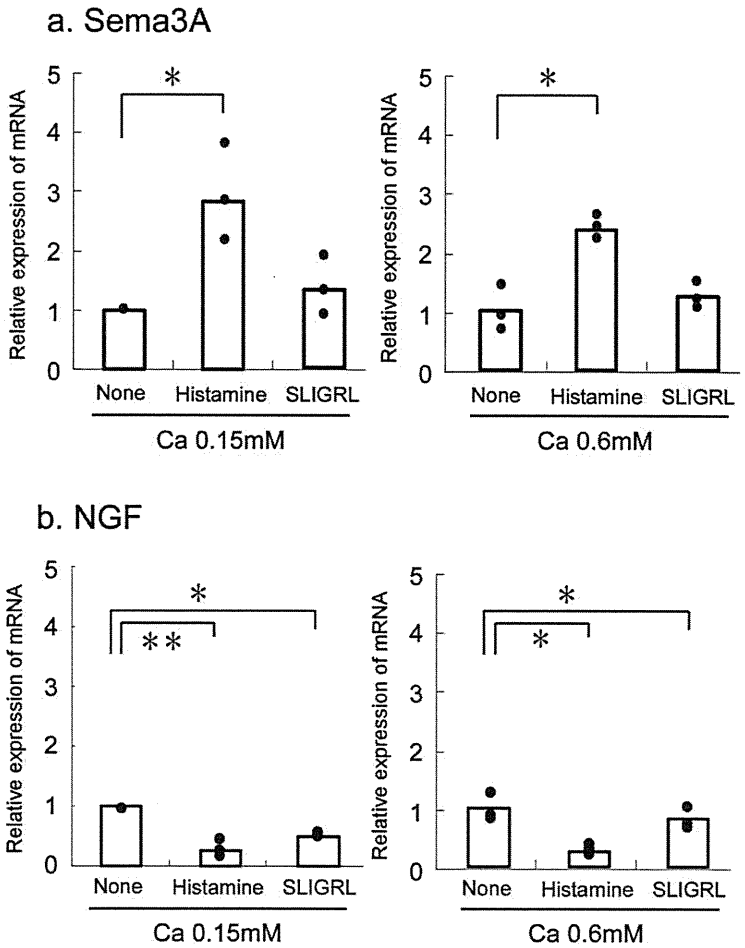

Fig. 4. Effects of histamine and SLIGRL on the expression of *SEMA3A* and *NGF* in NHEK. NHEK were cultured with histamine or SLIGRL under different Ca concentrations (0.15 or 0.6 mM) for 2 h. After harvesting, the expression of Sema3A and NGF was assessed by real-time PCR. The data are expressed as (expression level of stimulated group)/(expression level of no addition control). Bars represent the means of three independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$.

calcium are generated in that Ca^{2+}-induced differentiation [20]. Sema3A production might share the signaling pathway with many differntiation-asscoaited molecules. It has been reported that NGF secreted by basal keratinocytes causes hypertrophy of the peripheral nerve [7,21]. Our finding suggests that NGF is homogenously produced throughout the epidermis, and Sema3A determines the location of nerve endings and inhibits excess C-fiber elongation into the epidermis.

In accordance with the results from the NHEK culture study, our immunohistochemical study showed that keratinocytes in the prickle layer of the epidermis and the outer root sheath of the hair follicle were positive for Sema3A, while basal cells and suprabasal cells were negative in these tissues. The similar staining pattern was obtained in SCC, where Sema3A was expressed by squamoid epithelial cells in the tumor nests, sparing two to three layers of basal or adjacent suprabasal cells. Notably, cells of the sebaceous glands and arector pilli bore Sema3A, providing a possibility that sensory nerves cannot invade these apparatus tissues.

Histamine and SLIGRL not only stimulated NHEK to produce IL-8 and GM-CSF, but also modulated the expression of Sema3A and NGF in NHEK and NHFb. In NHEK, histamine increased Sema3A expression but decreased NGF expression, and SLIGRL did not increase Sema3A expression and decreased NGF expression to some degree. Thus, the modulatory effects of histamine on NHEK are greater than those of SLIGRL. These *in vitro* results suggest that histamine unexpectedly inhibits C-fiber elongation in the epidermis. The release of histamine from mast cells occurs upon antigenic stimulation *via* IgE and Fcε receptors and upon substance P stimulation *via* NK1 receptor [3,4,22]. Since these stimulatory events take place in itch-related allergic diseases, the histamine-enhanced *SEMA3A* and -reduced *NGF* expressions in keratinocytes might be a feedback phenomenon to suppress exaggerated pruritus. However, a recent paper has reported that H1 antagonist olopatadine hydrochloride increased the expression of Sema3A in the skin of NC/Nga mice with atopic dermatitis [23], providing a contradictory finding. Considering that the histamine-augmented Sema3A expression in NHEK was not inhibited by H1 blocker pyrilamine maleate salt (data not shown), the discrepancy between their and our findings might be due to different usage of the types of histamine receptors. In NHFb, histamine and SLIGRL depressed the expression of both *SEMA3A* and *NGF*. Considering the opposite actions of these two axon guidance factors, the final outcome remains unclear in this study. Concerning the chemorepellent, however, histamine seems to allow sensory fibers to elongate in the dermis. It should be noted that the opposite effects of histamine on NHEK and NHFb in the expression of *SEMA3A* may induce positive and negative sensory fiber elongation in the dermis and epidermis, respectively.

