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Senescent fibroblasts in melasma pathophysiology

Misun Kim[1] | So Min Kim[1] | Soohyun Kwon[1] | Tae Jun Park[2,3,4] | Hee Young Kang[1,4]

[1]Department of Dermatology, Ajou University School of Medicine, Suwon, Korea

[2]Department of Biochemistry and Molecular Biology, Ajou University School of Medicine, Suwon, Korea

[3]Chronic Inflammatory Disease Research Center, Ajou University School of Medicine, Suwon, Korea

[4]Department of Biomedical Science, The Graduate School, Ajou University, Suwon, Korea

Correspondence
Hee Young Kang, Department of Dermatology, Ajou University School of Medicine, Suwon, Korea.
Email: hykang@ajou.ac.kr

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Abstract

It has been proposed that melasma is a photoageing skin disorder. The photoaged fibroblasts have been suggested as an important source of melanogenic factors which are involved in the regulation of pigmentation. To investigate whether melasma includes senescent cells, lesional and perilesional normal skin from 38 melasma patients was assessed using a cell senescence marker, $p16^{INK4A}$. The results showed that lesional dermal skin had more $p16^{INK4A}$-positive senescent cells than perilesional skin. The impact of senescent fibroblasts was further investigated in a pilot study using radiofrequency (RF) intervention for melasma. It showed that the RF therapy decreased the number of senescent cells with increased expression of procollagen-1, which were associated with reduced epidermal pigmentation. This leads us to the speculation that senescent fibroblasts may contribute to drive melasma and might be considered as a potential therapeutic target.

KEYWORDS
melasma, photoageing, radiofrequency, senescent fibroblasts

1 | INTRODUCTION

Melasma is a common acquired hyperpigmentary disorder of sun-exposed skin. Although melasma is known as epidermal pigmentary disease, studies have suggested that it might be regarded as one of the phenotypes of photoageing. Immunohistochemical studies have shown that melasma shows prominent features of solar damaged skin compared with that in perilesional normal skin; lesional skin shows increased solar elastosis, vasculature and basement membrane disruption underlying increased epidermal pigmentation.[1–4] These findings suggested in melasma skin, not only melanocytes but other actors, especially dermal components such as photoaged fibroblasts or vasculature, probably have a key role in the development and the relapses of melasma.[5]

A pivotal role of photoaged fibroblasts in determining the pigmentation phenotype of ageing skin was recently highlighted.[6,7] Under ultraviolet (UV) irradiation, fibroblasts become senescent and produce more skin ageing-associated secreted proteins (SAASP) compared with normal fibroblasts.[8] These SAASP include variably expressed secreted factors controlling extracellular matrix production and also include melanogenic cytokines, such as stem cell factor (SCF), keratinocyte growth factor and hepatocyte growth factor.[9] It was further demonstrated that fibroblasts derived SCF or secreted frizzled-related protein 2 (SFRP2) play a role in the development of melasma.[10,11] Very recently, the direct role of senescent fibroblasts in the development of senile lentigo was shown.[12] These findings led to the speculation that senescent fibroblasts could be involved in the pathogenesis of melasma.

In this study, we investigated whether melasma includes senescent cells. As the results indicate that melasma lesional skin contains more dermal senescent cells, we further performed a pilot study to examine whether skin rejuvenation procedure targeting dermis may reduce the pigmentation of melasma. These data may provide a new therapeutic modality, such as senescent fibroblasts-targeting therapies to treat melasma.

2 | MATERIALS AND METHODS

2.1 | Biopsy collection

A retrospective study comprising 38 women with facial melasma with Fitzpatrick skin type III or IV (mean age 45) was performed. Diagnosis was based on physical examination and confirmed by histopathological findings. All patients underwent 2 mm skin biopsy, and samples were taken from lesional and perilesional normal areas (usually within 10 mm from the lesion margin).

2.2 | Participants and radiofrequency (RF) treatment

Three female volunteers with melasma (Fitzpatrick skin type III or IV, 32-45 years old) were included. Exclusion criteria were pregnancy/lactation, prior aesthetic medical procedures or use of topical depigmenting agents in the 3 months prior to the study. A bipolar pulsed-type RF device (SYLFIRM™, Viol, Gyeonggi, Korea) at a frequency of 2 MHz and with a disposable tip comprising 25 non-insulated microneedles was used in this trial. Parameters were set at level 2 with a penetration depth of 1.5 mm. Subjects received 5 sessions of RF treatments every 2 weeks. Mild pain and temporary erythema during and after the procedure were well tolerated in all participants. No serious adverse events were encountered. Skin pigmentation levels were measured using a chromameter (CR-300; Minolta, Osaka, Japan) and the values for lightness are denoted by L*. The lesional skin samples were taken at baseline and at 12 weeks for immunohistochemical study. This study was approved by the institutional review board of Ajou University Hospital (IRB number: AJIRB-DEV-DE3-15-491).

