

CONCISE COMMUNICATION

Age-related decrease in CD271⁺ cells in human skin

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ABSTRACT

According to recent studies, stem cells are found in various tissues in our bodies. It has been reported that stem cells can reside in the skin tissues, including the epidermis, dermis, hair follicles and subcutaneous tissues. Homeostasis of the skin is maintained because these stem cells collaborate with each other to form new cells. We previously identified the CD271(p75NTR)⁺ cell as a stem cell that was present in the epidermis, dermis and subcutaneous tissue, and further investigated the role of stem cells in wound healing and their association with skin disease. In this study, we investigated the localization of CD271⁺ cells in human skin (epidermis and dermis) and its age-related changes in stem cells using CD271⁺ cells. The study revealed that the number of CD271⁺ cells in the epidermis and dermis decreased with aging. It is possible that such an age-related decrease in stem cells causes impaired regenerative ability and is associated with various skin diseases. If the relationship between stem cells and skin aging and diseases can be elucidated by investigations such as this study, it may lead to the development of novel anti-aging technologies and medical treatments for skin diseases in the future.

Key words: aging, CD271 (p75NTR), dermal stem cell, epidermal stem cell, skin stem cell.

INTRODUCTION

Stem cell research for the skin, including the epidermis, first started in the 1970s. Experiments on the proliferation of mouse epidermal cells initially demonstrated the existence of cells (slow-cycling cells) that were either in a quiescent state or in an extremely slow cell cycle in the basal layer. Currently, stem cells have been found in regions including the basal layer of the epidermis, bulge and hair follicles. It is believed that these stem cells are associated with the maintenance of skin homeostasis.¹ Previous studies have also shown that stem cells express specific marker proteins. For example, Jones *et al.* found cells that strongly expressed integrin-β1 in the basal layer of the epidermis and suggested that these cells were stem cells.² It has also been reported that integrin-α6, melanoma-associated chondroitin sulfate proteoglycan, p63 and delta-like 1 are marker proteins for epidermal stem cells in the basal layer.³

Toma *et al.* succeeded in growing dermis-derived multipotent stem cells called skin-derived precursor (SKP) cells using a culture medium containing factors such as epidermal growth factor, basic fibroblast growth factor and B27. The known markers for dermal stem cells include Nestin, CD44, CD73, CD90 and CD105.⁴

Meanwhile, we have identified CD271 (p75NTR:p75 neurotrophin receptor) as a common stem cell marker for the epidermis and dermis and further investigated the characteris-

tics of cells that express CD271. Previous studies have demonstrated that CD271⁺ cells were characterized by excellent proliferation potency and multipotency.⁵ Thus, we speculated that CD271⁺ cells may play important roles in the maintenance of skin homeostasis. However, because most of the previous studies were conducted in mice, the roles of CD271⁺ cells in human skin have not yet been identified. In this study, we conducted an analysis of age-related changes in stem cells using CD271⁺ cells of human skin.

METHODS

Skin sample collection and histochemistry

Skin samples were collected from female subjects aged in their 10s to 80s (*n* = 23) at Fujita Health University Hospital (Aichi, Japan). Considering that the skin is susceptible to sex difference and ultraviolet light, we collected skin samples from unexposed areas (trunk) of the body of female subjects. This study was approved by the ethics committees of Fujita Health University and Nippon Menard Cosmetic Research Laboratories, and written informed consent was obtained from each subject or the subject's legal guardian prior to surgery. After surgery, discarded parts of the normal skin and subcutaneous tissue were collected and fixed with 4% paraformaldehyde for pathological analysis. Immunostaining for CD271 was performed in accordance with a standardized method.⁵

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RESULTS

Age-related changes in human skin

We observed age-related morphological changes in the normal skin of healthy female subjects aged in their 10s to 80s by hematoxylin–eosin staining. There were decreased numbers of cells in the epidermis and dermis, thinned epidermis and decreased collagen fiber bundles, which occurred with aging (data not shown). These results were consistent with the results of previous studies.⁶

Observation of CD271⁺ cells in the epidermis and dermis of human skin and their age-related change

Localization of CD271⁺ cell populations in human skin was carefully observed by pathological analysis using immunohistochemical staining. CD271⁺ cells were found in the basal layer of the epidermis of the young subjects, particularly in the uppermost parts of the projections of the epidermis (uppermost part of the papillary stratum). In the dermis, a number of CD271⁺ cells were found in the papillary stratum of the upper parts of the dermis (Fig. 1). On the other hand, the skin of the middle-aged and elderly subjects showed a decrease in the number of CD271⁺ cells and changes in the localizations in both the epidermis and dermis (Fig. 1).

