

## Signal Transducer and Activator of Transcription 3 in Keratinocytes Regulates Histaminergic Itch but Not Nonhistaminergic Itch

Takashi HASHIMOTO<sup>1,2</sup>, Kent SAKAI<sup>1</sup>, Gil YOSIPOVITCH<sup>1</sup> and Tasuku AKIYAMA<sup>1</sup>

<sup>1</sup>Dr. Phillip Frost Department of Dermatology and Cutaneous Surgery, Miami Itch Center, University of Miami Miller School of Medicine, Miami, FL 33136, USA, and <sup>2</sup>Department of Dermatology, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Tokyo, Japan. E-mail: takiyama@miami.edu

Accepted May 29, 2019; E-published May 29, 2019

Itch, an unpleasant sensation that evokes scratching behavior, is divided into two forms: histaminergic and nonhistaminergic itch. Histaminergic itch is caused by histamine that is secreted principally by mast cells and basophils, while nonhistaminergic itch is evoked by various pruritogens other than histamine from multiple origins. Recent findings suggest that not only the interaction between pruritogens and peripheral pruriceptor terminals, but also the interplay among peripheral pruriceptor terminals, immune cells, and epidermal keratinocytes play roles in itch induction (1, 2). However, the molecular mechanisms of this interplay are not fully understood.

Signal transducer and activator of transcription (STAT)3 is a transcription factor (3) that has recently been highlighted as a key player in itch sensation. In the spinal cord, STAT3 amplifies itch sensation through promoting astrogliosis (4). While STAT3 plays critical roles in the regulation of keratinocyte function (3), whether STAT3 in the skin is also important in itch sensation is still unclear. Since keratinocytes are capable of producing itch mediators (2), keratinocyte STAT3 may also modulate itch. The aim of this study was to elucidate the involvement of keratinocyte STAT3 in itch sensation.

### MATERIALS AND METHODS AND RESULTS

To examine the role of STAT3 in keratinocytes *in vivo*, we crossed *Stat3 flox* mice with *K5CreERT2* mice, and tamoxifen was injected to delete STAT3 in keratinocytes. Keratinocyte-specific STAT3-depletion was confirmed through immunohistochemistry (Fig. S1<sup>1</sup>). Vehicle-treated *K5CreERT2:Stat3 flox*<sup>+/+</sup> mice were used as controls. For further information on materials and methods, see Appendix S1<sup>1</sup>. All animal experiments were approved by the Institutional Committee of the University of Miami.

We initially assessed the *in vivo* effect of keratinocyte-specific STAT3 depletion on acute itch in mice. The acute itch was

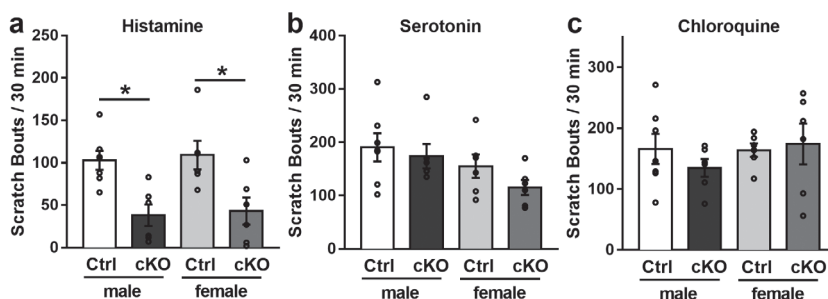
evoked by intradermally injecting either histaminergic or non-histaminergic pruritogen (histamine, serotonin, or chloroquine). All 3 pruritogens evoked scratching responses in control mice. Histamine-evoked scratching behavior was significantly attenuated in *STAT3* conditional knockout (cKO) mice while serotonin- and chloroquine-induced scratching responses did not change (Fig. 1). We also examined the impact of keratinocyte STAT3 on chronic itch using ovalbumin (OVA)-induced atopic dermatitis (AD) murine model, which has been shown to involve nonhistaminergic itch (5). We did not find any significant reduction of OVA-induced scratching responses in *STAT3* cKO mice (Fig. S2<sup>1</sup>). These results demonstrate that keratinocyte STAT3 contributes to histaminergic itch, but not nonhistaminergic itch.

To determine whether pruritogens elevate intracellular calcium in keratinocytes, we performed calcium imaging experiments on keratinocytes. In keratinocytes from control mice, intracellular calcium gradually increased and reached a maximum level 90–150 s after application of histamine and serotonin (Fig. S3a–c<sup>1</sup>). However, chloroquine did not increase intracellular calcium (Fig. S3d<sup>1</sup>), which is consistent with a previous report (1). The chloroquine receptor, Mas-related G protein coupled-receptor member A3, is expressed exclusively by sensory neurons, but not keratinocytes, suggesting that chloroquine evokes itch without affecting keratinocytes.

