

PIGMENTED NEVUS DIVIDED BETWEEN RECIPIENT AND DONOR SITES BY INTERMEDIATE SKIN GRAFTING PROCEDURE: A STUDY WITH FLUORESCENCE METHOD (FALCK & HILLARP)

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Abstract. Pigment freckles seen in donor and recipient sites at intermediate skin grafting were studied, chiefly by means of Falck & Hillarp's fluorescence method, with the following results. (1) An important role is played by the regenerative eccrine sweat ducts in the mechanism of recurrence of so-called "lentigines" after incomplete removal. (2) Junctional activity, not associated with the regeneration of epidermal appendages, can recommence in the epidermis of intradermal nevi. (3) B-type nevus cells once again actively produce melanin. (4) Specific fluorescence was observed corresponding to the melanin granules distributed in the manner of "nuclear caps" in keratinocytes around junctional activities. This latter finding may indicate that melanosomes which still contain DOPA are transferred from melanin-producing cells to keratinocytes.

Key words: Pigmented nevus; Fluorescence method; Skin grafting; Sweat duct, eccrine; Melanin-producing cells

It has long been known that pigment freckles which recur after incomplete removal of pigmented nevi show junctional nevi histopathologically (2, 10, 11). However, the origin of junctional nevus cells seen in regenerative epidermis has remained unresolved. Recently we (3) established that regenerative eccrine sweat ducts play an important role in the mechanism of the recurrence of spotted grouped pigmented nevi after use of the skin abrasion technique. Furthermore, we pointed out the possibility that a leading role may be played by epidermal appendages—especially by eccrine sweat ducts—in the mechanism of recurrence after incomplete removal not only of spotted grouped pigmented nevi but also of the other clinical types of nevi. In order to clarify this problem, so-called "lentigines" divided as to origin from recipient or donor site by an intermediate skin grafting procedure were studied, mainly by means of Falck & Hillarp's fluorescence method.

MATERIALS AND METHODS

A 24-year-old man had been treated with skin grafting at our hospital for one year under the diagnosis of extensive deep dermal burns on the chest, abdomen and limbs. Some time later, we noticed blackish pigment freckles in the grafted abdominal skin and in the donor site on the back (Fig. 1). The clinical course for the formation of these pigment freckles is recalled to be that shown in Fig. 2. Eight months earlier, intermediate split thickness skin containing so-called lentigines was taken from the back and grafted onto the granulation tissue of the abdomen. The pigment freckles persisted in the grafted skin and, furthermore, an insidious appearance of blackish pigment freckles was noted in the donor site corresponding to the dots where the lentigines had previously existed. Examination revealed many ricegrain-sized, dome-shaped, blackish nodules scattered on the upper back up to the shoulders. The blackish nodules, which are histopathologically nevus cell nevi, are generally called lentigo or lentigines in Japan. Three specimens were taken for biopsy: 1) from the pigment freckles of the recipient site, 2) from the freckles of the donor site, and 3) from three pigmentary nodules of the normal skin of the back. They

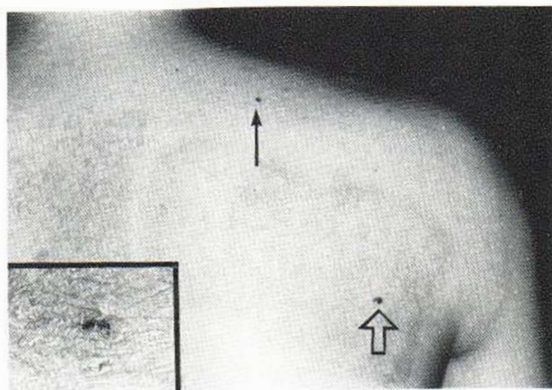


Fig. 1. Clinical picture of pigment freckles on both donor and recipient sites and of so-called lentigines on normal skin. Lentigo on normal skin surface (↑). Regenerative pigment freckle on donor site (↑). Inset indicates pigment freckle on recipient site.

