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Elevated IL-1β expression induces invasiveness of triple negative breast cancer cells and is suppressed by zerumbone

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ABSTRACT

Aberrant interleukin-1 beta (IL-1β) expression is associated with cancer development, metastasis, and poor prognosis. Here, we have investigated the regulatory mechanism of IL-1β expression, and the inhibitory effect of zerumbone (ZER) on IL-1β expression and IL-1β-induced signatures, including cell invasion and signaling activation in triple negative breast cancer (TNBC) cells. The basal IL-1β and cell invasiveness levels were significantly higher in TNBC cells, compared with non-TNBC cells. The invasiveness of TNBC cells was also increased following IL-1β treatment. In contrast, the invasiveness of TNBC cells was decreased following IL-1 receptor antagonist (IL-1RA) treatment. Additionally, the basal IL-1β level and the invasiveness of TNBC cells were decreased by Bay11-7085. In contrast, overexpression of NF-κB (p65) caused an increase in IL-1β expression in TNBC cells. Our results showed that treatment with ZER decreased the basal IL-1β expression level, and the phosphorylation level of NF-κB, in TNBC cells. Furthermore, we found that ZER completely suppressed IL-1β-induced NF-κB phosphorylation, but did not suppress IL-1β-induced Akt phosphorylation, in TNBC cells. Our results also demonstrate that IL-1β-induced cell invasion is suppressed by ZER in TNBC cells. Taken together, we demonstrated that IL-1β expression is regulated by the NF-κB-dependent pathway, and that elevated IL-1β is directly influencing the invasiveness of TNBC cells. ZER down-regulates IL-1β expression through the inhibition of NF-κB activity, and then suppresses cell invasiveness of TNBC.

1. Introduction

Breast cancer is the most common cancer in women, and is a genomically heterogeneous disease [1,2]. One breast cancer subtype, triple-negative breast cancer (TNBC), comprises about 15–20% of cases, and is a highly aggressive disease and carries the worst prognosis amongst all subtypes of breast cancer [3,4]. TNBC is defined by their lack of estrogen receptor (ER), progesterone receptor (PR), and human-epidermal growth factor receptor-2 (HER2) expression [3,4]. The clinical features of TNBC patients include a peak of recurrence risk within the first 3 years, a peak of cancer-related death in the first 5 years, and a high incidence of distant metastasis [5,6]. Systematic conventional chemotherapy is the only treatment option for TNBC patients, due to the absence of established targets, such as estrogen receptor and HER2 [7]. As such, many preclinical and clinical studies are focused on the development of effective therapeutic agents and target genes for TNBC patients.

Interleukins (ILs) are pro-inflammatory cytokines produced by monocytes, macrophages and epithelial cells. The interleukin-1 (IL-1) family consists of the cytokines IL-1α, IL-1β, and a specific receptor antagonist (IL1RA) [8–10]. In particular, IL-1β is highly expressed in various types of cancer, including melanoma, pancreatic, and colon cancers [11]. The IL-1β expression levels are regulated by several transcription factors, including retinoic acid receptor (RAR), AP-1, and NF-κB [12,13]. An upregulation of IL-1β eventually triggers the aggressive tumor biology associated with cell invasion, stemness, epithelial-mesenchymal transition (EMT) and metastasis in various cancers, including pancreatic and colon cancer [14–16].

Zerumbone (ZER) is derived from a Southeast Asian ginger sesquiterpene and has a variety of pharmacological effects, including anti-inflammatory and anti-oxidant activities [17–19].

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We have recently reported that ZER suppresses cell migration and invasion via the inhibition of IL-8 and MMP-3 expression, and reduces the motility and tumorigenecity of TNBC cells through the inhibition of the TGF-β1 signaling pathway [20,21]. In addition, ZER also inhibits cell growth through the induction of apoptosis and cell cycle arrest, and suppresses IL-6 secretion in ovarian and cervical cancer cells [22–25]. ZER has also been shown to inhibit tumor angiogenesis through the down-regulation of proangiogenic genes, such as VEGF and IL-8, in gastric and pancreatic cancer cells [26,27].

In this study, we focus on the regulatory mechanism of IL-1β expression, and the inhibitory effect of ZER on IL-1β-related signatures, in TNBC cells. Our results showed that IL-1β expression levels were higher in TNBC cells, compared with non-TNBC cells. Recombinant human IL-1β treatment triggered the invasiveness of TNBC cells, while IL-1RA (IL-1 receptor antagonist) suppressed the invasiveness of TNBC cells. Elevated IL-1β levels and the invasiveness of TNBC cells, were decreased by treatment with the NF-κB inhibitor, Bay11-7085. In contrast, NF-κB (p65) overexpression increased the IL-1β expression level, in TNBC cells. Interestingly, ZER completely suppressed aberrant IL-1β expression and the invasiveness of TNBC cells, through the inhibition of NF-κB phosphorylation. Taken together, we have demonstrated that ZER suppresses IL-1β expression through the inhibition of an NF-κB-dependent pathway, and then inhibits the invasiveness of TNBC cells. We therefore suggest that ZER may be a promising therapeutic agent for treating TNBC patients.

