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# Possible Involvement of Keratinocyte Growth Factor in the Persistence of Hyperpigmentation in both Human Facial Solar Lentigines and Melasma

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Dear Editor:

Acute pigmentation due to tanning is commonly understood as increased melanization of the epidermis observed in the skin after ultraviolet (UV) exposure, and the mechanisms underlying this condition are well understood now. Keratinocyte-derived gene products are upregulated by UV irradiation and act as paracrine factors in the skin to stimulate melanogenesis and melanin transfer by melanocytes[1]. Although acute pigmentation disappears over time, some types of hyperpigmentary disorders such as freckles, solar lentigines, and melasma, tend to persist if patients do not receive any treatments such as topical cosmetic products, medication, or laser.

Solar lentigines are dark brown spots that occur on sun-exposed areas[2], typically on the face, upper back, and shoulders. Multiple solar lentigines are considered a hallmark of aged skin. It is thought that cumulative UV exposure causes these spots. Therefore, pigmented spots of solar lentigines can be considered as indications of photoaging. Melasma is a common acquired symmetrical hypermelanosis on sun-exposed areas of the skin and is very common among Oriental women[3]. The major etiological factors include genetic influences, exposure to UV radiation, and sex hormones. However, the mechanisms underlying the persistence of hyperpigmentation in solar lentigines and melasma are not yet fully understood.

Keratinocyte growth factor (KGF) or fibroblast growth factor-7 (FGF-7) is a member of the FGF family[4]. KGF is secreted from cultured stromal fibroblasts derived from the skin and gastrointestinal tract, and is expressed *in vivo* in dermal cells, but not in epidermal cells[5]. In addition, this paracrine growth factor also plays a role in the stimulation of melanogenesis[5,6], proliferation of human melanoblasts, and differentiation of melanocytes[7]. A previous study reported higher levels of KGF in five patients with solar lentigines, suggesting the permeation of KGF from the dermis to the epidermis, which may result in the persistence of solar lentigines[6]. In this study, we quantitatively investigated the accumulation of KGF in the epidermis of patients with two major types of hyperpigmentary disorders, facial solar lentigines, and melasma to identify novel effective topical measures for their treatment.

We examined 24 Korean women with newly diagnosed facial solar lentigines and 13 others with newly diagnosed melasma, which were determined on physical examination and histological examination. This study was approved by the ethics committee of Ajou University Hospital (No. MED-KSP-12-171). Punch biopsies from lesions and perilesional normal skin were obtained from each patient. The perilesional normal skin was taken from the area within 1 cm away from the lesional border. Twenty-four pairs of facial solar lentigines and 13 pairs of melasma tissue were prepared for immunohistopathological examination.

We examined KGF protein accumulation in the epidermis

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of both facial solar lentigines and melasma. Paraffin-embedded sections of both lesional and perilesional normal skin were processed with monoclonal antibodies against KGF (dilution, 1:100; Abcam, Boston, MA, USA) for 20 min at 48°C. The stained area per epidermal area (SA/EA) and SA per single rate ridge length (SA/1R) of lesional and perilesional skin were measured. In both facial solar lentigines and melasma lesions, distinctly positive immunoreactivity against KGF was noticed in the epidermis, whereas perilesional normal skin only showed weak immunoreactivity (Fig. 1). The SA/EA of perilesional normal skin samples was $0.184 \pm 0.139$ for facial solar lentigines and $0.134 \pm 0.071$ for melasma, and that of lesional skin samples was $0.237 \pm 0.107$ for facial solar lentigines and $0.210 \pm 0.084$ for melasma (Fig. 2A). The differences were statistically significant ($p = 0.014$ for facial solar lentigines and $p = 0.016$ for melasma). The SA/1R of perilesional normal skin samples was $10.216 \pm 7.194$ for facial solar lentigines and $8.699 \pm 4.923$ for melasma, and that of lesional skin samples was $19.350 \pm 8.744$ for facial solar lentigines and $13.172 \pm 4.709$ for melasma (Fig. 2B). The differences were statistically significant ($p = 0.000035$ for facial solar lentigines and $p = 0.014$ for melasma).

