Progressive macular hypomelanosis (PMH) treatable but often misdiagnosed
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ULTRASTRUCTURAL FINDINGS IN PROGRESSIVE MACULAR HYPOMELANOSIS INDICATE DECREASED MELANIN PRODUCTION
ABSTRACT

Introduction: The pathogenesis of progressive macular hypomelanosis (PMH) is unknown. Recently, Westerhof et al. (2004) hypothesized that Propionibacterium acnes produces a depigmenting factor that interferes with melanogenesis in the skin, resulting in hypopigmented spots. The purpose of the study is to gain an insight into the pathogenesis of PMH.

Patients and Methods: We took a biopsy of 2-mm diameter from normal and lesional skin in eight PMH patients. Using electron microscopy, we compared melanization of melanosomes, melanosome transfer and amount of epidermal melanin in normal and lesional skin.

Results: Compared to non-lesional skin, we observed a decrease of epidermal melanin and less melanized melanosomes in lesional skin of all patients. When comparing normal and lesional skin of patients with skin type V and VI, we observed a difference in melanosome size and maturation and a switch of transferred melanosomes from single stage IV transferred melanosomes to aggregated stage I, II and III transferred melanosomes, as seen in healthy skin of skin type I to IV.

Conclusion: Hypopigmentation in PMH seems to be the result of an altered melanogenesis based on a decrease in melanin formation and a change in the distribution of melanosomes. In lesional skin of PMH patients with skin type V and VI less melanized, aggregated melanosomes in stead of single, mature melanosomes are transferred from melanocytes to keratinocytes. This results in a decrease of epidermal melanin. Further investigations are needed to determine the precise role of Propionibacterium acnes in this alteration of melanogenesis.
INTRODUCTION

Progressive macular hypomelanosis (PMH) is a skin disorder occurring in young adults. It is characterized by symmetrically distributed ill-defined nummular hypopigmented macules mainly on the trunk, sometimes extending to the neck and face, the buttocks and the upper half of the extremities (Figure 1). PMH is often misdiagnosed as pityriasis versicolor (PV) and pityriasis alba (PA).

The pathogenesis of PMH is unknown. Guillet et al. (1988) conducted ultrastructural studies of lesional and non-lesional skin in two PMH patients. They showed the presence of stage IV single melanosomes (the types of melanosomes normally present in black skin) in the healthy looking skin, and stage I–III aggregated melanosomes (the types of melanosomes normally present in white skin) in the hypopigmented spots. Because they observed PMH in racially mixed (Negroid-Caucasoid) patients, they concluded that it is caused by a ‘melting’ of genes of white and black parents. In 2006, Kumarasinghe et al. (2006) published an article which describes the clinico-pathological findings in PMH patients.

Recently, Westerhof et al. (2004) proposed that Propionibacterium acnes, by producing a depigmenting factor, might be the causative organism. This view is based on the fact that during inspection of the skin of PMH patients in a dark room with Wood’s lamp, a red follicular fluorescence was observed restricted to the lesional skin. P. acnes was cultured from biopsies taken from follicular lesional skin in 7 of 8 patients, whereas from healthy skin, no bacteria were cultured. This
hypothesis is supported by our finding that treatment of PMH patients with topical antibacterial agents leads to a significantly better improvement than treatment with anti-inflammatory agents (Relyveld et al. 2006).

From a theoretical point of view, a variety of genetic and environmental factors can be envisaged to lead to a decrease in epidermal melanin:

(1) Disorders in the melanization of melanosomes due to a defect in the structure of tyrosinase or an inhibition of tyrosinase: a decrease in the melanin synthesis leads to disorders in maturation, size and distribution of melanosomes, followed by hypopigmentation.

(2) Disorders of maturation of melanosomes: the formation of melanin is related to the structural maturing from premelanosomes to melanosomes. A decreased maturation of melanosomes leads to hypopigmentation.

(3) Disorders in melanosome transfer: microfibrils inside melanocyte dendrites and certain receptors are crucial for the transfer of melanosomes from melanocytes to keratinocytes. A decrease in the velocity of melanosome transfer from the melanocyte to the keratinocyte leads to hypopigmentation. Melanosome transfer may be disturbed by increased keratinocyte turnover and by all processes disturbing the interactions/contact between melanocytes and keratinocytes.

