

## Morphological Relationship between Nerve Fibers and Melanocytes in the Epidermis of Melasma

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### Abstract

A close contact between melanocytes and nerve fibers in the epidermis has been demonstrated in normal human skin. The interactions between the neural and immune systems play an important role in the pathophysiology of certain dermatoses. In this study, we investigated the intraepidermal nerve fibers and their contacts with melanocytes in the lesions of melasma using double labeled immunofluorescence and confocal laser scanning microscopy. We found that the percentage of contacts between intraepidermal nerve fibers and melanocytes in the lesion of melasma were 74%, significantly more than the 42% in the normal controls ( $p < 0.05$ ). The percentage of melasma lesions was also more than the 69% in the normal-appearing skin of the melasma patients ( $p < 0.05$ ). Our results of the increased contacts between the nerve fibers and melanocytes in the lesions of melasma suggest a potential coordination between the neural and cutaneous immune systems.

**Keywords:** Nerve fibers; Melanocytes; Melasma

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### Introduction

Melanocyte is the melanin-producing cell and is of neural crest origin. Melasma is a common acquired circumscribed hypermelanosis primarily occurring on the faces of women. It is known that the facial hyperpigmentation of melasma patients becomes more prominent when physically exhausted or emotionally distressed [1,2]. The precise feature of the connection between stress and exacerbation of skin inflammation has perplexed researchers. Studies have shown the evidence that stress travels to the skin through peripheral neuropeptidergic nerve fibers and exacerbates the neurogenic inflammatory aspect of dermatoses [3]. Sensory neurons of the peripheral nervous system send many primary afferent fibers to the skin. Melanocytes in human skin are known to have certain morphological links with the nerve fibers [4]. However, quantitative analysis of the morphological relationship between nerve fibers and melanocytes in the lesions of melasma have not been reported, which we delineated in this study.

### Materials and Methods

#### Skin biopsies

All subjects were enrolled into the study from No.1 Hospital of

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China Medical University, Shenyang, China, upon completion of the informed consent form. Normal control subjects were recruited with the exclusion of systemic and/or dermatological diseases within one year. The melasma patients were enrolled based on the clinical diagnostic criteria and pathological examination. Facial skin biopsies of the normal control subjects, and lesional skin and normal-appearing skin of the opposite side of the melasma patients were collected. The study conformed to the ethical guidelines of the 1975 Declaration of Helsinki, and was approved by our Institutional Medical Ethic and Human Research Committee.

## Biopsy and specimen processing

Incisional biopsy of 0.5 cm × 0.5 cm specimen embedded immediately in OCT Compound (Sakura Finetek, USA) with liquid nitrogen, and stored at -70°C until further processing. Blocks were cryosectioned at a thickness of 12 μm, dried at the room temperature, fixed by cold acetone for 10 min.

## Double staining for melanocytes and nerve fibers

The double labeled immunofluorescence was performed as described with a slight modification [5]. Briefly, anti-neurofilament H 200Kd rabbit serum (1:100; Serotec, UK), followed by tetraethyl rhodamine-conjugated goat anti-rabbit IgG (1:100; Southern Biotechnology, USA) was used to stain the cutaneous nerves. Anti-melanosome mouse IgG monoclonal antibody (1:5; NKI/beteb, Sanbio B.V., Netherlands), followed by fluorescein isothiocyanate labeled goat anti-mouse IgG (1:100; Serotec, UK) was used to stain melanocytes. The negative controls were performed by replacing the primary antibodies with normal rabbit or mouse serum. All slides were examined under a confocal laser scanning microscope (Leica, Germany). A total of 30 melanocytes were randomly examined. The percentage of green fluorescent melanocytes having contact with red fluorescent fibers was counted and calculated.

## Statistical analysis

Statistical analysis was performed using SPSS software (version 18.0, SPSS Inc., Chicago, IL, USA). Non-parametric test (Kruskal-wallis H) was performed to analyze the data. A p value < 0.05 was considered to be statistically significant.

## Results

Total of 31 facial skin biopsies from the normal control subjects were obtained from the clinic of plastic surgery. Biopsies of 36 lesional skin and 31 normal-appearing skin of the opposite side of the melasma patients were collected from the outpatient clinic of dermatology. All the subjects were female. The average age of melasma patients and normal subjects were 28-45 (34.2 ± 9.6) years and 25-50 (33.3 ± 10.4) years, respectively.