We further analyzed the age-related changes in the number of CD271⁺ cells in the epidermis and dermis.

Immunohistochemical staining was performed on 23 skin samples that were obtained from healthy female subjects aged in their 10s to 80s to analyze the number of CD271⁺ cells found per unit area ($500 \times 500 \mu\text{m}^2$). The results showed that the numbers of CD271⁺ cells in the dermis and epidermis were significantly decreased with aging (Fig. 2).

DISCUSSION

Previous studies have shown that the epidermis is continually replenished with keratinocytes that undergo a 28-day cell turnover. The connective tissue in the dermis is maintained with collagen, which is constantly produced by fibroblasts. However, the regulation of skin homeostasis declines with aging, thereby causing various signs of aging in the skin, such as wrinkles, sagging skin and dry skin. In recent years, the roles of stem cells in skin homeostasis have attracted increasing attention. In our previous studies, we have identified CD271⁺ cells as stem cells in the epidermis and dermis of mouse skin and demonstrated their excellent proliferation potency and multipotency.^{5–7} In this study, we conducted pathological analyses on the age-related changes in the CD271⁺ cells using human epidermis and dermis. The results showed that the number of CD271⁺ cells in the epidermis and dermis decreased with aging (Figs 1,2). When the skin sample of the subjects who were in their 60s was compared with that of the