Sema3A may play a crucial inhibitory role for C-fiber elongation/sprouting in the upper layers of the epidermis. Disruption of the physiological calcium gradient may induce the disordered Sema3A production by keratinocytes, resulting in C-fiber elonga-

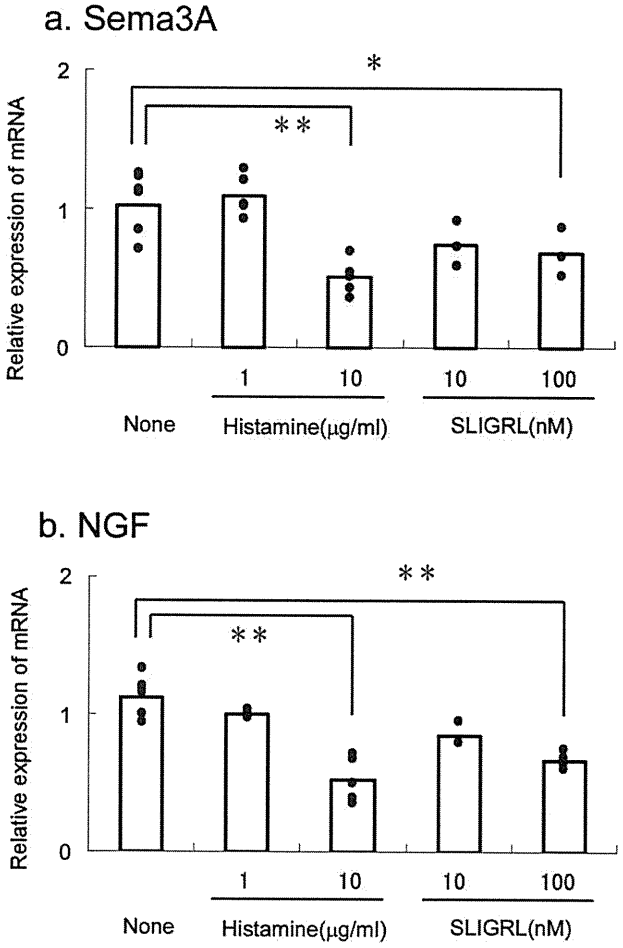

Fig. 5. Effects of histamine and SLIGRL on the expression of *SEMA3A* and *NGF* in NHFb. NHFb were cultured with histamine and SLIGRL at the indicated concentration for 2 h. After harvesting, the expression of *SEMA3A* (a) and *NGF* (b) was assessed by real-time PCR. The data are expressed as (expression level of stimulated group)/(expression level of no addition control). The bars represent the means of three to five independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$.

tion. In the epidermis of patients receiving hemodialysis and suffering from uremic pruritus, calcium concentration is not elevated from the inside to the outside of the skin, but distributed equally in all layers of the epidermis [24]. Considering that Sema3A production is regulated by calcium concentration, the disordered calcium gradient in such a pruritic disease might result in the elongation of C-fiber in the epidermis. In atopic dermatitis, C-fiber elongates into the upper epidermis as a result of a reduced production of Sema3A [9–11]. Our finding suggests that this epidermal elongation and sprouting of nerve endings is not promoted by histamine. It has been reported that mast cell-derived tumor necrosis factor (TNF) promotes nerve fiber elongation in the epidermis and dermis during contact hypersensitivity in mice [25]. This provides a possibility that mast cells contribute to nerve elongation by secreting TNF but not histamine. Future investigation may clarify this important issue.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jdermsci.2010.11.012.

