Glycative Stress Research

Online edition : ISSN 2188-3610
Print edition : ISSN 2188-3602
Received : July 26, 2022
Accepted : August 14, 2022
Published online : September 30, 2022
doi:10.24659/gsr.9.3_126

Review article

Skin stiffness due to decreased blood vessels induces epidermal stem cell senescence

Ryo Ichijo

Department of Biosystems Science, Institute for Frontier Life and Medical Science, Kyoto University, Kyoto, Japan

Abstract

Keratinocytes, which make up the majority of the skin epithelium, differentiate from epidermal stem cells and migrate from the basement membrane to the upper layers of the skin, where they are finally shed as grime. Epidermal stem cells play an important role for skin homeostasis by proliferative capacity and flexibility in response to wound healing and physiological changes. However, factors affecting epidermal stem cell proliferation remain unresolved. In this study, we focused on Piezo1, a mechano-ion channel that recognizes "stiffness" and becomes activated, and clarified the mechanism by which the mechanical environment induces epidermal stem cell senescence. A new mechanism of skin aging has been identified in which Ptx3 expression from dermal fibroblasts increases with aging, which induces vascular atrophy and dermal stiffening, and induces age-related alterations in epidermal stem cells due to long-term calcium influx via Piezo1. This finding may be useful for the development of new functional ingredients with a novel mechanism "Ptx3 regulation."

***KEY WORDS*:** epidermal stem cells, skin aging, dermal stiffening, vascular atrophy, Ptx3, Piezo1

Introduction

The skin has the epidermis, dermis, and hypodermis. The epidermis is composed of multiple cell layers at different stages of differentiation, starting from the upper layers: stratum corneum, granular layer, spinus layer, and basal layer. Basal cells in the basal layer maintain their function as epidermal stem cells attached to the basement membrane. Epidermal stem cells undergo a series of metabolic processes: they proliferate constantly, differentiate step by step by peeling off from the basement membrane and migrating to the upper layers, and finally peel off as grime. Epidermal stem cells respond flexibly to wound healing and physiological changes, and their proliferative capacity can be temporarily increased [1-3].

It is known that epidermal stem cells lose their function with aging, their adhesion to the basement membrane becomes weaker, and the direction of cell division becomes abnormal [4, 5]. This aging-related disorders of epidermal stem cells is thought to be caused by internal cellular changes, *i.e.*, DNA damage induced by oxidative stress [6]. However, the aging changes in the environment surrounding epidermal stem cells and their effects on epidermal stem cells have not yet been elucidated.

By focusing on the "stiffness" of the dermis under the epidermis, this study clarified the mechanism by which age-related alterations in the mechanical environment of epidermal stem cells induce aging [7].

Differences between young and aged skin

In the study of differences between young and aged mouse plantar epidermis, we found that epidermal stem cells in aged mice showed age-related alterations, including weakening of hemidesmosomes that support adhesion to the basement membrane, increased perpendicular division to the basement membrane, and ectopic expression of differentiation markers. To identify the cause of this aging disorders, gene expression in epidermal stem cells was compared between young and aged mice. The gene group

Correspondent to: Ryo Ichijo, PhD
Department of Biosystems Science, Institute for Frontier Life and Medical Science,
Kyoto University
Sakyo-ku, Kyoto 606-8507 Japan
TEL : +81-75-751-4040 FAX: +81-75-771-5699
e-mail: richijo@infront.kyoto-u.ac.jp

whose expression was elevated in aged mice was significantly enriched in genes related to calcium signaling. Regarding calcium, studies using cultured cells have shown that it induces keratinocyte differentiation and hemidesmosome disassembly [8].

Therefore, we performed calcium in vivo imaging of epidermal stem cells in the plantar region of living mice and found that calcium pulses of 20 seconds or longer duration were more frequent in aged mice than in young mice. We hypothesized that ion channels, which are activated by sensing mechanical changes in cells, may be involved in this phenomenon, and investigated the possibility of changes in tissue stiffness to find that the dermis was stiffer in aged mice than in young mice.

Next, we focused on Piezo1, a mechano-ion channel that recognizes and activates substrate "stiffness" [8]. When Piezo1 was knocked out specifically in epidermal basal cells, the prolonged calcium pulse induced by aging was suppressed, as was the aging disorders of epidermal stem cells. Thus, age-related stiffening of the dermis induces sustained activation of Piezo1 in epidermal basal cells, which in turn induces age-related disorders of epidermal stem cells through prolonged calcium influx.