In the present study, we demonstrated that KGF protein accumulation in the epidermis of facial solar lentigines and melasma was significantly increased. We performed the following two analytical techniques: first, the SA for KGF was normalized to the EA; second, the SA for KGF was normalized with 1R, which is independent from epidermal thickness. In both analyses, we found a statistical difference between perilesional normal and lesional skin of both facial solar lentigines and melasma. In this study, we did not take the duration and stage of solar lentigines and melasma into consideration because of the small number of patients, although they could have affected the results. We expect that a study with a larger number of patients in varied stages and duration of solar lentigines and melasma will demonstrate the correlation between KGF protein accumulation level and the duration or stage of solar lentigines and melasma.

Iriyama et al.[8] revealed that the degradation of heparan sulfate at the dermal–epidermal junction in photoaged skin impaired the function of the basement membrane, which regulates the transfer of several growth factors between the epidermis and dermis. Moreover, they demonstrated that heparan sulfate at the dermal–epidermal junction was specifically reduced in solar lentigines on the human back, and that the degradation of heparan sulfate en-

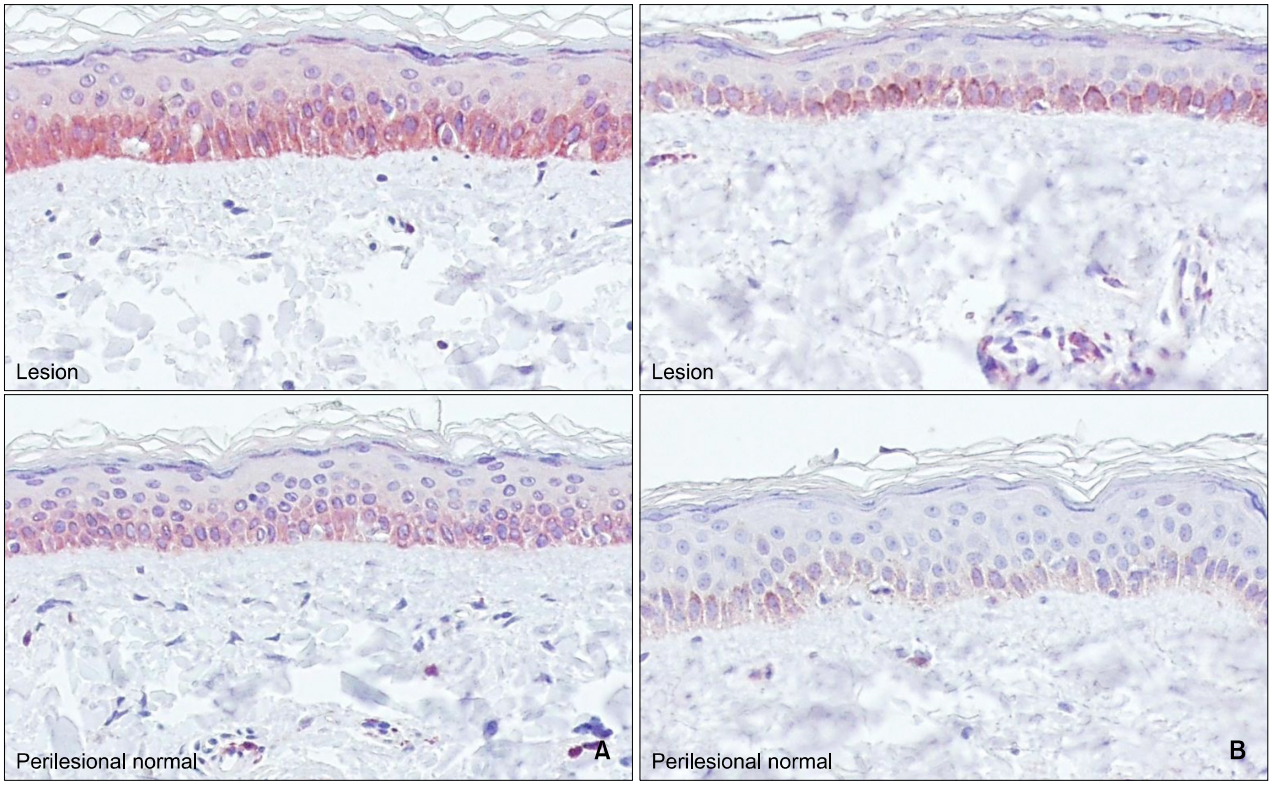


**Fig. 1.** Immunostaining with an antibody against keratinocyte growth factor (KGF). Significant amount of KGF protein was accumulated in the epidermis of lesional skin compared to that in perilesional normal skin of (A) solar lentigines and (B) melasma (A, B: ×400).

