PREFACE

Pigmentation is a complex and tightly regulated process shaped by thousands of years of evolution of the human kind. The color of the skin results from a subtle blend of pigments with variations linked to hemoglobin derivatives, quantitative and qualitative changes of melanin, thickness of the epidermis and abnormal presence of endogenous or exogenous pigments. Dyschromia is therefore the only or the main clinical sign of many dermatoses. Beyond usually well-known pigmentary disorders such as vitiligo or melasma, many other conditions present with pigmentary changes of the skin, hairs, nails, or mucous membranes. These pigmentary changes can be the manifestation of genetic defects and bring critical information for their diagnosis. They can be associated with systemic diseases or be limited to a disorders restricted to the skin. They may result from inflammatory, metabolic, infectious, tumor, toxic or iatrogenic causes. Being a window to genetic defects or systemic diseases, or limited only to a skin problem, these pigmentary changes have a frequent impact on the quality of life of affected individuals. Some of these pigmentary disorders are rare but many others, although often unrecognized, are quite frequent and must be known by physicians in order to provide adapted care.

Beyond the necessity to recognize these pigmentary disorders, their vast numbers and the diversity of their presentation can be an obstacle for making a proper diagnosis, even in trained dermatologists. Only a small number of pigmentary diseases are usually described in books or atlases for general dermatology. Yet, among all dermatological diseases, pigmentary disorders are probably the most appropriate to be fully described in a color atlas. Here, we have tried to gather pictures from most pigmentary disorders, from the most common ones to the rarest, emphasizing all their clinical presentations to help any physicians with recognition. After an introduction to the pigmentary system that will help our readers to understand the mechanisms involved in pigmentary changes, we propose a very practical approach to making proper diagnoses. The actual tools that can be used for evaluating pigmentary disorders are also described. An almost exhaustive description of pigmentary disorders is then proposed. Using a diagnostic approach, they are classified into acquired and genetic hyper- and hypomelanosis, drug-induced discoloration, non-melanic pigmentary disorders, and discoloration affecting the nails. Along with a rich color illustration for each disease, we synthesize the main information on epidemiology, genetics (if appropriate), pathophysiology, clinical dermatological presentation, extra-cutaneous signs, histopathology, differential diagnoses and treatment options. Key references are also provided for readers who would like to obtain further information on specific disorders.

We would like to thank all the contributors who shared their knowledge in the pigmentary field and provided synthetic descriptions of disorders. We would also like to thank our colleagues from around the world who kindly provided pictures of very rare conditions, allowing us to compile this unique collection of illustrations of common but also very rare pigmentary disorders.

We designed this atlas to be useful for students and trained physicians, not only dermatologists but also for general practitioners, geneticists, pediatricians, and everyone with a special interest in some aspect of pigmentation. We hope that this work will be helpful for them in this exciting field of pigmentation.

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T. Passeron and J.-P. Ortonne, *Atlas of Pigmentary Disorders*, DOI 10.1007/978-3-319-10897-1_1

This publication has been made possible through an educational grant from Galderma.

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FOREWORD

The skin has many special features. It is essential for life; as are just a few other organs, like the heart, lungs, and liver. But uniquely it is the most beautiful organ of the body. Everyone desires a perfect complexion whether their skin is very light, medium, or dark in color. The skin is subject to so many extraneous and intrinsic factors that can alter its color and make it lighter or darker than normal or produce hyper- or hypopigmented spots. Besides being disfiguring, the abnormalities can be important or critical clues to identifying exposures to noxious environmental agents or to worrisome internal disorders.

Although these problems affecting the skin are ubiquitous, the biology and clinical science of pigmentation are not commonly taught in many dermatology training programs and never in programs for non-dermatologist health care workers. Searching through a textbook with a soupçon of photographs showing skin discolorations is time consuming and frustrating. The author/editors of this Atlas of Pigmentary Disorders have put together literally a compilation of all the known and described pigmentary disorders, along with their clinical appearance and photographs. For each pigmentary disorder there is a concise description and information about its known genetics, pathophysiology, histology and treatment. Although these are brief, they are accompanied by several pertinent references. With this information, the clinician scholar can delve into all the other available textbooks, reference books and of course the online literature for additional information.

This atlas belongs on the desk of every dermatologist, general physician, pediatrician and all others involved in direct patient care. It will resolve so many diagnostic and therapeutic dilemmas presented by patients desiring a perfect complexion, a solution to a skin disorder or an explanation for an internal problem. It will be one of the most useful books on a health care worker's desk.

James J. Nordlund, MD

PIGMENTARY DISORDERS: PRACTICAL APPROACH

The color of the skin results from the presence of pigments in the epidermis and in the dermis. The melanins (eumelanin, dark brown, mostly produced by dark skin types, and pheomelanin, red-fair brown, mostly observed in fair skin types) are the most important pigments in human skin. However, other endogenous pigments such as hemoglobin and bilirubin also play a role in the color of the teguments.

Dyschromia can result from a darkening, a lightening, and the occurrence of an unusual skin color. Quantitative or qualitative defects in the production, or in the deposition of melanin, explain most of the pigmentary disorders. However, abnormal variations of other endogenous pigments and deposit of exogenous pigments also lead to dyschromic lesions. Those pigmentation disorders can be inherited or acquired.

HYPERMELANOSIS

An increased amount of melanin in the skin is called hypermelanosis or melanoderma. A brown hypermelanosis is caused by excessive amounts of melanin within the epidermis, whereas ceruloderma (or blue hypermelanosis) results from large amounts of melanin in the dermis. Mixed hypermelanosis, which is characterized by an excess of melanins in both epidermis and dermis, may also occur. Epidermal hypermelanosis may result from increased melanin production by a quantitatively normal melanocyte density in the epidermis (melanotic hypermelanosis), or by an increased number of epidermal melanocytes (melanocytic hypermelanosis). Dermal hypermelanosis can be due to the production of melanin by ectopic dermal melanocytes (dermal melanocytosis), or to an abnormal transfer of melanin from epidermal cells to the dermis (pigmentary incontinence). In this situation, melanin granules accumulate within melanophages, or may be free in the extracellular matrix of the dermis.

LEUKODERMA

Skin lightening or whitening (leukoderma, hypopigmentation) is most commonly the result of decreased melanin content in the skin (hypomelanosis). Epidermal hypomelanosis may be the result of at least two different pathogenic mechanisms:

- partial or total absence of epidermal melanocytes (melanocytopenic hypomelanosis);
- defect in melanin synthesis, in melanosome biogenesis, or in transport or transfer of melanosomes despite a normal number of epidermal melanocytes (melanopenic hypomelanosis).

Increases of epidermal turnover, or increased degradation of the melanin contained within keratinocytes can also induce hypomelanosis.

NON-MELANIC PIGMENTARY DISORDERS

Dyschromia may result from variation of the hemoglobin content within the skin (diffuse such as in anemia or in polycythemia, or localized such as in Bier spots).

Xanthoderma describes a yellow to orange macular discoloration of the skin. Jaundice and carotenoderma are the two main causes of xanthoderma.

Heavy metals (eg, iron, silver, gold, etc), and traumatic, medical, or esthetical tattoos are other sources of skin discoloration.

An increased thickness of the epidermis can lead to diffuse, patchy or reticulated light to dark brown hyperpigmentation.

The chronic avoidance of washing can also induce hyperpigmented and sometimes keratotic patches.

Finally, the discoloration of the skin cannot only be due to pigment abnormality within the skin, but also to an abnormal coloration of the sweat (called chromhidrosis or pseudo chromhidrosis).

DIAGNOSIS PROCESS

1. First to discriminate melanic and non-melanic pigmentary disorders
Hemoglobinopathies and vascular disorders can be easily distinguished from other pigmentary disorders as the change in color disappears with pressure.
Dirt dermatitis and sweat discoloration resolve with swabbing. However, the patches of the terra firma-forme dermatosis can be misleading as they are usually resistant to a regular wash with soap and water, and they require alcohol swabbing with substantial shearing force to be removed.
The clinical presentation of tattoos, and the yellow to orange coloration of skin induced by xanthodermas are highly suggestive of the diagnosis.
By contrast, ochronosis and heavy metal depositions can be difficult to diagnose. Careful interrogation of the patient on his exposure to drugs or substances containing heavy metals, and on the chronic use of topical agents with hydroquinone is mandatory.

