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Transcriptional regulation of *SLURP2*, a psoriasis-associated gene, is under control of IL-22 in the skin: A special reference to the nested gene *LYNX1*

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ABSTRACT

A novel nicotinic acetylcholine (ACh) receptor (nAChR)-mediated transduction pathway, regulating keratinocyte function, has been elucidated in studies of secreted mammalian Ly6/urokinase plasminogen activator receptor-related protein (SLURP)-1 and -2. SLURPs are members of Ly6/neurotoxin superfamily (Ly6SF) of proteins containing the unique three-finger domain in their three-dimensional structure. Some endogenously expressed Ly6SF proteins (such as LYNX1, SLURP-1, and SLURP-2) modulate the function of nAChR, either as allosteric and/or orthosteric modulators, or as antagonists. Although the expression and functions of SLURP-1 and SLURP-2 in keratinocytes are well documented, the expression and the modes of action of LYNX1 in keratinocytes are unknown. Additionally, a particular hybrid transcript, LYNX1–SLURP2, which contains both LYNX1 and SLURP-2 sequences, with unknown function, has been reported. Furthermore, although *SLURP2* is a gene strongly induced in psoriatic skin lesions, the mechanisms controlling *SLURP2* expression are largely unknown. To better understand the function of nAChRs in keratinocytes, we investigated the expression profiles of LYNX1, LYNX1–SLURP-2, and SLURP-2 in keratinocytes under various inflammatory conditions. We found that keratinocytes express *LYNX1* and *SLURP2*, but not *LYNX1–SLURP2*, at mRNA and protein levels. IL-22 treatment increased *SLURP2* expression in keratinocytes, but this effect was completely abolished by IFN-γ. Furthermore, the IL-22-induced up-regulation of *SLURP2* was completely suppressed by the inhibitor or siRNA for STAT3, a major transcriptional factor downstream of IL-22. These findings provide new insights into the nAChR-mediated regulatory mechanism of SLURP-2 expression in keratinocytes.

1. Introduction

Epidermal keratinocytes have the ability to synthesize acetylcholine (ACh), and express muscarinic and/or nicotinic ACh receptors (mAChRs and nAChRs, respectively) that respond to self-produced ACh. Keratinocytes express all five known mAChRs and several subtypes of nAChRs [reviewed in 1]. In the epidermis, keratinocytes differentially express these AChRs at various developmental or differentiation stages [reviewed in 1]. For example, α3 nAChRs are expressed in both basal and suprabasal keratinocytes and are reported to increase cell proliferation and migration, while α7 nAChRs, which are predominantly expressed by differentiating corneal keratinocytes, stimulate apoptosis.

An nAChR-mediated novel transduction pathway regulating keratinocyte function has emerged from studies of secreted mammalian Ly6/urokinase plasminogen activator receptor-related protein (SLURP)-1 and -2 [2–5]. SLURPs are members of the Ly6/neurotoxin superfamily (Ly6SF) of proteins that contain a characteristic pattern of four to five disulfide bonds that are essential for generating their unique three-dimensional structure [reviewed in 6,7].

Some endogenously expressed Ly6SF proteins (such as LYNX1, SLURP-1, and SLURP-2) modulate nAChR function, either as allosteric and/or orthosteric modulators, or as antagonists [reviewed in 6,7]. Chimienti et al. [8] showed that SLURP-1 potentiates signal transduction via α7 nAChRs and enhances the Ca^{2+} signaling induced by ACh, most likely by functioning as a positive allosteric ligand for α7 nAChRs.

Mutation of *SLURP1* has been implicated in the pathogenesis of Mal de Meleda (OMIM: 248300), a skin disease characterized by a hyperproliferative epithelium [2]. In addition, SLURP-1 exerts a proapoptotic effect by acting at the conventional ligand binding-site of keratinocyte nAChRs [3]. SLURP-2, which shares substantial protein sequence homology with SLURP-1, has also been found to be expressed in human epidermal and oral keratinocytes [4,5]. SLURP-2 competes more efficiently with epibatidine than nicotine for binding to α3 nAChRs,

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thereby delaying keratinocyte differentiation and preventing apoptosis [5].

LYNX1, the best-characterized Ly6SF protein, also functions as an endogenous antagonist against α7 nAChRs [reviewed in 7]. Although the expression and functions of SLURP-1 and SLURP-2 in keratinocytes are well documented, no study on the expression and the modes of action of LYNX1 in keratinocytes has been reported to date. In addition, SLURP-2 was sometimes referred to as LYNX1, and *SLURP2* was considered to be a nested gene of *LYNX1* (OMIM: 606110). Tsuji and colleagues [5] showed the presence of two transcripts, a 0.6-kb transcript in the esophagus and an 1.6-kb transcript in the stomach and duodenum, using a cRNA probe encompassing exon 1 to exon 3 of *SLURP2* (Fig. 1A(b)). According to the NCBI database, two alternative splicing variants, containing *SLURP2* exons 2 and 3 have been reported: one is a longer transcript (Fig. 1A(a), *LYNX1–SLURP2*) also containing *LYNX1* exons 1a, 2, and 3, and the other is a shorter form lacking these exons (Fig. 1A(b), *SLURP2*). Although SLURP-2 has been identified as a strongly induced gene in psoriatic skin lesions [4], it is not known whether the *LYNX1–SLURP2* chimeric transcript is also expressed in the skin. Furthermore, the mechanisms regulating expression of *SLURP2* are largely unknown.

