

# Effects of Sakakibara hot spring water (5T) on suppressing itching in mice

**Authors:** Yeunhwa Gu<sup>1\*</sup>, Takenori Yamashita<sup>2</sup> and Tota Inoue<sup>3</sup>

**Key Words:** Suppresses itching, IgE, IgG, IL-31, Histamine H1, Sakakibara hot spring water.

\* **Corresponding author:** Yeunhwa Gu<sup>1</sup>, email: gu.y@junshin-u.ac.jp

**Affiliated institutions:**

<sup>1</sup> Chairperson International Affairs Department of Radiological Science,  
Graduate School of Health Science, Faculty of Health Science Junshin Gakuen University  
[1-1-1 Chikushigaoka, Minami-ku, Fukuoka 815-8510 Japan.]

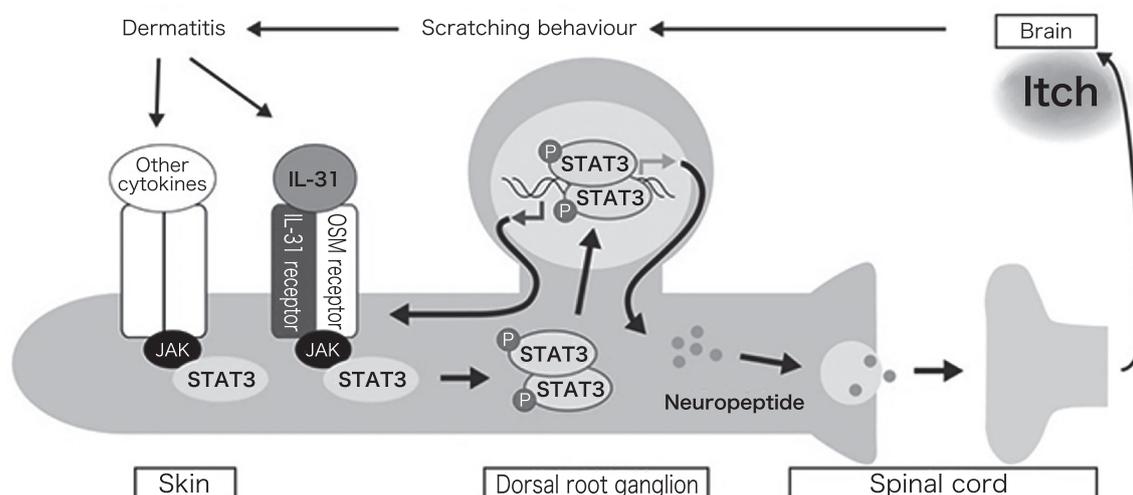
<sup>2</sup> Graduate School of Health Science, Suzuka University of Medical Science

<sup>3</sup> Mie breathing swallowing rehabilitation clinic

**Abstract**

In this study, we investigated the relationship between seven types of hot spring water and the itch-transmission mechanism of dermatitis and itching in mice.

It is clear that activation of the transcription factor STAT3 in sensory nerves plays an important role in the transmission of itch associated with dermatitis. In this study, we compared new natural hot spring waters for itch such as dermatitis, and it is expected to contribute to their development. We demonstrated that IL-31, which is deeply involved in dermatitis itch, causes itch by acting on receptors expressed in sensory nerves<sup>1-8</sup>). We also showed that activation of the transcription factor STAT3 downstream of the IL-31 receptor is important for itch induction. Furthermore, we found that STAT3 in sensory nerves is important for the expression of IL-31 receptors and neuropeptides involved in itch transmission. It was also suggested that STAT3 in sensory nerves is important not only for IL-31-dependent itch but also for IL-31-independent inflammatory itch<sup>9-14</sup>). These results suggest that the development and improvement of STAT3 inhibitors may lead to new improvements in itch.



**Figure 1.** Role of STAT3 in sensory neurons that transmit itch in relation to the inhibitory effect of hot spring water on itch

## 1. Purpose of study

Itch is defined as an unpleasant skin sensation that causes the desire to scratch, and is a sensation specific to the skin and some mucous membranes. Itch is one of the symptoms of various skin diseases (atopic dermatitis, contact dermatitis, urticaria, psoriasis, allergic diseases, etc.) and systemic diseases, especially visceral diseases (chronic renal failure, cholestasis, etc.). Itch is very unbearable, and scratching behavior caused by itching partially transforms the unpleasant sensation into a pleasant sensation, and partially promotes the release of endogenous prurigenic substances, leading to an increase and intensification of itching. This vicious cycle of itching is called the "itch-scratch cycle." Continued scratching due to this vicious cycle leads to further deterioration of symptoms such as dermatitis (cataracts in the eyes). In addition, because itching is an unpleasant sensation, it leads to a decrease in quality of life (QOL). For these reasons, suppressing itching is one of the most important treatment goals in clinical practice. However, in the case of such clinically problematic pruritus caused by skin diseases, antagonists of histamine receptors (H1 histamine receptors), which have long been considered a mediator of pruritus, are often ineffective. Therefore, the development of novel antipruritic drugs is desired.

In this study, we conducted objective evaluations using animals, focusing in particular on behavior, and investigated whether various hot spring waters have the effect of reducing itching substances that induce itching in mice.

In this study, we investigated the inhibitory effect of hot spring water on itch using ICR mice, NC/ Nga mice, and NC/ NgaSlc mice. In addition, H1 histamine receptor antagonists are often ineffective against itch in chronic pruritic skin diseases. Therefore, histamine is unlikely to be the main itch mediator, and we explored whether hot spring water is a new itch mediator. Therefore, based on the results of itch research using mice, we also investigated the background to our focus on optimal hot spring water, rather than mast cells, and the involvement of optimal hot spring water in the occurrence of itch.

## 2. Research method

Itching is one of the main symptoms of many skin diseases. If itching cannot be suppressed, it becomes painful, and scratching due to itching aggravates the skin symptoms. Therefore, suppressing itching and scratching is an important treatment goal for pruritic skin diseases. Until now, histamine released from mast cells has been thought to play an important role as an endogenous itch factor, but H1 histamine receptor antagonists are often ineffective against the itch of such skin diseases. This suggests that there are mediators and mechanisms of itch other than the mast cell-histamine system<sup>15-20</sup>.

Therefore, we established an evaluation system for itch using mice, and research using various itch model mice revealed that hot spring water optimal for the epidermis produces and releases leukotriene B4, thromboxane A2, nociceptin, nitric oxide, and hydrogen peroxide, which are responsible for itch.

