Ras promotes the epithelial-mesenchymal transition via a ‘leukotriene B₄/BLT2’-linked inflammatory axis

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The epithelial-mesenchymal transition (EMT) has been implicated in cancer invasion and metastasis. Depending on the cellular context, oncogenic Ras can induce EMT in the presence of transforming growth factor-β (TGF-β). By synergistically promoting the EMT in cooperation with TGF-β, oncogenic Ras promotes cell migration in vitro and tumor invasion and metastasis in vivo. Despite these observations, the mechanism by which oncogenic Ras contributes to the EMT is not well understood. We recently demonstrated that BLT2, a G protein-coupled receptor for the inflammatory lipid mediator leukotriene B₄ (LTB₄), lies downstream of Ras and mediates oncogenic Ras-induced EMT in mammary epithelial cells. Thus, these inextricable networks involving the Ras oncogene, EMT and inflammatory lipid mediators play critical roles in the tumor microenvironment.

Keywords: Ras; epithelial-mesenchymal transition; BLT2; inflammation; cancer; ROS; NF-κB

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In the tumor microenvironment, inflammation and inflammatory mediators are closely linked with cancer progression through complex networks [1, 2]. The levels of LTB₄, an inflammatory lipid mediator, and its receptor BLT2 are markedly up-regulated by oncogenic Ras, and these factors mediate Ras-associated tumorigenic activities [3, 4, 5]. BLT2 expression in ovarian and breast cancer tissues is also increased in advanced stages and is associated with poor clinical outcome [6, 7, 8]. Furthermore, autocrine or paracrine BLT2 signaling mediates ovarian, bladder and breast cancer cell invasion and metastasis [7, 9, 10]. Despite these reports implicating BLT2 as a potential mediator of aggressive metastatic cancer, its mechanism of action in the epithelial-mesenchymal transition (EMT) has not been characterized.

In our recent paper entitled “Ras Promotes Transforming Growth Factor-β (TGF-β)-induced Epithelial-Mesenchymal Transition via a Leukotriene B₄ Receptor-2-linked Cascade in Mammary Epithelial Cells” [11], we demonstrated that BLT2 lies downstream of Ras and collaborates with TGF-β to induce the EMT in mammary epithelial cells. Compared with MCF-10A immortalized human mammary epithelial cells, MCF-10A/Hras cells expressing oncogenic H-Ras and treated with TGF-β exhibited a highly branched morphology in a three-dimensional culture matrix concomitant with reduced E-cadherin expression and increased vimentin expression, which is characteristic of the EMT. In addition, MCF-
10A/Hras cells stimulated with TGF-β were more invasive than TGF-β-stimulated MCF-10A cells. However, BLT2 depletion using a BLT2-specific small interfering RNA (siBLT2) in MCF-10A/Hras cells greatly reduced the morphological alterations and the changes in epithelial and mesenchymal marker expression. Additionally, siBLT2 knockdown remarkably reduced the invasiveness of MCF-10A/Hras cells in response to TGF-β. Thus, these results suggest that BLT2 contributes to the EMT. In support of the notion that BLT2 acts as a potential contributor to the EMT, LTBA4 treatment or BLT2 overexpression enhanced EMT induction in the presence of TGF-β in MCF-10A cells. Together, these results suggest that an inflammatory BLT2-linked axis contributes to the EMT in mammary epithelial cells.

In our work, we examined the signaling pathways involved in BLT2-mediated stimulation of the EMT and discovered that ‘NADPH oxidase-1 (Nox1)-derived production of reactive oxygen species (ROS) and subsequent NF-κB activation’ acted downstream of BLT2 and promoted the EMT in the presence of TGF-β in MCF-10A/Hras cells [11]. We also found that Nox1 acted downstream of BLT2, and Nox1-induced ROS generation accentuated TGF-β-driven EMT via NF-κB activation. Consistent with the role of Nox1 in the EMT, Nox1 depletion via siRNA knockdown attenuated ROS generation as well as TGF-β-induced EMT [11]. These results are consistent with previous reports suggesting that ROS are involved in the EMT [12]. However, it was recently reported that the TGF-β-Smad3-Nox4 pathway could also contribute to ROS production, which may be critical for the progression of the EMT [13, 14]. In fact, we found that Nox4 mRNA levels, but not Nox1 mRNA levels, were markedly increased upon exposure to TGF-β and that TGF-β-induced up-regulation of Nox4 was not inhibited by BLT2 knockdown [11]. Therefore, we suspect that ‘Ras-BLT2-Nox1-derived ROS’ and ‘TGF-β-Smad-Nox4-derived ROS’ may additively stimulate NF-κB activation, which is thought to be the critical point where the Ras-BLT2 and TGF-β cascades converge to drive the EMT. In support of this hypothesis, siRNA-mediated depletion of both Nox1 and Nox4 in TGF-β-treated MCF-10A/Hras cells led to further inhibition of NF-κB activation and changes in EMT marker expression (e.g., E-cadherin and vimentin), whereas knockdown of either gene alone (Nox1 or Nox4) caused partial inhibition [11].

These results demonstrate for the first time that a BLT2-linked inflammatory pathway contributes to the EMT. BLT2 is stimulated by a few inflammatory lipid ligands; in addition to LTBA4, ligands of BLT2 include 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) [15, 16]. Ongoing research in our lab suggests that BLT2 is associated with many aspects of cancer progression [16]. Particularly, ROS generation via Nox has been closely associated with BLT2-mediated cancer progression [16]. Further understanding of the detailed roles of BLT2 and the downstream signaling cascade may provide valuable insight into the EMT and cancer progression. In addition, this knowledge will offer new perspectives and targets for the development of novel anti-cancer therapeutics.

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Conflict of interests

The author declares no conflict of interests.

References


