Activation of the Leukotriene B₄ Receptor 2- Reactive Oxygen Species (BLT2-ROS) Cascade following Detachment Confers Anoikis Resistance in Prostate Cancer Cells

Jin-Wook Lee and Jae-Hong Kim

From the College of Life Sciences and Biotechnology, Korea University, Seoul 136-701, Korea

Background: Despite the critical role of anoikis resistance in prostate cancer progression, the molecular mechanism causing anoikis resistance remains unclear.

Results: BLT2 protects prostate cancer cells against anoikis via a NOX-ROS-NF-κB-linked pathway.

Conclusion: Activation of the BLT2-linked pathway following cell detachment confers anoikis resistance.

Significance: Our findings suggest BLT2 as a key regulator of anoikis resistance in prostate cancer cells.

The majority of prostate cancer-related deaths are associated with advanced and metastatic malignancies. Although anoikis resistance has been recognized as one of the hallmarks of metastatic prostate malignancies, the molecular events that cause anoikis resistance are poorly understood. In this study, we found that the detachment of PC-3 prostate cancer cells caused a time-dependent increase in the expression level of the leukotriene B₄ receptor-2 (BLT2) and that BLT2 played a critical role in establishing anoikis resistance in these cells. Blocking BLT2 with the pharmacological inhibitor LY255283 or with RNAi knockdown clearly abolished anoikis resistance and resulted in severe apoptotic death. Additionally, we demonstrated that the activation of NADPH oxidase (NOX) and subsequent generation of reactive oxygen species (ROS) were downstream of BLT2 signaling and led to the activation of NF-κB, thus establishing anoikis resistance during cell detachment. Furthermore, we observed that the ectopic expression of BLT2 in normal prostate PWR-1E cells rendered the cells resistant to anoikis and apparently diminished apoptotic cell death following detachment. Taken together, our results suggest that BLT2-NOX-ROS-NF-κB cascade induction during detachment confers a novel mechanism of anoikis resistance in prostate cancer cells and potentially contributes to prostate cancer progression.

Prostate cancer is the most frequently diagnosed cancer and one of the leading causes of cancer-related death in men (1). The majority of prostate cancer-related deaths are associated with advanced and metastatic malignancies (1). Although radical prostatectomy, radiotherapy, and androgen ablation have been accepted as applicable medical treatments for tumors that are confined to the prostate, these therapies are not effective to metastatic disease. As a result, metastasis remains a major clinical challenge in the treatment of prostate cancer (2). Resistance to detachment-induced cell death known as anoikis resistance is a major contributor to the emergence of metastatic prostate cancer (3). Anoikis resistance is associated with a high degree of cancer metastasis and advanced stage cancers, which suggests that anoikis resistance is a hallmark of metastatic malignancies (4). The underlying mechanisms that render prostate cancer cells resistant to anoikis are not fully understood, but it has been postulated that tumor cell resistance to anoikis is driven by genetic alterations and aberrant signaling responses that are unique to the tumor microenvironment; these alterations could lead to the constitutive activation of integrin/growth factor signaling pathways and the inactivation of the core apoptotic machinery (5, 6). For example, several lines of evidence point to the NF-κB signaling pathway and the up-regulation of interleukin-6 (IL-6), CXCL-8, and epidermal growth factor (EGF) as initiators of molecular cross-communications that inhibit tumor cell anoikis within the tumor microenvironment (2, 7). Additionally, inflammatory responses in the tumor microenvironment can modulate the anoikis resistance of metastatic cancers (8–10).

Arachidonic acid metabolism by the COX or lipooxygenase (LOX)² pathways has been implicated in inflammatory pathogenesis and is thought to play critical roles in tumor promotion and prostate cancer progression (11, 12). For example, leukotriene B₄ (LTB₄), a 5-LOX-catalyzed product, and 12(S)-hydroxyeicosatetraenoic acid (HETE), a 12-LOX-catalyzed product, are overexpressed in prostate cancer tissues, and these eicosanoids have been suggested to act in an autocrine or paracrine manner in the tumor microenvironment to regulate cell survival, growth, and the metastatic potential of prostate cancer (11, 13–15). Additionally, growing evidence demonstrates that

---

* This work was supported by Bio & Medical Technology Development Program Grant 2012M3A9C5048709, Basic Science Research Program Grant 2012R1A2A2A01044526 through the National Research Foundation funded by the Ministry of Science, Information and Communication Technologies (ICT) and Future Planning, Republic of Korea, and the National Research and Development for Cancer Control Grant 1220020, Ministry for Health and Welfare, Republic of Korea. This work was also supported by Korea University Grant G1300090.