References

[1] Schmelz M, Schmidt R, Bickel A, et al. Specific C-receptors for itch in human skin. J Neurosci 1997;17:8003–8.
[2] Sugiura H, Omoto M, Hirota Y, et al. Density and fine structure of peripheral nerves in various skin lesions of atopic dermatitis. Arch Dermatol Res 1997;289:125–31.
[3] Liu JY, Hu JH, Zhu QG, et al. Substance P receptor expression in human skin keratinocytes and fibroblasts. Br J Dermatol 2006;155:657–62.
[4] Dallos A, Kiss M, Polyanka H, et al. Effects of the neuropeptides substance P, calcitonin gene-related peptide, vasoactive intestinal polypeptide and galanin on the production of nerve growth factor and inflammatory cytokines in cultured human keratinocytes. Neuropeptides 2006;40:251–63.
[5] Yosipovitch G, Greaves MW, Schmelz M. Itch. Lancet 2003;361:690–4.
[6] Levi-Montalcini R. The nerve growth factor 35 years later. Science 1987;237:1154–62.
[7] Albers KM, Wright DE, Davis BM. Overexpression of nerve growth factor in epidermis of transgenic mice causes hypertrophy of the peripheral nervous system. J Neurosci 1994;14:1422–32.
[8] Dontchev VD, Letourneau PC. Nerve growth factor and semaphorin 3A signaling pathways interact in regulating sensory neuronal growth cone motility. J Neurosci 2002;22:6659–69.
[9] 9 Ikoma A, Steinhoff M, Stander S, et al. The neurobiology of itch. Nat Rev Neurosci 2006;7:535–47.
[10] Tominaga M, Ogawa H, Takamori K. Decreased production of semaphorin 3A in the lesional skin of atopic dermatitis. Br J Dermatol 2008;158:842–4.
[11] Yamaguchi J, Nakamura F, Aihara M, et al. Semaphorin 3A alleviates skin lesions and scratching behavior in NC/Nga mice, an atopic dermatitis model. J Invest Dermatol 2008;128:2842–9.
[12] Tominaga M, Tengara S, Kamo A, et al. Psoralen-ultraviolet A therapy alters epidermal Sema3A and NGF levels and modulates epidermal innervation in atopic dermatitis. J Dermatol Sci 2009;55:40–6.
[13] Hennings H, Michael D, Cheng C, et al. Calcium regulation of growth and differentiation of mouse epidermal cells in culture. Cell 1980;19:245–54.
[14] Menon GK, Elias PM. Ultrastructural localization of calcium in psoriatic and normal human epidermis. Arch Dermatol 1991;127:57–63.
[15] Steinhoff M, Neisius U, Ikoma A, et al. Proteinase-activated receptor-2 mediates itch: a novel pathway for pruritus in human skin. J Neurosci 2003;23:6176–80.
[16] Giustizieri ML, Albanesi C, Fluhr J, et al. H1 histamine receptor mediates inflammatory responses in human keratinocytes. J Allergy Clin Immunol 2004;114:1176–82.
[17] Santulli RJ, Derian CK, Darrow AL, et al. Evidence for the presence of a protease-activated receptor distinct from the thrombin receptor in human keratinocytes. Proc Natl Acad Sci USA 1995;92:9151–5.
[18] Johnson CL, Johnson CG, Bazan E, et al. Histamine receptors in human fibroblasts: inositol phosphates, Ca2+, and cell growth. Am J Physiol 1990;258: C533–C543.
[19] Rattenholl A, Steinhoff M. Proteinase-activated receptor-2 in the skin: receptor expression, activation and function during health and disease. Drug News Perspect 2008;21:369–81.
[20] Xie Z, Chang SM, Pennypacker SD, et al. Phosphatidylinositol-4-phosphate 5-kinase 1alpha mediates extracellular calcium-induced keratinocyte differentiation. Mol Biol Cell 2009;20:1695–704.
[21] Pincelli C, Sevignani C, Manfredini R, et al. Expression and function of nerve growth factor and nerve growth factor receptor on cultured keratinocytes. J Invest Dermatol 1994;103:13–8.
[22] Arck PC, Slominski A, Theoharides TC, et al. Neuroimmunology of stress: skin takes center stage. J Invest Dermatol 2006;126:1697–704.
[23] Murota H, El-latif MA, Tamura T, et al. Olopatadine hydrochloride improves dermatitis score and inhibits scratch behavior in NC/Nga mice. Int Arch Allergy Immunol 2010;153:121–32.
[24] Momose A, Kudo S, Sato M, et al. Calcium ions are abnormally distributed in the skin of haemodialysis patients with uraemic pruritus. Nephrol Dial Transplant 2004;19:2061–6.
[25] Kakurai M, Monteforte R, Suto H, et al. Mast cell-derived tumor necrosis factor can promote nerve fiber elongation in the skin during contact hypersensitivity in mice. Am J Pathol 2006;169:1713–21.