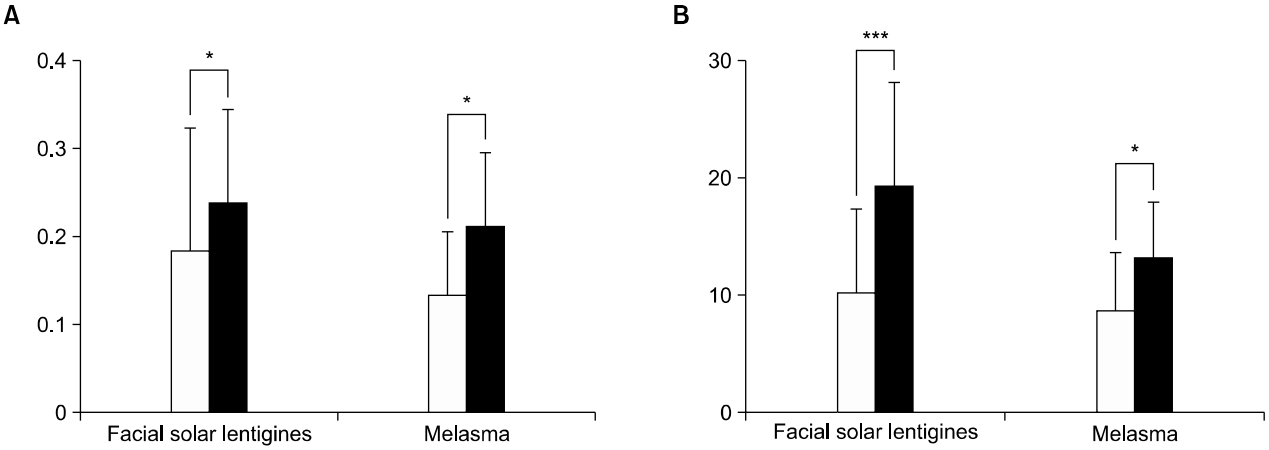


**Fig. 2.** Quantitative analysis of immunostaining. Immunohistochemical analysis for keratinocyte growth factor (KGF) was quantified in two ways: the stained area per epidermal area (SA/EA) (A) and the stained area per single rate ridge length (SA/1R) (B). Each measurement was taken under constant magnification. For each frame, the tracing was repeated three times, and the mean ± standard deviation was used for evaluation. The image was analyzed by using Image Pro Plus ver. 4.5 (Media Cybernetics Inc., Silver Spring, MD, USA). Comparisons of SA/EA and SA/1R between lesional and perilesional normal skin were done by using two-sided paired Student's t-test. A *p*-value of $<0.05$ was considered statistically significant. SPSS ver. 11.0 statistical program (SPSS Inc., Chicago, IL, USA) was used for the analysis. White bar: perilesional normal skin, black bar: lesional skin. Statistically significant differences: $*p<0.05$, $***p<0.001$.

hanced melanogenesis in a skin equivalent model. In addition, recent histological studies on melasma described changes in the basement membrane in the lesional skin of melasma[9,10]. The basement membrane structure in lesional skin was not intact and appeared disrupted. Thus, the loss of heparan sulfate at the dermal–epidermal junction of facial solar lentigines and the loosening of the basement membrane in melasma may enhance the transfer of dermis-derived factors such as KGF. Our present study strongly supports the hypothesis that in hyperpigmentary disorders, such as facial solar lentigines and melasma, the fibroblast-derived KGF is transferred into the epidermis in which it accumulates, thereby leading to the persistence of hyperpigmentation. Thus, targeting the dermal-derived KGF itself and the degradation of heparan sulfate at the dermal–epidermal junction, which allow an excess amount of KGF to transfer into the epidermis, could be effective topical measures like cosmetic or medication use for treating hyperpigmentary disorders.