2. A melanic pigmentary disorder is suspected
• Are the lesions congenital or acquired?
Be aware of the fact that although presented since birth some lesions can be noticed only after one or two summers when the child has begun to tan. Familial history of pigmentary disorders, and disposition along Blaschko's lines can help to diagnose genetic pigmentary disorders.
• What is the pattern of the dyschromic lesions?
Dyschromic lesions can be diffuse, reticulated, acral or localized. Lesions following Blaschko's lines are highly suggestive of mosaicisms. Always examine the folds, nails, hairs and mucous membranes as they provide useful clues for the diagnosis.
• Has any topical substance been applied?
Many topical agents (alone or in combination with sun exposure) can lead to dyschromic lesions. They may also have changed the clinical presentation, or have hidden the preexisting inflammation.

3. Wood's lamp examination
Any patient with a pigmentary disorder should be fully examined under both visible and UVA light (ie, Wood's lamp).

• Leukodermas
Under visible light it is sometimes difficult to distinguish between hypomelanosis and amelanosis in individuals who have very lightly pigmented skin (types I or II), and neonates. Due to the greater loss of epidermal pigmentation, the contrast under Wood's lamp examination is more pronounced in amelanosis as compared to hypomelanosis. Acquired amelanotic lesions are in most cases vitiligo. If the lesions are congenital, other diagnoses such as piebaldism or Waardenburg syndrome have to be ruled out. Hypomelanotic lesions are in most cases post-inflammatory.
This technique is also helpful in differentiating hypomelanotic macules from hemoglobin-related leukodermas; for example, nevus anemicus becomes inapparent. Wood's lamp also facilitates the diagnosis of tinea versicolor by showing a yellowish green fluorescence of the hypopigmented macules.

• Hypermelanosis
Hyperpigmented lesions should also be examined under Wood's lamp. When the contrast between lesional and non-lesional skin is increased under Wood's lamp examination, as compared to visible light, the hyperpigmentation is mostly epidermal. However, when the contrast is decreased under Wood's lamp, the hyperpigmentation is mostly in the dermis. This is most helpful as it can help to discriminate between some diagnoses, and also has a therapeutic value as dermal hyperpigmentation cannot be treated with topical depigmenting agents.

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THE PIGMENTARY SYSTEM

Skin color is the result of a subtle blend of pigments. Hemoglobin derivatives or the abnormal presence of endogenous or exogenous pigment thus change the color of skin tissue. Thickening of the epidermis can also result in color variations. However, most pigmentation of skin, hair and eyes result from quantitative and qualitative changes of the melanin pigment. Melanin is produced and secreted by specialized cells called melanocytes. Melanocytes are present in the skin and in hair follicles but are also found in some sensory organs such as the retina or inner ear and the central nervous system (leptomeninges). In the skin, these cells are located in the basal layer of the epidermis or in the inferior part of the hair follicles (Figure 1). Melanoblasts are precursors of melanocytes. During embryonic life, they migrate from the neural crest to their distal territories, then proliferate and differentiate into melanocytes. They then acquire the ability to synthesize and transport melanin in specific organelles, called melanosomes. Finally, these melanosomes are distributed to adjacent keratinocytes in order to play their physiological role.

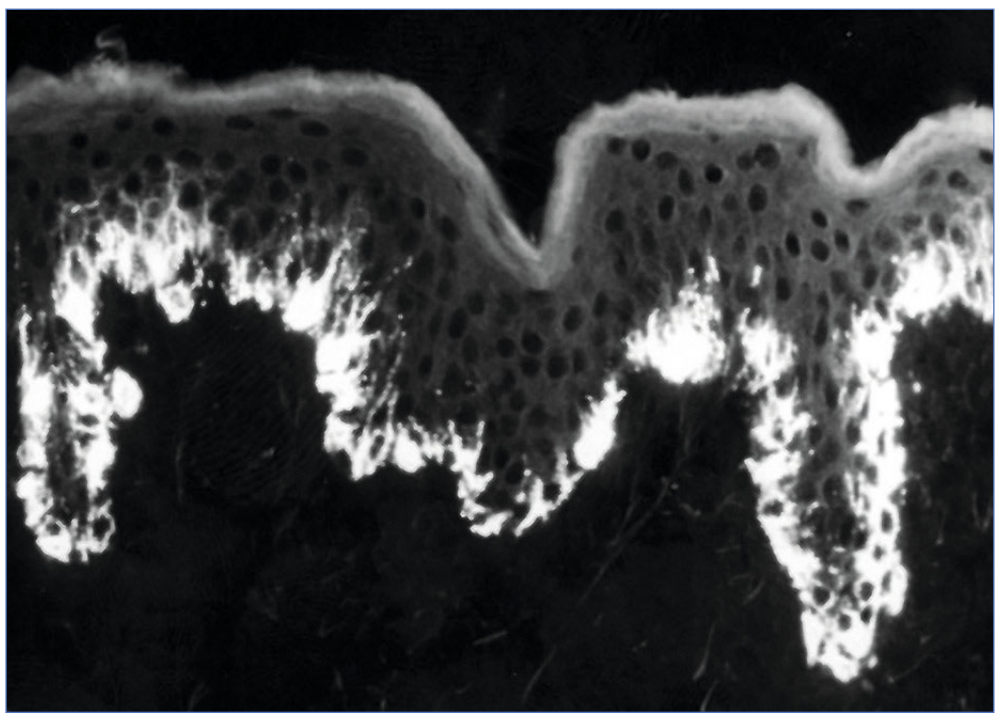

Figure 1. Section of human skin immunolabeled with anti-tyrosinase antibodies to locate melanocytes. Reproduced with permission from © Springer.

The acquisition of sufficient and homogeneous pigmentation is therefore a complex process, only possible if melanocytogenesis (embryonic development of the pigment system) is successful and all elements involved in the process of pigmentation (melanogenesis, biogenesis and transport of melanosomes, as well as transfer of melanosomes to keratinocytes) are functional.

Melanin pigmentation is genetically predetermined. However, it may be affected by ultraviolet (UV) radiation, as well as numerous other agents (hormones, peptides, chemical mediators), which can stimulate or inhibit skin pigmentation.

MELANOCYTOGENESIS

Melanocytes are cells derived from the dorsal part of the neural crest that then migrate into the dermis and the epidermis. It has been shown that nerves also contain stem cells capable of differentiating into either Schwann cells or melanocytes depending on cytokine stimulation [1]. This possible new origin of melanocytes is very interesting, as it highlights the close relationship observed clinically between nerves and pigmentation.

During embryogenesis, the cells of the neural crest are subjected to a multitude

of stimulations, finally to become functional melanocytes. The agents of this stimulation and their exact roles are not yet fully understood; however, the importance of specific molecules has now been clearly identified. Microphthalmia-associated transcription factor (MITF) is a transcriptional factor that activates the transcription of certain melanocyte proteins including the key enzymes of melanogenesis, tyrosinase and tyrosinase-related protein 1 (TRP1) [2]. The PAX3 gene also plays a crucial role in melanocyte differentiation. SOX10, which encodes a transcription factor that with PAX3 regulates MITF transcription, is also involved in the survival of cells from the neural crest [3]. The tyrosine kinase receptor located on the surface of melanocytes, called c-kit, and its ligand, stem cell factor (SCF), produced by keratinocytes, is also involved in the proliferation and survival of melanoblasts. Finally, the product of the SLUG gene (a transcription factor expressed by neural crest cells, including melanoblasts) and also the endothelin B receptor (EDNRB), and its ligand, endothelin 3 (EDN3), also appear to be involved in melanocyte differentiation and survival during embryogenesis. Clinically, mutations in the genes encoding these proteins cause piebaldism (cKIT gene mutations) and Waardenburg syndrome (mutations in the other aforementioned genes) [4–13].

MELANOGENESIS

The main function of differentiated melanocytes is melanin synthesis (melanogenesis). This process involves different enzymes that catalyze each of the reactions leading to the formation of melanin pigments in specialized organelles called melanosomes. The key enzymes are tyrosinase, TRP1 and dopachrome tautomerase (DCT). Tyrosinase is the rate-limiting enzyme of melanogenesis. These enzymes must be correctly synthesized and then transported in melanosomes to be active (Figure 2).

An anomaly occurring at this stage is responsible for oculocutaneous albinism (OCA) types 1–4. OCAs are now considered to be diseases affecting the intracellular transport of tyrosinase rather than impaired catalytic functions of melanogenesis enzymes. Indeed, even minor mutations of TYR or TYRP1 induce the same clinical phenotype as mutations responsible for the loss of protein function. All muteins are indeed recognized as abnormal by the quality control system of the endoplasmic reticulum, and are then directed to the proteasome to be degraded [14, 15]. Similarly in OCA2 and OCA4, mutations of the P and MATP genes prevent the transfer of tyrosinase (and TYRP1 in OCA4) from the trans-Golgi network to melanosomes [16–18]. Only a small proportion of enzymes are correctly transported to the melanosomes and the quantity of melanin thus produced is very low.