Intradermal injection of 5T hot spring water in mice induces scratching behavior with the hind paw at the injection site, which is an itch-related behavior, and nitric oxide enhances the scratching behavior induced by intradermal injection of a prurigen. Thromboxane A2 and nociceptin produced and released from optimal hot spring water may enhance itch in addition to their direct effect on primary sensory nerves. It is generally known that IgE plays an important role in allergic itch. Recently, it has been revealed that high-affinity IgG receptors are expressed in primary sensory nerves, and that in addition to the direct effect of antigen-IgG complex formation on primary sensory nerves, the existence of a mechanism for the generation of itch responses due to the activation of optimal hot spring water has been revealed<sup>21-26</sup>. Therefore, in this study, we will study the effects of hot spring water from Sakakibara Onsen (5T) on suppressing itch in mice and examine its improving effects.

### 2-1. Research Method

#### (1) Immunoglobulins

It is well known that the IgE mast cell system is involved in immediate allergic itch. However, in a mouse model of atopic dermatitis, serum IgE levels were examined. In a mouse model of mosquito-induced

allergic itch, the inhibitory effect of H1 histamine receptors on the allergic scratching behavior was also examined. In addition, serum IgE and IgG1 levels were measured in comparison with non-sensitized mice. Mice without mast cells (mast cell-deficient mice) were also sensitized and showed the same degree of scratching behavior as healthy mice with mast cells that had been sensitized to the same antigen. These findings suggest the existence of a mechanism for the development of allergic itch other than the IgE mast cell system. We have revealed that high-affinity IgG receptors are expressed in primary sensory nerves, and that in a sensitized state, antigens bind to primary sensory nerves in the skin, that the formation of antigen-IgG1 complexes stimulates the primary sensory nerves, and that substance P is released from the nerve terminals<sup>27-31</sup>.

Therefore, in this study, we will investigate the effects of hot spring water from Sakakibara Onsen (5T) on suppressing itching in mice and examine its ameliorative effects.

## (2) Animal rearing environment

In order to allow the animals to become accustomed to the breeding environment (room temperature  $22 \pm 3^\circ\text{C}$ , humidity 60%, indoor lighting on for 14 hours, off for 10 hours), they were kept for one week as preliminary breeding, and the experiment began when they were 6 weeks old. Water (Tap water) and food (CA-1: CLEA Japan) were given free access.

## (3) Research on IgG and IgE

IgE were measured using ICR mice, NC/Nga mice, and NC/NgaSlc mice. The total IgG and IgE in mouse serum were measured by enzyme immunoassay (hereinafter referred to as ELISA) using Mouse IgE ELISA Quantitation Kit and Mouse IgG ELISA Quantitation Kit manufactured by Eppendorf Laboratories, Inc. The ELISA method uses antibodies and enzymes as detection agents for target substances (IgG and IgE in this experiment) and determines the concentration of the target substance by the antigen-antibody reaction of antibodies that specifically bind to a specific substance and the degree of color development by the enzyme. The specific measurement procedure is as

follows: first, whole blood is collected from the heart of an anesthetized mouse using a Terumo syringe (needle: 23G), and then heparinized (5 units/mL) to prevent blood coagulation, and then centrifuged (Time: 15 min,  $1.5 \times 1000$  rpm) to separate only serum from the whole blood. 100  $\mu\text{L}$ /well of solid-phase antibodies diluted 1/100 with Coating Buffer were dispensed into a 96-well microplate and incubated at room temperature for 60 minutes.

After incubation, the coating buffer was discarded and the plate was washed twice with wash solution. Next, 200  $\mu\text{L}$  of postcoat solution was dispensed per well to immobilize the antibody. After incubation for 30 minutes at room temperature, the postcoat solution was discarded and the plate was washed twice with wash solution. 100  $\mu\text{L}$  of serum diluted 50-fold with sample dilution solution and standard serum of known concentration were dispensed per well and incubated for 60 minutes at room temperature. After incubation, the serum was discarded and the plate was washed four times with wash solution.

100  $\mu\text{L}$  of enzyme-labeled antibody diluted 1/120,000 with conjugate dilution solution was dispensed per well to perform enzyme labeling. After incubation for 60 minutes, the enzyme solution was discarded and the plate was washed four times with wash solution. After washing, 100  $\mu\text{L}$  of enzyme substrate solution was dispensed per well and incubated for 15 minutes to develop the enzyme color. After dispensing 100  $\mu\text{L}$  of reaction stop solution per well to stop the color reaction, the absorbance was measured using a microplate reader MPR A4 (wavelength: 450 nm) manufactured by Toyo Soda Co., Ltd. A standard curve was created from the absorbance of the standard serum to determine the concentrations of total IgG and IgE in serum. For statistical analysis, because the concentrations of total IgG and IgE in serum showed normality, a parametric *t*-test between two groups was used to test for significant differences in the concentrations of IgG and IgE. In this study, we investigated the effects of hot spring water from Sakakibara Onsen (5T) on the suppression of itching in mice and investigated its improving effects<sup>32-38</sup>.

## (4) Research on IL-31

Serum IL-31 was measured using ICR mice, NC/ Nga mice, and NC/ NgaSlc mice. IL-31 in mouse serum was measured by enzyme immunoassay using Mouse IL-31 ELISA Quantitation Kit manufactured by Eppendorf Laboratories, Inc. ELISA is a method that uses antibodies and enzymes as detection agents for IL-31, and measures the concentration of the target substance by the antigen-antibody reaction of the antibody that specifically binds to a specific substance and the degree of color development by the enzyme.

The specific measurement procedure is as follows: first, whole blood is collected from the heart of an anesthetized mouse using a Terumo syringe (needle: 23G), and heparinized (5 units/mL) to prevent blood coagulation, and then centrifuged (Time: 15 min, 1.5 × 1000 rpm) to separate only serum from the whole blood. 100 µL/well of solid-phase antibody diluted 1/100 with Coating Buffer was dispensed into a 96-well microplate and incubated at room temperature for 60 minutes. After incubation, the Coating Buffer was discarded and the plate was washed twice with Wash Solution.

Next, 200µL of postcoat solution was dispensed per well to immobilize the antibody. After 30 minutes of incubation at room temperature, the postcoat solution was discarded and the plate was washed twice with wash solution. Serum diluted 50-fold with sample dilution solution and standard serum of known concentration were dispensed at 100µL per well and incubated at room temperature for 60 minutes. After incubation, the serum was discarded and the plate was washed four times with wash solution. Enzyme-labeled antibodies diluted 1/120,000 with conjugate dilution solution were dispensed at 100µL per well for enzyme labeling. After 60 minutes of incubation, the enzyme solution was discarded and the plate was washed four times with wash solution. After washing, 100µL of enzyme substrate solution was dispensed per well and incubated for 15 minutes to develop the enzyme color. After dispensing 100 µL of stop solution per well to stop the color reaction, the absorbance was measured using a microplate reader MPR A4 (wavelength: 450 nm) manufactured by Toyo Soda Co., Ltd.

A standard curve was created from the absorbance of

the standard serum, and the serum IL-31 concentration was calculated from the standard curve<sup>39-42</sup>. For statistical analysis, since the serum IL-31 concentration showed normality, a parametric *t*-test between two groups was used to test for significant differences in the IL-31 concentration. In this study, we investigated the effects of hot spring water from Sakakibara Onsen (5T) on suppressing itching in mice and investigated its improving effects.