1 To whom correspondence should be addressed: College of Life Sciences and Biotechnology, Korea University, 5-1 Anam-dong, Sungbuk-gu, Seoul 136-701, Korea. Tel.: 82-2-3290-3452; Fax: 82-2-927-9028; E-mail: jhongkim@korea.ac.kr.

2 The abbreviations used are: LOX, lipooxygenase; BLT2, leukotriene B₄ receptor; DCFDA, 2’,7’-dichlorofluorescein diacetate; DMSO, dimethyl sulfoxide; DPI, diphenyleneiodonium; HETE, hydroxyeicosatetraenoic acid; LTB₄, leukotriene B₄; NOX, NADPH oxidase; PI, propidium iodide; ROS, reactive oxygen species; WST-1, water-soluble tetrazolium salt-1.
the LTB₄ cognate receptor BLT2 is highly expressed in various cancers, including colon, ovarian, and pancreatic cancers (16–19). BLT2 signaling has been suggested to be a potential mediator of metastasis in cancer progression, but the role of BLT2 in anoikis resistance has not been reported previously (20–22).

In this study, we have now found that increased BLT2 expression following detachment could confer anoikis resistance in prostate cancer cells and that the activation of NAPDH oxidase (NOX) and the subsequent generation of reactive oxygen species (ROS) acted downstream of BLT2 to confer anoikis resistance. Together, our observations suggest that activation of the BLT2-ROS cascade following detachment results in the prevention of anoikis and contributes to the progression of prostate cancer.

**EXPERIMENTAL PROCEDURES**

**Cell Culture, Chemicals, and Plasmids**—The immortalized human prostate epithelial cell line PWR-1E was obtained from the American Type Culture Collection (ATCC, Rockville, MD) and maintained in keratinocyte serum-free medium (In Vitrogen) supplemented with 0.05 mg/ml bovine pituitary extract, 5 ng/ml human recombinant EGF, 10% fetal bovine serum (FBS) (Hyclone), and antibiotic-antimycotic solution (Invitrogen) at 37 °C in a humidified atmosphere with 5% CO₂. The human prostate cancer cell line PC-3 was obtained from the Korean Cell Line Bank (Seoul, Korea) and maintained in RPMI 1640 medium (Invitrogen) supplemented with 10% FBS and antibiotic-antimycotic solution. Diphenyleneiodonium (DPI) and the appropriate ROS products.

**Semiquantitative RT-PCR and Real-time PCR Analysis**—Total cellular RNA was extracted with Easy Blue™ (Intron Company, Sungnam, Korea), and the extracted RNA was reverse-transcribed with M-MLV reverse transcriptase (Beams Biotechnology, Kyunggi, Korea). The resulting cDNA was then subjected to semiquantitative RT-PCR analysis with an RT-PCR PreMix kit (Intron) and specific primers (forward and reverse) respectively for human BLT2, NOX1, and NOX4 mRNAs were normalized to GAPDH for a quantitative analysis, and the meltcurves were visualized by staining with ethidium bromide. The PCR products were purified by agarose gel electrophoresis and visualized by staining with ethidium bromide.

For real-time PCR analysis, cDNA derived from total RNA was subjected to PCR, and the data were analyzed with Light-Cycler software 3.3 (Roche Applied Science). The following primers (forward and reverse, respectively) were used for real-time PCR analysis: 5' -CTCTCATCGGGCATCACAG-3' and 3' -ATCTTCGTCGCTAGCAG-3' for human BLT2, 5' -AAGCTGGTGAAGTCTCC-3' and 5' -TCCAGACTGGAATATCGGTGAC-3' for human NOX1, and 5' -TCACGAGGAATCACTCGTGTG-3' and 5' -AGAGAACACCACAATCGCGCCT-3' for human NOX4. The amounts of BLT2, NOX1, and NOX4 mRNAs were normalized to GAPDH mRNA for a quantitative analysis, and the melt curves were analyzed to ensure the specificity of the amplified PCR products.

**ROS Measurement**—Intracellular ROS levels were determined by measuring dichlorofluorescein diacetate with a flow cytometer (FACSCalibur™, BD Biosciences) as described previously (25). Cells subjected to intracellular H₂O₂ measurement were incubated for 15 min in the dark at 37 °C with 10 μM DCFDA, and dichlorofluorescein fluorescence was measured through flow cytometry with a FACSCalibur instrument.

