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Induction of thymic stromal lymphopoietin by a steroid alkaloid derivative in mouse keratinocytes

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ABSTRACT

Thymic stromal lymphopoietin (TSLP) plays critical roles in inducing and exacerbating allergic diseases. Chemical compounds that induce TSLP production can enhance sensitization to antigens and exacerbate allergic inflammation. Hence, identifying such chemicals will be important to prevent an increase in allergic diseases. In the present study, we found, for the first time, that a steroid alkaloid derivative, code no. 02F04, concentration and time dependently induced mRNA expression and production of TSLP in a mouse keratinocyte cell line, PAM212. In particular, the activity of 02F04 was selective to TSLP. As an analogue of the liver X receptor (LXR) endogenous ligand, 02F04 rapidly increased ATP-binding cassette transporter A1 (ABCA1) expression by regulating the nuclear receptor of LXR. However, instead of being inhibited by the LXR antagonist, 02F04-induced TSLP production was delayed and markedly suppressed by inhibitors of phospholipase C (PLC), pan-protein kinase C (PKC), PKCδ, Rho-associated protein kinase (ROCK), extracellular signal-regulated kinase (ERK) 1/2, and IκB kinase 2 (IKK2). Treatment with 02F04 caused the formation of F-actin filaments surrounding the nucleus of PAM212 cells, which then disappeared following addition of ROCK inhibitor. 02F04 also induced phosphorylation of ERK1/2 from 2 h after treatment, with a maximum at 24 h, and increased nuclear factor-κB (NF-κB) promoter activity by 1.3-fold. Taken together, these results indicate that 02F04-induced TSLP production is regulated via distinct signal transduction pathways, including PLC, PKC, ROCK, ERK1/2, and NF-κB but not nuclear receptors. 02F04, with a unique skeletal structure in inducing TSLP production, can represent a potential new tool for investigating the role of TSLP in allergic diseases.

1. Introduction

Thymic stromal lymphopoietin (TSLP), an interleukin-7 (IL-7)-like cytokine [1] derived mainly from epithelial cells such as keratinocytes [2], is regarded as a master regulator in the pathogenesis of allergic disorders, such as atopic dermatitis, allergic rhinitis, and asthma [3]. High TSLP expression was reported in skin lesions of patients with atopic dermatitis [4]. Moreover, increased TSLP activated dendritic cells, which primed naive T cells to release the pro-inflammatory cytokine tumor necrosis factor-α (TNF-α) and the Th2 cytokine IL-13 [5], and further accelerated allergic skin diseases [6,7]. Certain exogenous factors, such as viral infections, allergen exposure, cigarette smoke, diesel exhaust, and ligation of Toll-like receptors have been found to trigger the production of TSLP [8,9]. In addition, exposure to several chemical compounds can also induce TSLP production and exacerbate allergies [10]. For example, phthalate ester increases TSLP production in vivo [11]. In our previous study, some chemical compounds in the environment, such as xylene, toluene and trimethylbenzene, were shown to increase the production of TSLP and exacerbate contact sensitizers in allergic dermatitis [12]. Recently, we also found that several

Abbreviations: TSLP, Thymic stromal lymphopoietin; LXR, Liver X receptor; RXR, retinoid X receptor; RAR, retinoic acid receptor; PPAR, peroxisome proliferator-activated receptor; VDR, vitamin D receptor; GR, glucocorticoids receptor; 22R–HC, 22(*R*)-hydroxycholesterol; TNF-α, tumor necrosis factor-α; IL-1β, interleukin-1β; IL-13, interleukin-13; IL-25, interleukin-25; ABCA1, ATP-binding cassette transporter A1; PLC, phospholipase C; PKC, protein kinase C; ROCK, Rho-associated protein kinase; MAPK, mitogen-activated protein kinase; ERK1/2, extracellular signal-regulated kinase 1/2; JNK, Jun NH2-terminal kinase; NF-κB, nuclear factor-κB; IKK2, IκB kinase 2; p-ERK, phosphorylated ERK1/2; p-IKKα/β, phosphorylated IKKα/β; TPA, 12-*O*-tetradecanoylphorbol-13-acetate

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short/medium-chain fatty acids, including pentanoic acid [13], octanoic acid, nonanoic acid, and decanoic acid [14] markedly increased TSLP production in PAM212 cells or in vivo. TSLP-inducing compounds have been proposed to enhance sensitization to antigens, such as pollens and mites, and exacerbate allergic inflammation, which classifies them as chemical allergo-accelerators [14]. At present, it remains unclear which chemicals have the potential to induce TSLP production and how. Therefore, identifying such chemicals and the molecular mechanisms involved in TSLP production has the potential to prevent a rise in allergic diseases.

To find potent compounds that can affect TSLP production, a murine keratinocyte cell line, KCMH-1, which can produce a large amount of TSLP without stimulation [15], was set up in our lab. High-throughput screening of 2169 compounds yielded a steroid alkaloid derivative, code no. 02F04, with the IUPAC name of (4S,6aR,8aS,8bR,9S,9aR,12-S,16aS,17aS,17bS)-6a,8a,9,12-tetramethyl-1,3,4,5,6,6a,6b,7,8,8a,8-b,9,9a,10,11,12,13,15,16a,17,17a,17b-docosahydronaphtho[2′,1′:4,5]indeno[1,2-*f*]pyrido[1,2-c][1,3]oxazepin-4-ol. 02F04 significantly increased the production of TSLP. With chemical formula of $C_{28}H_{45}NO_2$ and molecular weight of 427.67, 02F04 has a similar chemical structure (shown in Fig. 1A) as oxysterols, the endogenous ligands of nuclear receptor of liver X receptor (LXR). Indeed, glucocorticoids such as dexamethasone, which has a steroidal structure, are known to inhibit TSLP production [16] and to possess strong anti-inflammatory effect through multiple mechanisms including regulation of the nuclear receptor of glucocorticoids receptor (GR) [17]. However, there are hardly any reports on steroidal compounds inducing TSLP production. Therefore, in the present study, we investigated in detail whether 02F04 increased TSLP production and mRNA expression in mouse keratinocytes, and whether the nuclear receptor participated in this process. Furthermore, we clarified the underlying molecular mediators and mechanisms of action contributing to this effect.