#### (5) Observation of mouse skin

##### (5)-1. Microscopic image analysis of mouse dorsal skin

Skin photography was performed using a Canon Eos KISS Digital IV equipped with an EF-S18-S 5mF 3.5-5.611 USM lens. Corneal cell exfoliation was measured at Pola Chemical Industries, Ltd. after the preparation of the exfoliated specimens.

##### (5)-2. Analysis of mouse skin by H&E staining

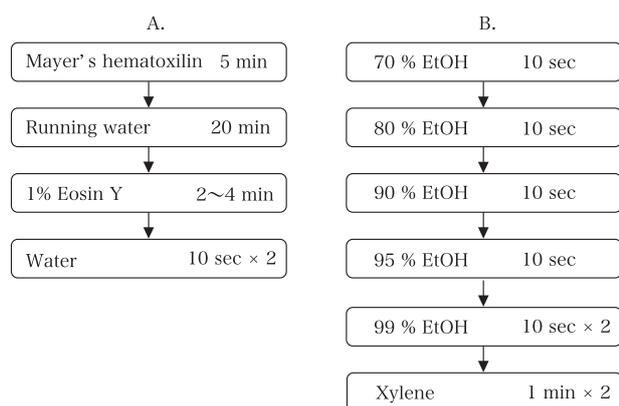
###### (5)-2-1. Preparation of frozen slide specimens

After 18 weeks of oral administration, mice from each group were cervically dislocated, blood was removed by perfusion of saline (1 mL/min) through the left ventricle, and fixation was performed by perfusion of 10% formalin solution (1 mL/min, 15 min). The brains were removed and immersed in 10% formalin solution for 24 hours (4°C), 10% sucrose/0.1 M PBS (4°C) for 4 hours, 20% sucrose/0.1 M PBS for 4 hours (4°C), and finally immersed in 30% sucrose/0.1 M PBS overnight (4°C)<sup>43-44</sup>.

Next, the brains were embedded in OCT compound (embedding medium for freezing), sliced into 9 µm slices using a cryostat, and attached to glass slides. After that, the skin was dried with cold air for more than 1 hour, immersed in 50% EtOH for 30-60 minutes, washed with running water for 2-4 minutes, and then histological examination was performed using each staining method. In this study, the skin was thinly sliced according to the method described by David *et al.*

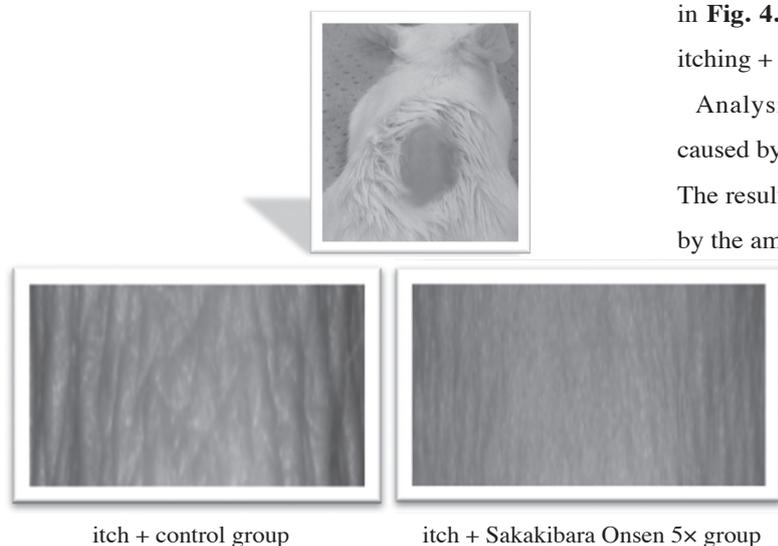
###### (5)-2-2. Staining method for skin sections (hematoxylin and eosin (HE) method)

The procedure for HE staining is shown in **Fig. 2A**, and

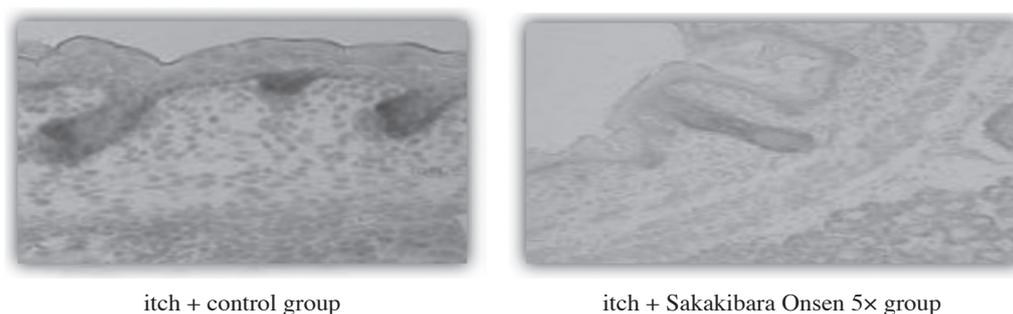


**Fig. 2.** Protocol of HE stain (A) and alcohol dehydration and penetration (B).

the method for alcohol dehydration and clearing is shown in **Fig. 2B**. After preparing mouse brain slices from each group, they were stained with Mayer's hematoxylin solution for 5 minutes and washed with running water for 20 minutes. Next, they were stained with 1% eosin solution for 2-4 minutes, lightly washed with water for about 10 seconds to wash off excess eosin solution, and this procedure was repeated twice. The specimens were immersed in 70%, 80%, 90%, 95%, and 99% (I and II)



**Figure 3.** Microscopic image analysis of itchy skin



**Figure 4.** Image analysis of H&E stained skin induced by itching.

ethanol for 10 seconds each to distinguish and dehydrate them, and then cleared with xylene I and II, and mounted using Eukit.

## 2-2. Statistical analysis

All results are shown as mean  $\pm$  standard error. In the short-term administration study, the significance test was performed using paired Dunnett test and *t*-test, with a significance level of 5% or less in a two-sided test.

## 3. Research result

### 3-1. Microscopic image analysis of mouse dorsal skin

Microscopic images of the skin on the back of the mice are shown in **Fig. 3**. Compared to the ① itch + control group, ② itch + Sakakibara Onsen 5 $\times$  group showed significant improvement.

### 3-2. Histopathological analysis of H&E stained images of itchy skin

Histopathological analysis of H&E stained images of mouse skin that had been induced to be itchy are shown in **Fig. 4**. Significant improvement was observed in the itching + Sakakibara Onsen 5 $\times$  group.

