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Mechanisms and Managements of Intractable Itch in Atopic Dermatitis

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Increased epidermal nerve density is involved in intractable itch in atopic dermatitis. Epidermal innervation is regulated by the balance between nerve growth factors (e.g. NGF) and nerve repulsion factors (e.g. semaphorin 3A, *Sema3A*) in keratinocytes. Emollient ointment, UV-based therapy, *Sema3A* ointment and cyclosporine administration decreased the density of epidermal nerve fibers, resulting in improvement of itch. Retinoid-related orphan receptor α (*ROR* α) agonist and LL-37 which induce *Sema3A* in keratinocytes may be a useful treatment of atopic dermatitis.

Key words: atopic dermatitis, intractable itch, epidermal nerve fiber, semaphorin 3A, UV-based therapy

Introduction

Atopic dermatitis (AD) is a chronic relapsing inflammatory skin disease characterized by eczematous skin lesions and chronic severe itch. Although AD is a common skin disease its pathogenesis remains unclear. One major symptom of AD which is resistant to conventional treatments is intractable itch. The itch in AD is induced by ordinary nonpruritic stimuli (alloknesis) and severe itch is induced by weak stimuli (hyperknesis)¹⁾. These findings suggest that nerve fibers are related to itch induction in AD. Other properties of this itch include induction of itch by pain stimuli and resistance to antihistamines. In this paper the mechanisms and treatment of intractable itch in AD were discussed.

Mechanisms of itch in AD

Itch in AD is resistant to conventional treatments including antihistamines. Proposed mechanisms of antihistamine-resistant itch include 1) involvement of chemical mediators other than histamines such as protease, eosinophil products and cytokines (IL-1, 2, 31, TNF- α , TSLP, etc.)²⁾, 2) involvement of H4 receptor, 3) heightened itch perception caused by increased epidermal nerve density³⁾ and 4) opioid peptide-receptor mediated itch⁴⁾. Proposed itch related mediators in AD include substance P, CGRP, PAR-2/tryptase system, TRPV-1, TRPA-1, Mrgprs, GRP/GRPR, Nppb/Npra, opioid, epidermal opioid²⁾⁻⁴⁾, cannabinoids, cytokines such as IL-31, TSLP and histamine H4R. Recently there has been lot of focus on IL-31, TSLP and H4R as new itch-related factors³⁾. Nerve fibers in the epidermis are easily stimulated by exogenous triggers such as

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Figure-1 Interactions between pruritogen-producing cells and nerve fibers in the skin

Nerve fibers in the epidermis are easily stimulated by exogenous triggers, pruritogens from keratinocytes and immune cells such as mast cells and T cells, resulting in itch perception in the brain via the spinal cord.

Figure-2 Distribution of nerve fibers in normal healthy skin (NHS) and AD skin

In NHS, many nerve fibers are located in the dermis and terminate around the dermo-epidermal junction. In AD skin, many nerve fibers penetrate the epidermis.

(Tominaga M, *et al*: Br J Dermatol, 2008; 158: 842-844⁶⁾)

physical, chemical and biological stimuli and pruritogen from keratinocytes and immune cells, resulting in perception in the brain via the spinal cord⁵⁾ (Figure-1). Therefore increased density of nerve

fibers in the epidermis is responsible for itch sensitization.

Figure-3 Expression of Sema3A in epidermis

The expression level of Sema3A in epidermis is abundant in NHS, whereas it is significantly decreased in AD skin. (Tominaga M, *et al*: Br J Dermatol, 2008; 158: 842-844⁶⁾)

Figure-4 The regulation of epidermal nerve density by nerve elongation factor (NEF) and nerve repulsion factor (NRF) in NHS and AD skin

In normal healthy skin (NHS), expression level of NRF such as Sema3A is dominant than that of NEF such as NGF. In this case nerve fibers do not penetrate epidermis. In AD skin, expression level of NEF (NGF) is more dominant than that of NRF (Sema3A). In this condition, nerve fibers penetrate epidermis.

Regulatory mechanisms of epidermal nerve fibers in AD

1. Epidermal nerve fibers in itch of AD

The distribution of nerve fibers in normal healthy skin differs from that in AD skin. In normal healthy skin, many PGP 9.5-immunoreactive nerve fibers are located in the dermis and terminate around the dermo-epidermal junction. In contrast, in AD skin many nerve fibers penetrate the epidermis

(Figure-2).

2. How/why do nerve fibers penetrate the epidermis?

Another question is, how do we prevent the penetration of nerve fibers into the epidermis? Epidermal nerve density is regulated by nerve elongation factors such as NGF and nerve repulsion factors such as Sema3A⁵⁾. Among these factors, Sema3A is important in the regulation of epidermal

nerve density. The expression level of Sema3A in epidermis is abundant in normal healthy skin (NHS), whereas it is significantly decreased in AD skin⁶⁾ (Figure-3). The expression level of Sema3A mRNA in skin of AD patients decreased significantly compared with that in NHS. Expression level of NGF in AD skin is more increased compared to that in NHS. These findings suggest that decreased Sema3A expression induced the penetration of nerve fibers into the epidermis⁵⁾. The relationship between the expression level of Sema3A and NGF in the epidermis regulates the intraepidermal penetration of nerve fibers (Figure-4). Nerve fibers penetrate the epidermis with up-regulation of NGF and down-regulation of Sema3A in keratinocytes.

