News Release
Kao Corporation

Kao
Kirei–Making Life Beautiful

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FOR IMMEDIATE RELEASE

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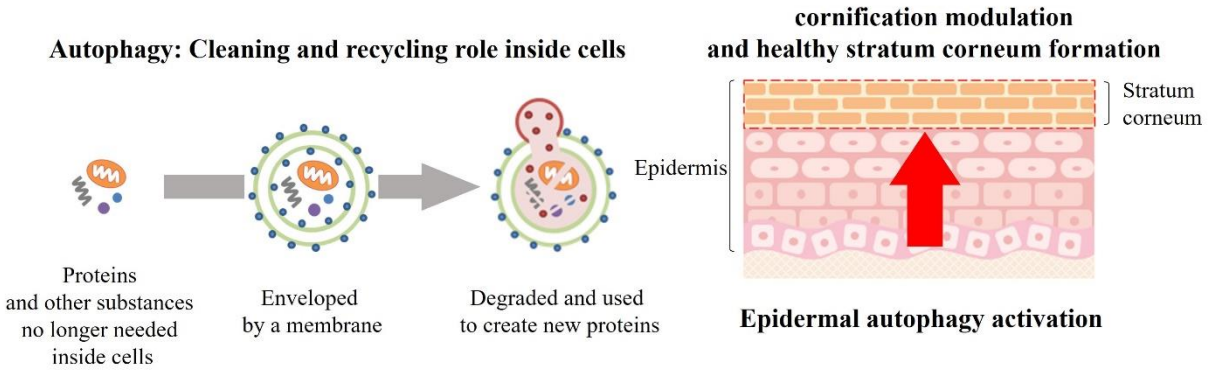
Activation of Skin Autophagy for Healthy Stratum Corneum Formation
Discovery of Autophagy Activation with Eucalyptus and Bilberry Extracts

Research performed by the Biological Science Research Division of Kao Corporation has revealed that the decline of autophagy activity in skin adversely affects epidermal homeostasis and disturbs formation (cornification) of the stratum corneum, which functions as a barrier and retains hydration in the skin. Furthermore, this research also suggests the potential to maintain homeostasis of epidermal cornification by increasing autophagy activity. Based on these findings, Kao has found promising plant extracts that promote epidermal cornification by increasing autophagy activity.

A portion of the research findings have been published in the International Journal of Molecular Sciences*1 and announced at the 143rd Annual Meeting of the Pharmaceutical Society of Japan (March 25–28, 2023, Hokkaido).

*1 "Autophagy declines with premature skin aging resulting in dynamic alterations in skin pigmentation and epidermal differentiation" *Int. J. Mol. Sci.* 21: 5708 (2020).



Technology to form a healthy stratum corneum through autophagy activation (conceptual image)

Background

Autophagy (literal meaning "self-eating") is a natural process that takes place within all cells in the human body, where unwanted or dysfunctional proteins, which are component of body organs, are degraded into amino acids. These amino acids are recycled to create new proteins. Cells are able to maintain a constant state of self-renewal and the health of the individual through autophagy performing this recycling role inside cells.

Researchers at Kao began studies of skin autophagy in 2010 and have been leading research studies on autophagy in the field of skin science as well as examining applications related to skin care. In 2019, in consultation with Dr. Tamotsu Yoshimori, a distinguished Osaka University professor, Kao

successfully measured autophagy activity using human skin tissue, and reported that autophagy activity declines not only in chronological aging but also in photoaging due to ultraviolet (UV) light exposure*2. It is thought that beneficially harnessing the skin autophagy process that naturally occurs in the human body can contribute to the health of skin cells and more beautiful skin.

Kao has conducted research based on the hypothesis that autophagy also plays a crucial role in the epidermis, where old cells are constantly being replaced with new cells. In the epidermis, keratinocytes mature and undergo cornification to form the stratum corneum. In this process, components key to hydration are created and the barrier that protects the skin from physical and chemical stressors from external environment is also attained. This is why the cornification process is so important to skin health and beauty.