In the present study, we investigated the expression profiles of *LYNX1*, *LYNX1–SLURP2*, and *SLURP2* in normal human epidermal keratinocytes (NHEKs). NHEKs expressed *LYNX1* and *SLURP2*, but not *LYNX1–SLURP2* at mRNA and protein levels. SLURP-2 expression, but not LYNX1, was increased by IL-22 stimulation. The enhancement was inhibited by IFN-γ, actinomycin D, or STAT3 inhibitor. These results suggest that IL-22-induced SLURP-2 expression, but not that of LYNX1 or the LYNX1–SLURP-2 chimera, may be involved in homeostasis of the skin or the pathogenesis of skin disorders.

2. Materials and methods

2.1. Cell culture, treatment with pharmacological inhibitors, and transient transfection of siRNAs

Second-passage neonatal foreskin NHEKs were purchased from Kurabo Industries (Osaka, Japan) and were cultured in serum-free keratinocyte growth medium, HuMedai-KG2 (Kurabo Industries), containing human epidermal growth factor (0.1 ng/mL), insulin (10 μg/mL), gentamicin (50 μg/mL), amphotericin B (50 ng/mL), and bovine pituitary extract (0.4%, *v*/*v*), at 37 °C under an atmosphere of 95% air/5% CO_2. Cells were passaged at 60–70% confluence to avoid differentiation, and the experiments were conducted using subconfluent passage 3 cells in the proliferative phase, at 60–80% confluence.

The following pharmacological inhibitors and concentrations were used: the STAT3 inhibitor S3I-201 (50 μM, BioVision, Bilpitas, CA, USA), the STAT5 inhibitor 573108 (100 μM, Merck Millipore, Billerica, MA, USA), the MAP kinase kinase (also known as MAPK/ERK kinase or MEK kinase) inhibitor PD98059 (30 μM, Cayman Chemical Co, Ann Arbor, MI, USA), cycloheximide (0.3 μg/mL, Sigma-Aldrich, St Louis, MO, USA), and actinomycin D (0.25 μg/mL, Sigma-Aldrich).

NHEKs were seeded in a 6-well plate at a density of 2×10^4 cells/well and cultured for 5 days. The cells were transfected with 100 nM of siRNA against *STAT3* (Cell Signaling Technology, Inc., Danvers, MA, USA), *STAT5* (Cell Signaling Technology, Inc.), or a scrambled siRNA, using lipofectamine 2000 (Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions. Forty-eight hours after siRNA transfection, NHEKs were transferred to growth medium with or without 50 ng/mL IL-22, and were cultured for another 24 h.

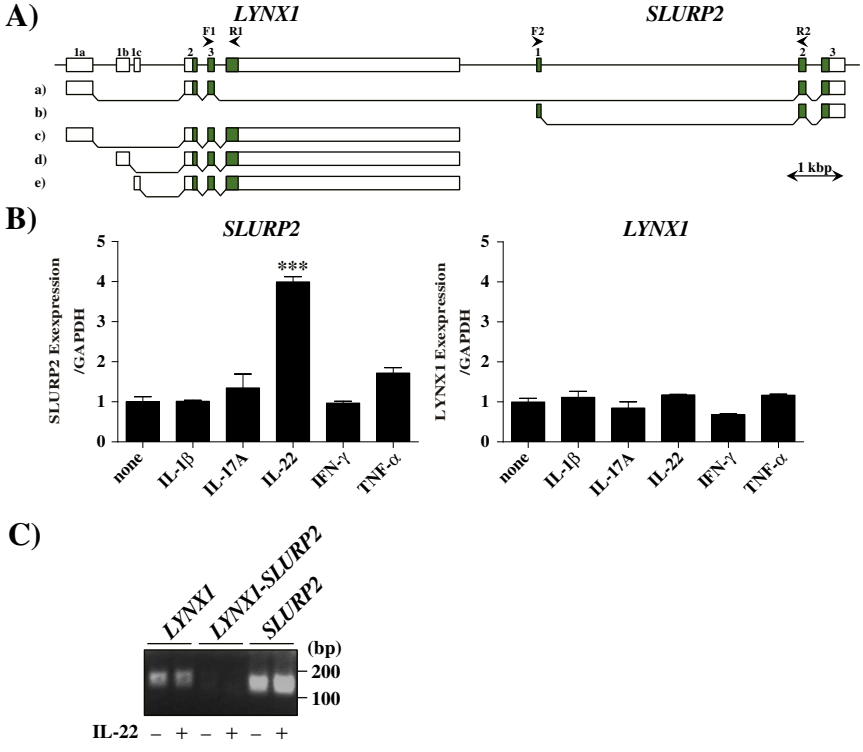

Fig. 1. Expression of *LYNX1* and *SLURP2* in cytokine-stimulated NHEKs. A) Genomic organization and transcript variants of human *LYNX1* and *SLURP2*. Arrows indicate the location and orientation of the primers used in PCR analyses. Exons are represented by boxes; coding regions by solid boxes, and untranslated regions by open boxes. Primers F1 and R1 were used to amplify *LYNX1* transcripts (c, d, e; 191 bp), primers F2 and R2 were used to amplify the *SLURP2* transcript (b; 153 bp), and primers F1 and R2 were used to amplify the *LYNX1–SLURP2* transcript (a; 158 bp). B) NHEKs were stimulated with a panel of cytokines (each 50 ng/mL) for 24 h. RNA was isolated from the cells and subjected to quantitative real-time PCR analysis for *SLURP2* (b) and *LYNX1* (c, d, e) mRNAs. Values represent the mean ± S.D. ($n = 3$). ***$p < 0.001$. The p-values were calculated using one-way ANOVA. C) PCR products of (B) and *LYNX1–SLURP2* (a) were resolved on agarose gels (1.5%).