We next examined whether STAT3 is involved in intracellular calcium elevations by pruritogens in keratinocytes. The increased responses of keratinocytes to histamine and serotonin were significantly reduced in *STAT3* cKO mice (Fig. S3a–c<sup>1</sup>). This result was confirmed using a STAT3 inhibitor, STA-21. Keratinocytes treated with STA-21 for one hour showed a significant reduction of intracellular calcium responses compared to vehicle-treated keratinocytes (Fig. S3e, f<sup>1</sup>).

A recent report has shown that histamine-induced Ca<sup>2+</sup> influx in keratinocytes is dependent on transient receptor potential cation channel subfamily V member 4 (TRPV4), a highly calcium selective ion channel, and TRPV4-mediated Ca<sup>2+</sup> influx is required for histaminergic itch (2). Thus, we examined the effects of a selective TRPV4 inhibitor, GSK205, on the intracellular calcium responses to histamine and serotonin in keratinocytes. The magnitude of intracellular calcium responses of keratinocytes from naive mice was significantly reduced by the application of GSK205 (Fig. S3e, f<sup>1</sup>).

To investigate whether STAT3 is involved in TRPV4 expression, we performed immunostaining experiments on epidermal sheets. In epidermal sheets from control mice, TRPV4 was detected



**Fig. 1. Effects of *in vivo* depletion of keratinocyte STAT3 on scratching behaviors.** (a) *K5CreERT2:Stat3 flox*<sup>+/+</sup> mice were injected with either tamoxifen (cKO) or vehicle (Ctrl). cKO mice showed attenuated histamine-induced scratching behavior in comparison with Ctrl mice. (b,c) Scratching bouts in response to serotonin (b) and chloroquine (c) did not change in cKO mice compared with Ctrl mice. Vertical bars indicate mean + SEM ( $n = 6-7$  mice/group). \*  $p < 0.05$  versus Ctrl mice, two-tailed unpaired *t*-test.

on the cell surface of keratinocytes (Fig. S4a<sup>1</sup>). This signal was considerably weaker in keratinocytes of *STAT3* cKO mice, while TRPV4 mRNA expressions were detected in the epidermal sheets from control mice as well as *STAT3* cKO mice (Fig. S4b<sup>1</sup>).

## DISCUSSION

In this work, we showed the importance of keratinocyte *STAT3* in histaminergic itch. We also detected that serotonin- and chloroquine-induced itch were not significantly affected by *in vivo* depletion of keratinocyte *STAT3*. These two types of itch are also implicated in chronic itch, and this result seems to be compatible with the data that scratching behavior of OVA-induced AD was not attenuated in *STAT3* cKO mice.

It is somewhat surprising that depletion of keratinocyte *STAT3* significantly attenuated serotonin-evoked calcium responses of the keratinocytes, but not serotonin-evoked scratching behavior. A possible explanation for these results may be that intracellular calcium increases in keratinocytes and trigger the release of the itch enhancers that can modulate itch evoked by histamine, but not serotonin. Histaminergic itch is mediated through TRPV1 in sensory neurons, whereas serotonin-induced itch is independent of TRPV1 (6). It can, therefore, be assumed that TRPV1 sensitizers (e.g., nerve growth factor, IL-6, and granulocyte-macrophage colony-stimulating factor; (7–9)) enhance histaminergic itch without affecting serotonin-induced itch. Consistent with this idea, histamine is reported to release all above-mentioned chemical messengers from keratinocytes (10–12).

Another possible explanation for the mismatch of reduced calcium signaling/unchanged scratch behavior for serotonin may be that chemical mediators produced from keratinocytes are different between histamine and serotonin. This hypothesis is supported by the finding that histamine and serotonin differently affect on keratinocytes in epidermal barrier recovery (13). Keratinocyte may release itch mediators by histamine, but not serotonin.

The intracellular calcium responses to histamine and serotonin were reduced in both keratinocytes from *STAT3* cKO mice and in keratinocytes treated with the *STAT3* inhibitor as well as the TRPV4 antagonist. While TRPV4 mRNA expressions were detected in the epidermal sheets from *STAT3* cKO mice, TRPV4 expression was faint in keratinocytes from *STAT3* cKO mice. These results suggest that *STAT3* might be involved in the trafficking of TRPV4 to the plasma membrane. TRPV4-containing vesicles derived from mitochondria fuse to the plasma membrane within a few minutes to ensure constant turnover of TRPV4 (14). *STAT3* may reduce TRPV4 expression by disturbing this exocytosis process. In accordance with this idea, mitochondrial *STAT3* has a critical role in exocytosis in mast cells (15).