(4) Disorders in degradation of melanosomes: increased rate of melanosome destruction might lead to hypopigmentation

The purpose of our study is to gain an insight into the mechanisms involved in the pigmentation disturbance of PMH. We conducted an electron microscopic study to find out which of the four mechanisms underlies the hypopigmentation in lesional skin of PMH by comparing lesional and non-lesional skin of these patients.

**PATIENTS AND METHODS**

**Patients and settings**

Patients with PMH were seen at the Dermatology Department of the Saint Franciscus Hospital in Rotterdam, the Netherlands and at the Institute for Pigment Disorders in Amsterdam, the Netherlands. The diagnosis of PMH was based on a combination of the clinical signs, the presence of red fluorescence coinciding with the pilosebaceous ducts of lesional skin when examined with a Wood’s lamp in a dark room, negative KOH tests and the absence of spores and mycelia on histological examination.
Diagnostic biopsies
Two biopsies of 2 mm for routine examination and electron microscopy were taken under local anaesthesia with xylocaine 2%, one from the affected skin and the other from the adjacent normal-appearing skin from the back of the patients.

Light microscopy
A part of each biopsy was fixed in 10% formaldehyde solution and embedded in paraffin. Paraffin sections were histologically stained with haematoxylin and eosin, and the difference in the amount of melanin in the lesional and normal skin was assessed.

Electron microscopy
For electron microscopy, part of the biopsies was fixed in Karnovsky’s fixative, post-fixed in 1% osmium tetroxide (OsO₄) and further processed according to standard procedures.

Assessment of the number of melanosomes, the size of the melanosomes and melanosome transfer was done by comparing electron microscopic pictures of lesional and non-lesional skin. In order to confirm our clinical findings and more important, to improve our understanding of the pigment dynamics in PMH, the difference in total epidermal melanin between normal and lesional skin was estimated in one visual field according to the following assessment scale: > 50%, more than 50% less melanin in lesional skin than in non-lesional skin; 0% to 50%, between 0% and 50% less melanin in lesional skin than in non-lesional skin; 0, lesional and non-lesional skin have same amount of melanin. At a magnification of 4500×, three keratinocytes from lesional and from normal skin were selected. Each keratinocyte was then investigated at a magnification of ×44 000. Melanosome transfer type (single or aggregated) was determined and melanosome size was measured.

Former studies indicate a correlation between melanosome size and the distribution pattern of melanosomes within secondary lysosomes of keratinocytes. Melanosomes larger than 1 µm are singly distributed, whereas those smaller than 1 µm in long axis are aggregated to form melanosome complexes (Szabo 1969, Toda et al. 1972, Wolff et al. 1974, Yamamoto et al. 1994, Jimbow et al. 1998).

Based on these findings, we assessed the length of the long axis of the 10 largest melanosomes in each keratinocyte. Per patient the median of the length of the melanosomes in lesional and normal skin was then computed. The same calculations were also done for the diameter of the melanosomes.
The median and 25th and 75th percentile of the length and diameter of the melanosomes were described, but additional statistical tests are meaningless for a sample size of eight patients. Researchers performing the calculations were all blinded.

RESULTS

Subjects

Eight patients with PMH between 15 and 41 years (mean age, 26 years) were included. All except one were female. Baseline characteristics of the patients are presented in Table I.

<table>
<thead>
<tr>
<th>Table I Baseline characteristics of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female, no.</td>
</tr>
<tr>
<td>Male, no.</td>
</tr>
<tr>
<td>Mean age in years +/- SD</td>
</tr>
<tr>
<td>Skin type *, no.</td>
</tr>
<tr>
<td>III</td>
</tr>
<tr>
<td>IV</td>
</tr>
<tr>
<td>V</td>
</tr>
<tr>
<td>VI</td>
</tr>
</tbody>
</table>

*Skin type according to Fitzpatrick.

Light microscopy

A comparison of the pigmentation in normal and lesional skin revealed a decrease in the amount of melanin in the epidermis of the lesional skin of all patients. The dermis showed no abnormalities (Figure 2A,B).

Electron microscopy

General epidermal changes

In all patients, electron microscopic pictures of low magnification, and light microscopy showed a decrease in pigmentation of the epidermis in the lesional skin compared with the normal skin.