Analysis of the biochemical mechanism of itching caused by histamine H1 receptor levels (% control)

The results of the analysis of the biochemical mechanism by the amount of histamine H1 receptors (% control) that

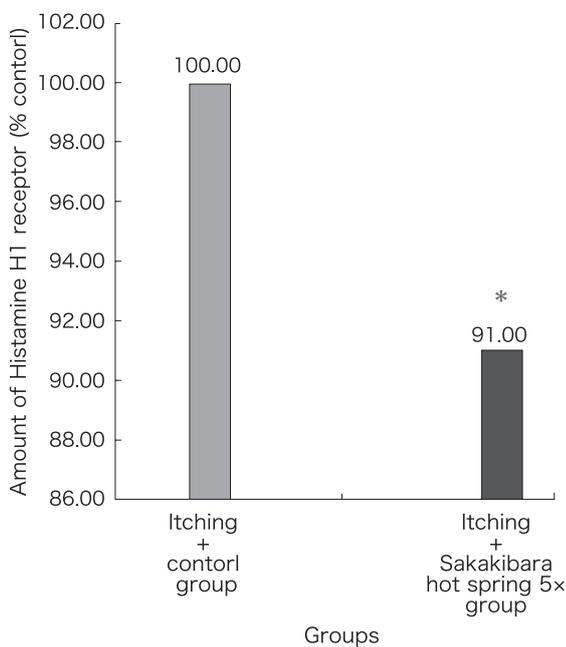
caused itching in mouse skin are shown in **Fig. 5**. Compared to 100% of the itching + control group, ② 91 % of the itching + Sakakibara Onsen 5 $\times$  group showed significant improvement.

Analysis of the biochemical mechanism of serum IgE -induced itch The results

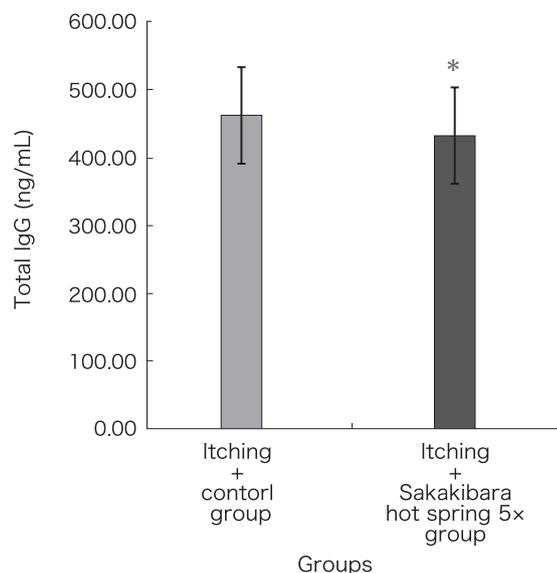
of the analysis of the biochemical mechanism of serum IgE that caused itching in mouse skin are shown in **Fig. 6**. Compared to ① the itching + control group, ② the itching + 5× Sakakibara Onsen group showed a statistically significant difference and marked improvement.

### 3-6. Analysis of the biochemical mechanism of serum IgG causing itching

The results of the analysis of the biochemical mechanism of serum IgG that caused itching in mouse



**Figure 5.** Analysis of the biochemical mechanism of itching by the amount of histamine H1 receptor (% control)

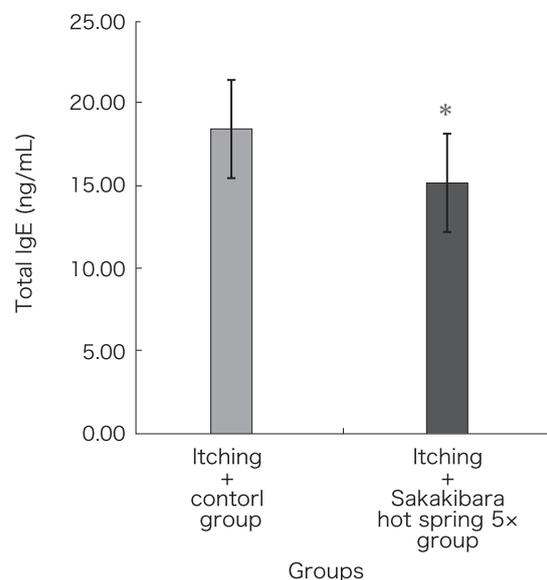


**Figure 7.** Analysis of the biochemical mechanism of serum IgG inducing itch.

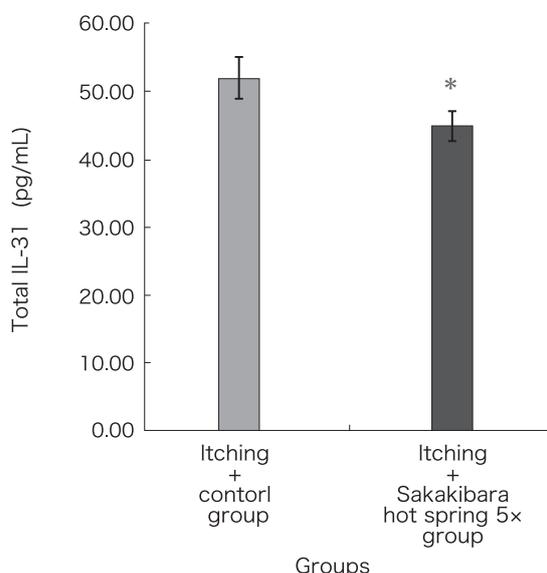
skin are shown in **Fig. 7**. Compared to ① the itching + control group, ② the itching + Sakakibara Onsen 5× group showed a statistically significant difference and marked improvement.

### 3-7. Analysis of the biochemical mechanism by which IL-31 induces itch

The results of the analysis of the biochemical mechanism of IL-31 inducing itching in mouse skin are shown in **Fig. 8**. Compared to ① the itch + control group, ② the itch + Sakakibara Onsen 5× group showed a statistically significant difference and marked improvement.



**Figure 6.** Analysis of the biochemical mechanism of serum IgE -induced itch.



**Figure 8.** Analysis of the biochemical mechanism by which IL-31 induces itch

#### 4. Discussion

Itching is defined as an unpleasant skin sensation, and is a sensation specific to the skin and some mucous membranes. Itching is one of the symptoms of various skin diseases (atopic dermatitis, contact dermatitis, urticaria, psoriasis, allergic diseases, etc.) and systemic diseases, especially visceral diseases (chronic renal failure, cholestasis, etc.). Itching promotes the release of endogenous pruritic substances, which increases itching. In addition, itching is an unpleasant sensation, which leads to a decrease in QOL. For such clinically problematic itch caused by skin diseases, antagonism of the histamine receptor (H1 histamine receptor), which has been considered a mediator of itch, acts as an antipruritic agent. In this study, it is hoped that natural hot spring water can be used as an antipruritic agent by long-term use<sup>11-21)</sup>.