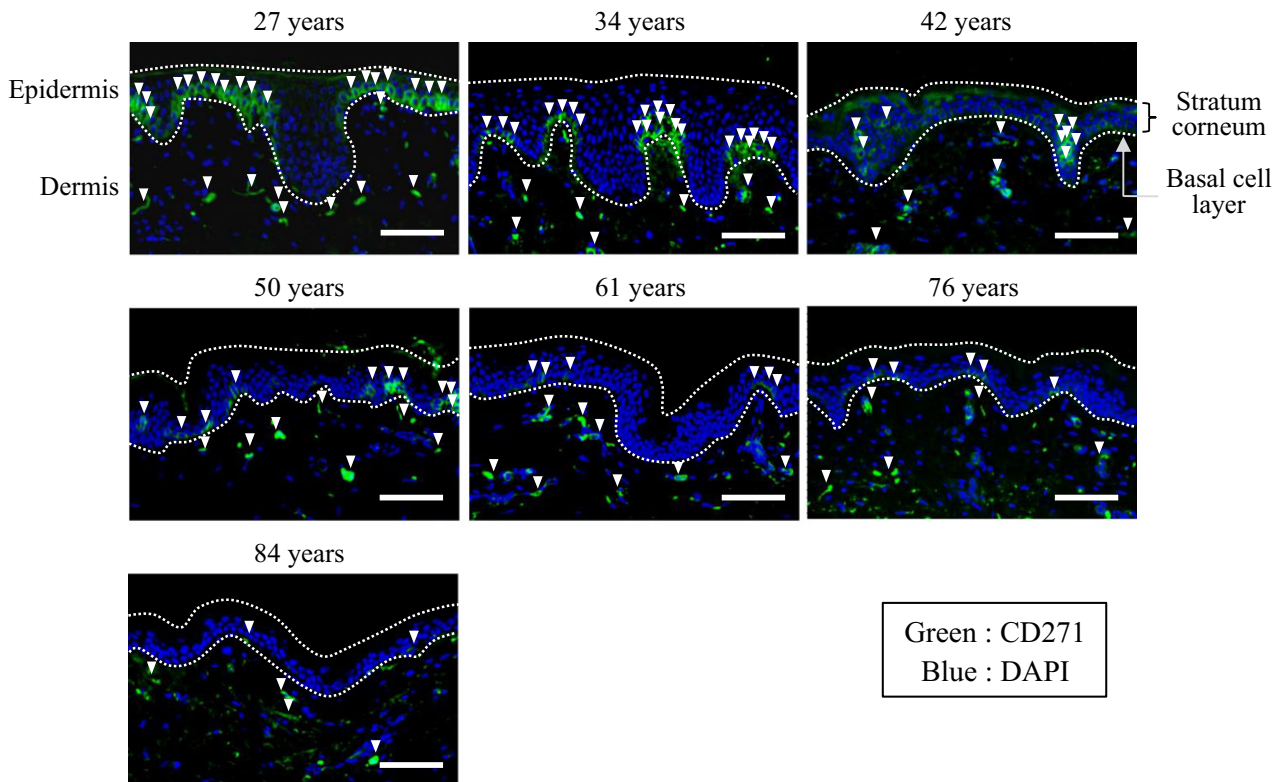


Figure 1. Among 23 subjects, we showed a representative example of the immunostaining in each generation (20s, 30s, 40s, 50s, 60s, 70s and 80s). Arrowheads indicate CD271⁺ cells (green). 4',6'-Diamidino-2-phenylindole dihydrochloride (DAPI) was used for nuclear staining (blue) (bars = 100 μm).

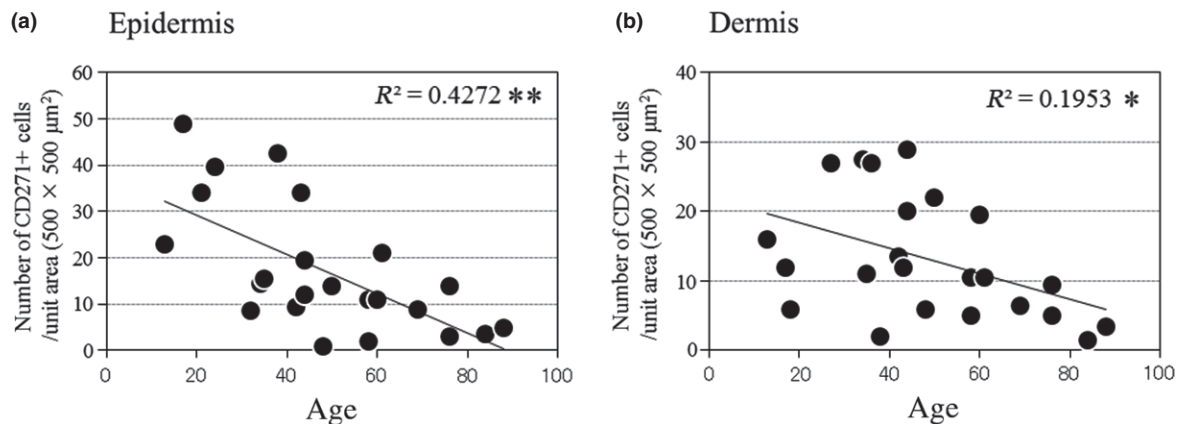


Figure 2. The number of CD271⁺ cells/500 μm × 500 μm unit area in the (a) epidermis and (b) dermis were analyzed using NIH Image. Statistical analysis was performed using ANOVA with Student's *t*-test. The significance level was set at **P* < 0.05, and ***P* < 0.01 indicated a strongly significant difference.

subjects in their 20s, the number of CD271⁺ cells per unit area of the older subjects was approximately one-third of that of the younger subjects (Fig. 2).

A decrease in the stem cells in the skin may have a major effect on skin homeostasis. For example, Potten *et al.*⁸ have suggested that stem cells played an important role in the turnover of the epidermis. Based on our results, we speculated that the age-related decrease in stem cells may cause an insufficient production of keratinocytes, resulting in a delayed cell turnover. Gunin *et al.* have shown that the number of fibroblasts per unit area (1 mm²) of the dermis decreased with aging.⁹ Furthermore, Castelo-Branco *et al.*¹⁰ showed a decreased production of collagen. It is possible that such a decrease in fibroblasts and production of collagen is attributed to a decrease in the number of dermal stem cells.

Recently, Truzzi *et al.*¹¹ and Vaculik *et al.*¹² have also demonstrated that CD271⁺ cells had an important role in the maintenance of skin homeostasis. Based on the results of our study, the age-related decrease in CD271⁺ cells may be a key factor that causes impaired skin homeostasis. Our previous study demonstrated that CD271⁺ cells were also decreased in cases of chronic ulcers in human skin.¹³ Thus, we concluded that the decreased number and deteriorated function of CD271⁺ cells were closely associated with skin aging and diseases. According to recent studies, a number of stem cell markers other than CD271 have been identified.^{2–5} We believe that these stem cell-like cells in the skin play important roles in skin homeostasis and skin renewal. Further investigation on the function of CD271⁺ cells and stem cell-like cells that express other stem cell markers and the analysis of various skin diseases may spur the development of novel treatments for skin diseases and drug discovery research.

CONFLICT OF INTEREST: None declared.

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