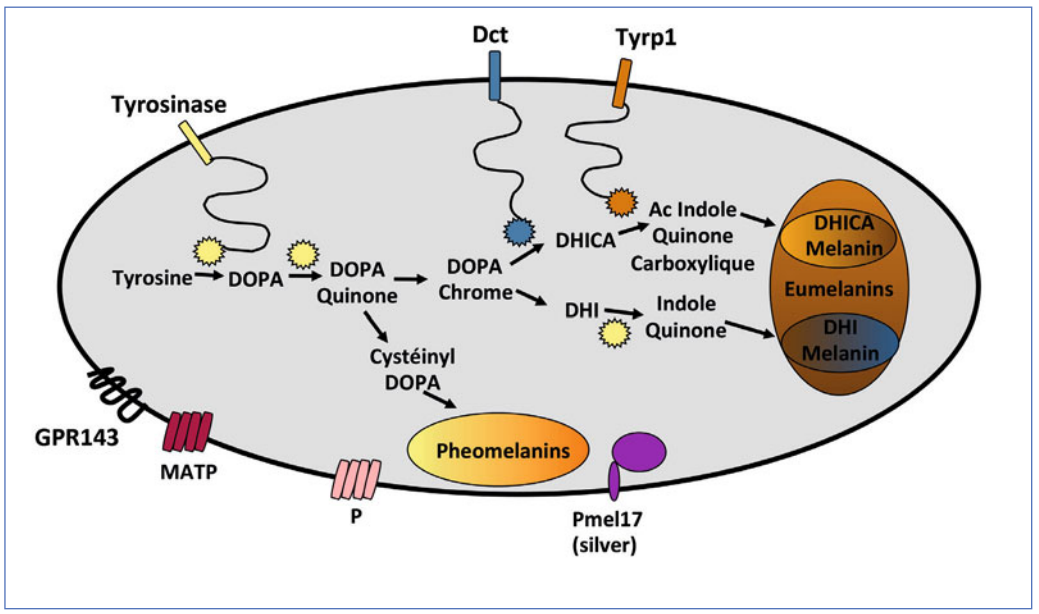

Figure 2. Enzyme machinery dedicated to the production of the melanins within melanosome.

BIOGENESIS OF MELANOSOMES

Melanosomes form part of the family of secretory lysosomes. They result from the combination of membrane structure proteins and different melanogenic enzymes. The study of melanosomes by electron microscopy has revealed several stages of maturation: 'premelanosomes' at stage I are spherical in shape and at stage II are oval with a filamentous matrix; stage III melanosomes have opaque matrix deposits of melanin that are dense with electrons (beginning of synthesis); stage IV 'mature' melanosomes have a uniformly opaque matrix.

Of the proteins involved in the biogenesis of melanosomes, lysosomes and dense platelet granules, the LYST protein involved in the pathogenesis of Chediak–Higashi syndrome must be mentioned [19]. Hermansky–Pudlak syndrome is also caused by abnormalities in melanosome biogenesis [20–26]. These similarities between melanosomes and other secretory lysosomes explain the association in these genetic disorders of extracutaneous symptoms (such as bleeding disorders or immunodeficiencies) and pigment dilution.

TRANSPORT OF MELANOSOMES

Melanocytes have cytoplasmic expansions called dendrites that enable them to interact with keratinocytes of the suprabasal layers. Each melanocyte interacts with about 36 keratinocytes, thus forming an 'epidermal unit of melanization'. While melanin is synthesized, melanosomes are transported to the end of the melanocyte dendrites. Melanosomes are transported along actin and tubulin fibres. Motor proteins are associated with microtubules and are involved in the migration of melanosomes. Kinesin thus enables anterograde transport of melanosomes while dynein is involved in retrograde transport. The importance of transport along actin fibers was demonstrated by the better understanding of another genetic hypomelanosis known as Griscelli syndrome (GS). Transport on actin fibres is carried out by means of a molecular complex involving at least one molecular motor, myosin Va (mutated in GS1), a small GTPase, Rab27a (mutated in GS2) and melanophilin (mutated in GS3) (Figure 3) [27–29]. Again, the involvement of muteins in other physiological mechanisms explains the phenotype observed depending on the different types of GS. All people with GS will thus have pigment dilution (mild skin hypopigmentation with silvery grey hair), but some will also have neurological damage (Va myosin mutations), or immunological damage (Rab27a mutations), whereas skin appendage damage is only observed in cases of melanophilin gene mutations.

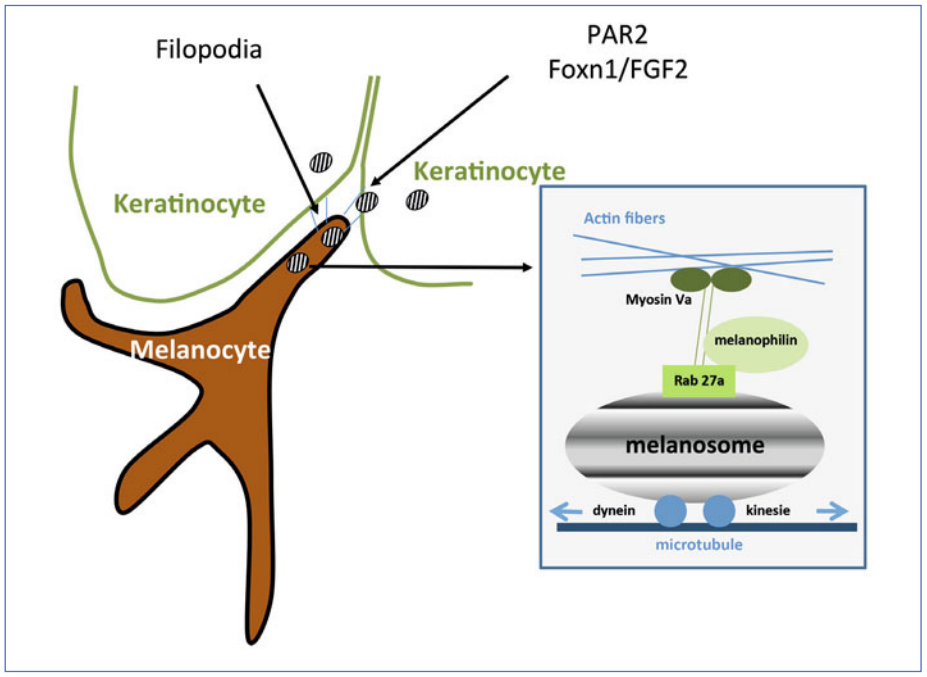

Figure 3. Players involved in the transport of the melanosomes within melanocytes and their transfer to the surrounding keratinocytes.

KERATINOCYTE TRANSFER

After having reached the end of the dendrites, the melanosomes are then transferred to the keratinocytes (Figure 3). The mechanisms involved in this transfer are still poorly defined. The protease activated receptor 2 protein (PAR-2) as well as lectins and surface glycoproteins, still unidentified, are thought to facilitate this transfer. It has also been demonstrated that a transcription factor, Foxn1, plays a key role in these transfer phenomena by first identifying the cells receiving the pigments and then recruiting adjacent melanocytes so that they connect to them by means of dendrites and transfer their pigments [30]. The transfer of melanosomes from melanocytes to keratinocytes might be carried out by small growths on the cell extremities, known as filopodia [31]. Once transferred, the melanosomes are then gradually eliminated with the keratinocytes as they ascend to the epidermal surface.

INTRACELLULAR SIGNALING

The main signaling pathway involves cyclic adenosine monophosphate (cAMP), the intracellular increase of which activates protein kinase A (PKA). The inducing agent of this pathway is α-melanocyte stimulating hormone (αMSH), a keratinocyte agent, which binds to its MC1R receptor on the surface of melanocytes and that is positively coupled to adenylate cyclase by the intermediary of a Gas protein. cAMP regulates gene expression by means of transcription factors from the family of cAMP-responsive element binding proteins, which bind to specific sequences called CRE (cAMP responsive elements). cAMP thus induces an increase in the expression of MITF and SOX9 (SRY-box containing gene 9) leading to the expression of genes and key proteins for melanogenesis, especially DCT and tyrosinase, in order finally to induce melanin synthesis [2, 32].

MELANINS AND THEIR ROLES

Two types of melanins are produced: eumelanins and pheomelanins. In humans, melanins are generally a mixture of eumelanins and pheomelanins in different proportions. Eumelanins are brown or black-colored melanins. Pheomelanins are yellow-orange in color. Eumelanins and pheomelanins derive from the enzymatic conversion of tyrosinase into dihydroxyphenylalanine (dopa), then into dopaquinone through tyrosinase activity. Thereafter, the synthesis pathways diverge, involving either TRP-1 and TRP-2 (also known as DCT) in eumelanogenesis, or incorporating sulphur derivatives for pheomelanogenesis (Figure 2).