**Immunoblot Analysis**—Protein samples were heated at 95 °C for 3 min and subjected to SDS-PAGE on acrylamide gels, followed by transfer to polyvinylidene difluoride (PVDF) membranes with a wet transfer unit (NOVEX; 1 h at 100 V). The membranes were blocked for 1 h in TBS with 0.05% (v/v) Tween 20 plus 5% (w/v) nonfat dry milk and incubated for 2 h with the appropriate primary antibodies in TBS with 0.05% (v/v) Tween 20 plus 3% (w/v) BSA followed by 1 h with HRP-conjugated secondary antibodies. The immunoreactive bands were developed with an ECL kit (Amersham Biosciences).

**Water-soluble Tetrazolium Salt-1 (WST-1) Assay**—Prostate cells were incubated for the indicated times in adherent or suspension cell cultures. WST-1 solution (Daellab Service, Seoul, Korea) was added to each well, and the plates were incubated at 37 °C for 1 h. The absorbance of each well was detected at 450 nm with a microplate reader.

**Hoechst Staining**—Cells were incubated with 50 μg/ml Hoechst 33258 (Sigma) in the dark for 15 min at 37 °C. Then, the cells were blindly scored for apoptotic morphological features using fluorescence microscopy (Carl Zeiss, Oberkochen, Germany).

**RNA Interference**—BLT2-specific (5' -CCACGCAGUCAACCUCUCG-3') (18) and control (scrambled) siRNAs were obtained from Ambion (Austin, TX). The mammalian expression vectors pSuper (OligoEngine, Seattle, WA) and pSuper encoding NOX4 (pSuper-siNOX4) or NOX1 siRNA (pSuper-siNOX1) were kindly provided by Dr. Yoon-Soo Bae; the NOX4 and NOX1 target sequences were described previously (26, 27). The siRNAs and siRNA vectors were introduced into cells by transfection with Oligofectamine and Lipofectamine 2000 reagents (Invitrogen), respectively, for the indicated times in Opti-MEM (Invitrogen).
Measurement of LTB₄ and 12(S)-HETE—PC-3 cells were detached and subsequently incubated for the indicated times on poly-HEMA-coated plates. The culture supernatants were collected and freeze-dried overnight, and reconstituted with assay buffer supplied with ELISA kits for LTB₄ and 12(S)-HETE (Assay Designs, Ann Arbor, MI). The concentrations of LTB₄ and 12(S)-HETE were measured with the ELISA kits.

Flow Cytometry Analysis of BLT2 Expression—To evaluate BLT2 expression, cells were fixed with 2% paraformaldehyde and incubated with a rabbit polyclonal anti-BLT2 antibody (1:200 dilution; Cayman Chemical) followed by fluorescein isothiocyanate (FITC)-conjugated secondary Ab (IgG, 1:200 dilution; Sigma). Rabbit IgG was used as an isotype control. The labeled cells (10,000/sample) were subjected to flow cytometry on a FACSCalibur instrument, and the mean fluorescence intensities were determined with Cell Quest software.

Detection of Apoptosis via FITC-Annexin V/Propidium Iodide (PI) Staining—PWR-1E cells were transfected with pcDNA3.1 or pcDNA3.1-BLT2 (GenBank accession no. NM_019839.1) using Lipofectamine (Invitrogen) for 36 h, and the cells were then detached and treated with BLT2 ligands. The cells were collected and suspended in 100 μl 1 Annexin V binding buffer (BD Biosciences). 2 μl of FITC-Annexin V (BD Biosciences) and 5 μl PI (BD Biosciences) were added, and the cells were incubated at room temperature for 15 min in the dark. Then, 400 μl of Annexin V binding buffer was added, and flow cytometry was performed on a FACSCalibur flow cytometer. Cells were considered to be apoptotic if they were Annexin V⁺/PI⁻ (early apoptotic). At least 10,000 events were collected for each analysis.