About 42.3 ± 5.6% of melanocytes in epidermis of normal control subjects had the intimate contacts with nerve fibers (**Figure 1**). The morphological contacts of melanocytes with the intraepidermal nerve fibers were significantly increased not only in the melasma lesions (74.5 ± 6.7%), but also in the normal-appearing skin of the opposite sites of melasma lesions (69.4 ± 7.3%) (**Figure 2**). Significant difference was seen between normal control skin and melasma lesion (p < 0.05) or between normal control skin and normal-appearing skin of the melasma patients (p < 0.05). The difference in the number of neural-melanocytic contacts between the lesional and normal-appearing skin of the melasma patients was also statistically significant (p < 0.05).

## Discussion

Human melanocytes are dendritic cells of neural crest cell origin with monophenolase activity and distinctive melanosomes. The skin melanocytes have a close contact with the surrounding keratinocytes via their dendritic processes. This physical connection

enables melanocytes to transfer melanin into keratinocytes to define the skin color and protect against damage from ultraviolet radiation. Several environmental and physiological factors such as ultraviolet radiation and α-melanocyte stimulating hormone are known to regulate the quantity and quality of melanin in the skin of various species [6]. A balance in these regulatory factors is essential for the normal melanocytic development and function. Any derangement that causes a deficiency or over stimulation has an abnormal effect on melanocytic function. It is generally accepted that skin is a target organ of the stress reaction [7-9]. The function of melanocytes are also affected by physical and emotional stresses [10,11].

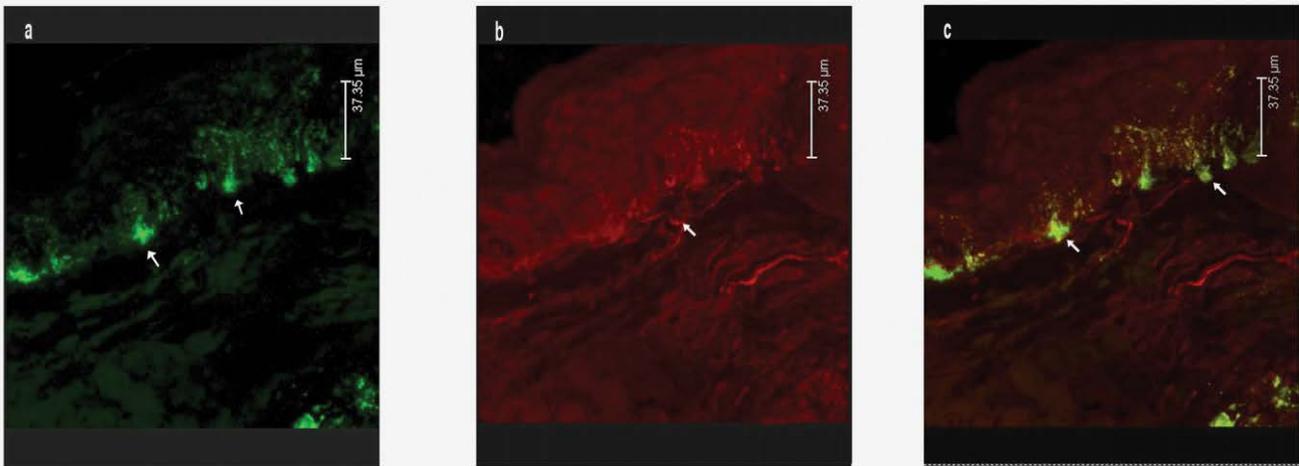
Melasma is an acquired circumscribed facial hyperpigmentation commonly seen in Asian women. A number of anecdotal clinical observations have shown that when the melasma patients are physically exhausted or emotionally distressed, the facial skin hyperpigmentation becomes more obvious [12-14]. The psychoneural impact on melasma was described as early in 1742 in an ancient textbook of Chinese traditional medicine entitled "Golden Mirror for Original Medicine-Surgical Secrets". It says that "Dark dyschromia comes to face due to the blood deficiency and malnutrition resulting from anxiety and depression". Anecdotal reports related the emotional changes, depression, athymia and neurasthenia to the facial discoloration. It has demonstrated close interactions between the nervous and immune systems that regulate peripheral inflammation and link psychosocial stress with chronic somatic disease [12,13].

Although the biological significance of the intimate contacts between the nerve fibers and melanocytes remains speculative, our finding provides evidence that neural system may directly regulate certain biological functions of melanocytes. Moreover, how the morphological contact between the nerve fibers and melanocytes contributes to the skin pigmentation remains unclear and needs further research.

