PRESS RELEASE

October 2022
Shiseido Company, Limited

Shiseido Discovers that Capillaries Promote Epidermal Regeneration

- CITRUS JUNOS FRUIT EXTRACT regulates the distance between capillaries and epidermis -

Shiseido Company, Limited ("Shiseido") has become the world's first to discover that pericytes*1, which adhere to the outer side of dermal capillaries, may promote epidermal regeneration by moving from the capillary loop to the epidermis and transforming into epidermal stem-like cells (Figure 1). We also found that aging causes variance in the direction of capillary loop running toward the epidermis, resulting in the expansion of distance between the capillaries and the epidermis (Figure 2, 4). After verifying this mechanism, we revealed that Netrin-1*2, which is released from the epidermis, increases abnormally with aging and repels the Netrin-1 receptor (UNC5B) expressed at the top of the capillary loop, thereby distancing the capillaries from the epidermis (Figure 5). This phenomenon would inhibit pericyte migration from capillaries in aging skin and reduce the supply of epidermal stem cells. These findings suggest that the supply of epidermal stem cells derived from pericytes may be inhibited in aging skin. Furthermore, we found that CITRUS JUNOS FRUIT EXTRACT suppresses the expression of Netrin-1. Part of these study results will be presented at the 47th Annual Meeting of the Japanese Society for Investigative Dermatology held from December 2 to 4, 2022 and the 30th Annual Meeting of the Japanese Vascular Biology and Medicine Organization held on December 16 and 17, 2022.

This research is being conducted based on the Inside/Outside approach of our R&D philosophy "DYNAMIC HARMONY". Going forward, we will continue to create new approaches to beauty by actively elucidating the relationship between beautiful skin and blood vessels, which play an important role in vital functions.

*1 A cell that adheres to the vascular endothelial cells of capillaries and stabilizes the capillary structure.

*2 It is known as a protein that controls the attraction and repulsion of nerve axons and the migration of nerve-related cells. However, it was not known that Netrin-1 acts to regulate the distance between the epidermis and capillaries in the skin.

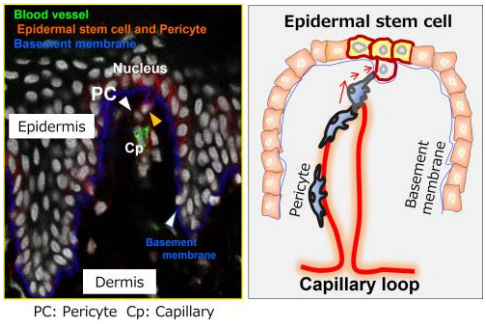


Figure 1. Positional relationship between epidermal stem cells (red) and pericytes (PC; red) around the top of the capillary loop (Cp; green)

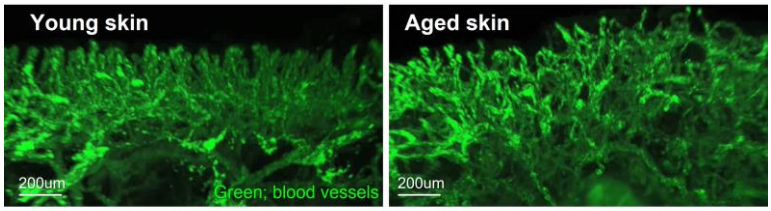


Figure 2. Difference in the direction of capillary loop between young skin and aged skin.

Research Background

We have previously revealed that healthy capillaries may contribute to the suppression of wrinkles and sagging and to the maintenance of skin elasticity through more than 20 years investigating the molecular mechanisms by which skin capillaries maintain dermal homeostasis. Since capillaries are present in the dermis, capillaries were thought to only be loosely associated with the epidermis, which is responsible for the beauty of the skin surface. However, through detailed observation of the positional relationship between capillaries and epidermal cells, we found that the loop structure of dermal capillaries, which run toward the epidermis, exists much closer to the epidermis than expected (Figure 1), indicating the possibility that capillaries indirectly control epidermal homeostasis. Given that blood vessels are known to form a niche for stem cells in organs throughout the body and promote tissue regeneration, we hypothesized that dermal capillaries have a function that maintains epidermal stem cells and verified the hypothesis using our own advanced visualization technology and molecular biological approach.