It is a peptide of 11 amino acids that is widely distributed from the peripheral to central nervous system. In particular, the dorsal horn of the spinal cord is known to be involved in the transmission of pain information. It has been suggested that it may be involved in pruritic skin diseases such as atopic dermatitis. One of the mechanisms suggested to be a pathway mediated by histamine released by degranulation of mast cells (36-42). This study also showed that scratching behavior is an itch-related behavior in mice, it is unclear whether this behavior is entirely a reaction caused by itch. Therefore, we focused on whether scratching behavior in animals is a reaction caused by itch. Studies using H1 histamine receptor antagonists and mast cell-deficient mice revealed that the mast cell-histamine system is not very involved in inducing this scratching behavior. These findings suggest the existence of a previously unknown pathway for the generation of itch (44-47).

It is well known that the IgE mast cell system is involved in immediate allergic itch, but in a mouse model of atopic dermatitis, it is known that serum IgE levels and the number of scratching movements are related to it.

In a mouse model of mosquito-induced allergic pruritus, the allergic scratching behavior was not suppressed by antagonism of H1 histamine receptors. Furthermore, serum IgE did not increase significantly compared to

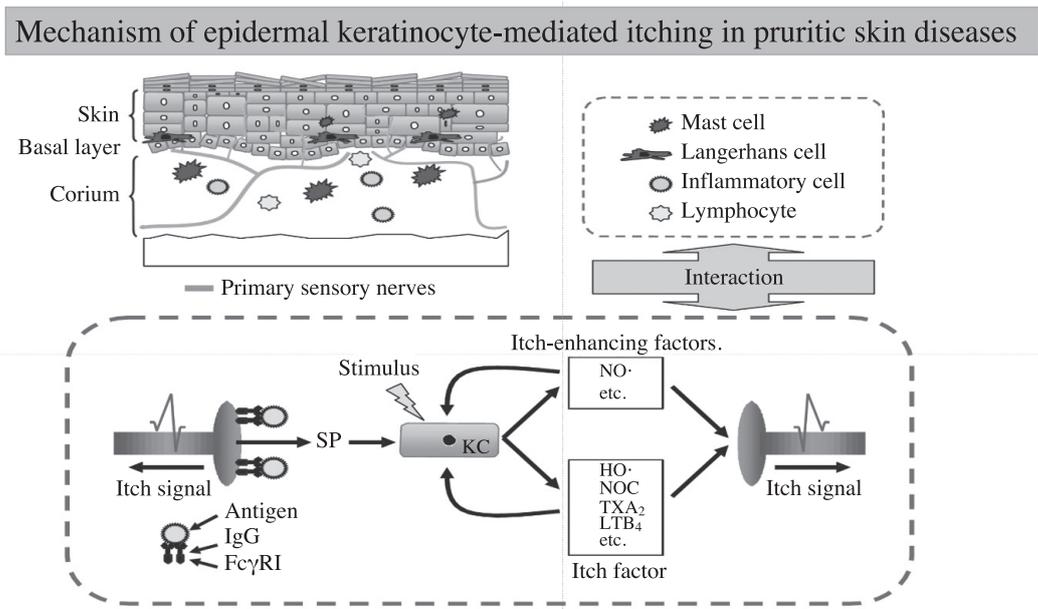
non-sensitized mice, and instead the amount of IgG increased significantly. Mice without mast cells (mast cell-deficient mice) were also sensitized and challenged with an antigen, and exhibited scratching behavior to the same extent as healthy mice with mast cells that had been similarly sensitized. These findings suggest the existence of a mechanism for the development of allergic pruritus other than the IgE mast cell system<sup>45-47)</sup>.

IgG receptor RI is expressed in primary sensory nerves, and that in a sensitized state, antigens bind to the primary sensory nerves in the skin, the formation of antigen-IgG complexes stimulates the primary sensory nerves, and substance P is released from the nerve terminals<sup>48-50)</sup>.

In allergic pruritus mice with predominant antigen-specific IgG production, scratching induced by antigen stimulation was suppressed by NK1 receptor antagonists and BLT1 receptor antagonists. This suggests that allergic itch may be caused by two pathways: one in which antigen stimulation directly stimulates the primary sensory nerves, causing itch, and the other in which the primary sensory nerves are mediated by the substance P keratinocyte system. In skin with chronic pruritic skin diseases such as atopic dermatitis, the primary sensory nerves extend into the epidermis, suggesting that they are involved in skin irritation caused by scratching and increased sensitivity to substances released from keratinocytes. These nerves, particularly those containing substance P and calcitonin gene-related peptide, may be important pathways for the induction and augmentation of itch, in addition to the action of substances released from keratinocytes on the primary sensory nerves, as in the allergic reaction described above<sup>37,45,49)</sup>.

Regarding the direct action of IL-31 on sensory nerves to induce itch, the above experimental results demonstrated for the first time that IL-31 acts directly on sensory nerves to induce itch. On the other hand, it was suggested that the action of IL-31 on keratinocytes has little contribution to the induction of itch, at least in mice without dermatitis.

Therefore, this research has demonstrated that hot spring water may have a variety of benefits for the health of not only people, but also pets. Below, we can expect hot springs to have a positive effect on skin diseases



**Figure 9.** Mechanism of itching mediated by epidermal keratinocytes in pruritic skin diseases

(KC: keratinocyte, SP: substance P, NO: nitric oxide, HO: hydroxyl radical, NOC: nociceptin, TXA2: thromboxane A2, LTB4: leukotriene B4)

in pets. However, depending on the ingredients and water quality of the hot spring, it is thought that some hot springs may be effective against dermatitis and skin diseases. Simply washing the skin in a hot spring, as if gently massaging it, may cleanse the skin and relieve itching and dandruff. In fact, there have been reported cases where the symptoms of pet dogs with skin diseases have improved<sup>47-52</sup>.

Are also effective in alleviating neurological disorders, and for dogs with joint inflammation or pain or motor paralysis, the sedative and buoyant effects of hot springs may be effective. Drinking hot spring water may improve decreased gastrointestinal function. In particular, when using hot springs for pets, it is important to be careful of the temperature. Also, if your dog has a medical condition, you should consult a veterinarian. Hot springs can have a positive effect even if you don't bathe your dog's entire body, just bathing its hands and feet, so it is a good idea to use them to promote the health of your dog. Highly alkaline hot springs in particular have a natural soap effect, so they easily wash away excess fat

and dirt from the surface of your dog's skin, cleaning and sterilizing the skin. They are also very effective against bacterial and fungal skin diseases such as pyoderma and seborrhea, and other such diseases. When introducing your dog to a hot spring facility, it will be necessary to have the dog have had rabies and mixed vaccine injections within a year<sup>50-51</sup>. Also, dogs that are in heat or pregnant, or infected with infectious diseases or parasites such as fleas or ticks, are not allowed to enter the facility. For dogs, bathing in a hot spring has a relaxing effect and helps relieve stress, and it is also effective in treating neuralgia and skin disorders (**Fig. 9.**)<sup>48-59</sup>.