UV radiation increases the synthesis of melanin and accelerates its transfer to keratinocytes. The production of melanin is an adaptive response of the body to prolonged exposure to sunlight. Therefore, after UV stimulation, melanocytes produce an optional pigment reflecting the ability of each individual to develop a tan, the skin's natural protective mechanism. Melanin pigment is the most important photoprotective system. It absorbs more than 90% of UV rays that have crossed the stratum corneum. Despite processes of absorption, approximately 15% of UVB still manages to reach the basal layer of the epidermis and 50% of UVA reaches the dermis. UVB induces the formation of dimers in DNA chains, causing metabolic defects (ageing), cell death by apoptosis or the acquisition of disordered proliferation properties (cancers). It is now known that UVA plays a role at least as important as UVB in these phenomena, particularly in the production of free radicals. Melanins filter visible and UV rays. During irradiation, melanosomes gather around the nucleus (the capping phenomenon) and thus protect the genetic material of keratinocytes. Eumelanins have a photoprotective power about 1000 times greater than that of pheomelanins. They can absorb free radicals generated in cells by UV radiation, preventing the DNA from damage, and thus protect the skin from the harmful effects of UV radiation. Recent work suggests the involvement of pheomelanins, both as a result of UVA but also independently of UV rays (for individuals with a fair skin type), in the genesis of melanoma through mechanisms of oxidative damage on DNA [33, 34].

Although the quantity, quality and distribution of melanin in keratinocytes are important in protecting from UV-induced damage, the DNA repair mechanisms might play an even more crucial role [35]. After UV irradiation, individuals with dark skin types have less DNA damage of the basal keratinocytes than those with fair skin types, confirming the greater efficiency of melanin filtering in patients with high skin types [36]. In addition, these same individuals with dark skin types have a higher rate of keratinocyte apoptosis and faster removal of UV-induced dimers, suggesting more effective detection and DNA damage repair mechanisms [36].

REGULATION OF MELANOGENESIS

Melanogenesis is regulated mainly by UVA and UVB radiation from sunlight. UVA and UVB rays penetrate to the basal layer of the epidermis and can therefore affect melanocytes and keratinocytes. Experimental evidence demonstrates that UV rays, particularly UVB, can act directly on melanocytes in order to stimulate melanogenesis, including the activation of USF-1 transcription factor by the p38 stress protein (Figure 4) [38]. Furthermore, it is clear that exposure of keratinocytes to UVB induces the production of many agents that regulate differentiation of melanocyte, dendrite growth and melanogenesis. The coordinated action of these factors as well as the direct effect of UV on melanocytes leads to the final effects of UV, namely the stimulation of the growth of melanocytes and of their melanogenic activity, resulting in increased skin pigmentation, ie, tanning (Figure 5).

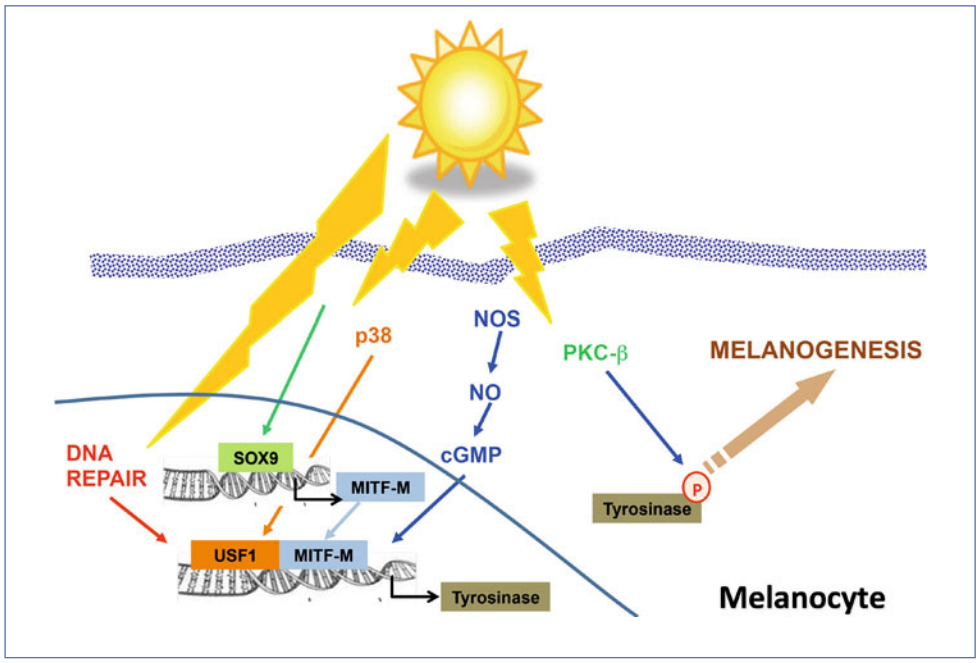

Figure 4. Direct action of ultraviolet (UV) radiations on melanocytes to stimulate melanogenesis.

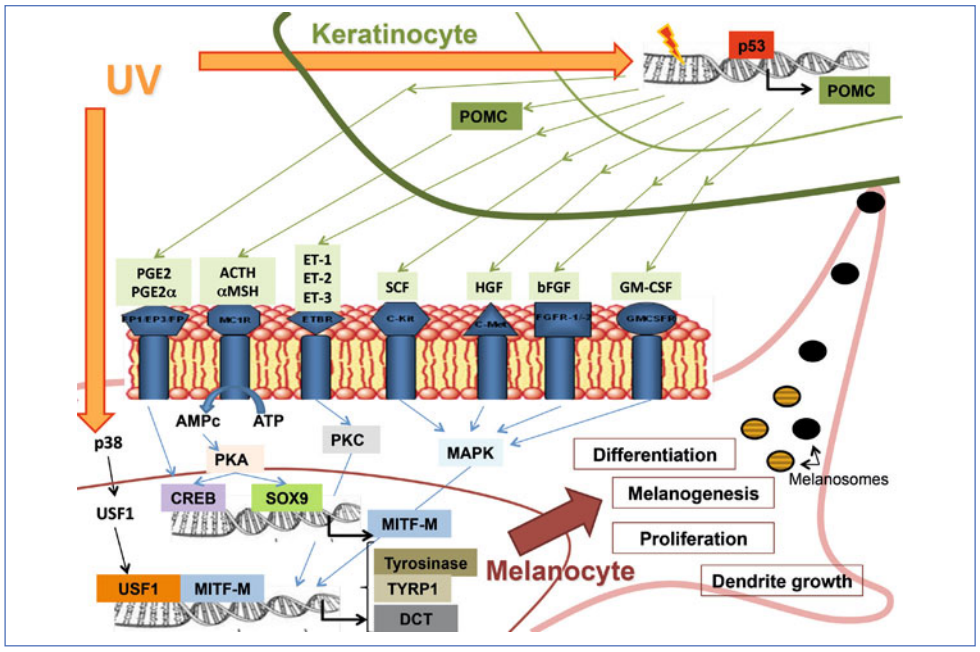

Figure 5. The key role of secreted keratinocyte factors in UV-induced pigmentation.

Of the agents of keratinocyte origin, in which production is stimulated by UV, αMSH and adrenocorticotropic hormone (ACTH) are the most potent activators of melanogenesis. αMSH and ACTH are polypeptide hormones generated by the split of a high molecular weight precursor, proopiomelanocortin (POMC).

Activation of the p53 stress protein under the effect of UV rays on keratinocytes appears to play an important role in inducing POMC transcription [39]. The association of skin hyperpigmentation with Addison's disease and Cushing's syndrome is the consequence of ACTH hypersecretion. The effects of αMSH and ACTH are initiated by binding the hormone to a seven-transmembrane domain receptor located on the surface of melanocytes, known as MC1R [40]. Allelic variations in the gene encoding MC1R are associated with the red phenotype and the presence of freckles [41, 42]. It has been demonstrated that some of these allelic variations were related to an independent risk of the occurrence of melanoma [43]. The MC1R receptor is coupled to a Gas protein, which activates adenylate cyclase and increases intracellular cAMP concentration. Extensive in-vitro data have illustrated the crucial role of cAMP in the regulation of melanogenesis, but also the transport of melanosomes [44, 45]. The increase in intracellular cAMP levels activates several downstream signaling pathways, including SOX9 and MITF, which play a key role in melanogenesis (Figure 6) [2, 32].