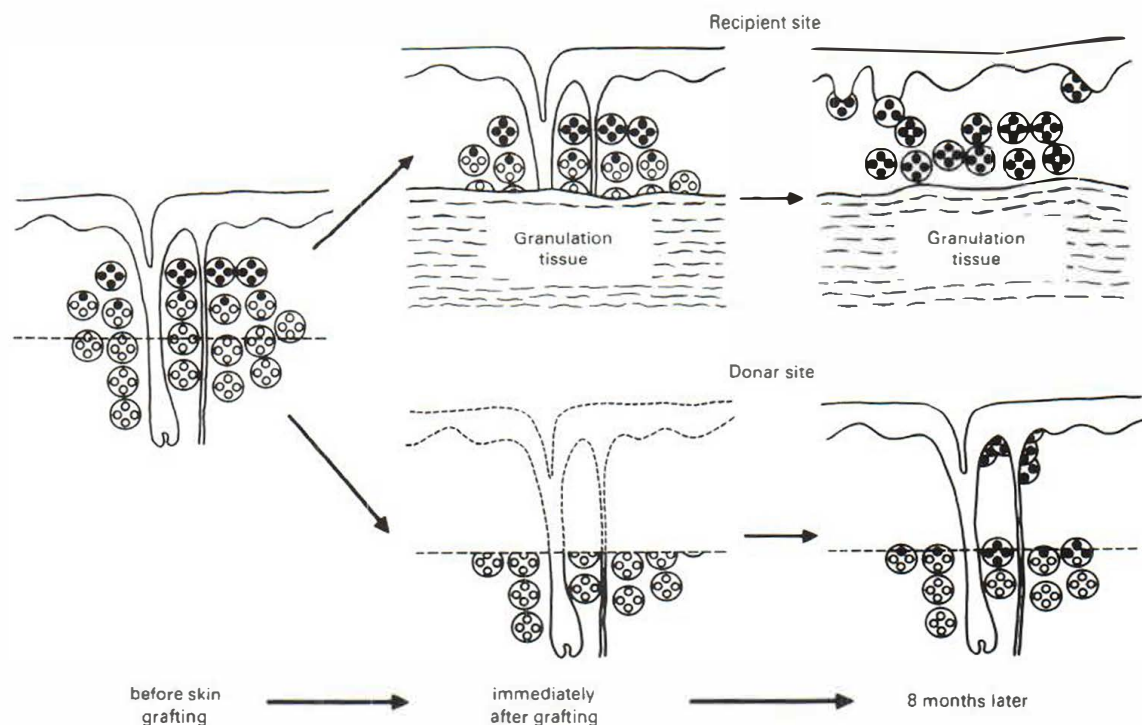


Fig. 2. Scheme of process of pigment freckle formation seen on donor and recipient sites.

were sectioned into halves. One half-specimen was fixed in 10% formalin solution and stained with hematoxylin-eosin, Masson-silver and Weigert-van Gieson stain. The other half-specimens were frozen in dry ice-cooled isopentanol immediately after excision, freeze-dried for 7 days, and treated with formaldehyde vapour at 80°C for 1 hour. After being embedded in paraffin, these specimens were sectioned at a thickness of 10 μ m and mounted for fluorescence microscopy (3, 7). The specific fluorescence observed in melanin-producing cells is thought to be based on DOPA or DOPA-containing compounds. Recent biochemical studies on melanoma have shown the presence of 5-S-cysteinylidopa (1, 9, 12).

RESULTS

Light microscopic and fluorescence microscopic findings

1. Pigmentary nodules (so-called lentigines) on normal skin surface

All the pigmentary nodules were revealed to be intradermal nevi. Hair follicles and eccrine sweat ducts running along them were situated at times in the central portion of the lesion. Fluorescence microscopy showed that melanin-producing cells,

which have indistinct dendritic processes and emit weak green specific fluorescence, were distributed more densely in the basal layer of epidermis than in normal skin, but no junctional activity was found. No melanin-producing cells were found in eccrine sweat duct walls. Yellowish-green to yellow specific fluorescence was observed in the cytoplasm of A-type nevus cells situated in the upper dermis (Fig. 3). Green specific fluorescence emitted by B-type nevus cells in the mid-dermis was found to wane or to disappear, depending on the depth of location.