Changes in melanocytes

In both the lesional and the non-lesional skin of PMH patients, the melanocyte cell bodies contained melanosome precursors in all stages of development. In lesional
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skin; however, mature melanosomes were distinctly smaller and less melanized than those in non-lesional skin. The melanocytic dendrites in the lesional skin of patients with skin types V and VI contained smaller, less melanized melanosomes than those in non-lesional skin. In patients with skin types III and IV, on the other hand, the melanocytic dendrites in normal as well as lesional skin showed similar melanosomes.

Changes in keratinocytes

Non-lesional skin Keratinocytes in patients with skin type V and VI showed numerous single, large, melanosomes that were ellipsoidal and intensely melanotic (Figure 3A). Patients with skin type III and IV showed single large stage IV melanosomes as well as clustered, immature stage II and III, aggregated melanosomes.

Lesional skin Keratinocytes in patients with skin type V and VI showed smaller and less dense melanosomes (stage II and III melanosomes) that were clustered in membrane bound groups (Figure 3B).

The median length as well as the median diameter of the melanosomes was longer in normal skin than in lesional skin of all skin types, except for the median length in skin type IV. Differences in melanosome size are presented in Table II. The keratinocytes in patients with skin type III and IV had the same aspect as described above, although the differences between normal and lesional skin were

Figure 2 (A) Light microscopy of normal skin in a PMH patient with skin type V. (B) Light microscopy of lesional skin in the same patient.
Figure 3  (A) Magnification ×44 000. A keratinocyte in non-lesional skin of a patient with skin type V, showing numerous, single, large melanosomes that are ellipsoidal and intensely melanocytic. (B) Magnification ×44 000. A keratinocyte in lesional skin of the same patient, showing smaller, less dense melanosomes that are clustered in membrane-bound groups. (C) Magnification ×6100. Bacteria present in a hair follicle of lesional skin.

Table II Electron microscopic observations in keratinocytes in PMH patients

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Skin type</th>
<th>Amount of melanin LS vs non-LS*</th>
<th>Distribution non-LS**</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>III</td>
<td>&gt;50%</td>
<td>Single, large, intensely melanotic, and few smaller, less melanotic in membrane bound groups</td>
</tr>
<tr>
<td>2</td>
<td>III</td>
<td>0-50%</td>
<td>Single, large, intensely melanotic as well as small, less melanotic in membrane bound groups</td>
</tr>
<tr>
<td>3</td>
<td>IV</td>
<td>0</td>
<td>Single, large, intensely melanotic</td>
</tr>
<tr>
<td>4</td>
<td>V</td>
<td>&gt;50%</td>
<td>Single, large, intensely melanotic</td>
</tr>
<tr>
<td>5</td>
<td>V</td>
<td>0-50%</td>
<td>Single, large, intensely melanotic</td>
</tr>
<tr>
<td>6</td>
<td>V</td>
<td>0-50%</td>
<td>Single, large, intensely melanotic</td>
</tr>
<tr>
<td>7</td>
<td>VI</td>
<td>&gt;50%</td>
<td>Single, large, intensely melanotic</td>
</tr>
<tr>
<td>8</td>
<td>VI</td>
<td>&gt;50%</td>
<td>Single, large, intensely melanotic</td>
</tr>
</tbody>
</table>

LS: lesional skin; non-LS: non-lesional skin. *Amount of melanin in keratinocyte of LS compared with non-LS. 50%, more than 50% less melanin than in non-lesional skin; 0% to 50%, between 0% and 50% less melanin than in non-lesional skin; 0, lesional and non-lesional skin have same amount of melanin. (Percentages are based on estimations). **Distribution of melanosomes in keratinocytes of non-LS. ***Distribution of melanosomes in keratinocytes of LS. (P25–P75): 25th to 75th percentile.
less obvious than in patients with skin type IV to VI. In one patient with skin type V, bacteria could be identified in the sebaceous duct next to the hair follicle of the lesional skin (Figure 3C). These bacteria were not observed in the normal skin.