Numerous factors can very finely regulate the production of melanin pigments and/or the growth and differentiation of melanocytes. Therefore, nitric oxide, but also some growth factors, such as basic fibroblast growth factor (bFGF), stem cell growth factor, hepatocyte growth factor (HGF), endothelin-1 (ET1) and some prostaglandins, present in the circulation or secreted by keratinocytes, act to varying degrees on melanocyte growth and the melanogenic activity

of melanocytes [46]. The role of fibroblasts in melanocytogenesis and melanogenesis has been demonstrated. Palmoplantar fibroblasts thus express high levels of Dickkopf 1, which reduces melanocyte proliferation and differentiation by acting on MITF, explaining (at least partly) the lower pigmentation generally observed on the palms and soles [47–49].

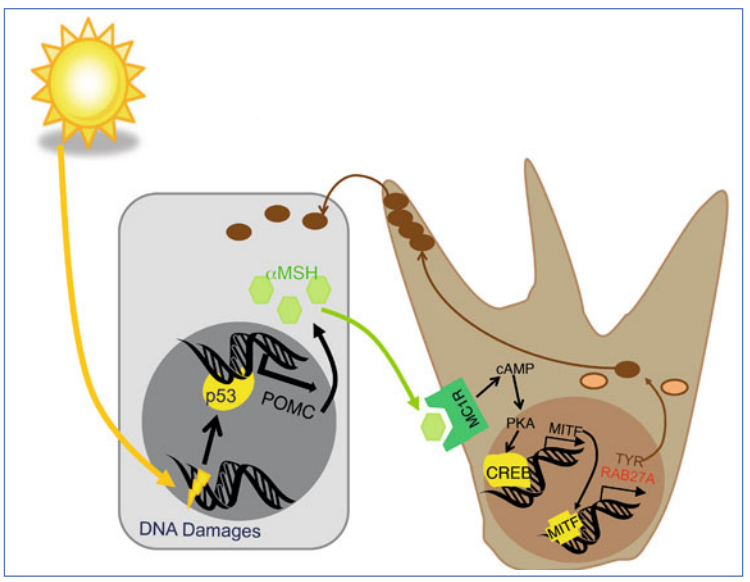

Figure 6. Simplified scheme of the main pathway involved in UV induced pigmentation.

Ultraviolet (UV), and particularly UVB, induce DNA damage in keratinocytes. This cellular stress activates the p53 protein, which binds to the promoter of the POMC gene (responsible for proopiomelanocortic hormone production) in order to activate it. This results in an increase in the production of α-melanocyte stimulating hormone (αMSH) by keratinocytes. αMSH then binds to its receptor (MC1R) located at the surface of the melanocytes. This binding activates the cyclic adenosine monophosphate (cAMP) pathway, which then activates protein kinase A (PKA), ultimately leading to the increased expression of microphthalmia-associated transcription factor (MITF) protein in melanocytes. MITF then activates both melanin production, by stimulating the synthesis of tyrosinase in particular, but also the transport of melanosomes to the end of melanocyte dendrites by increasing Rab27a. This cAMP pathway also stimulates the transfer of these melanosomes to adjacent keratinocytes. This results in an optional pigmentation, more commonly known as tanning. CREB, cAMP-responsive element binding protein.

More recently, it has been shown that fibroblasts were capable of producing factors acting on melanogenesis, which differ according to the skin type of the individual [50]. Therefore, one of these factors, known as neuregulin-1 (NRG1), secreted by fibroblasts in black skin (skin type VI), significantly increases the pigmentation of human melanocytes in culture. These results demonstrate the involvement of fibroblasts in melanogenesis.

More recently, it has been shown that visible light can also induce a tan [50]. However, not all wavelengths of the visible spectrum have the same photobiological effects on pigmentation. Therefore, short wavelengths (415 nm: blue–violet) can induce intense and prolonged pigmentation over 3 months, while red light (630 nm) has little effect on skin pigmentation (T. Passeron et al, 2014, submitted). This involvement of visible light in pigmentation could play a role in certain pigmentary disorders, such as melasma or post-inflammatory hyperpigmentation.

PRINCIPAL ETHNIC DIFFERENCES

Few studies are yet available on the differences between white skin and black skin. Color differences are caused by the intensity of melanin pigmentation. In the darkest phenotypes, melanin pigment is found throughout the entire basal membrane and continues in the stratum corneum. If the number of melanocytes is identical, the type and number of melanosomes will vary depending on the skin type. In white skin, melanosomes are few and their maturation is often incomplete (stages I–III). They are also rapidly degraded. In black skin, their number is increased and, above all, most are at stage IV. The distribution of melanosomes in keratinocytes also plays a key role in skin color. The type of melanin is different, with a much larger proportion of eumelanin in black skin while lighter skin types have predominantly pheomelanins. Finally, individuals with high skin types seem to have a more effective repair and elimination mechanism of UV-induced damage than those with lighter skin [36].

To date, 378 loci are known to induce pigmentation abnormalities in mice when mutated (see http://www.espcr.org/micemut). However, the gene involved is identified in only approximately half of cases. These genes control the complex mechanisms that account for the diversity of skin, appendage and eye color, and reflect the adaptation of humans to the climate changes faced over the course of evolution [51–54]. The study of these genes has led to a better understanding of pigment genodermatoses and associated systemic diseases. The close relationship between the major genes involved in pigmentation and their roles in melanoma clearly show that the study of these mechanisms will eventually enable clinicians to obtain a better understanding of and perhaps control pigmentation, and to define new therapeutic approaches targeting melanoma [55–58].

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MEASURING SKIN COLOR AND PIGMENTATION DISORDERS

L. DUTEIL, K. ROUSSEL, P. BAHADORAN

INTRODUCTION

The color of the skin depends mainly on its pigment content, on the spectrum of the illuminating light and on the quality of the cutaneous surface. The pigments of the skin, also called chromophores, are mainly represented by melanin in the epidermis and by hemoglobin in the dermis. Other molecules such as bilirubin, amino acids, nucleic acids, porphyrins and carotenoids (endogenously produced) may participate at different levels to the absorbing and reflecting process of the light [1-3]. The need for assessment of the skin color and pigmentation extends from the dermato-cosmetology domain (including sunscreens, skin pigmenting or depigmenting products, antiaging agents and make-up evaluations) to the clinical dermatology for the characterization of the various types of skin pigmented lesions. Although the human eye is able to distinguish between hundreds of colors, the results of visual assessment remain subjective and qualitative with a poor reproducibility in time. Since the last decades, several objective methods and technologies have been developed [4-6] to measure skin color and some were proposed as commercially available devices. Two main types of skin color investigation systems exist. One is based on different approaches of analysis of reflected light by the skin, which gives only spectral information. The second is based on the skin imaging technologies which offers, beside the color and spectral aspects, spatial information, taking into account, for example, the lesion borders or heterogeneity on a large skin area (eg, the whole face). The first type of systems is based on the spectrophotometric analysis of the reflected light by the skin, the illuminating light being either a continuous spectrum (white light) [7, 8] or single bands specific of absorption of melanin and hemoglobin [9-12]. These objective methods are easy to use but inconveniently produce color information only on small areas (0.5 to 1 cm^2). They are very useful in skin pharmacology; where the information is needed on small test surfaces. With the progress of optical instrumentation, digital imaging techniques and the development of dedicated image analysis processing, the spatial component of the skin color can be integrated in the measurement [13, 14]. The recent development of dermoscopy has been shown to improve the diagnostic accuracy of pigmented skin lesions [15]. Multispectral imaging [16], which takes into account the combination of spectral and spatial information, aims to determine the severity of skin pathologies. Finally, reflectance confocal microscopy (RCM) [17] represents a very promising tool that allows the dermatologist to perform a noninvasive analysis of pigmented skin lesions at a histological level.

VISUAL ASSESSMENT OF SKIN COLOR AND PIGMENTATION

The eye is the first diagnostic tool for the dermatologist. Color perception is the result of the perception of the 'visible' wavelength range 400 to 700nm collected by the eye and interpreted by the brain [18, 19]. The evaluation of skin pigmentation by visual examination depends not only on the subjective perception of colors by the observer but also on the nature of the illuminant and on the geometric position of the observer relative to the skin surface. Various scales and indexes have been developed to reduce potential interobserver variability and enable more objective assessment of skin pigmentation. For example, in the case of melasma, the MASI [20]. Using a standardized environment and grading skin color with either standard color references, such as Munsell standards or very precise rating scales or color charts [4, 21] may lead to reliable visual color assessments.