2. Materials and methods

2.1. Materials

T0901317 and 22 (*R*)-hydroxycholesterol (22R-HC) were purchased from Cayman Chemical Company (Ann Arbor, MI, USA). GSK2033 and TPCA-1 were acquired from Sigma-Aldrich (St. Louis, MO, USA). Manoalide, rottlerin and GF109203X were obtained from Santa Cruz Biotechnology, Inc. (Heidelberg, Germany), R&D Systems (Minneapolis, MN, USA) and Funakoshi Co., LTD. (Tokyo, Japan), respectively. 12-*O*-tetradecanoylphorbol-13-acetate (TPA), U0126 and Y-27632 were purchased from Wako Pure Chemical Industries (Osaka, Japan), Promega (Madison, WI, USA), and Nacalai Tesque Inc. (Kyoto, Japan), respectively. 02F04 was acquired from InterBioScreen LTD. (Cat. No. STOCK1N-53172; Moscow, Russia).

2.2. Cell culture

KCMH-1 is a murine keratinocyte-derived squamous cell carcinoma cell line isolated from CBA/j mouse skin [18]. PAM212, a BALB/c mouse skin-derived murine keratinocyte cell line [19] was kindly provided by Dr. Yuspa (National Institutes of Health, Bethesda, MD, USA). Cells were cultured at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air in alpha minimal essential medium, containing 10% heat-inactivated fetal bovine serum, penicillin G potassium (15 μg/mL), and streptomycin (50 μg/mL) (Meiji Seika, Tokyo, Japan). All cells were used within 6 to 15 passaging cycles, and cell cultures were passaged

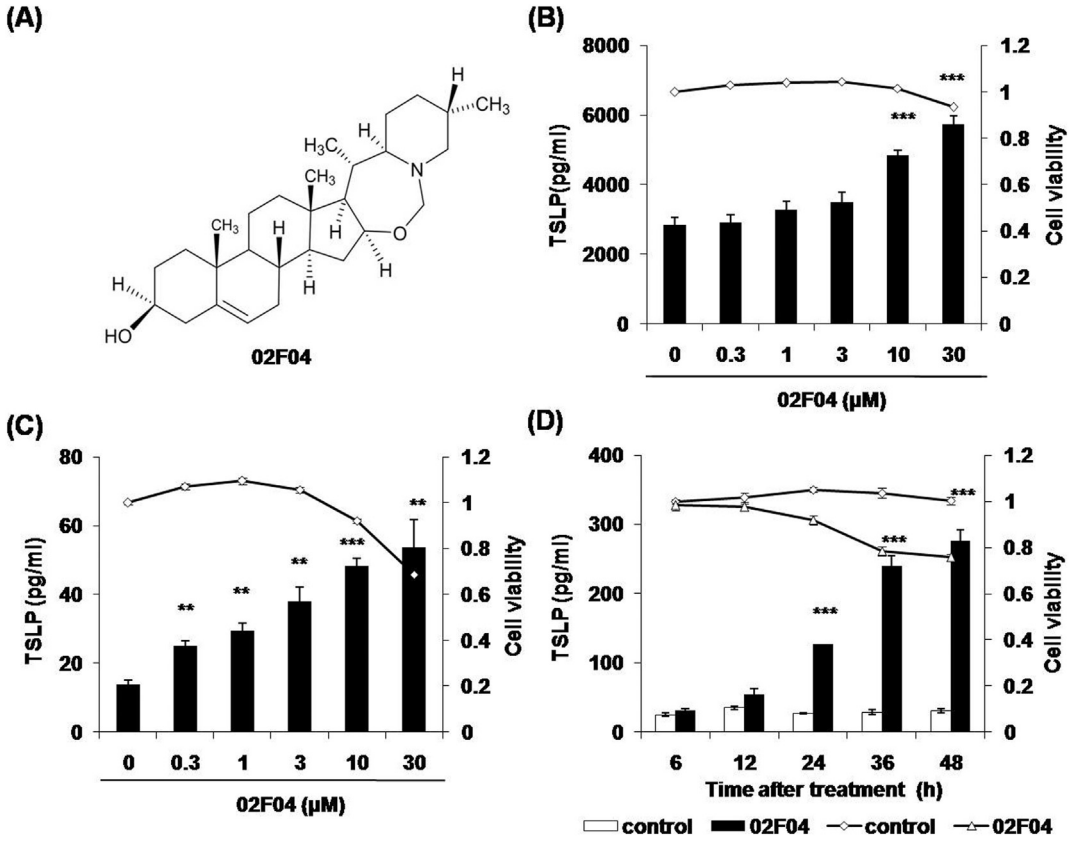

Fig. 1. Induction of TSLP production by 02F04 in mouse keratinocytes. (A) Chemical structure of 02F04. KCMH-1 cells (B) and PAM212 cells (C, D) were stimulated with 02F04 for 24 h at the indicated concentrations (B, C) or for the indicated time at 10 μM (D). TSLP levels in culture supernatant and cell viability were determined by ELISA and the MTT assay, respectively. Data are shown as the mean ± SEM of four samples. Significance: $^{**}p < 0.01$, $^{***}p < 0.001$ vs. the corresponding control. (■, □: TSLP production; -△-, -◇-: Cell viability).