**DISCUSSION**

We showed in PMH a deficiency in the melanization of melanosomes, leading to changes in the maturation, size, number and distribution of melanosomes in lesional skin of patients with skin type V and VI. This seems to be the underlying microanatomy of hypopigmentation. In skin type III and IV, the differences between normal and lesional skin were less obvious because in those skin types, melanosomes are already smaller, less melanized and predominantly packed in aggregated groups. In one patient with skin type III, lesional skin showed 50% less melanin than normal skin. This was unexpected, because we would expect a smaller difference in total amount of melanin in this skin type (Table II). This may be a coincidence related to the effect of tanning, because the study was conducted during summertime, but it can also be explained by the fact that in EM studies only a limited part of the 2-mm

<table>
<thead>
<tr>
<th>Distribution LS***</th>
<th>Median length non-LS in µm (P25-P75)</th>
<th>Median length LS in µm (P25-P75)</th>
<th>Median diameter non-LS in µm (P25-P75)</th>
<th>Median diameter LS in µm (P25-P75)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small, less melanotic, in membrane bound groups</td>
<td>1.2 (1.0-1.6)</td>
<td>0.7 (0.7-1.1)</td>
<td>0.4 (0.4-0.4)</td>
<td>0.3 (0.3-0.3)</td>
</tr>
<tr>
<td>Small, less melanotic, in membrane bound groups</td>
<td>1.4 (1.1-1.4)</td>
<td>1.2 (1.0-1.5)</td>
<td>0.8 (0.6-0.8)</td>
<td>0.7 (0.7-0.7)</td>
</tr>
<tr>
<td>Small, less melanotic, in membrane bound groups</td>
<td>1.1 (1.0-1.2)</td>
<td>1.0 (0.9-1.2)</td>
<td>0.8 (0.7-0.8)</td>
<td>0.6 (0.4-0.6)</td>
</tr>
<tr>
<td>Single smaller, less melanotic, as well as in membrane bound groups</td>
<td>1.5 (1.5-1.6)</td>
<td>1.2 (1.0-1.4)</td>
<td>0.7 (0.6-0.7)</td>
<td>0.4 (0.4-0.4)</td>
</tr>
<tr>
<td>Small, less melanotic, in membrane bound groups</td>
<td>1.5 (1.3-2.0)</td>
<td>1.0 (0.9-1.1)</td>
<td>0.7 (0.6-0.7)</td>
<td>0.5 (0.5-0.5)</td>
</tr>
<tr>
<td>Small, less melanotic, in membrane bound groups</td>
<td>1.6 (1.4-1.9)</td>
<td>1.1 (0.9-1.3)</td>
<td>0.7 (0.6-0.7)</td>
<td>0.5 (0.4-0.5)</td>
</tr>
<tr>
<td>Single smaller, less melanotic, as well as in membrane bound groups</td>
<td>1.9 (1.8-1.9)</td>
<td>1.7 (1.5-1.9)</td>
<td>1.0 (0.8-1.0)</td>
<td>1.1 (0.8-1.1)</td>
</tr>
<tr>
<td>Single smaller, less melanotic, as well as in membrane bound groups</td>
<td>1.8 (1.8-2.2)</td>
<td>1.4 (1.3-1.5)</td>
<td>0.8 (0.7-0.8)</td>
<td>0.7 (0.5-0.7)</td>
</tr>
</tbody>
</table>

LS: lesional skin; non-LS: non-lesional skin. *Amount of melanin in keratinocyte of LS compared with non-LS. 50%, more than 50% less melanin than in non-lesional skin; 0% to 50%, between 0% and 50% less melanin than in non-lesional skin; 0, lesional and non-lesional skin have same amount of melanin. (Percentages are based on estimations). **Distribution of melanosomes in keratinocytes of non-LS. ***Distribution of melanosomes in keratinocytes of LS. (P25–P75): 25th to 75th percentile.
biopsy specimen is investigated and therefore may not be representative for the whole biopsy specimen.

There were no structural defects (tyrosinase functional defects can not be demonstrated by EM) nor were there disturbances in the melanosomal transfer because the tips of the dendrites of the melanocytes did not show any accumulation of melanosomes and keratinocytes were not devoid of melanosomes. Furthermore, there were no defects in melanosomal degradation because there were no signs of disintegrated melanosomes in the lysosomal compartments.

We conclude that a decrease in melanin synthesis, which leads to an altered distribution of melanosomes must be the explanation for hypopigmentations in PMH. This leaves us with the question in which way P. acnes can play a role in this alteration.

Possible mechanisms behind the altered distribution are 2-fold:

1) It is known that melanosomal structure correlates with the type of melanin within (Szabo 1969, Toda et al. 1972, Wolff et al. 1974, Yamamoto et al. 1994, Jimbow et al. 1998). Jimbow et al. (1998) described two types of melanosomes (large and single vs. small and aggregated). According to them, the morphology of the pigment granules, responsible for the size of the melanosomes, depends on the type of melanin formed (i.e. eumelanin or pheomelanin). In skin type I only, pheomelanin is formed, whereas in skin type VI, pheomelanin and eumelanin are formed, but the latter is present in much higher concentrations.