## ACKNOWLEDGMENT

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## REFERENCES

1. Gilchrest BA. Molecular aspects of tanning. J Invest Dermatol 2011;131:E14-E17.
2. Hodgson C. Senile lentigo. Arch Dermatol 1963;87:197-207.
3. Kang HY, Ortonne JP. What should be considered in treatment of melasma. Ann Dermatol 2010;22:373-378.
4. Finch PW, Rubin JS, Miki T, Ron D, Aaronson SA. Human KGF is FGF-related with properties of a paracrine effector of epithelial cell growth. Science 1989;245:752-755.
5. Chen N, Hu Y, Li WH, Eisinger M, Seiberg M, Lin CB. The role of keratinocyte growth factor in melanogenesis: a possible mechanism for the initiation of solar lentigines. Exp Dermatol 2010;19:865-872.
6. Lin CB, Hu Y, Rossetti D, Chen N, David C, Slominski A, et al. Immuno-histochemical evaluation of solar lentigines: The association of KGF/KGFR and other factors with lesion development. J Dermatol Sci 2010;59:91-97.
7. Hirobe T, Hasegawa K, Furuya R, Fujiwara R, Sato K. Effects of fibroblast-derived factors on the proliferation and differentiation of human melanocytes in culture. J Dermatol Sci 2013;71:45-57.
8. Iriyama S, Ono T, Aoki H, Amano S. Hyperpigmentation in human solar lentigo is promoted by heparanase-induced loss of heparan sulfate chains at the dermal-epidermal junction. J Dermatol Sci 2011;64:223-228.
9. Lee DJ, Park KC, Ortonne JP, Kang HY. Pendulous mela-

nocytes: a characteristic feature of melasma and how it may occur. Br J Dermatol 2012;166:684-686.
10. Torres-Álvarez B, Mesa-Garza IG, Castanedo-Cázares JP, Fuentes-Ahumada C, Oros-Ovalle C, Navarrete-Solis J, et al. Histochemical and immunohistochemical study in melasma: evidence of damage in the basal membrane. Am J Dermatopathol 2011;33:291-295.

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# A Case of Focal Eosinophilic Myositis Associated with Hypereosinophilic Syndrome: A Rare Case Report

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Dear Editor:

Hypereosinophilic syndrome (HES) is classically defined as (i) persistent eosinophilia of >1,500 eosinophils/$mm^3$ for >6 months; (ii) the absence of any other evident cause of eosinophilia, including allergic diseases and parasitic infections; and (iii) signs or symptoms of organ involvement by eosinophilic infiltration. Skin involvement and cutaneous findings are frequently seen in these patients. Although many other organs other than the skin can also be affected by HES, myopathies associated with HES have rarely been reported[1]. Here, we report a rare case of focal eosinophilic myositis associated with HES. A 49-year-old woman visited our clinic with a solitary ovoid subcutaneous tender nodule on her right palm that appeared 2 weeks before her visit (Fig. 1). She denied any history of an insect bite or trauma at the site. Routine laboratory tests showed marked elevations in the eosinophil counts (6,730/$mm^3$; reference range, 50~500/$mm^3$), platelet counts ($562\times10^3$/$mm^3$; reference range, $150\sim350\times10^3$/$mm^3$), and C-reactive protein levels (1.97 mg/dl; reference range, 0~0.6 mg/dl); the other test results were normal. Chest radiography showed mild bilateral pleural effusion. Skin biopsy was then performed, and the patient was referred to the department of allergy to check for the cause of blood eosinophilia. Thorough medical history taking, laboratory examinations, and imaging studies excluded any known causes of hypereosinophilia such as allergic diseases, allergic drug reactions, parasitic infections, human immunodeficiency virus infections, and solid tumors. The skin biopsy showed marked infiltration of eosinophils and lym-

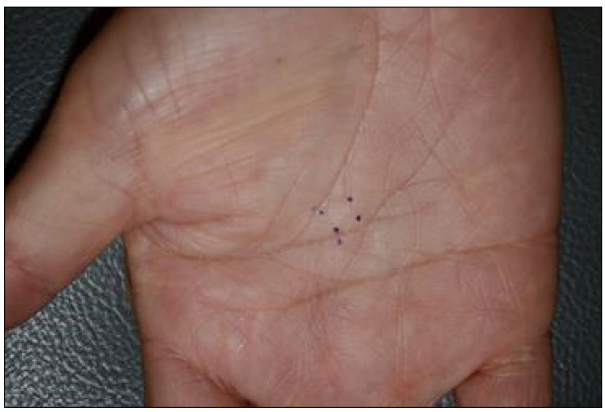

**Fig. 1.** A solitary well demarcated erythematous to skin colored ovoid subcutaneous nodule on the right palm.

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