102

EMERGENCE OF MELANIN-OVERPRODUCING CELLS AND ITS SUPPRESSION BY ALARIA PRAELONGA EXTRACT

T Yamada[1], *S Hasegawa*[1], *Y Inoue*[1], *Y Date*[1], *N Hotta*[1], *T Sakaida*[1], *Y Yashiro*[1], *H Mizutani*[1], *S Nakata*[1]
[1]*Research Laboratories, Nippon Menard Cosmetic Co., Japan*

ABSTRACT

Purpose

Solar lentigines are characterized by hyperpigmentated spots caused by photo-aging and are commonly seen on the parts of the body frequentlyexposed to sunlight such as face and arms. Because of a negative impact it has on skin appearance, it is expected to elucidate the pathogenic mechanism and develop an effective treatment especially for skin whitening. Many of previous studies on skin whitening focused on symptomatic treatments such as inhibition of melanin synthesis and acceleration of melanin elimination. According to recent studies, it is suggested that melanocyte stem cells in hair follicles differentiated into melanocytes through a stage of melanoblasts. In other words, to discover a more fundamental, causal treatment for Solarlentigines it is necessary to understand melanocyte stem cells and melanoblasts and control them as well as mature melanocytes which previous studies had targeted. In this study, we elucidated the process of differentiation of melanocyte stem cells into melanoblasts and melanocytes and further investigate a novel skin whitening technology.

Methods

Using healthy human skin tissues, localization of melanocyte stem cells, melanoblasts and melanocytes was analyzed by immunohistological study in which Frizzled-4 (FZD4), microphthalmia-associated transcription factor (MITF), Melanoma Antigen Recognized by T cells-1 (MART-1) and Tyrosinase (TYR) were used as indicators. After endothelin-1(EDN1), stem cell factor (SCF) and dibutylyl cyclic AMP (dbcAMP) were added to a human melanoblast culture system to induce differentiation into melanocytes, the time-course changes in gene expression were analyzed by real-time PCR. Furthermore, we searched for materials that may control the differentiation of melanoblasts into melanocytes and examined the clinical efficacy of the materials that suppress the differentiation of melanocytes in skin.

Results

In analysis of localization of melanocyte stem cells, melanoblasts and melanocytes in human skin tissues, we confirmed the presence of melanocyte stem cells ($FZD4^+/MITF^+$) in the bulge area, melanoblasts in lower hair infundibulum ($MART\text{-}1^+/TYR^-$) and melanocytes in upper hair infundibulum and epidermis ($MART\text{-}1^+/TYR^+$) (Fig. 1a-f). We also found a great difference in a level of TYR expression, a rate-limiting enzyme for melanin synthesis, among the mature melanocytes in epidermis (Fig. 1g-i). These findings suggested that melanocyte stem cells existing in hair follicle bulges differentiate into mature melanocytes through a stage of melanoblasts. It was also considered that the melanogenic potential of each mature melanocytes may greatly vary.

It was shown in *in vitro* analysis of differentiation of melanoblasts into melanocytes that the expressions of melanin synthesis-related genes were enhanced in a time-dependent manner. It was also observed that the amount of synthesized melanin varies in each melanocyte. More specifically, there was a variety of melanocytes which actively or hardly synthesize melanin after induction of differentiation (Fig. 2a,b). We refer to melanocytes which actively synthesize melanin as melanin-overproducing melanocytes. In screening for materials to control the differentiation of melanocytes using the culture system, we found *AlariaPraelonga*extract reduces melanin sysnthesis in mature melanocytes. From the results of microscopic observation and gene expression analysis, we considered that *AlariaPraelonga*extract suppressed the emergence of melanocytes from melanoblasts. Currently, we are conducting a clinical study on the skin-whitening effect of *AlariaPraelonga*extract.

Conclusion

Melanogenic potential of melanocytes which are differentiated from melanocyte stem cells and melanoblasts varies for each melanocyte. This is probably because melanin-overproducing melanocytes may emerge depending on the differentiation and maturation environment. Therefore, it was concluded that such differentiation and maturation process from melanocyte stem cells to melanocytes may be related to the formation of solar lentigines. *AlariaPraelonga*extract was considered to be effective in treatment of solar lentigines as it suppresses the differentiation into melanin-overproducing melanocytes.

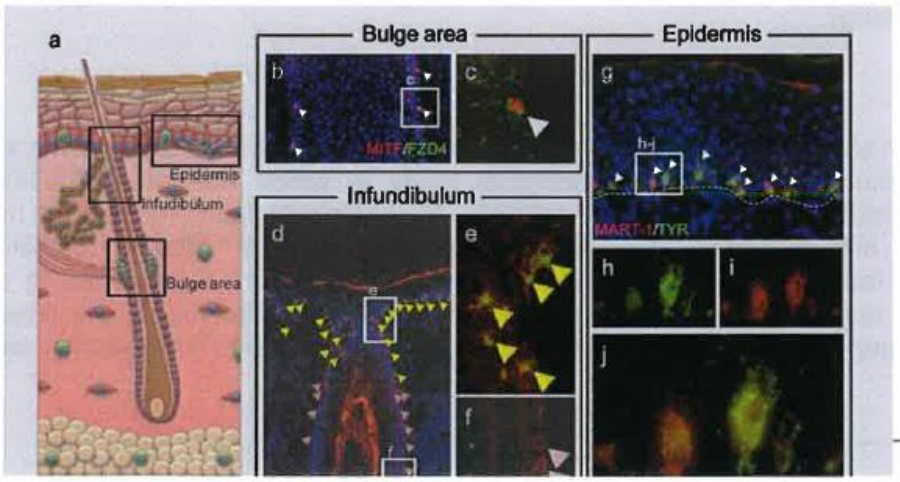

Fig.1 Immunostaining analysis of localization of melanocyte lineage The boxed regions of the diagram of a hair follicle indicate bulge area, epidermis and infundibulum (a). The presence of melanocyte stem cells ($FZD4^{+}/MITF^{+}$) in the