In this study, based on the results of research into itching using mice, we focused on the optimal hot spring water rather than mast cells, and also conducted research into the involvement of the optimal hot spring water in the occurrence of itching. A significant improvement in the itching of the mouse skin was observed in the ② Itch + 5× Sakakibara Hot Spring group compared to the ① Itch + control group.

## 参考文献

1. KM Kang, YN Kang, IB Choi, Y Gu, T Kawamura, Y Toyoda, A Nakao: Effects of drinking hydrogen-rich water on the quality of life of patients treated with radiotherapy for liver tumors. *Medical gas research*, 1: 1-8, 2011.
2. S Mishima, K Saito, H Maruyama, M Inoue, T Yamashita, T Ishida, Y Gu: Antioxidant and immuno-enhancing effects of *Echinacea purpurea*. *Biological and Pharmaceutical Bulletin*, **27**(7): 1004-1009, 2004.
3. UH Jin, TW Chung, SK Kang, SJ Suh, JK Kim, KH Chung, YH Gu, I Suzuki: Caffeic acid phenyl ester in propolis is a strong inhibitor of matrix metalloproteinase-9 and invasion inhibitor: isolation and identification. *Clinica. Acta*, **362**(1-2): 57-64, 2005.
4. M Oshima, Y Gu, S Tsukada: Effects of *Lepidium meyenii* Walp and *Jatropha macrantha* on blood levels of estradiol-17 $\beta$ , progesterone, testosterone and the rate of embryo implantation in mice. *Journal of Veterinary Medical Science*, **65**(10): 1145-1146, 2003.
5. UH Jin, KH Song, M Motomura, I Suzuki, YH Gu, YJ Kang, TC Moon: Caffeic acid phenethyl ester induces mitochondria-mediated apoptosis in human myeloid leukemia U937 cells. *Molecular and cellular biochemistry*, **310**: 43-48, 2008.
6. Y Gu, CS Huang, T Inoue, T Yamashita, T Ishida, KM Kang, A Nakao: Drinking hydrogen water ameliorated cognitive impairment in senescence-accelerated mice. *Journal of Clinical Biochemistry and Nutrition*, **46**(3): 269-276, 2010.
7. Y Gu, Y Takagi, T Nakamura, T Hasegawa, I Suzuki, M Oshima: Enhancement of radioprotection and anti-tumor immunity by yeast-derived  $\beta$ -glucan in mice. *Journal of medicinal food*, **8**(2): 154-158, 2005.
8. Y Takagi, IS Choi, T Yamashita, T Nakamura, I Suzuki, T Hasegawa, YH Gu: Immune Activation and Radioprotection by Propolis. *The American Journal of Chinese Medicine* **33**(02): 231-240, 2005.
9. Y Gu, M Kai, T Kusama: The embryonic and fetal effects in ICR mice irradiated in the various stages of the preimplantation period. *Radiation research*, **147**(6): 735-740, 1997.
10. M. Oshima, Y. Gu: Pfaffia Paniculata -induced changes in plasma estradiol-17 $\beta$ , progesterone and testosterone levels in mice. *Journal of Reproduction and Development*, **49**(2): 175-180, 2003.
11. S Takagi, T Miura, C Ishibashi, T Kawata, E Ishihara, Y Gu: Effect of corosolic acid on the hydrolysis of disaccharides. *Journal of nutritional science and vitaminology*, **54**(3): 266-268, 2008.
12. Yeun-Hwa Gu, Hyunju Choi, Takenori Yamashita, Ki-Mun Kang: Pharmaceutical Production of Anti-tumor and Immune-potentiating *Enterococcus faecalis*-2001  $\beta$ -glucans: Enhanced Activity of Macrophage and Lymphocytes in Tumor-implanted Mice. *Current Pharmaceutical Biotechnology*, **18**(8): 653-661, 2017.
13. YH Gu, Y Fujimiyama, Y Itokawa, M Oshima, JS Choi, T Miura, T Ishida: Tumoricidal effects of  $\beta$ -glucans: Mechanisms include both antioxidant activity plus enhanced systemic and topical immunity. *Nutrition and cancer*, **60**(5): 685-691, 2008.
14. YH Gu, T Yamasita, KM Kang: Subchronic oral dose toxicity study of *Enterococcus Faecalis* 2001 (EF 2001) in mice. *Toxicological Research*, **34**: 55-63, 2018.
15. Y Gu, KM Kang, T Hasegawa, H Tanabe, I Suzuki, BO Choi, IB Choi: Electrochemical cancer therapy induces apoptosis in SCC-7 tumor of mice. *Journal of the IABC*, **1**: 22-32, 2003.
16. YH Gu, T Yamashita, H Yamamoto, T Matsuo, N Washino, JH Song: Plant Enzymes Decrease Prostate Cancer Cell Numbers and Increase TNF- $\alpha$  In Vivo: A Possible Role in Immunostimulatory Activity, *International Journal of Food Science*, **2019**(1): 8103480, 2019.
17. YH Gu, T Yamasita, DJ Choi, H Yamamoto, T Matsuo, N Washino: The anticancer effect of plant enzymes on mouse breast cancer model. *Adv. Medi. Plant Res.*, **6**(4): 70-77, 2018.
18. YH Gu, KM Kang, T Yamashita, JH Song: Effects of Vitamin E on the immune system and tumor growth during radiotherapy, *Journal of Cancer Research and Therapeutics*, **17**(1): 211-217, 2021.
19. T Nakamura, Y Itokawa, KH Cho, JS Choi, I Suzuki, T Miura, YH Gu: Effects of Propolis on peripheral white cells, antioxidant activity and tumor growth in irradiated mice. *Journal of traditional chinese medicine*, **26**(4): 299-305, 2006.
20. YH Gu, T Hasegawa, I Suzuki, Y Yamamoto, Y Yoon, SY Rhee: A study of the radioprotection effect of guarana (*Paullinia cupana*) on the fetuses of ICR mice. *J. Kor. Radiat. Prot.*, **26**: 347-356, 2001.
21. T Yamashita, T Kato, T Isogai, Y Gu, T Ito, N Ma: Taurine deficiency in tissues aggravates radiation-induced gastrointestinal syndrome. *Taurine 12: A Conditionally Essential Amino Acid*, 113-120, 2022.
22. YH Gu, T Yamashita, T Inoue, KM Kang: Inhibition of radioactive depletion of hemocytes and antitumor effects of flavonoid. *Archives of Clinical and Medical Case Reports*, **5**(1): 118-128, 2021.
23. Y Gu, T Kaida, K Kaida: Immunostimulating and Antitumor Effects by *Inonotus obliquus* (Ach. Pers.) Pilat. *International Journal of Medicinal Mushrooms*, **7**: 2, 2005.
24. Gu Y, Ukawa Y, Oshima M, Suzuki I, Maenaka T: Radioprotection and Antitumor Effect by *Lyophyllum decastes* Singer and Propolis in Mice. *International Journal of Medicinal Mushrooms*, **7**(3): 2, 2005.
25. IS Choi, Y Itokawa, T Maenaka, T Yamashita, M Mitsumoto, Y Gu: Antioxidant activity and anti-tumor immunity by Propolis in mice. *Advances in Traditional Medicine*, **5**(2): 100-109, 2005.
26. S Mishima, YH Gu, K Saito, T Yamashita, H Maruyama, M Inoue, KS Ahn: Immune activation and radioprotection by *Echinacea purpurea* (American herb). *Oriental pharmacy and experimental medicine*, **4**(3): 163-170, 2004.
27. Y Gu, M Oshima, T Hasegawa: Dose dependence of the severity of radiation-induced thymic lymphoma in mice. *Environmental Mutagens and Carcinogens*, **22**(4): 266-273, 2002.
28. T Hasegawa, YH Gu: Non-invasive temperature monitoring using small coils during radio-frequency heating. *Thermal Medicine (Japanese Journal of Hyperthermic Oncology)*, **17**(1): 33-43, 2001.
29. K. Miyata, T. Hasegawa, K. A. Maeda, M. Amano, A. Fukuyama, H. Monzen, and G. Yeunhwa, Takahashi T, Yamamoto: The Activation of Immunological Activity and Anti-tumor Effects by Mild-Hyperthermia. *Japanese Journal of Hyperthermic Oncology*, **21**(1): 2001.
30. YH Gu, T Yamashita, T Matsuo, N Washino, JH Song, KM Kang, T Inoue: Improve the intestinal bacterial flora environment on renal function recovery in chronic kidney disease by efficacy of administration of plant enzymes. *Asian J. Complement Altern. Med.*, **8**(8): 9-16, 2020.
31. Suzuki I, Tanamachi M, Tomida M, Gu Y, Ukawa Y: Antihypertensive effect of *Lyophyllum decastes* Sing in Spontaneously