Although keratinocytes express a variety of itch mediators, how keratinocytes contribute to itch is still unclear. This study has shown that keratinocyte *STAT3* play a

major role in histaminergic itch, but not nonhistaminergic itch. A further study could assess the role of other signaling pathway molecules in itch via keratinocytes.

## ACKNOWLEDGEMENTS

This work was supported by Pfizer grant (W1203521: GY and TA) and National Institutes of Health grant (AR063228: TA). TH has received scholarships from the Uehara Memorial Foundation, the Japanese Society of Allergology.

*Conflicts of interest:* GY has served in advisory board and consultant for Pfizer, Novartis, Eli Lilly, Sanofi, Galderma, Trevi, Menlo, Sienna, UCB.

## REFERENCES

- Chen Y, Fang Q, Wang Z, Zhang JY, MacLeod AS, Hall RP, et al. Transient receptor potential vanilloid 4 ion channel functions as a pruriceptor in epidermal keratinocytes to evoke histaminergic itch. *J Biol Chem* 2016; 291: 10252–10262.
- Schwendinger-Schreck J, Wilson SR, Bautista DM. Interactions between keratinocytes and somatosensory neurons in itch. *Handb Exp Pharmacol* 2015; 226: 177–190.
- Sano S, Chan KS, DiGiovanni J. Impact of *Stat3* activation upon skin biology: a dichotomy of its role between homeostasis and diseases. *J Dermatol Sci* 2008; 50: 1–14.
- Shiratori-Hayashi M, Koga K, Tozaki-Saitoh H, Kohro Y, Toyonaga H, Yamaguchi C, et al. *STAT3*-dependent reactive astrogliosis in the spinal dorsal horn underlies chronic itch. *Nature Medicine* 2015; 21: 927–931.
- Yatsuzuka R, Inoue T, Jiang S, Nakano Y, Kamei C. Development of new atopic dermatitis models characterized by not only itching but also inflammatory skin in mice. *Eur J Pharmacol* 2007; 565: 225–231.
- Imamachi N, Park GH, Lee H, Anderson DJ, Simon MI, Basbaum AI, et al. TRPV1-expressing primary afferents generate behavioral responses to pruritogens via multiple mechanisms. *Proc Natl Acad Sci U S A* 2009; 106: 11330–11335.
- Shu X, Mendell LM. Acute sensitization by NGF of the response of small-diameter sensory neurons to capsaicin. *J Neurophysiol* 2001; 86: 2931–2938.
- Donatien P, Anand U, Yiangou Y, Sinisi M, Fox M, MacQuillan A, et al. Granulocyte-macrophage colony-stimulating factor receptor expression in clinical pain disorder tissues and role in neuronal sensitization. *Pain Rep* 2018; 3: e676.
- Fang D, Kong LY, Cai J, Li S, Liu XD, Han JS, et al. Interleukin-6-mediated functional upregulation of TRPV1 receptors in dorsal root ganglion neurons through the activation of JAK/PI3K signaling pathway: roles in the development of bone cancer pain in a rat model. *Pain* 2015; 156: 1124–1144.
- Giustizieri ML, Albanesi C, Fluhr J, Gisondi P, Norgauer J, Girolomoni G. H1 histamine receptor mediates inflammatory responses in human keratinocytes. *J Allergy Clin Immunol* 2004; 114: 1176–1182.
- Kohda F, Koga T, Uchi H, Urabe K, Furue M. Histamine-induced IL-6 and IL-8 production are differentially modulated by IFN- $\gamma$  and IL-4 in human keratinocytes. *J Dermatol Sci* 2002; 28: 34–41.
- Kanda N, Watanabe S. Histamine enhances the production of nerve growth factor in human keratinocytes. *J Invest Dermatol* 2003; 121: 570–577.
- Denda M. Physical and Chemical Factors that Improve Epidermal Permeability Barrier Homeostasis, Atopic Dermatitis. In: Esparza-Gordillo J, ed. *Disease Etiology and Clinical Management*: InTech, London, 2012.
- Baratchi S, Keov P, Darby WG, Lai A, Khoshmanesh K, Thurgood P, et al. The TRPV4 Agonist GSK1016790A Regulates the Membrane Expression of TRPV4 Channels. *Front Pharmacol* 2019; 10: 6.
- Erllich TH, Yagil Z, Kay G, Peretz A, Migalovich-Sheikhet H, Tshori S, et al. Mitochondrial *STAT3* plays a major role in IgE-antigen-mediated mast cell exocytosis. *J Allergy Clin Immunol* 2014; 134: 460–469.