*2 Kao news release, November 18, 2019
Quantification of autophagy in skin: Autophagy decline associated with intrinsic aging and photoaging

Skin with disturbed cornification has lower autophagy activity

To study the relationship between skin cornification and autophagy, Kao researchers compared the elbow skin and arm skin around the elbow in healthy individuals between the ages of 30 and 50 experiencing skin problems at the elbow such as dryness and roughness*3. The results confirmed hyperkeratosis (increased thickness of the stratum corneum) at the elbow. Staining the proteins loricrin and filaggrin*4, which perform important roles in cornification in the epidermis, as well as LC3*5 protein with fluorescent dye visually showed that their distribution was severely disturbed (Fig. 1). In addition, the metabolic rate of LC3 in the elbow skin was lower than in the arm skin, and autophagy activity was markedly lower.

These findings suggest that lower autophagy activity may be one cause of cornification disturbance.

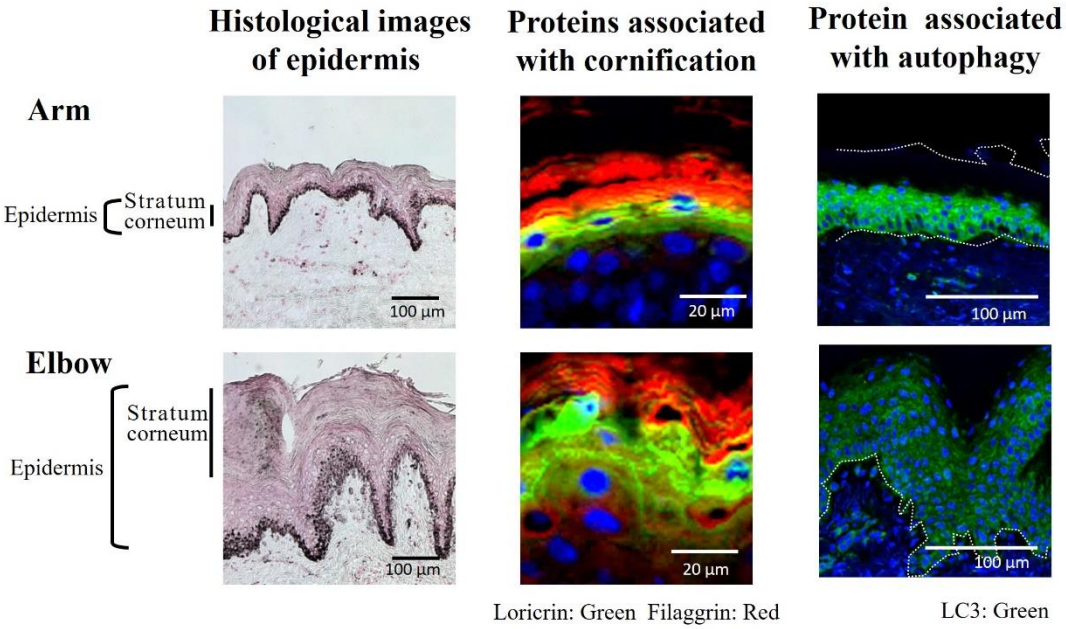


Fig. 1. Comparison of cornification and autophagy in elbow and arm skin tissue

*3 This research was conducted using a protocol previously approved by an institutional review board (IRB) in the United States and the Human Research Ethics Committee of Kao Corporation.

*4 Loricrin: One substance comprising the insoluble membrane structure (cornified envelope) enveloping stratum

corneum cells. Loricrin acts to strengthen the stratum corneum. Filaggrin: Strengthens the skin's barrier function in stratum corneum cells and maintains hydration.

*5 LC3: An important protein in autophagy existing only within the a small bubble-like structure (autophagosome) that wraps around intracellular components. Autophagy activity can be measured by detecting the metabolic rate of LC3 protein (flux assay).

Confirmation that autophagy activation inhibits cornification disturbance

To further study the effects of autophagy on cornification disturbance, a reagent that activates autophagy was added to cultured elbow skin. The results show that reagent treatment in the cultured hyperkeratosis skin tissues were normalized, and that proteins associated with cornification were also more normally distributed than they had been before (Fig. 2).

This suggests that increasing autophagy activity can help modulate disturbed cornification and healthy stratum corneum formation.