2.3 | Immunohistochemical analysis

Immunohistochemical staining was performed on 4% paraformaldehyde-fixed, paraffin-embedded sections. Antibodies against a cell senescence marker, $p16^{INK4A}$, (Santa Cruz Biotechnology, Dallas, TX, USA) and procollagen-1 (The Developmental Studies Hybridoma Bank, Iowa City, IA, USA) were used for protein detection. Fontana-Masson staining was performed to measure the amount of melanin pigmentation. Image signals were evaluated using Image Pro Plus Version 4.5 (Media Cybernetics Co., Rockville, MD, USA). The number of $p16^{INK4A}$-positive cells in the dermis within 200 μm from the epidermal-dermal junction was determined.

2.4 | Statistics

Statistical significance was determined using Student's *t* test. A *P* value of <0.05 was considered statistically significant. All results are presented as mean ± SD.

3 | RESULTS

3.1 | Melasma lesional skin contains more dermal senescent cells

Biopsy samples of lesional and perilesional normal skin of 38 melasma were assessed using $p16^{INK4A}$, a cell senescence marker.[13] The number of $p16^{INK4A}$-positive cells was significantly higher in the dermis of lesional skin than in that of perilesional skin (5.58 ± 4.55 vs 2.05 ± 1.71, $P < 0.001$; Figure 1). Most $p16^{INK4A}$-positive cells were observed in the superficial dermis near the epidermal-dermal junction, not in the deep dermis, suggesting the influence of UV irradiation on cellular senescence. There was no change in the number of $p16^{INK4A}$-positive cells in the epidermis of the lesional skin compared to perilesional normal skin (lesional skin: 1.39 ± 2.79 vs perilesional skin: 0.79 ± 1.60, $P = 0.08$). These results indicate that melasma includes the accumulation of senescent cells in the dermis.

3.2 | Reduction of senescent cells are associated with improvement of pigmentation

Radiofrequency technique has been used as an anti-ageing treatment and the RF electro-thermal injury induces vigorous dermal remodelling and generation of new collagen.[14,15] Moreover, fractional laser resurfacing therapy was shown to decrease the proportion of senescent fibroblasts in aged dermis.[16] Three female volunteers with melasma underwent microneedle fractional RF treatment. All subjects showed clinical improvement after RF treatment compared with baseline (Figure 2A). The L* (lightness) value increased from the baseline (54.67 ± 6.20 vs 60.87 ± 4.32). Histologic analysis revealed that the RF treatment showed a reduction in $p16^{INK4A}$-positive cells and appeared to reverse the senescence-dependent decrease in collagen response, that is, there was an increase in the density of procollagen-1 after RF treatment. These changes were associated with

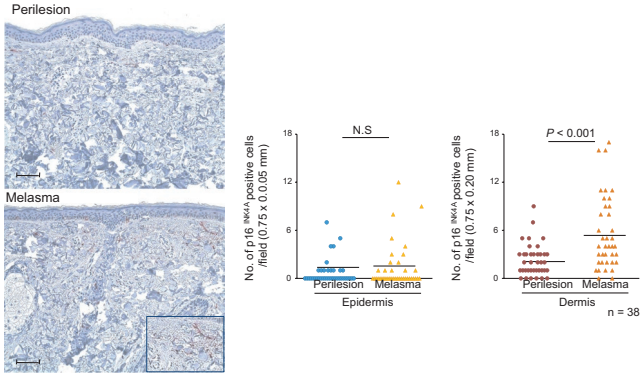

FIGURE 1 Melasma lesional skin contains more dermal senescent cells. Biopsies of lesional and perilesional normal skin of melasma were assessed using a marker of cell senescence, $p16^{INK4A}$. The scale bar indicates 200 μm. Statistical significance was determined using Student's *t* test