Studies by Del Marmol et al. (1996) and Smit et al. (1997) showed that inhibition of tyrosinase while promoting cysteine, leads to a switch from eumelanogenesis to pheomelanogenesis resulting in the formation of smaller, aggregated melanosomes. Inhibition of tyrosinase by a hypothetical factor produced by P. acnes might have the same effect. Smit et al. (1997) showed that a high concentration of l-tyrosine was always connected with increased pigmentation. In combination with a low l-cysteine content, there was an increase in tyrosinase activity and the highest melanin content. At high concentrations of both l-tyrosine and l-cysteine, the melanocytes showed reduced tyrosinase activity and they produced notably more pheomelanin. Strongly increased concentrations of pheomelanin were maintained in high l-tyrosine medium compared with those grown with low l-tyrosine. This was especially true for the combination with low l-cysteine showing that the l-tyrosine content of the medium strongly influences not only the eumelanin but also the pheomelanin production. They concluded that variations in the concentrations of l-tyrosine and l-cysteine could be used to regulate the melanogenetic phenotype under in vitro conditions.
(2) Wolff et al. (1972) showed that an important criterion for the way of melanosome distribution is the actual size of the melanosomes itself. Studies on the phagocytosis of latex beads by epidermal keratinocytes of guinea pigs showed that the mode of uptake of these melanosome like particles is size dependent. Large latex beads were incorporated singly into cells, whereas small particles were taken up in groups. As this model puts an emphasis on the active role of the keratinocyte, rather than the melanocyte, in this uptake of melanosomes, the authors suggest that the size of the individual melanosomes seemed to be the decisive factor that determines the distribution of pigment organelles.

Minwalla et al. (2001), however, showed that in a co-culture of keratinocytes and melanocytes, melanosome size does not correlate with the ultimate pattern of distribution within the keratinocyte. Studies showed that recipient melanosomes, regardless of skin type, are predominantly distributed individually by keratinocytes from dark skin and in membrane-bound clusters by those from light skin. Melanosome size was not related to whether the melanosomes were distributed individually or clustered. The authors suggested that regulatory factors within the keratinocyte determine recipient melanosome distribution patterns.

A hypothetical factor produced by P. acnes might influence such regulatory factors within the keratinocyte that determine recipient melanosome distribution patterns as described by Minwalla et al. (2001).

Further research on the L-tyrosine and L-cysteine content of the skin and the role of regulatory factors in melanosome distribution in PMH is apparently necessary. With this study, the findings of Guillet et al. (1988) who studied only two patients were confirmed; in addition, the dynamics of PMH on the ultrastructural level were further elucidated. We conducted a more specified and detailed study with a larger patient population and a greater diversity in skin types, also performing (semi)quantitative measurements of the melanosome size. Like Kumarasinghe et al. (2006) who also observed PMH in Chinese, Mongoloid and Indian type people we disagree with Guillet et al.’s conclusion that the hypopigmentations are caused by a ‘melting’ of genes of white and black parents, because we also diagnosed PMH in patients of other ethnicities and with a skin colour ranging from skin type III to VI. The results presented facilitate a sharper delineation of PMH from other hypopigmentation disorders like PV and PA. The keynote findings in PV are the presence of yeast cells and hyphae, degenerative altered melanocytes (Galadari et al. 1992, Breathnach et al. 1975) and partial blocking of the transfer of melanosomes (Charles et al. 1973), all these phenomena lacking in PMH. The keynote findings
in PA are characteristics of dermatitis, also lacking in PMH (Urano-Suehisa et al. 1985). Electron microscopic studies in PA are rare, although Zaynoun et al. (1983) conducted electron microscopic studies in what they called ‘extensive pityriasis alba’ (EPA). However, in our view, their study is not valid for comparison, because we believe EPA to be identical with PMH (Relyveld et al. 2006).

The histological and electron microscopic findings presented assist in further defining the unique clinical picture of PMH. We believe further investigations are necessary to further unravel the pathogenesis of PMH and to make a connection between our present ultrastructural findings and our hypothesis of a microbial factor being the cause of the hypopigmentations.