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Membrane translocation of transient receptor potential ankyrin 1 induced by inflammatory cytokines in lung cancer cells

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ABSTRACT

Transient receptor potential ankyrin 1 (TRPA1) is known as one of the nociceptors expressed in sensory neurons. It also plays a role in non-neural cells in inflammatory sites. However, the regulatory mechanisms for the reactivity of TRPA1 in these cells under inflammatory conditions are not clear. To clarify these mechanisms, we examined the effects of inflammatory cytokines (interleukin [IL]-1α, IL-1β and tumor necrosis factor α [TNFα]) on TRPA1 reactivity and expression in the endogenously TRPA1-expressing lung tumor cell line A549. Treatment with IL-1α, but not IL-1β or TNFα, increased the number of cells responding to allyl isothiocyanate, a TRPA1 agonist, in a dose- and time-dependent manner. The IL-1α-induced increase of TRPA1 responsiveness was inhibited by an extracellular-regulated kinase (Erk) inhibitor (PD98059) but not by inhibitors of *c*-Jun kinase, p38 mitogen-activated protein kinase or phosphatidylinositol-3 kinase. Phosphorylation of Erk gradually increased at 24 h after its transient induction in cells treated with IL-1α. IL-1α increased the TRPA1 levels on biotinylated cell surface proteins. These results suggest that IL-1α enhances the translocation of TRPA1 to the plasma membrane via the activation of Erk in A549. TRPA1 may have a pathophysiological role in non-neural lung cells under inflammatory conditions.

1. Introduction

Transient receptor potential ankyrin 1 (TRPA1), a member of the TRP family, is a nociceptive ion channel mainly expressed on sensory neurons. It is activated by a wide variety of endogenous ligands, physical stimuli, pungent chemicals and environmental irritants eliciting pain [1–4]. TRPA1 is also profoundly involved in inflammation because many inflammatory agents (reactive oxygen species, prostaglandin derivatives, heavy metals, exhaust gas components, and bacterial components) cause pain and the release of inflammatory neuropeptides via TRPA1 activation [3,5–7]. In addition, an increasing number of reports have demonstrated that TRPA1 is also expressed in non-neural tissues [8–12]. TRPA1 is expressed in non-neural cells of the human and mouse respiratory tracts [5]. Icilin, a TRPA1 agonist, induces the gene expression of bone morphogenetic protein 7 and growth differentiation factor 15, which are related to cell proliferation and cell differentiation in epidermal keratinocytes [8]. Cinnamaldehyde and acrolein, TRPA1 agonists, increased the intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$) in human airway epithelial cells, lung fibroblasts and bronchial smooth muscle cells [5]. Increases of plasma protein extravasation caused by cigarette smoke inhalation in the lungs of TRPA1 gene-deficient mice are significantly reduced compared to in wild-type mice [5]. Therefore, TRPA1 involved in the inflammatory pathology of respiratory tissues. However, the mechanisms of change in TRPA1 reactivity in non-neurons are not clear.

Interleukin (IL)-1α belongs to the danger-associated molecular patterns that are released from damaged cells during necrosis. It induces inflammation similar to IL-33 and high mobility group box protein 1 [13]. IL-1α is constitutively present in the state of its precursor in the epithelial layers of the entire gastrointestinal tract, lung, liver and kidney, endothelial cells and astrocytes. The released IL-1α precursor is fully active and functions as a tissue injury alarm molecule, an alarmin [14]. In inflammatory bowel disease, IL-1α from intestinal epithelial cells activates fibroblasts that produce IL-6 and IL-8, amplifying the inflammation [15]. In the lung, microparticles and photodynamic stimuli induce inflammation through the release of IL-1α from epithelial cells [16,17]. It has been reported that IL-1α induces the expression of TRPA1 via the activation of

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hypoxia-inducible factor-1α in human synoviocytes [18]. Although TRPA1 and IL-1α seem to closely interact during inflammation, the changes in TRPA1 reactivity caused by inflammatory cytokines in lung are not determined.

In this study, to clarify the functional changes in TRPA1 in the early inflammatory lung in vitro, we examined the effects of inflammatory cytokines on the reactivity and expression of TRPA1 in A549, human lung adenocarcinoma cells expressing TRPA1 endogenously.

2. Materials and methods

2.1. Chemicals

The following chemicals were used (vehicle, concentration for stock solution). IL-1α (0.1% bovine serum albumin [BSA] solution, 10 μg/mL) was purchased from PeproTech (Rocky Hill, NJ, USA). Allyl isothiocyanate (AITC) (dimethyl sulfoxide [DMSO], 1 M) was from Nacalai Tesque, Inc. (Kyoto, Japan). PD98059 (DMSO, 10 mM), SB203580 (DMSO, 10 mM), and JNK inhibitor II (DMSO, 10 mM) were obtained from Calbiochem (Merck Millipore Co., Tokyo, Japan). LY294002 (DMSO, 10 mM) and HC-030031 (DMSO, 0.1 M) were from Wako Pure Chemicals (Osaka, Japan). Other chemicals were purchased from Wako Pure Chemicals. These stock solutions were diluted more than 1000-fold with either culture medium or HEPES-buffered solution (in mM: 134 NaCl, 6 KCl, 1.2 $MgCl_2$, 2.5 $CaCl_2$ and 10 HEPES, pH 7.4).