Immunofluorescence Staining of the NF-κB p65 Subunit—PC-3 cells in suspension or adherent culture were subjected to immunofluorescence staining of the NF-κB p65 subunit. The suspended cells were washed with cold PBS and resuspended in PBS with 1% BSA. Cytospins of the cells were prepared by centrifuging slides at 1000 rpm for 5 min. Then, both the adherent and suspended cells were fixed in 2% paraformaldehyde, permeabilized with 0.1% Triton X-100, and incubated with an anti-p65 Ab (1:100 dilution; Santa Cruz Biotechnology) followed by a FITC-conjugated secondary Ab (1/200 dilution; Molecular Probes). The integrity of the nuclei was confirmed with DAPI (4',6-diamidino-2' phenylindole dihydrochloride) staining. The localization of the NF-κB p65 subunit was monitored with a confocal laser scanning microscope (Carl Zeiss).

Soft Agar Assay—PC-3 cells (100, 000) were suspended in 0.4% top agar over a bottom layer of 0.8% base agar in 6-well plates. The solidified soft agar was overlaid with RPMI 1640 medium containing 10% FBS. The medium was changed every 4–5 days, and inhibitors were added to the top agar. After 3 weeks of incubation, PC-3 colonies were stained with 0.05% crystal violet and counted in triplicate wells.

Statistical Analysis—The data are presented as the mean ± S.D. and analyzed by one-way analysis of variance or Student’s t test for comparisons among multiple or between two groups, respectively. A p value of < 0.05 was considered statistically significant.

RESULTS

BLT2 Confers Anoikis Resistance in Prostate Cancer Cells—Previously, PC-3 cells were shown to be resistant to anoikis and have highly aggressive properties (10). Similarly, we observed that PC-3 prostate cancer cells remained viable after detachment, although the viability of normal prostate epithelial PWR-1E cells was significantly diminished, suggesting that PC-3 cells were able to escape anoikis (Fig. 1A). To investigate the potential role of BLT2 in establishing anoikis resistance, we first examined the levels of BLT2 expression in PC-3 cells following detachment. Semiquantitative RT-PCR, real-time PCR, immunoblot analysis, and FACS analysis showed that detachment of the cells caused a time-dependent increase in BLT2 expression levels (Fig. 1, B–D). Next, the effects of BLT2 knockdown by siRNA were tested to determine whether BLT2 played a role in anoikis resistance. BLT2 knockdown significantly reduced viability (Fig. 1E) and increased the frequency of apoptotic cells after detachment (Fig. 1F), indicating that the knockdown abolished anoikis resistance in PC-3 cells. BLT2 inhibition did not cause any apoptotic death in adherent states (Fig. 1, E and F). We further analyzed the effects of BLT2 inhibition on apoptosis-related proteins. As shown in Fig. 1G, BLT2 knockdown by siRNA led to a reduction in anti-apoptotic Bcl-2 levels, elevations in pro-apoptotic Bax and Bad levels in the detached, suspended PC-3 cells without affecting the levels in adherent cells. In addition, BLT2 blockade resulted in the cleavage of caspase-9 and poly(ADP-ribose) polymerase, typical markers of Bax/Bad-dependent cell death, in suspended PC-3 cells (Fig. 1G). Similarly, BLT2 blockade with the pharmacological inhibitor LY255283 induced apoptotic cell death and altered the expression levels of apoptosis-related proteins in detached PC-3 cells (Fig. 1, H–J). Furthermore, we observed that the number of surviving LY255283-treated cell clones was markedly reduced compared with untreated cells, suggesting that BLT2 established anoikis resistance in the absence of cell attachment (Fig. 1K).