3. The next question is, how do nerve fibers penetrate the basement membrane (BM) ?

The main component of the BM is type IV collagen. Type IV collagenase such as metalloproteinase (MMP)-2 or MMP-9 was shown to be related to the penetration of nerve fibers into the BM. Experimental results demonstrated that nerve fibers penetrated the BM using NGF-activated MMP-2 in the growth cone of nerve fibers⁷⁾.

Management of intractable itch in AD targeting epidermal nerve fibers

1. Effect of topical emollient on epidermal nerve fibers

Topical emollients such as heparinoid, petrolatum and steroid ointments decreased dry skin-induced epidermal nerve fibers by down-expression of NGF in keratinocytes⁸⁾.

2. Effect of UV-based therapy on epidermal nerve fibers

PUVA therapy significantly suppressed itch in AD patients. PUVA therapy decreased the density of epidermal nerve fibers similar to topical emollient application. PUVA therapy restored the decreased expression level of Sema3A to normal level in keratinocytes in AD, resulting in a reduction of epidermal nerve fibers⁹⁾. Other UV-therapies such as narrow band-UVB and excimer lamp, also significantly decreased the epidermal nerve fibers like PUVA therapy¹⁰⁾ (Figure-5). UV-based therapy decreases epidermal nerve fibers by restoring

Figure-5 Effect of UV-based therapy on epidermal nerve density in dry skin

PUVA, NB-UVB, Excimer lamp significantly decreased the density of epidermal nerve fibers. BV; betamethasone valerate, PUVA; psoralen ultraviolet A, NB-UVB; narrow-band ultraviolet B.

(Kamo A, *et al*: J Dermatol Sci, 2011; 62: 91-97¹⁰⁾)

Sema3A and suppresses itch. UV-based therapy is useful for the treatment of AD.

3. Effects of cyclosporine A (CsA) and Sema3A ointment on pruritus in AD

CsA is an immunosuppressive drug isolated from fungus. Clinically CsA is widely used in the treatment of severe AD with intractable itch and suppresses pruritus. However the antipruritic mechanisms of CsA is unclear. Therefore we investigated the suppression mechanism of itch in AD. CsA administration intraperitoneally reduced the density of epidermal nerve fiber dose-dependently. In a mouse model of AD, 5 mg/kg CsA significantly reduced scratching bouts. CsA suppressed scratching behavior through reduction of epidermal nerve fibers¹¹⁾.

The effect of Sema3A ointment on itch was investigated using AD model mouse. Sema3A expression in AD skin was decreased. Rescue of Sema3A reduced epidermal nerve density, attenuated pruritus, inhibited scratching behavior and finally ameliorated dermatitis¹²⁾.

Regulation of endogenous Sema3A in human keratinocytes

Penetration of nerve fibers in the skin is regulated by axon-guidance molecules such as

Sema3A which is produced in keratinocytes. Therefore it is important to investigate the regulation of endogenous Sema3A in keratinocytes. We found that ROR α agonist and LL-37 were associated with induction of Sema3A in keratinocytes.

1. ROR α agonist

Sema3A promotor activities were significantly reduced by deletion of ROR α /AP-1 binding site. The binding site of ROR α /AP-1 is the most important site in Sema3A expression. ROR α is involved in the induction of Sema3A gene expression in normal human epidermal keratinocytes. Knockdown of ROR α in keratinocytes using siRNA reduced Sema3A mRNA expression. Cholesterol sulfate, an ROR α agonist, induced up-regulation of Sema3A mRNA expression and Sema3A protein. Activation of ROR α induced Sema3A expression in keratinocytes¹³⁾.

2. LL-37

LL-37 is an antimicrobial peptide produced by cleavage of human cathelicidin. Of the antimicrobial peptides such as human β -defensins and LL-37, only LL-37 induced Sema3A expression in epidermal keratinocytes¹⁴⁾.

ROR α agonist and LL-37 induced Sema3A in keratinocytes and reduced the density of epidermal nerve fibers, attenuating itch.

Conclusions

1. Epidermal hyperinnervation is related to induction of intractable itch in AD.
2. Epidermal hyperinnervation is caused by an imbalance between nerve elongation factors such as NGF and repulsion factors such as Sema3A.
3. Emollient, UV-based therapies (PUVA, narrow band-UVB, excimer lamp), and cyclosporine administration are useful treatments for pruritus involving epidermal nerve density.
4. The promotor region of Sema3A gene was ROR α . ROR α agonist and antimicrobial peptide

LL-37 increased the production of Sema3A in keratinocytes. These molecules may be useful in treatment of intractable itch in AD.

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