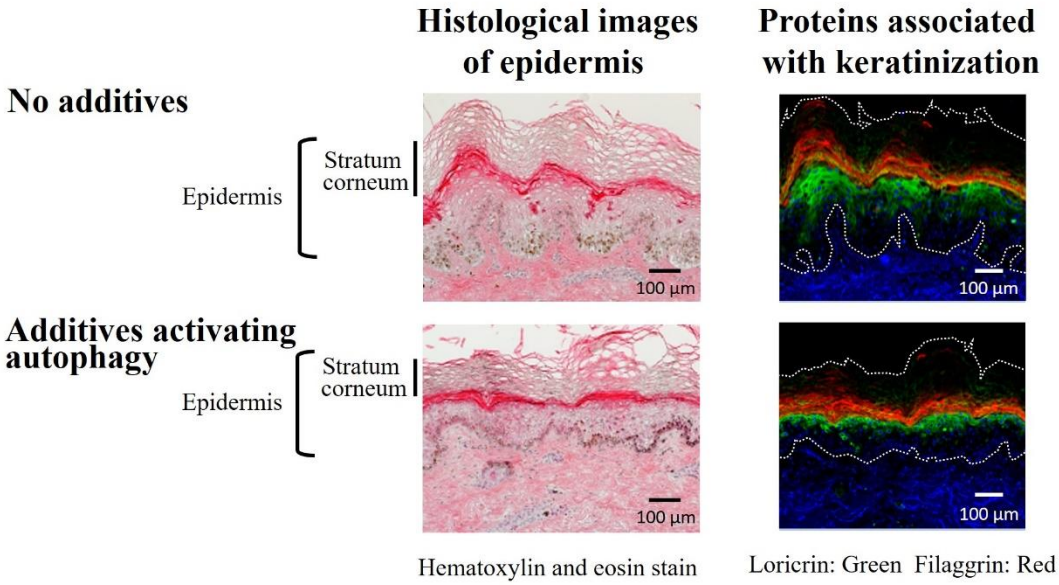


Fig. 2. Changes in cornification from autophagy activation in cultured elbow skin tissue with hyperkeratosis

Discovery of plant extracts that activate autophagy and help modulate cornification

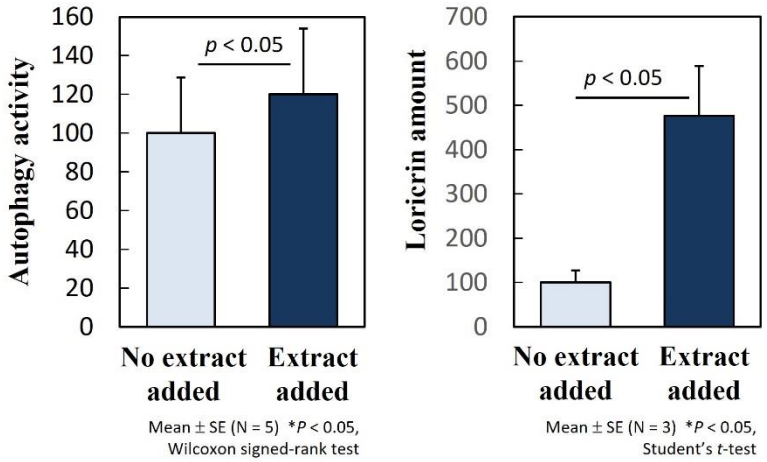
Among more than 200 ingredients that can be applied to skin, Kao investigated which ingredients beneficially activate autophagy for healthy stratum corneum formation.

Kao confirmed that when eucalyptus extract and bilberry extract are added to keratinocytes at the same time, autophagy activity increases and the amount of loricrin protein increases (Fig. 3). This suggests that the combination of eucalyptus extract and bilberry extract has the ability to modulate cornification.

Future Prospects

Kao's research has revealed the importance of activating autophagy, a natural process that protects life present in all living creatures from yeast to humans, in the formation of a healthy stratum corneum, which functions as a barrier and retains hydration in human skin. The findings also show the potential of simultaneous use of eucalyptus extract and bilberry extract to increase autophagy activation and

stimulate healthy cornification. Kao will continue conducting research on the important function of autophagy in our bodies, aiming to further improve its technologies able to realize the healthy and beautiful skin that people want to attain.



Extracts: Eucalyptus extract + bilberry extract
Relative value when no extracts added is 100

Fig. 3. Autophagy activation and increase in proteins associated with cornification from eucalyptus extract and bilberry extract

About Kao

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