BLT2 Overexpression Confers Anoikis Resistance in PWR-1E Normal Cells—Because BLT2 was shown to protect PC-3 cells against apoptosis after detachment, we explored whether the ectopic overexpression of BLT2 in the anoikis-sensitive normal prostate epithelial PWR-1E cell line could render these cells resistant to anoikis in the absence of attachment. Indeed, both BLT2 overexpression and stimulation by its agonist (CAY10583) rendered PWR-1E cells partly resistant to anoikis after detachment (Fig. 2A). Similarly, stimulation with another BLT2 ligand, 12(S)-HETE, significantly diminished anoikis-associated apoptotic cell death in detached BLT2-overexpressing PWR-1E cells (Fig. 2B). Under these experimental conditions, BLT2 expression was elevated in BLT2-transfected PWR-1E cells compared with control PWR-1E cells (Fig. 2C); and BLT2 overexpression and stimulation by its ligand, 12(S)-HETE, led to an elevation in Bcl-2 levels and a reduction in Bax/Bad levels in detached, suspended PWR-1E cells (Fig. 2D). Together, these results implicate BLT2 as a crucial determinant of anoikis resistance in detached cells.
12(S)-HETE, Together with BLT2, Is Involved in the Anoikis Resistance of Prostate Cancer Cells—Next, we determined which BLT2 ligands affected the establishment of anoikis resistance. Our results showed that the LTB4 production in PC-3 cells did not increase after detachment (Fig. 3A), whereas the level of 12(S)-HETE was markedly increased after detachment (Fig. 3B). However, in adherent cells, we did not detect any 12(S)-HETE up-regulation (Fig. 3B). In agreement with our observation, several previous studies showed that 12-LOX is critical to the survival and metastatic potential of prostate cancer (14, 15) and that 12(S)-HETE is the ligand that acts on BLT2 in androgen receptor-positive prostate cancer cells (28). Thus, we hypothesized that 12(S)-HETE might be the principal ligand for BLT2 and that the 12(S)-HETE-BLT2 axis might be up-regulated after PC-3 cell detachment. Next, we tested the effects of baicalein, a 12-LOX inhibitor, on anoikis resistance in PC-3 cells. As expected, baicalein significantly decreased cell viability and triggered apoptotic cell death after detachment (Fig. 3, C–E). Moreover, a soft agar colony formation assay showed that 12-LOX inhibition promoted a significant reduction in the...
number of surviving clones (Fig. 3F). Together, these results suggest that the increased expression of 12(S)-HETE after detachment contributes to the protection of prostate cancer cells from anoikis.

**NOX-dependent ROS Generation Is Downstream of BLT2 Signaling**—Because BLT2 was previously reported to generate NOX-mediated ROS (28–33), we tested whether NOX was modulated by BLT2 after detachment. We found that the tran-
script levels of NOX1 and NOX4 were significantly increased after detachment (Fig. 4, A and B) but markedly attenuated by BLT2 siRNA knockdown in detached, suspended PC-3 cells (Fig. 4, C and D). Additionally, Fig. 4, E and F, show that BLT2 inhibition by siRNA knockdown caused a marked reduction in ROS generation and that 12-LOX inhibition by baicalein treatment also significantly diminished ROS generation in detached, suspended cells, suggesting that the 12(S)-HETE-BLT2-NOX axis plays a role in generating ROS after detachment. Next, we investigated the role of NOX in PC-3 cell anoikis resistance and tested the effects of DPI, an inhibitor of flavoproteins, such as NOX, in the absence of adhesion. As shown in Fig. 4, G and H, treatment with DPI reduced cell viability and the number of surviving clones in the absence of attachment, suggesting that DPI-sensitive NOX might be involved in the establishment of anoikis resistance. Indeed, the RNAi-mediated depletion of NOX1 or NOX4 significantly abrogated detachment-induced ROS generation (Fig. 4I) and reduced cell viability (Fig. 4J) in the absence of adhesion of PC-3 cells, indicating that increased ROS levels were due to NOX1 and NOX4 up-regulation and that NOX-dependent ROS generation is essential for cell survival after detachment. Furthermore, the ectopic expression of either NOX1 or NOX4 partially rescued cell death following detachment in PWR-1E cells (Fig. 4K). Taken together, these results suggest that the BLT2-NOX1/4-ROS cascade is up-regulated following detachment and contributes to anoikis resistance in prostate cancer cells.

NF-κB Lies Downstream of BLT2 to Induce Anoikis Resistance—NF-κB, a well known redox-sensitive transcription factor, has been shown to be activated following cell detachment and pro-
vide an anti-apoptotic signal (34, 35). Thus, we hypothesized that the BLT2-ROS cascade could result in NF-κB activation in detached suspension. Fig. 5A shows that BLT2 inhibition through siRNA knockdown resulted in down-regulated p65 nuclear translocation and phosphorylated IkBα levels in detached, suspended PC-3 cells but not in adherent cells. Moreover, 12-LOX inhibition through baicalein treatment also clearly diminished p65 nuclear translocation and phosphorylated IkBα, implicating the BLT2 cascade in the stimulation of NF-κB activity after detachment (Fig. 5B). Next, we analyzed the effects of Bay11-7082, an NF-κB inhibitor, on cells in the absence of adhesion. Treatment with Bay11-7082 reduced cell viability and triggered apoptotic cell death in suspension (Fig. 5, C–E), demonstrating the functional importance of NF-κB in anoikis resistance (34). In addition, we tested whether the activation of NF-κB protected PWR-1E cells from anoikis. To activate NF-κB, pCMV-p65 (23) was transfected into PWR-1E cells, and p65 transfection markedly stimulated NF-κB activity, as measured by luciferase assay (data not shown). As shown in Fig. 5F, NF-κB activation by p65 transfection rescued cell death, at least in part, following detachment in PWR-1E cells. Collectively, these results suggest that NF-κB is a crucial signaling mediator of anoikis resistance downstream of the BLT2-ROS cascade.