REFLECTANCE TRISTIMULUS CIE COLORIMETRY

The colorimetry tristimulus system is based on the following two principles: the first is that each color can be matched by a suitable mixture of three selected light radiations, the second is that if two colors are matched by three radiations, the mixture of these two colors is found additive by suitable optical means. In this system, each color is defined with a set of three tristimulus primary values (X: red content, Y: green content, Z: blue content). The L*, a*, b* coordinates are also calculated from the X, Y, and Z tristimulus primary values. In the L*a*b* color space, L* is the

lightness ranging from 0 (black) to 100 (white), a* is the balance between red (positive values) and green (negative values) and b* is the balance between yellow (positive values) and blue (negative values). All the different types of basal skin color for a selected body site (the back for example) are located inside a vertical arched volume in the L*a*b* color space [19, 20] (Figure 1). Thus, sectors of skin color categories have been delineated [22, 23] to correspond roughly to the skin phototypes [24]. The sectors are delimited by radii originating from L*=50 and b*=0 and constitute defined angles with the b* axis. Therefore, a subject can be characterized by the so-called individual typology angle (ITA°), which is calculated by: ITA°= Arctangent ((L*-50)/b*)x180/Π. The values proposed for the angles of skin categories boundaries were: Very light skin > 55° > Light skin > 41° > Intermediate skin > 28° > Tanned skin > 10°. Colorimetry has been extensively used to assess the process of UV-induced erythema/pigmentation [25, 26)], skin typology [27] and photoprotection factors [28-31].

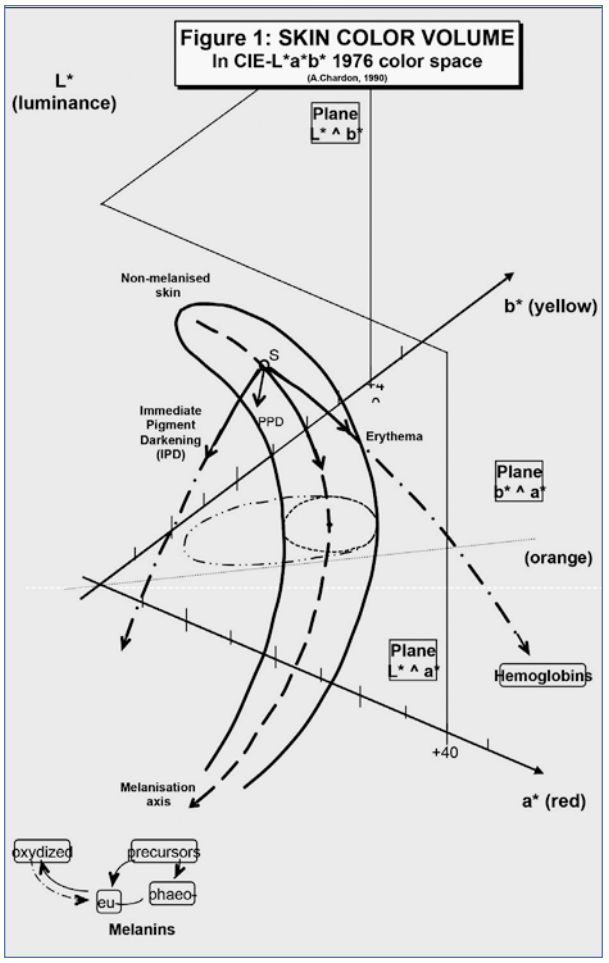


Figure 1. The skin color volume in the cie l*, a*, b* color space (with permission of Chardon et al, 1993 [22]).

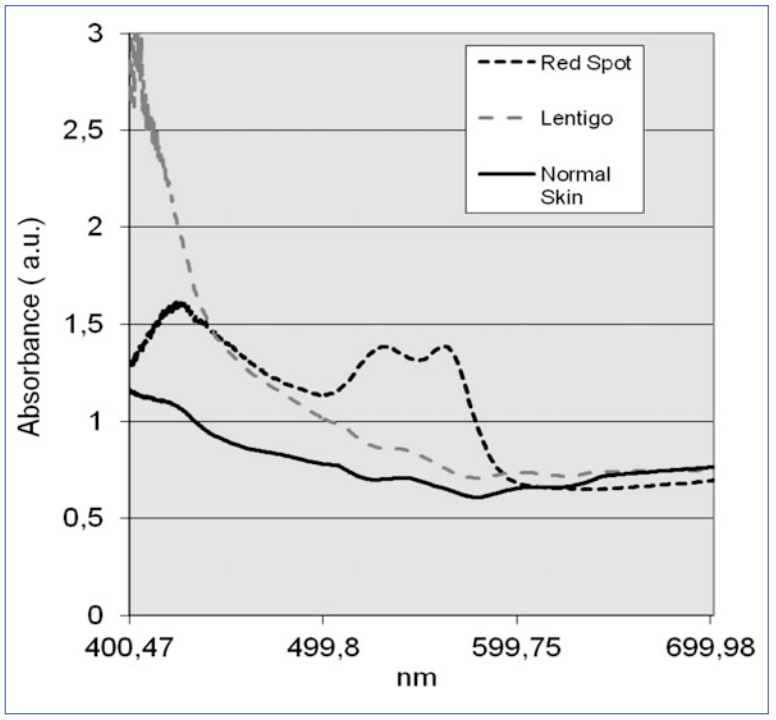


Figure 2. DRS absorbance spectra (arbitrary unit) of three types of skin area: a pigmented lentigo (shape of typical melanin absorbance curve), a raised red spot (shape of typical hemoglobins absorbance curve, absorption peaks of oxyhemoglobin visible at 542 and 577 nm) and a normal skin area. Measurements performed with a diffuse reflectance spectrometer (Canfield Scientific System, Fairfield, NJ, US).

REFLECTANCE SPECTROPHOTOMETRY

REFLECTANCE SPECTROSCOPY

The scanning reflectance spectroscopy [7, 32] analyses the spectrum of light reflected by the skin, typically between 400 and 700 nm, and allows the measurement of skin color in order to obtain information on skin chromophore content. The optical properties of the skin are determined by the spectral absorption, reflection and scattering of the light as it strikes and penetrates the different cutaneous structures. The scanning reflectance spectrometers are built to measure the diffuse reflectance of the light, ie, the part of the light which is modified by the absorption, reflection and scattering processes inside the skin, and which is re-emitted from the skin. This technique is also called diffuse reflectance spectroscopy or DRS [32]. Different skin modeling simulations have been used to analyze spectral data [33, 34]. For example, the reflectance spectra of the human skin in visible and near-infrared (NIR) spectral region have been calculated using the Monte Carlo technique [35]. An example of skin DRS reflectance spectra (presented as absorption spectra) is illustrated in Figure 2. In this figure, compared to the normal skin spectrum, the differences of a heavy pigmented skin lesion (lentigo) spectrum and a deeply vascularized lesion (raised red spot) are clearly observable.

Wallace et al [36] aimed to document the optical reflectance (range 320 to 1100 nm) characteristics of pigmented skin lesions in order to evaluate their potential for improving the differential diagnosis of malignant melanoma from benign pigmented skin lesions. Characteristic differences

in spectra from benign and malignant lesions were studied and showed significant differences between lesion groups classified by histology.

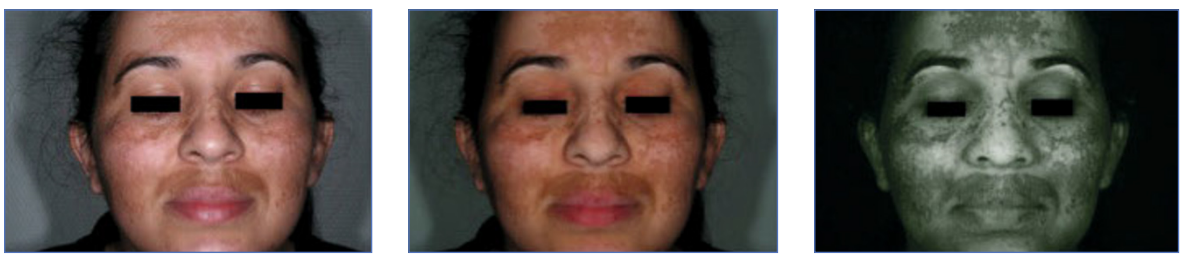
NARROW BAND REFLECTANCE SPECTROSCOPY

The reflectance spectrophotometers are known to be expensive, cumbersome and not well adapted for routine clinical uses. Since the spectrophotometric measurements often result in the analysis of some specific narrow bands or peaks of spectra corresponding to the absorption bands of the main chromophores of the skin, the use of simpler and cheaper devices based on narrow-band analysis was developed. The Mexameter MX16® (Courage-Khazaka, Elelectronik, Köln, Germany) is equipped with 16 light emitting diodes (LED) arranged circularly and emitting at 568 nm (green), 660 nm (red) and 880 nm (infrared). The system is based on the principles described by Diffey and coworkers [37]. The melanin index (mx) is measured at two wavelengths (660 and 880 nm). These wavelengths have been chosen in order to achieve different absorption rates by the melanin pigments. For the erythema index (ex), two different wavelengths are used to measure the absorption capacity of the skin. One of these wavelengths corresponds to the spectral absorption peak of hemoglobin (568 nm) and the other wavelength (660 nm) has been chosen to avoid other color influences (eg, bilirubin). Several studies [38, 39] have been performed to compare narrow-band spectrophotometers to tristimulus colorimeter. The results showed that both kind of instruments are able to detect very small changes in skin color. The correlation between the instruments was found to be moderately good between L* and the melanin indexes and good between a* and the erythema indexes. A recent study [40] showed that the Mexameter, the Colorimeter, and the DSL II colorimeter provided reliable color data on normal skin and scars. Various type applications of assessment of the cutaneous pigmentation by narrow-band spectrophotometers were performed including, for example, UV-induced pigmentation [41, 42], efficacy of depigmenting agent [43,44], and protection of vitiligo [45, 46]. Skin typology [47, 48] and epidemiology [49] are the subject of numerous studies.