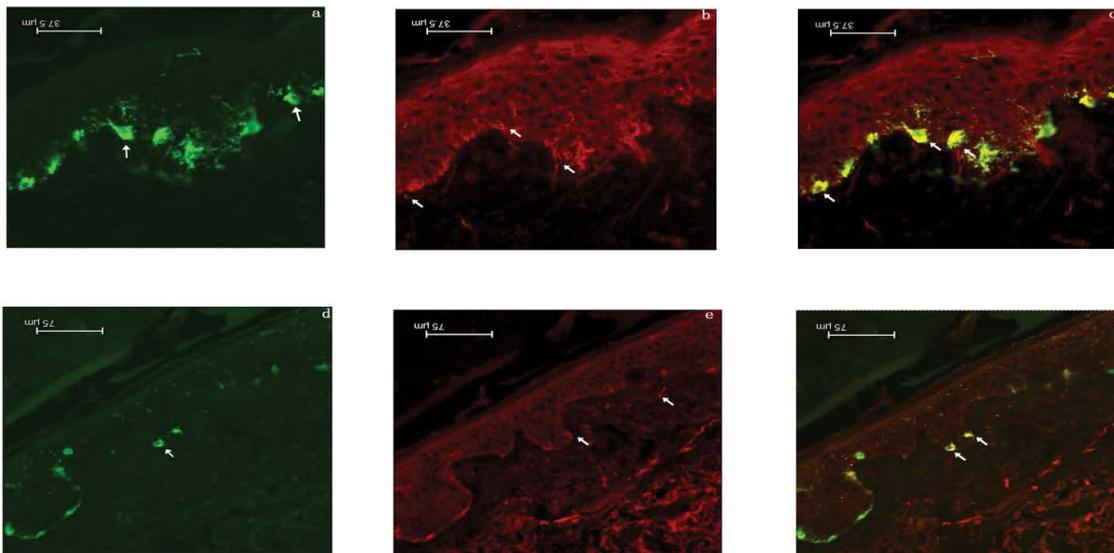
As is well known, the skin color of certain species of frogs and fish changes with surroundings, which has been found to be relevant to the excitation of sympathetic nerve and/or parasympathetic nerve. The color changes of human skin are also related to autonomic nerve. The inhibitory factors generated while sympathetic nerve is excessively active have rivalry effects on α-melanocyte stimulating hormone and reduce pigment, while parasympathetic nerve can deepen pigment. It is generally thought that onset of vitiligo is a kind of disorder of immune function, psychological effects and endocrine secretion under the stimulus of various factors [10,15].

In the skin, nerve fibers may secrete neuromediators, such as calcitonin-gene related peptide, substance P, vasoactive intestinal peptide, and α-melanocyte stimulating hormone. Neuromediators are also found to be expressed in the skin to modulate the functions of skin cells [16,17]. The nerve system, endocrine system, and cutaneous immune system are not independent respectively but closely associated and interact with each other via the same language of cytokines and neurotransmitters.

Crosstalk between the skin and its microbial environment is highlighted and they cooperate to maintain the homeostasis of the skin via the so-called neuro-immuno-cutaneous network. These neuromediators cooperate to maintain the homeostasis of the skin, via the so-called neuro-immuno-cutaneous network [18]. A number of common dermatologic diseases have some form of psychomediated pathogenesis that partially explains the



**Figure 1** Confocal laser scanning microscopy of melanocytes and nerve fibers in normal human facial skin a, Melanocytes (in bright green) are present in the epidermis.



**Figure 2** The representative photograph of the confocal laser scanning microscopy of melanocytes and never fibers in lesional and normal-appearing skin of a melasma.

development of skin lesions. There is a link between emotional stressors (acute or chronic), psychiatric diseases, and dermatoses. Our study provides a direct evidence for the morphological links of this network.

At present the creature-psychology-community medicine formula has gradually become the main medical trend, and psychological elements have been receiving increasing attention. Nerve system and skin are closely linked anatomically and physiologically. Psychological elements may affect the flare-up and severity of skin diseases via the neuro-immuno-cutaneous-endocrine network. Therefore, the further investigation of the neuro-immuno-cutaneous-endocrine network will provide new insights into etiologies of and the diagnosis and therapeutic modalities for dermatological diseases. Clarification of the role of this alternative stress axis may enable the design of novel therapeutic strategies [9].

In summary, our study provides morphological evidence of the contacts between intraepidermal nerve fibers and melanocytes

in melasma. The neural-melanocytic contacts in the lesional skin of the melasma patients were statistically more than those in the normal-appearing skin of the opposite side of the same individuals and those in normal controls. Our finding also provides evidence that the skin color is linked to the central nervous system via the peripheral nerve fibers in the skin, corroborating the proverbs “The skin is the mirror which reflects the state of the mind” and “The skin is the window of the mind”.

### Conflict of Interest

The authors state no conflict of interest.

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