Effects of aging on the skin dermis

We have attempted to elucidate the causes of age-related dermal stiffening and previously clarified that blood vessels are important for epidermal stem cell proliferation [9]. Therefore, we examined the blood vessels in the dermis of aging mice and found that the number of blood vessels was reduced in the dermis of aging mice. Furthermore, artificially increasing the number of blood vessels in transgenic mice restored the age-related stiffening of the dermis and also the aging disorders of epidermal stem cells. Conversely, genetic modification of young mice to reduce vascularity induced a phenotype of dermal stiffening and age-related transformation of epidermal basal cells.

Next, we attempted to identify the cause of age-related vascular atrophy. Using a single cell RNA sequencing of the dermis of young and aged mice, it revealed the increased cluster of fibroblasts expressing high levels of Pentraxin 3 (Ptx3), a secreted factor that inhibits angiogenesis [10]. In Ptx3 knockout mice, age-related vascular atrophy was suppressed, dermal stiffness was improved, and age-related disorders of epidermal stem cells were alleviated. Furthermore, analysis of human skin samples showed that Ptx3 accumulated more in the skin dermis of older adults than that of younger adults.

Conclusion: Decreased blood vessels and dermal stiffening

These results reveal a novel mechanism of skin aging in which Ptx3 expression increases with aging from fibroblasts in the dermis, which induces vascular reduction and dermal stiffening, thus inducing age-related disorders of epidermal stem cells by long-term calcium influx via Piezo1 (***Fig. 1***). The results may be useful for the development of drugs and cosmetics with new mechanisms of action "Ptx3 regulation."

Conflict of interest declaration

The authors have no conflicts of interest to declare with respect to this study.

Acknowledgment

This work was supported by JSPS KAKENHI Grant Number 21K16209.

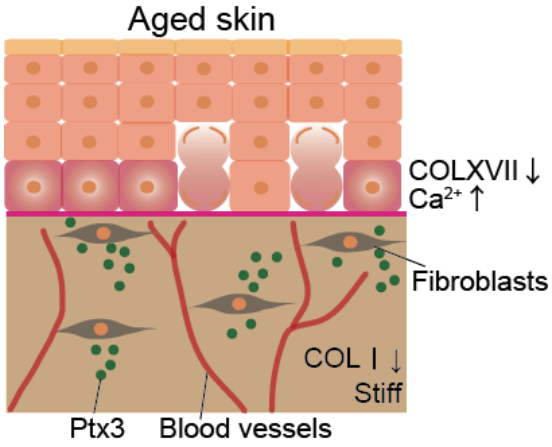

Fig. 1. **Schematic of the mechanism of age-associated IFESC dysregulation.**

References

1) Hsu YC, Li L, Fuch E. Emerging interactions between skin stem cells and their niches. *Nat Med*. 2014; 20: 847-856.
2) Aragona M, Dekoninck S, Rulands S, et al. Defining stem cell dynamics and migration during wound healing in mouse skin epidermis. *Nat Commun*. 2017; 8: 14684.
3) Ichijo R, Kobayashi H, Yoneda S, et al. Tbx3-dependent amplifying stem cell progeny drives interfollicular epidermal expansion during pregnancy and regeneration. *Nat Commun*. 2017; 8: 508.
4) Watanabe M, Natsuga K, Nishie W, et al. Type XVII collagen coordinates proliferation in the interfollicular epidermis. *Elife*. 2017; 6: e26635.
5) Liu N, Matsumura H, Kato T, et al. Stem cell competition orchestrates skin homeostasis and ageing. *Nature*. 2019; 568(7752): 344-350.
6) Behrens A, van Deursen JM, Rudolph KL, et al. Impact of genomic damage and ageing on stem cell function. *Nat Cell Biol*. 2014; 16: 201-207.
7) Ichijo R, Maki K, Kabata M, et al. Vasculature atrophy causes a stiffened microenvironment that augments epidermal stem cell differentiation in aged skin. *Nat Aging*. 2022; 2: 592-600.
8) Bikle DD, Xie Z, Tu CL. Calcium regulation of keratinocyte differentiation. *Expert Rev Endocrinol Metab*. 2012; 7: 461-472.
9) Ichijo R, Kabata M, Kidoya H, et al. Vasculature-driven stem cell population coordinates tissue scaling in dynamic organs. *Sci Adv*. 2021; 7: eabd2575.
10) Rusnati M, Camozzi M, Moroni E, et al. Selective recognition of fibroblast growth factor-2 by the long pentraxin PTX3 inhibits angiogenesis. *Blood*. 2014; 104: 92-99.