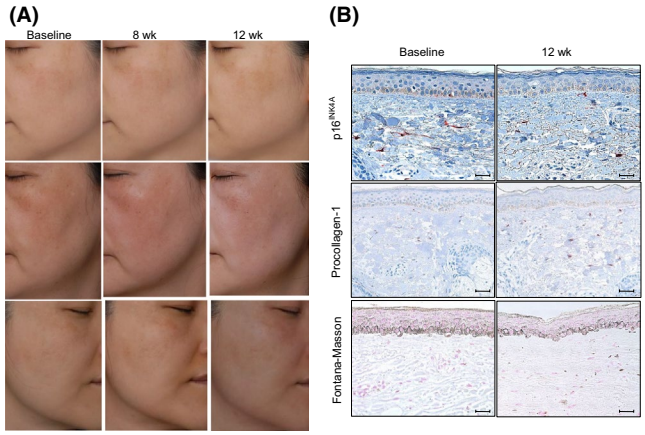

FIGURE 2 Reduction of senescent cells is associated with improvement of pigmentation. A, Clinical photographs of participants at 0, 8 and 12 wks of microneedle fractional radiofrequency (RF) treatment, showing gradual improvement in pigmentation. B, Changes in the number of $p16^{INK4A}$-positive cells and expression of procollagen-1 and melanin pigmentation in lesional skin after RF treatment. The scale bar indicates 200 μm

decreased pigmentation in the epidermis, that is, Fontana-Masson staining demonstrated a marked decrease in pigmentation of lesional skin compared with baseline values (Figure 2B). The above findings were similar in all three subjects with variable degrees.

4 | DISCUSSION

The present study demonstrated that melasma is characterized by accumulation of senescent cells in lesional dermis. The increased number of spindle-shaped $p16^{INK4A}$-positive fibroblasts is thought to originate from chronic UV irradiation. These findings are in line with the previous observations that melasma shows features of sun-damaged skin and the in vitro UV-induced senescence like phenotype of fibroblasts leads to the increased production of the melanogenic factors.[1–11] The senescent cells were mainly observed in the upper layer of the dermis, which have the advantage of easy crosstalk with nearby melanocytes. The impaired basement membrane in melasma may also contribute to the interaction between senescent fibroblasts and epidermal melanocytes.[3,4] All these observations suggest that senescent fibroblasts may contribute to increased pigmentation in melasma, and that dermal targeting therapies could be considered for melasma treatment.

Potential strategies with regard to the ageing process include interfering with the adverse effects of senescent cells by targeting the secretory phenotype and eliminating senescent cells.[17] In the present pilot study, the impact of senescent fibroblasts on skin pigmentation was suggested when reducing senescent cells with RF intervention, which was associated with reduced pigmentation. The removal of senescent fibroblasts might be due to dermal remodelling by inducing a mild wound-healing response of RF therapy; the RF may increase the percentage of replicating fibroblasts and these newly recruited young fibroblasts actively produce new collagen and may also modify skin microenvironmental milieu towards balanced normal pigmentation. Indeed, the skin lightening effects of anti-ageing procedures including anti-ageing cocktail hydroporation or microneedling as well as RF in melasma were previously suggested.[18–20] These observations may highlight the importance of the dermal microenvironment in regulating melanocyte biological activity. However, it should be noted that three subjects only were used for the RF treatment and any conclusions from this work are necessarily limited.

The major classical aetiological factors of melasma include genetic influences, sun exposure and female sex hormones.[21] Under exposure to the sun, the network of cellular interactions between keratinocytes, fibroblasts and perhaps the vasculature and melanocytes may play a role in the development of epidermal hyperpigmentation in melasma. Especially, the senescent change of the fibroblasts and their phenotype switching may contribute to the increased pigmentation of melanocytes. In this regards, although Kligman formula is still a golden standard for melasma treatment, the microenvironment around melanocytes is another important target for a more efficient treatment of melasma and a better prevention of the relapses. Melasma is no more simple melanocytic disease and is considered as a part of photoageing.

In summary, this study shows that melasma involves senescent fibroblasts accumulation in the dermis and the aged dermis including senescent cells might be a therapeutic target for melasma.

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CONFLICT OF INTEREST

The authors have declared no conflicting interests.

AUTHOR CONTRIBUTION

M. Kim, S.M. Kim and S. Kwon performed the research and analysed the data. T.J. Park and H.Y. Kang designed the research study and wrote the paper.

ORCID

Hee Young Kang http://orcid.org/0000-0001-8697-4292

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