- Hypertensive Rats. *International Journal of Medicinal Mushrooms*, **3**(2-3): 2001.
32. YH Gu, T Hasegawa, T Mori, Y Yamamoto, T Kusama: Combined effects of ionizing radiation and ultrasound on malformation in ICR mice at organogenesis stage. *Journal of Radiation Protection and Research*, **24**(1): 23-30, 1999.
  33. Y Gu: Malformation and embryonic death in ICR mice after mild hyperthermia. *Thermal Medicine (Japanese Journal of Hyperthermic Oncology)*, **14**(4): 235-243, 1998.
  34. YH Gu, T Kusama, M Kai: Embryonic effects of radiation on ICR mice depending developmental stages. *Journal of radiological science and technology*, **18**(1): 91-95, 1, 1995.
  35. Y Gu, T Yamashita, T Inoue: Immunostimulatory effect and detox effect of spasm. crispa fruiting body. *New Food Industry*, **66**(2), 86-99, 2024.
  36. Yeunhwa Gu, Takenori Yamashita and Tota Inoue: Immunostimulatory and detox effects of spasm. crispa fruiting body. *New Food Industry*. **66**(2): 86-99, 2024.
  37. Yeun-Hwa Gu, Takenori Yamashita, Tatsuhiko Matuo, Noriyuki Washino, Ki- Mun Kang and Tota Inoue: Oxidative stress in the pathogenesis of Alzheimer's disease: Improvement of memory deteriorating disease by administration of plant enzyme. GSC Advanced Research and Reviews (GSCARR), eISSN: 2582-4597. CODEN (USA):GARRC2, Cross Ref DOI:10. 30574/ gscarr, P140-156,2023.
  38. Yeunhwa Gu, Takenori Yamashita and Tota Inoue: Radioprotective effect of Guarana water extract against radiation-induced teratogenicity in ICR mice. *New Food Industry*. **65**(9): 514-524, 2023.
  39. Yeun-Hwa Gu, Ryo Matsumoto, Takenori Yamashita: Effects of Vitamin E Derivative TMG on the Radiation Protector and Tumor Growth during Radiotherapy. *Journal of Radiation Protection and Research*, **48**(1): 1-8, 2023.
  40. Yeunhwa Gu, Takenori Yamashita and Tota Inoue: The mechanism of radiation protective effect in propolis against radiation teratogenicity. *New Food Industry*. **65**(4): 215-225, 2023.
  41. Yeunhwa Gu, Takenori Yamashita and Tota Inoue: Presence or absence of immune enhancement effect and body fat reduction effect by mushroom complex. *New Food Industry*. **64**(12): 779-786, 2022.
  42. Yamashita T, Kato T, Isogai T, Gu Y, Ito T, Ma N: Taurine Deficiency in Tissues Aggravates Radiation-Induced Gastrointestinal Syndrome. *Adv. Exp. Med. Biol.*, **1370**:113-120. 2022.
  43. Yeunhwa Gu, Takenori Yamashita and Tota Inoue: Radiation protective effect in blood cells and anti-inflammatory effect of stomatitis during radiation therapy by flavonoid. *New Food Industry*. **64**(6): 382-390, 2022.
  44. Gu, Yeun-Hwa, Yamashita, Takenori, Kang, Ki- Mun, Inoue, Tota: Radioprotective and Anticancer Effects of Ganoderma Lucidum in a Mouse Tumor Model. *Current Traditional Medicine*, **7**(6): 56-65(10), 2021.
  45. Yeunhwa Gu, Takenori Yamashita and Tota Inoue: Anti-type 2 diabetic effect in fuscoporia obliqua, coffee and cocoa combinations. *New Food Industry*. **63**(6): 427-436, 2021.
  46. Yeun-Hwa Gu, Takenori Yamashita, Tota Inoue: Tatsuhiko Matsuo, Noriyuki Washino, Ki- Mun Kang: Plant Enzymes Decrease Pancreatic Cancer Cell Numbers in vivo Through an Immunomodulatory Mechanism. *Arch. Clin. Case. St. Case. Rep.*, **2**(4): 199-207, 2021.
  47. Yeun-Hwa Gu, Takenori Yamashita, Tota Inoue, Ki- Mun Kang: Inhibition of Radioactive Depletion of Hemocytes and Antitumor Effects of Flavonoid. *Arch. Clin. Med. Case. Rep.*, **5**(1): 108-118, 2021.
  48. Yeun-Hwa Gu, Takenori Yamashita, Tota Inoue, and Ki- Mun Kang: The Mechanism of Radioprotective Effect and Immunoreactive Effect on Vitamin C. *Am. J. Med. Sci. Res.* **10**(3): 238-245. 2020. (AJBSR. MS.ID.001505. DOI: 10.34297/AJBSR.2020.10.001505).
  49. Yeunhwa Gu: Clinical study on immune function, antidiabetic and diet effects in sericite (Tellus-Gizer). *New Food Industry*. **62**(12): 882-894, 2020.
  50. Yeunhwa Gu: Clinical test related to bloodstream, anti-oxidant, anti-metabolic syndrome in human by oriental medicine lust (YS). *New Food Industry*. **62**(7): 483-496, 2020.
  51. Yeun-Hwa Gu, Takenori Yamashita, Tatsuhiko Matsuo, Noriyuki Washino, Jin -Ho Song, Ki- Mun Kang, Tota Inoue: Improve the intestinal bacterial flora environment on renal function recovery in chronic kidney disease by efficacy of administration of plant enzymes. *A- J.Compl. Alter. Medi.*, **8**(1): 9-16, 2020.
  52. Yeunhwa Gu: Clinical study on antioxidant activity and body calcium absorption index in enzyme. *New Food Industry*. **62**(4), 255-260, 2020.
  53. Yeun-Hwa Gu, Takenori Yamashita, Hajime Yamamoto, Tatsuhiko Matsuo, Noriyuki Washino, Jin -Ho Song and Ki- Mun Kang: Plant Enzymes Decrease Prostate Cancer Cell Numbers and Increase TNF- $\alpha$  In Vivo: A Possible Role in Immunostimulatory Activity. *International Journal of Food Science*. Hindawi, Volume **2019**, Article ID 8103480, 1-7, <https://doi.org/10.1155/2019/8103480>.
  54. Yeunhwa Gu: Clinical trial research on the immunopotentiating and anti-inflammatory effect of cat's claw and enzyme treated cat's claw. *New Food Industry*. **61**(10): 733-740, 2019.
  55. Yamashita T, Kato T, Isogai T, Gu Y, Ma N: Protective Effects of Taurine on the Radiation Exposure Induced Cellular Damages in the Mouse Intestine. *Adv. Exp. Med. Biol.*, **1115**: 443-450, 2019.
  56. Ma N, Kato T, Isogai T, Gu Y, Yamashita T: The Potential Effects of Taurine in Mitigation of Radiation Nephropathy. *Adv. Exp. Med. Biol.*, **1115**: 497-505, 2019.
  57. Yeun-Hwa Gu, Takenori Yamasita, Tota Inoue, Jin -Ho Song and Ki- Mun Kang: Cellular and Molecular Level Mechanisms against Electrochemical Cancer Therapy. *Journal of Pathogens*. Hindawi, Volume **2019**: pages 1-11. Article ID3431674, <https://doi.org/10.1155/2019/3431674>, 2019.
  58. YeunHwa Gu: Improved memory and cognitive function in Hericium erinaceum fruit body. *New Food Industry*. **61**(6): 437-452, 2019.
  59. YH Gu, T Yamasita, DJ Choi, H Yamamoto, T Matsuo, N Washino, JH Song and KM Kang: The anticancer effect of plant enzymes on mouse breast cancer model. *Adv. Medi. Plant Res.*, **6**(4): 70-77, 2018.