2. Regenerative pigment freckle in donor site

A study with H. E., Masson-silver and Weigert-van Gieson staining showed that the papillary to midreticular layer is replaced by scar tissue and the lower dermis is normal in appearance. In the regenerative epidermis, melanin is found to be increased in the whole layers and numerous clear cells are observed in the basal layer surrounding the epidermal appendages, especially the eccrine sweat ducts (Fig. 4a). At fluorescence microscopy, the



Fig. 3. Findings of pigmentary nodules on normal skin surface. A-type nevus cells in the upper dermis emit strong specific fluorescence, whereas the specific fluorescence emitted by B-type nevus cells in the mid-dermis is weak and has a tendency to wane, depending on the depth of location. Fluorescence method, $\times 35$.

scar tissue looks dark, as it does not contain elastic fibers and the regenerative collagen fibers lack in autofluorescence, whereas the underlying connective tissue emits autofluorescence as seen in normal skin. In the basal layer of regenerative epidermis, specific fluorescence ranging in color from green to yellow is emitted by many melanin-producing cells, which are larger than melanocytes of normal skin. Melanin-producing cells are prominently distributed in the basal layer surrounding an eccrine sweat duct, and several of these cells aggregate to form junctional activity (Fig. 4b). These dendritic, melanin-producing cells are observed in eccrine sweat duct walls of the upper dermis. In the scar tissue and around the epidermal appendages of the lower dermis, fluorescing nevus cells are found in small aggregations.

3. Pigment freckle in recipient site

In the epidermis, rete ridges are elongated irregularly, and junctional activities are formed in the rete ridges at the peripheral portion of the lesion (Fig. 5a). Nevus cell nests containing abundant melanin are present in the upper to middle reticular layer, whereas the lower reticular layer is scar tissue and lacks epidermal appendages. The findings at fluorescence microscopy are different from those of pigmentary nodules on normal skin (Fig. 5b). Both A- and B-type nevus cells located in the upper to middle reticular layer emit yellowish-green to yellow specific fluorescence. In the basal cell layer covering these nevus cell nests, dendritic melanin-producing cells are observed which emit yellowish-green to yellow specific fluorescence, and junctional activities are formed to some extent. Formation of junctional activities is clearly seen in the rete ridges of the peripheral portion of the lesion. Yellowish-green-yellow fluorescence is observed in "nuclear caps" within keratinocytes in the basal layer to the upper squamous layer of the epidermis surrounding the junctional activities. As these findings were not obtained in those specimens not treated with formaldehyde gas, the fluorescence within the keratinocytes is thought to be specific. The presence of specific fluorescence within the keratinocytes was also ascertained in the specimen taken from the pigment freckle of the donor site. Masson-silver stain revealed that the specific fluorescence emitted in these specimens corresponds to the melanin within the keratinocytes.

DISCUSSION

Pigmented nevi are histopathologically rather uniform and are classified into junctional nevi, compound nevi and intradermal nevi, but their clinical appearances are multiform. Some of the regenerative pigment freckles, after incomplete removal of pigmented nevi, are thought to derive from the formation of junction nevi histopathologically (2, 10, 11). This transformation is understood to be the nevus cell's reliving of the early part of its natural history (10). But the mode of recurrence varies and depends on the clinical type of pigmented nevi. It may therefore be an aid to know the pathogenesis of these nevi in order to pursue the mechanism of recurrence after incomplete removal of pigmented nevi, according to their clinical type.