WOOD'S LIGHT AND DERMOSCOPY

WOOD'S LIGHT

The classical photography technique performed in Wood's light is a simple and useful method to visualize skin pigmentation [50-52]. The Wood's lamp has a UVA band pass filter which blocks most visible light and allows through UV. It emits mostly long wave (UV-A) ultraviolet light. The hyperpigmented skin areas such as solar lentigos appear darker on the black and white image compared to 'normal skin' due to the fact that melanin absorbs heavily in the UVA domain. On the other hand, depigmented lesions, such as vitiligo macules are displayed as white areas on



A B C

Figure 3. Enhancement of melasma visualization using polarised (B) and Wood's light (C) compared to white light (A).

the skin [53]. UV photography of facial pigmented lesions can also be performed through facial complexion analysis systems such as Visia® (Canfield®, USA). The processing of these kind of images by dedicated gray levels analysis software could increase the objective quantification level of this technique. Figures 3A, B, and C illustrate the enhancement of melasma visualization using Wood's light and crossed polarized light.

DERMOSCOPY

Dermoscopy (dermatoscopy, epiluminescence microscopy) is one of the major in vivo noninvasive diagnostic techniques used in the diagnosis of melanoma and other pigmented skin lesions [15, 46]. The technical set up consists of a magnifying optical system (surface microscope, stereomicroscope, hand-held scope) allowing magnification of the lesion image. The lesion is covered with immersion oil or any kind of liquid including water and alcohol in order to eliminate surface reflections of the illuminating light. This makes the stratum corneum translucent, enabling the

visualization of pigmented structures of the epidermis and of the dermal-epidermal junction and superficial papillary dermis, which are impossible to observe with the naked eye. The vessels of the superficial vascular plexus can also be observed. Various diagnostic systems have been proposed for assessing dermoscopic images. For all the diagnostic systems, the color aspect of the pigmentation of the lesion is crucial, including number of different colors, aspect of pigment

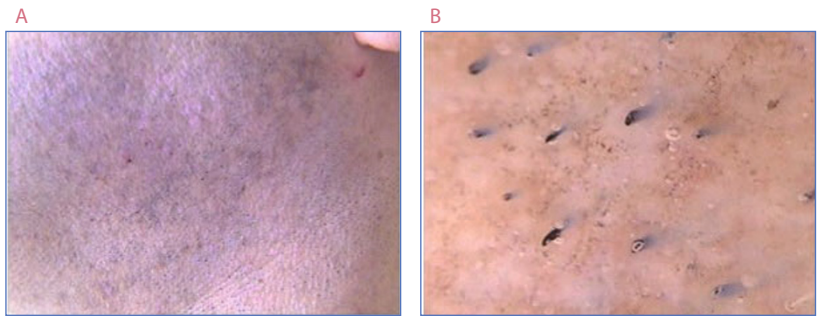


Figure 4. Dermoscopy of post-inflammatory pigmentation.
A. Clinical aspect of a diffuse pigmentation of the cheek in the context of folliculitis barbae.
B. Dermoscopic aspect: diffuse blue-gray dots realizing a 'peppering' pattern and corresponding histologically to melanophages.

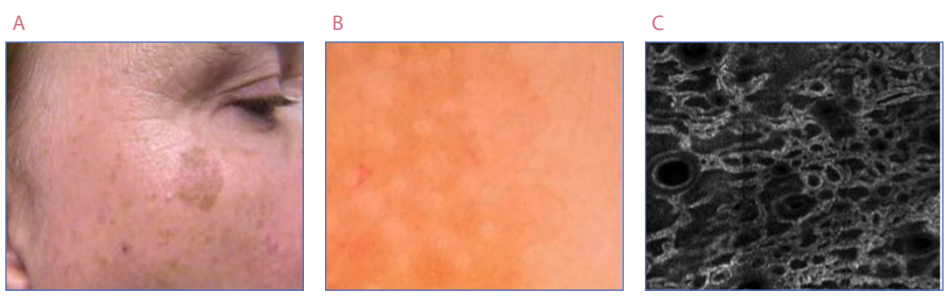


Figure 5. Reflectance confocal microscopy imaging of actinic lentigo.
A. Clinical aspect.
B. Dermoscopic aspect.
C. Confocal aspect: increased and distorded dermal papillae (DP), cord-like elongated rete-ridges (≥) realizing a 'donut-like' pattern around hair follicles (HF).

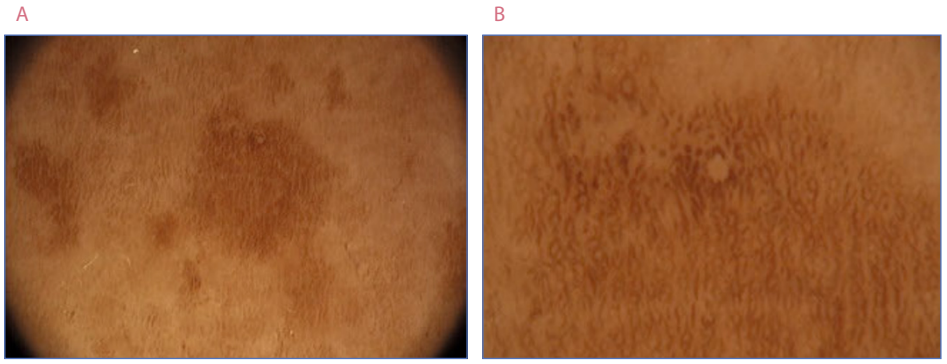


Figure 6. Dermoscopy of solar lentigos (Ax20; Bx70).

networks, distribution of pigments and shape margin of pigmented areas. The use of dermoscopy by trained dermatologists allows the diagnosis of melanoma with a sensitivity of 89% and a specificity of 84%. Since the last decade, numerous computerized image analysis models have been proposed in order to maximize the diagnostic accuracy, in particular in the field of lesion border detection [15, 54, 55], color quantification [56, 57] and pattern classification [58]. Regarding pigmentation disorders, dermoscopy is useful to distinguish some forms of dermal (Figure 4) versus epidermal (Figure 5B) pigmentation (see also Figures 6A and B).

REFLECTANCE CONFOCAL MICROSCOPY (RCM)

Reflectance confocal microscopy (RCM) is a recent skin imaging technique that allows in vivo, non-invasive, real time, and almost histological visualization of the skin [59]. Confocal microscopy is a technique that enables virtual optical sections through an object of interest. Confocal microscopy was first used in biology to visualize cellular organelles, and it was adapted recently for clinical practice, especially in dermatology. Reflectance means that the images are obtained by reflection of a laser by endogenous molecules. Since melanin is the strongest contrast agent in the skin, RCM is particularly suitable to investigate skin pigmentation. Paradoxically, melanized structures look very bright on RCM images, a feature that can be easily seen on RCM sections of the dermal-epidermal junction, where bright (pigmented) keratinocytes surround dark (non-

pigmented) dermal papilla (Figure 7). Interestingly, based on morphological criterias, RCM can discriminate between the different pigment cell populations in the skin [60]. The inte-

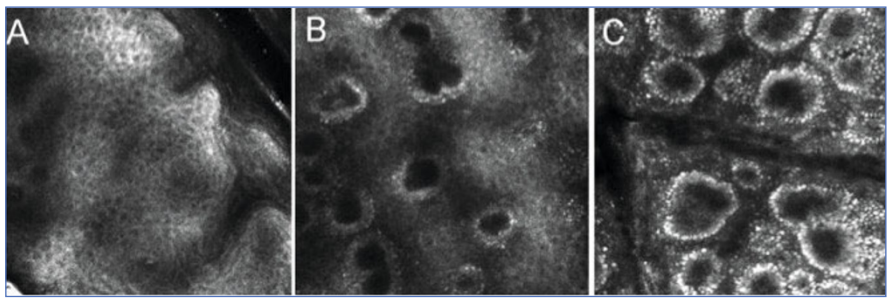


Figure 7. Reflectance confocal microscopy imaging of vitiligo. Confocal images (500 x 500 microns) at the dermal epidermal showing increasing brightness of peri-papillar cells in (A) vitiligo lesion, (B) perilesional skin, and (C) distant normal skin. Abdomen skin, non-exposed. Adapted from: Kang, et al [62].