2. Materials and methods

2.1. Reagents

All cell culture media and antibiotics were purchased from Life Technologies (Rockville, MD, USA). Fetal bovine serum (FBS) was purchased from Hyclone (Logan, UT, USA). Rabbit monoclonal *anti*-phospho and total-NF-κB, and Akt antibodies were purchased from Epitomics (Burlingame, CA, USA). β-actin antibody was purchased from Abfrontier (Seoul, Korea). Secondary horseradish peroxidase (HRP)-conjugated antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Recombinant human IL-1β was purchased from Peprotech (Rocky Hill, NJ, USA). IL-1 receptor antagonist was purchased from ProSpec (Ness Ziona, Israel). Zerumbone was a generous gift from Dr. Murakami Akira (Kyoto University, Japan) [17,28]. LY294002, Bay11-7085, and SB253580 were purchased from TOCRIS (Ellisville, MO, USA). Rapamycin was purchased from Calbiochem-EMD Biosciences, Inc. (La Jolla, CA).

2.2. Cell culture and drug treatment

MCF7, Hs578T, and MDA-MB231 breast cancer cells were cultured in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% FBS, 100 IU/ml penicillin, and 100 μg/ml streptomycin. SKBR3 breast cancer cells were cultured in RPMI1640 supplemented with 10% FBS, 100 IU/ml penicillin, and 100 μg/ml streptomycin. Cells were grown in a humidified atmosphere with 5% CO_2, at 37 °C. In the drug treatment experiment, after serum starvation for 24 h, cells were treated with ZER, or the various specific inhibitors, for 24 h. Cell number was not significantly changed by drug treatment. After serum starvation to synchronize the cell status for 24 h, cells were pretreated with ZER for 1 h, and then treated with IL-1β for 24 h.

2.3. Boyden chamber assay

Cell invasive capacity was analyzed using a Boyden chamber assay, as described previously [29]. 24-well Boyden chambers with matrigel-coated filters (8 μm pore size) were purchased from Becton-Dickinson (San Diego, CA, USA). MCF7, SKBR3, Hs578T and MDA-MB231 breast cancer cells to be tested for invasion were resuspended in culture media (5×10^4 cells/well), and then added to the matrigel-coated upper compartment of the invasion chambers. Fresh culture media with 5% FBS was added to the lower compartment of the invasion chamber. In addition, Hs578T and MDA-MB231 cells were resuspended in culture media (5×10^4 cells/well), and then added to the matrigel-coated upper compartment of the invasion chambers, in the presence or absence of 10 ng/ml IL-1β, 10 ng/ml IL-1RA, 5 μM Bay11-7085, and 20 μM ZER. Fresh culture media with 5% FBS was added to the lower compartment of the invasion chamber. After 24 or 48 h incubation, the cells on the upper side of the filter were removed using cotton swabs. The underside of the filter was fixed in 100% methanol, washed in 1 × PBS, and stained using hematoxylin and eosin (H&E). Cells that had invaded through the matrigel were located on the underside of the filter. These cells were analyzed using a Scanscope XT (Aperio Technologies, CA, USA).

2.4. Real-time polymerase chain reaction

Total RNA was extracted from cells using TRIzol reagent (Invitrogen, CA, USA), according to the manufacturer's protocol. Isolated RNA samples were then used for RT-PCR. Samples of total RNA (1 μg) were reverse-transcribed into cDNA, in 20 μl reaction volumes, using a first-strand cDNA synthesis kit for RT-PCR, according to the manufacturer's instructions (MBI Fermentas, MD, USA). Gene expression levels were quantified by real-time PCR, using a SensiMix SYBR kit (Bioline Ltd., London, UK), and 100 ng of cDNA per reaction. The primer sequence used for this analysis was as follows: human IL-1β: forward, 5′-GCC CTA AAC AGA TGA AGT GCT C-3′ and reverse, 5′-GAA CCA GCA TCT TCC TCA G-3′; and human IL-1R1: forward, 5′-AGA GGA AAA CAA ACC CAC AAG G-3′ and reverse, 5′-CTG GCC GGT GAC ATT ACA GAT-3′. An annealing temperature of 60 °C was used for all primers. PCRs were performed in a standard 384-well plate format, with an ABI 7900HT real-time PCR detection system (Foster City, CA, USA). For data analysis, the raw threshold cycle (C_T) value was first normalized to a housekeeping gene for each sample in order to obtain a ΔC_T. The normalized ΔC_T was then calibrated to the control cell samples, resulting in a $\Delta\Delta C_T$.

2.5. Enzyme-linked immunosorbent assay

ELISA assays were performed on the culture media (200 μl) collected from Hs578T, and MDA-MB231 breast cancer cells. IL-1β protein levels were measured using an ELISA kit (R&D Systems, MN, USA), according to the manufacturer's instructions. Secreted protein levels were analyzed at a wavelength of 450 nm on a spectrometer (Spectra Max 190; Molecular Devices, CA, USA).

2.6. Western blotting

Cell lysates were prepared for the detection of *anti*-phospho and total-NF-κB, Akt, and β-actin expression. Equal protein amounts (50 μg) were boiled for 5 min in Laemmli sample buffer, and then electrophoresed in 10% sodium dodecyl sulfate polyacrylamide (SDS-PAGE) gels. Separated proteins were transferred to polyvinylidene fluoride (PVDF) membranes, and the membranes were blocked with 10% skimmed milk, in Tris buffered saline (TBS) containing 0.01% Tween-20 (TBS/T), for 15 min. The blots were washed 3 times in TBS/T, and then incubated with *anti*-phospho and total-NF-κB, Akt, and β-actin antibodies in TBS/T buffer at 4 °C, overnight. The blots were washed 3 times in TBS/T, and subsequently incubated with secondary HRP-conjugated antibodies (1:2000 dilution) in TBS/T buffer. After 1 h incubation at room