Discovery of a system in which capillaries promote epidermal regeneration

Using our original technology that makes tissues, such as skin, transparent and visualizes their specific structures in three dimensions, we observed the positional relationship between the capillary network and epidermal stem cells. As a result, it was found that epidermal stem cells exist around the uppermost part of the capillary loop structure, which is distinctively present just below the epidermis, as if they were covering caps. Then, we further conducted detailed observation of a single capillary loop by staining the blood vessel, epidermal stem cell, and basement membrane, and discovered that there are cells that cross-link between epidermal stem cells and pericytes adhering to capillaries (Figure 1, yellow arrows). This suggests that pericytes attached to capillary loop in the dermis may be detached and supplied to the epidermis as epidermal stem cells. To verify the function of pericytes in this phenomenon, we isolated pericytes from the skin by using FACS (Fluorescence-Activated Cell Sorting) technology*[3] and cell surface proteins that specifically express on pericytes, as an indicator, and cultured them under the conditions in which epidermal cells are cultured. Consequently, we found that gene expression of p63 and keratin 5, which are characteristics of epidermal cells, increased in pericytes, and that the morphology of pericytes change to epidermis-like cells (Figure 3). Based on these results, we found for the first time in the world that pericytes supplied from the dermal capillary loop may act as epidermal stem cells, contributing to the maintenance of epidermal cells.

*[3] A method to separate and acquire specific cell types from a mixture of various cells according to their light scattering and fluorescence characteristics.

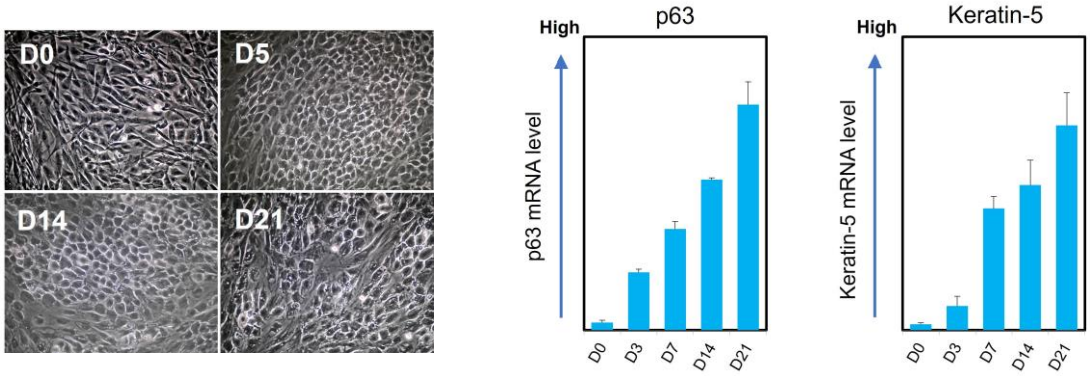


Figure 3. Morphological changes in pericytes (left) and changes in expression level of characteristic genes in epidermal cells (right)

Effect of CITRUS JUNOS FRUIT EXTRACT on maintaining adequate distance between capillaries and epidermis

Next, we observed age-induced changes in capillary loop structure. Three-dimensional analysis via our skin transparency technology revealed that in young skin, capillary loops are aligned orderly toward the epidermis, whereas in aged skin, the direction of the loops varies (Figure 2), and the distance between the epidermis and capillaries becomes further apart (Figure 4). To elucidate the mechanism of this phenomenon at the molecular level, we measured the expression level of Netrin-1, which is known to inhibit cell migration, as a factor that may affect capillaries, and found that the Netrin-1 expression increased with aging in epidermis (Figure 5 left). Then, we analyzed the expression of UNC5B, which is known as Netrin-1 receptor and has a repulsive effect on each other, and found that UNC5B appears at the top of the capillary loop (Figure 5 right).

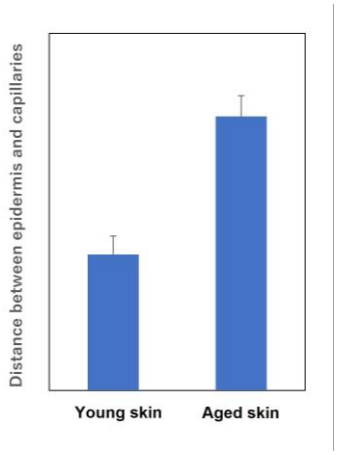
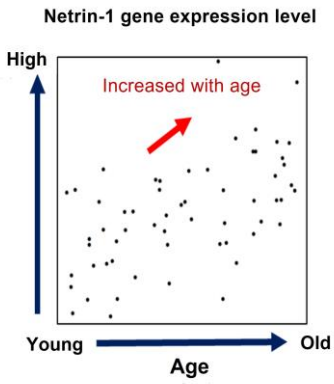


Figure 4. The distance between capillaries and epidermis expands in aged skin

In other words, it suggests that in normal skin, Netrin-1 released from the epidermis acts on and repels UNC5B expressed at the top of the capillary loop, preventing the capillaries from invading the epidermis and maintaining the adequate distance. However, the aged skin shows an abnormal level of Netrin-1 production, causing the capillaries to be farther apart from the epidermis than necessary and reduces its ability to pass pericytes from the capillaries to the epidermis, which results in a decrease in the number of epidermal stem cells.