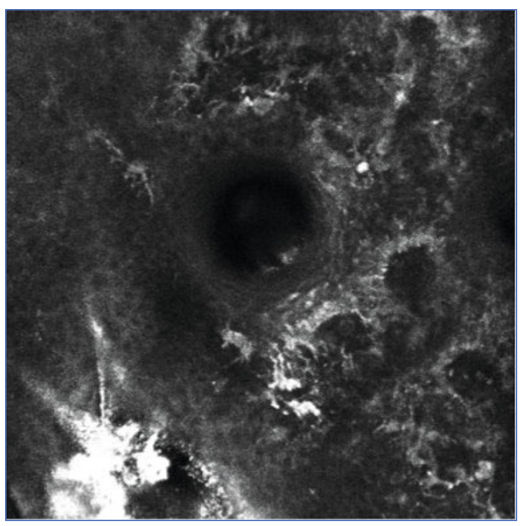


Figure 8. Reflectance confocal microscopy imaging of vitiligo. Confocal images of vitiliginous skin during UVB therapy showing bright dendritic melanocytes around hair follicles (O) and dermal papillae (0).

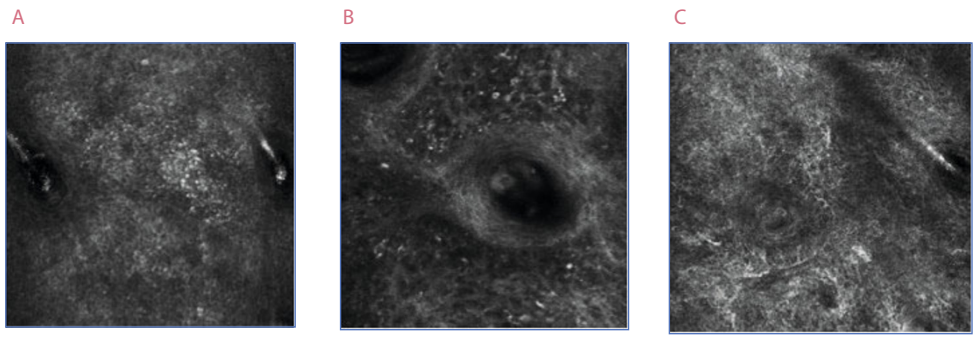


Figure 9. Reflectance confocal microscopy imaging of melasma.
(A) Epidermal melasma: clusters of small bright cells corresponding to hyperpigmented keratinocytes in the epidermis. (B) Dermal melasma: plump bright cells corresponding to melanophages in the dermis. (C) Melasma with activated melanocytes: dendritic bright cells in the epidermis.

rest of RCM for the diagnosis of pigmented lesions, especially pigmented tumors has largely been reported [61]. RCM is also interesting for the evaluation of pigmentation disorders such as vitiligo [62] (Figures 8, 9) melasma [63] (Figure 10) and lentigines (Figure 11). In addition regarding normal pigmentation, it was reported recently that RCM can quantify variations of skin pigmentation [64]. One of the objectives of this work was to identify RCM parameters able to quantify in vivo epidermis pigmentation potentially applicable in clinical studies. The study included 111 healthy female volunteers with phototypes I-VI. The authors proposed an index called 'papillary contrast' and defined as the difference in brightness between the cellular ring around the papilla zone and the central dermal papilla zone.
The mean papillary contrast (PC) was estimated according to the following function:

$$\mathrm{PC} = \frac{\sum(\overline{B\_\mathrm{ring}} - \overline{B\_\mathrm{center}})}{Nb\_\mathrm{pap}}$$

where PC, papillary contrast; B_ring, brightness of the ring papilla; B_center, brightness of the central dermal papilla; and Nb_pap, number of papillae. PC measured at the dermo-epidermal junction appeared to be a reliable marker of epidermis pigmentation and showed a strong correlation with skin pigmentation assessed clinically using the Fitzpatrick's classification. However, additional studies comparing RCM with established methods of skin color measurement, such as colorimetry are needed in the future to confirm the interest of this approach. Another limitation worth considering is the price of the RCM device ~100 000 euros, though it can be used for other purposes. Despite these limitations, RCM represents a significant advance in the field of pigment measurement since it might allow a quantification of melanin along with a morphological identification of melanized cells.

SPECTRAL IMAGING AND IMAGE PROCESSING

Spectral imaging is the integration of spectroscopy with spatial measurement; thus, it extends the image into spectroscopy by allowing measurement of the spectrum at each point of the image. As described above, spectroscopy yields quantitative information and extends understanding of skin pigmentation. Images give morphological information, such as lesion texture, borders, and area. Spectral imaging of the skin surface is then a way to extract both of these two crucial aspects of pigmented lesions. The information captured by spectral imaging is similar to that acquired by the clinician. So spectral imaging provides global and sufficiently rich information to support diagnosis. In addition, this information is quantitative, objective, and reproducible. The spectral information is more detailed than information provided by human eye or contained in color images. The human eye, like the dermatoscope, only has the capacity to capture in three spectral bands with its sensors for red, green, and blue (RGB). Multispectral imaging (MSI) can capture spectral information in wavelengths from several tens up to one hundred for hyperspectral imaging (HIS). The high definition of the skin spectral response, which can be out of the visible light range, offers a new possibility for identifying and quantitatively characterizing a cutaneous lesion leading to a more precise and robust diagnosis. Spectral imaging methods can be divided into four different methods [65]. The selection of the most appropriate method depends on the capture constraints (eg, time, sample movement, resolution need). The larger the number of wavelengths or the higher the spatial resolution, the longer will be the capture. Multispectral technology has been applied to dermatology mainly for noninvasive diagnosis of melanomas. It has also been proposed for analysis of bruised skin [66], vascular lesions, [67], acne vulgaris [68] and various pigmented lesions; including melasma [69], naevi, and pigmented skin cancers [70]. For non-invasive melanoma diagnosis, band selection in spectral imaging has been tested. Some authors have used a predefined set of bands [71-74]. Other authors have applied methods and algorithms derived from the image processing field [65]. The most currently used method is the independent component analysis (ICA) introduced in computerized skin analyses by Tsumura et al [75].The aim was to isolate the spectral components linked to melanin and hemoglobin chromophores using ICA. The principle was to obtain the spatially independent spectral components of the image expecting that some of these independent features would correspond to certain molecules present in the skin. Melanin and hemoglobin, the two principal chromophores involved in skin spectrum, are independently distributed. Tsumara et al proved that the two features obtained by ICA correspond to these two skin chromophores representing the healthy skin spectral print. ICA is commonly implemented to multispectral imaging seeking specific characterization of skin pigmented pathology, for example for melasma diagnosis [69]. A large number of different approaches have been proposed in the literature: implementation of statistical method based on blind source separation (BSS) as non-negative matrice factorization (NMF) to quantified melanin and hemoglobin [76], band selection based on on machine learning algorithms support vector machine (SVM) [77], and geometric method. Although further investigation is expected, multispectral imaging is an effective tool for capturing the information useful in dermatology.

CONCLUSION

Skin color and pigmentation can be assessed using a wide variety of techniques. The choice depends on the objectives of the investigator. Two main families of methods can be identified, the first is related to the measure of skin color and pigmentation as a whole, ie, as it can be needed in clinical pharmacology for example, where only the information on skin erythema or pigmen-

tation intensities are needed on small test surfaces. The associated technologies are based on different approaches of analysis of reflected light by the skin and gives only spectral information. The second family concerns the diagnostic assessments of pigmented skin lesions in which the accurate measurement of hue and chroma of the substructures of the pigmented lesion are very important, including integration of morphological components in the assessments. In that sense, dermoscopy has been shown to improve the diagnostic accuracy of pigmented skin lesions and some other techniques, such as the multispectral imaging, taking into account the combination of spectral and spatial information, are still in development. Finally, thanks to the improvement of digital image processing technology and to the high quality of the available optics, techniques of epiluminescence microscopy, such as dermoscopy and reflectance confocal microscopy, represent very promising tools allowing the dermatologist to perform a very accurate noninvasive diagnosis of pigmented skin lesions.

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