DISCUSSION

In this study, we found that BLT2 is a determining factor in the establishment of anoikis resistance and is thus a potential contributor to prostate cancer progression. Our data showed that activation of the BLT2-linked pathway after cell detachment conferred anoikis resistance and that NOX-derived ROS generation and subsequent NF-κB activation occurred downstream of BLT2. Collectively, our findings provide important insights into the mechanism of anoikis resistance during prostate cancer progression.

BLT2 is a G protein-coupled receptor for LTB₄ and 12(S)-HETE, which are major pro-inflammatory local acting lipid mediators that are produced in the 5-LOX and 12-LOX pathways, respectively (36). The tumor-promoting roles of these LOX-derived eicosanoids and their cognate receptors, including BLT2, are well recognized in the tumor microenvironment (2, 8, 9, 21, 37, 38). Additionally, recent studies have suggested that BLT2 and its ligands critically regulate tumor progression by promoting cell proliferation, survival, migration, and metastasis (17, 18, 20, 22, 28–30). For example, it was shown that LTB₄ and 12(S)-HETE levels were increased during cancer progression, and in particular, 12(S)-HETE was reported to be critical to the survival and metastatic potential of prostate cancer cells (11, 13–15, 39, 40). Moreover, our previous data demonstrated that a 12(S)-HETE-BLT2-linked cascade induced the up-regulation of androgen receptor, which contributed to cell survival in androgen receptor-positive prostate cancer cells (28). Despite reports implicating BLT2 as a potential marker for aggressive cancers, the role of BLT2 in anoikis resistance has not been reported previously. In the present study, our results...
clearly demonstrate that BLT2 up-regulation during cell detachment significantly contributed to anoikis resistance in aggressive PC-3 prostate cancer cells. Additionally, we found that 12(S)-HETE was a principal ligand for BLT2 and that the levels of 12(S)-HETE were significantly increased after cell detachment. We further determined that treatment with baicalein, an inhibitor of 12-LOX, significantly abolished anoikis resistance and induced marked levels of apoptotic cell death. In contrast to the up-regulated synthesis of 12(S)-HETE, we did not detect any increases in LTB₄ levels after PC-3 cell detachment. Additionally, we observed that inhibition of the LTB₄ receptor-1, BLT1, by U75302 did not affect PC-3 cell anoikis resistance (data not shown). The actual in vivo levels of 12(S)-HETE or LTB₄, however, might be more significantly increased in the tumor microenvironment, likely due to the enhanced accumulation of leukocytes and hypoxic conditions, which were shown to enhance the levels of these lipid mediators (41). For instance, the recruitment of leukocytes to the tumor microenvironment would augment the production of LTB₄ or 12(S)-HETE and cause the subsequent accumulation of BLT2 ligands within the tumor microenvironment. Therefore, our proposed scheme (Fig. 5G) is likely to operate more efficiently in vivo than in cell culture to confer anoikis resistance to cancer cells. Indeed, increased LTB₄ levels were detected in prostate cancer tissues relative to corresponding normal tissues (13). Such amplification of the action of BLT2 ligands due to recruitment of leukocytes in the inflammatory microenvironment has been proposed to operate in other pathological situations (42). Further studies are necessary to elucidate the exact effects of the tumor microenvironment on BLT2-driven prostate cancer cell anoikis resistance.