Furthermore, we searched for ingredients that inhibit Netrin-1 expression and found that very effect in CITRUS JUNOS FRUIT EXTRACT (Figure 6). It is expected that CITRUS JUNOS FRUIT EXTRACT will regulate the delivery of epidermal stem cells from capillaries by appropriately suppressing the excessive expression of Netrin-1 and maintaining the distance between capillaries and epidermis.



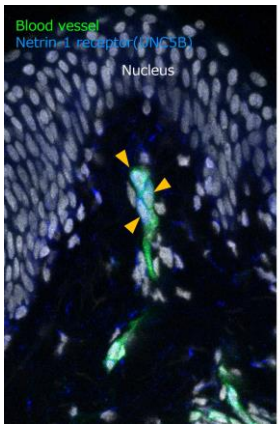


Figure 5. Netrin-1 is increased in aged epidermis (left).
Its receptor (blue) is expressed at the top of capillary loop (green) (right, arrows)

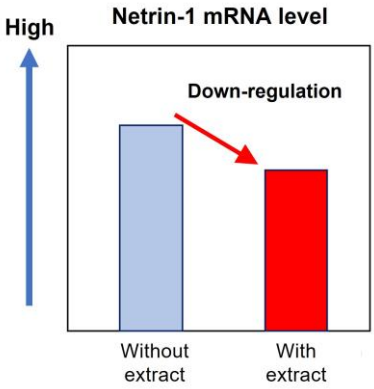


Figure 6. CITRUS JUNOS FRUIT EXTRACT has suppressing effect on Netrin-1 gene expression.

Future prospects

To date, the skin capillaries have attracted attention for their ability to supply nutrients and oxygen, and much research have been conducted focusing on their role in the dermis. In this study, we revealed that capillaries also play an important role in the regeneration of epidermis, even without being structurally attached to each other. Going forward, we will further pursue research to be a Skin Beauty Company from various perspectives, including proposing approaches to realize healthy skin based on blood vessel research.

Shiseido's R&D philosophy "DYNAMIC HARMONY"

Shiseido Formulates its Unique R&D Philosophy "DYNAMIC HARMONY" (2021)
https://corp.shiseido.com/en/news/detail.html?n=00000000003252
DYNAMIC HARMONY special website:
https://corp.shiseido.com/en/rd/dynamicharmony/

Related news release

Shiseido Elucidates that Age-Induced Deterioration of Skin Capillaries Involves in Skin Aging (2009)
https://corp.shiseido.com/jp/newsimg/archive/00000000001072/1072_s2e08_jp.pdf (Japanese only)
Shiseido Reveals the Relevance of Capillaries in Skin Elasticity (2019)
https://corp.shiseido.com/en/news/detail.html?n=00000000002780
Shiseido Elucidates Mechanism by Which Capillaries Maintain Skin Elasticity (2020)
https://corp.shiseido.com/en/news/detail.html?n=00000000002911

<Reference>

Researchers' challenges

■ Focusing on the regenerative ability of blood vessels that run through all organs in the body

Blood vessels form a network, connecting all organs including the skin and keep them healthy by delivering nutrients and oxygen. In addition, blood vessels have the function of maintaining stem cells, which are the source of organ regeneration, and are attracting attention in the field of regenerative medicine. At the Max Planck Institute in Germany, where I studied for two and a half years, I joined a laboratory that was active in researching the unique functions of blood vessels in various organs and the system in which blood vessels maintain tissue stem cells. While I was there, I started thinking that there must be a distinctive role for blood vessels that would promote skin regeneration. After returning to Japan, I started research at Shiseido with the aim of regenerating the skin surface morphology through blood vessels, which is a research theme directly related to beauty.

Researcher, Mika Sawane, Ph.D.

■ The moment when a cell appeared to move from the capillaries to the epidermis

At first, I was working on the hypothesis that capillaries indirectly send out signals to maintain stem cells in the epidermis. To support this theory successfully, we needed to have beautiful, clear images of capillaries and epidermis. I spent seven hours a day in a dark room looking through a microscope and observing capillaries and the epidermis, day after day. One day, a single cell in the vicinity of the capillaries appeared to get absorbed into the epidermis. It was a "pericyte", a cell with a stem cell-like function, and it existed as a bridge between the capillaries and the epidermis. Based on this observation, we reached the hypothesis that pericytes were passing stem cells from capillaries in the dermis to the epidermis. Then, we spent the next three years attempting to demonstrate a new mechanism by which capillaries directly regenerate the epidermis.

■ Developing a new approach to skin aging by identifying minute changes in capillaries

In the past 20 years, Shiseido has proven that capillaries play various roles in creating healthy, beautiful skin. In this study, we found that blood vessels, which are connected to the inside of the body, help create the beautiful skin surface. Going forward, we will further deepen vascular research from the holistic beauty standpoint and investigate how changes in people's daily lives and lifestyles pose an influence on skin aging from the perspective of blood vessels, which connect the inside and outside of the skin, and would like to further develop this research into creating a new approach to beautifying the skin from the inside.