# マウスに対する榊原温泉 (5T) の温泉水と 痒みの抑制効果に関する研究

具 然和 (GU Yeunhwa)<sup>1\*</sup>, 山下 剛範 (YAMASHITA Takenori)<sup>2</sup>, 井上 登太 (INOUE Tota)<sup>3</sup>

Key Words: かゆみ抑制, IgE, IgG, IL-31, ヒスタミン H1, 榊原温泉水

## 要旨

本研究では、マウスに対する7種類の温泉水と痒みや皮膚炎のかゆみ伝達機序を解明するために研究を行った。皮膚炎に伴うかゆみの伝達に、感覚神経における転写因子 STAT3 の活性化が重要な役割を果たしていることが明らかである。本研究では、皮膚炎などのかゆみに対する新たな天然温泉水を比較し、開発に貢献すると期待される。皮膚炎のかゆみに深く関わる IL-31 が、感覚神経に発現する受容体に作用することで、かゆみを引き起こしていることを実証した。また、IL-31 受容体の下流で、転写因子 STAT3 が活性化されることが、かゆみ誘導に重要であることを示した。さらに、感覚神経の STAT3 は、IL-31 受容体の発現や、かゆみ伝達に関わる神経ペプチドの発現にも重要であることも見いだした。感覚神経の STAT3 は、IL-31 依存的なかゆみだけでなく、IL-31 非依存的な炎症性のかゆみにも重要であることも示唆された。これらの結果から、STAT3 の阻害薬が開発・改良されれば、新たなかゆみの改善となる可能性が示された。

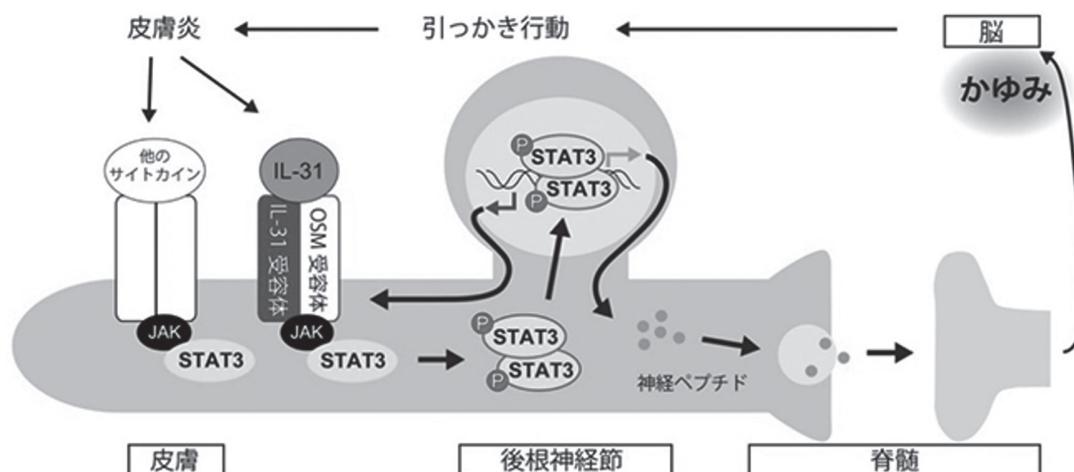


図1 温泉水と痒みの抑制効果に関するかゆみを伝達する感覚神経細胞における STAT3 の役割

\* 責任著者: 具 然和 (Yeunhwa Gu)<sup>1</sup>

<sup>1</sup> 純真学園大学保健医療学部放射線技術科学科 〒815-8510 福岡県福岡市南区筑紫丘1丁目1-1  
TEL: 092-554-1255 e-mail: gu.y@junshin-u.ac.jp

<sup>2</sup> 鈴鹿医療科学大学大学院保健衛生学研究科 〒510-0293 三重県鈴鹿市岸岡町1001番地1

<sup>3</sup> みえ呼吸嚙下りハビリクリニック 〒519-0171 三重県亀山市アイリス町14-7