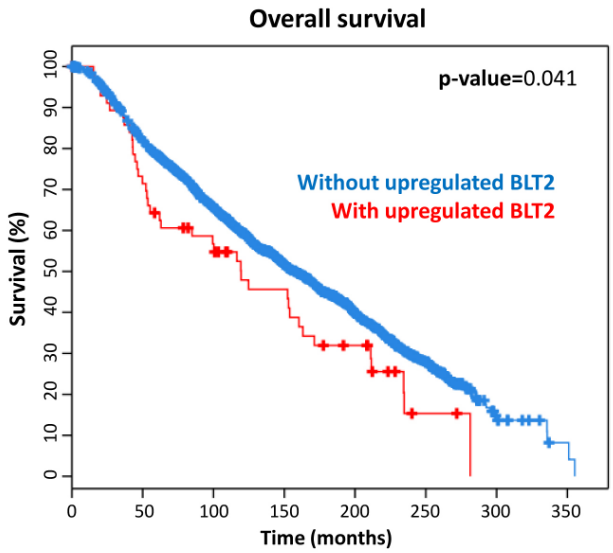


Fig. 2.

Figure 3



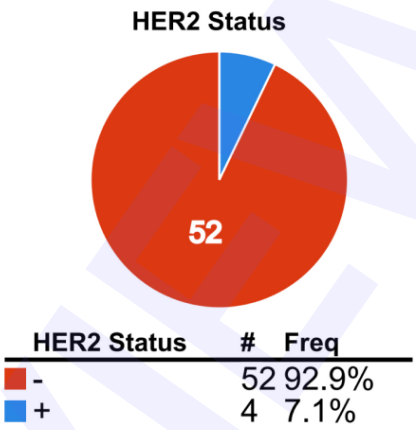


Fig. 3.