Recently we (3) made a chronological study using



Fig. 4. Findings of regenerative pigment freckle on donor site. Melanin-producing cells are densely distributed in the basal layer of epidermis surrounding eccrine sweat ducts. (a) Masson-silver, $\times 75$; (b) Fluorescence method, $\times 75$.

the fluorescence method on the regenerative pigment freckles after skin abrasion of spotted, grouped, pigmented nevi and summarized the mechanism of recurrence as follows. Almost simultaneous with the epidermal regeneration, dendritic melanin-producing cells derived from epi-

dermal appendages, especially eccrine sweat ducts, appear in the basal and prickle cell layers of regenerative epidermis. These cells then, form junctional activities in the basal layer of the epidermis and epidermal appendages, particularly the eccrine sweat duct walls, and finally, they drop off along

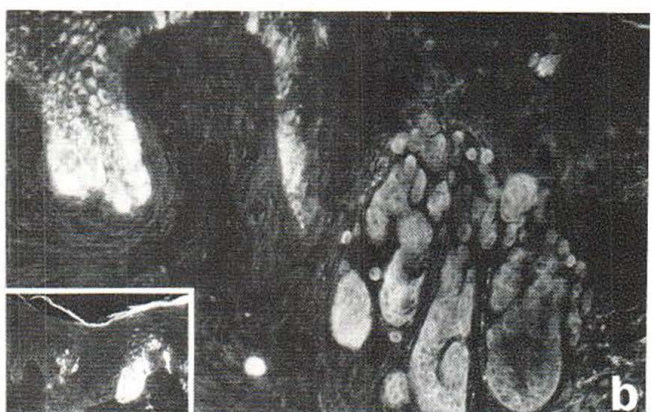


Fig. 5. Findings of pigment freckle on recipient site. A- and B-type nevus cells in the dermis emit specific fluorescence and contain abundant melanin. Junctional activities are seen in the rete ridges of the peripheral portion of the lesion. Specific fluorescence is observed in "nuclear caps" within keratinocytes which surround junctional activities. Inset in (b) shows junctional activity in another specimen. (a) H. E., $\times 71$; (b) Fluorescence method, $\times 71$.

the epidermal appendages. It is of interest to study whether or not such a mechanism applies to the recurrence after incomplete removal of clinical types of pigmented nevi other than the type "spotted grouped pigmented nevus" (8). From the results obtained in our present study, it was clear that eccrine sweat ducts play a leading role in the recurrence following incomplete removal of so-called lentigines, the smallest scale of pigmented nevi.

Intradermal nevus cells are called A-type, B-type, and C-type nevus cells according to their location. On DOPA reaction, it is known that A-type nevus cells are positive, B-type nevus cells mostly negative, and C-type nevus cells are negative (5, 6). But it is reported that B-type nevus cells also show a positive DOPA reaction after ultraviolet ray irradiation (4, 6). In specimens taken from pigment freckles at the recipient site, we also found that most of the nevus cells situated in the upper to mid-dermis emit a specific fluorescence and contain abundant melanin. These findings suggest that not only after ultraviolet ray irradiation but also after trauma B-type nevus cells are again able to produce melanin copiously.

In the specimen taken from pigment freckle of the grafted skin, formation of junctional activities was observed in the basal layer of rete ridges. The preoperative histopathological findings of this pigment freckle are supposed to be those of intradermal nevi, in view of the results obtained with so-called lentigines on normal skin. If this be true, the above-mentioned junctional activities might be thought to be formed *not* in association with the regeneration of epidermal appendages, since there are no epidermal appendages in the grafted skin or in the underlying granulation tissue. In other words, it is suggested that, though a pigmented nevus is histopathologically an intradermal nevus, junctional activity may be formed *de novo* in the overlying epidermis *not* associated with epidermal appendages, in case inducement occurs.

In the specimens taken from the pigment freckles of both donor and recipient sites, yellowish-green to yellow specific fluorescence is observed in "nuclear caps" within keratinocytes around junctional activities. Generally speaking, melanosomes transferred from melanocytes to keratinocytes lack tyrosinase activity. Rorsman et al. (9) demonstrated biochemically the presence of 5-S-cysteinyl-dopa in the fully keratinized red and black hairs of guinea pigs, and assumed that hair keratinocytes ingest

premelanosomes together with the melanosomes. The above-mentioned findings suggest the possibility that DOPA-containing melanosomes are transferred from melanin-producing cells to keratinocytes, in the regenerative pigment freckles with profuse melanin formation.

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