2.2. Cell culture

A549, a human lung adenocarcinoma cell line, was provided by RIKEN BRC through the National Bio-Resource Project of MEXT, Japan. Cells were cultured in DMEM medium (Sigma-Aldrich Japan, Tokyo, Japan) supplemented with 10% heat inactivated fetal bovine serum (Life Technologies Japan, Tokyo, Japan), 100 μg/mL streptomycin (Meiji Seika Pharma Co., Ltd., Tokyo, Japan) and 100 U/ml

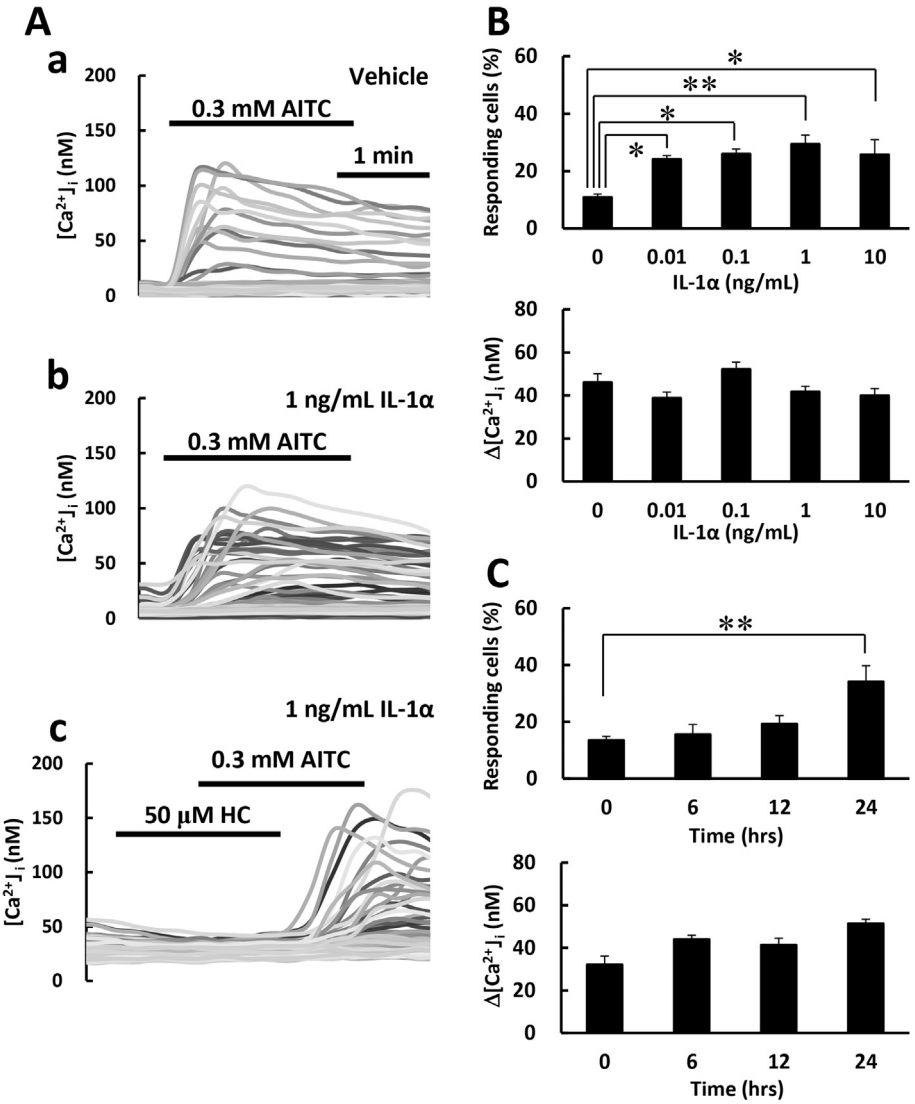

Fig. 1. IL-1α increases TRPA1 responsiveness to AITC in A549 cells. (A) Actual traces of $[Ca^{2+}]_i$ responses to 0.3 mM AITC in cells treated with the vehicle (a) or 1 ng/mL IL-1α for 24 h (b). (c) 50 μM HC-030031 (HC) was applied 1 min before and during 0.3 mM AITC in A549 treated with 1 ng/mL IL-1α for 24 h. (B) Relationship between IL-1α-dose and the percent of cells responding to AITC (top), and increment of $[Ca^{2+}]_i$ (bottom) (n = 44–126). Cells were treated with various doses of IL-1α for 24 h. (C) Time-course of changes in percent of cells responding to AITC (top) and increment of $[Ca^{2+}]_i$ (bottom) (n = 104–200). Cells were treated with 1 ng/mL IL-1α. Data were obtained from three separate experiments. Columns and vertical lines show mean ± SEM. *: $p < 0.05$, **: $p < 0.01$ vs. IL-1α-untreated control, one-way ANOVA with the Tukey-Kramer test.

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