We found that NOX-derived ROS generation was induced downstream of BLT2 and acted as a mediator of BLT2-associated anoikis-resistance. Previously, it was recognized that tumors exhibit an excessive and persistent elevation of ROS levels and utilize a redox-based mechanism to evade death by anoikis (43, 44). For example, ROS were shown to inhibit the anoikis of cancer cells through the inhibition of caveolin-1 degradation in lung carcinoma (4). In prostate cancer, ROS have been reported to be responsible for the redox-mediated activation of Src, which trans-phosphorylates the EGF receptor and thus mediates survival effects upon the loss of extracellular matrix contact (10, 45). Furthermore, Zhu et al. clearly demonstrated that NOX1 is the predominant oxidase that causes anoikis resistance through angiopoietin-like 4 (ANGPTL4) (43). Recently, ANGPTL4 was established as a regulator of lipid metabolism and was implicated in prostaglandin E₂-mediated cancer progression (46). Previously, ROS have been suggested to be critical to anoikis resistance in aggressive human cancers, but the signaling mechanisms that led to the generation of ROS were poorly understood. Here, we found that BLT2-NOX1/4 cascade is a critical mediator of the generation of ROS, which contributed to cell survival during detachment. When the previously reported roles for ROS in anoikis resistance are considered, ROS generated by the BLT2-NOX cascade could mediate cross-communication between multiple pathways, such as the caveolin-1, EGF receptor, and ANGPTL4 pathways. Additionally, we showed that NF-κB, a well known downstream target of ROS (47), affects the survival of suspension-cultured cancer cells. Previously, NF-κB has been reported to modulate anoikis, which is consistent with our results (34, 35). Cell detachment activates NF-κB, which activates an inflammatory response through the induction of inflammatory mediators, such as IL-6 and IL-1β, and thus delays apoptosis in intestinal epithelial cells (35). Furthermore, NF-κB activation induces Bcl-2 and IAP-1 expression after detachment, which acts to suppress anoikis (34). There might be other potential mechanisms through which BLT2 protects prostate cancer cells against anoikis. One such potential downstream component would be Akt. Indeed, we detected that BLT2 inhibition significantly diminished the levels of phosphorylated Akt in suspended PC-3 cells, suggesting that Akt is also downstream of BLT2 in mediating anoikis resistance (data not shown). Clearly, further studies are necessary to elucidate the detailed downstream mechanisms of BLT2 during the detachment of prostate cancer cells.

Tumor cells can also escape from detachment-induced apoptosis by controlling the ECM-integrin cell survival pathway and the mitochondrial-mediated apoptosis pathway (3). Some integrins are expressed on the most aggressive tumor cells, and the up-regulated expression of these integrins can increase colony-forming abilities, even in the absence of specific ligands, indicating that integrin plays a role in anchorage independence (48). Because 12(S)-HETE was previously reported to promote tumor cell survival by enhancing the expression of integrins αvβ₃ and αvβ₅, we hypothesized that the BLT2-linked pathway might contribute to integrin expression following the detachment of tumor cells (49). However, we could not detect any effects of BLT2 inhibition on the transcript levels of integrins αvβ₃ or αvβ₅ in prostate cancer cells (data not shown). Nevertheless, there might be possible mechanisms through which the BLT2 pathway could be linked to integrin activation. Recent studies have implicated an active role for Bcl-2 family proteins in the modulation of anoikis through the mitochondrial-mediated apoptosis pathway (3). Consistent with these findings, inhibition of the BLT2 pathway triggered a dramatic down-regulation of Bcl-2 and up-regulation of Bax and Bad during detachment, suggesting that BLT2 could act to suppress the classical apoptotic pathway during the detachment of prostate cancer cells.

In the present study, we report a previously unrecognized anti-anoikis role for BLT2 in prostate cancer cells. As anoikis resistance is critical to cancer progression, our findings will contribute to a better understanding of the molecular mechanisms of cancer progression. Additionally, the results of this study could provide potential targets for the development of new prostate cancer therapeutics.

REFERENCES


OCTOBER 18, 2013 • VOLUME 288 • NUMBER 42 JOURNAL OF BIOLOGICAL CHEMISTRY 30061
BLT2-ROS Cascade in Anoikis Resistance

cer cell anoikis through the inhibition of caveolin-1 degradation. Am. J. Physiol. Cell Physiol. 300, C235–C245


ment to the skin is essential for allergic skin inflammation. Immunity 37, 747–758


Activation of the Leukotriene B₄ Receptor 2-Reactive Oxygen Species (BLT2-ROS) Cascade following Detachment Confers Anoikis Resistance in Prostate Cancer Cells

Jin-Wook Lee and Jae-Hong Kim

J. Biol. Chem. 2013, 288:30054-30063. doi: 10.1074/jbc.M113.481283 originally published online August 28, 2013

Access the most updated version of this article at doi: 10.1074/jbc.M113.481283

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 49 references, 16 of which can be accessed free at http://www.jbc.org/content/288/42/30054.full.html#ref-list-1