Melanosome Distribution in Keratinocytes in Different Skin Types: Melanosome Clusters Are Not Degradative Organelles

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The melanosome pattern was characterized systematically in keratinocytes in situ in highly, moderately, and lightly pigmented human skin, classified according to the individual typological angle, a colorimetric measure of skin color phenotype. Electron microscopy of skin samples showed qualitatively and quantitatively that in highly pigmented skin, although melanosomes are mostly isolated and distributed throughout the entire epidermis, clusters are also observed in the basal layer. In moderately and lightly pigmented skin, melanosomes are concentrated in the first layer of the epidermis, isolated—but for most of them, grouped as clusters of melanocores delimited by a single membrane. Electron tomography resolving intracellular three-dimensional organization of organelles showed that clustered melanocores depict contacts with other cellular compartments, such as endoplasmic reticulum and mitochondria. Additionally, immunogold labelling showed that clusters of melanocores do not correspond to autophagosomes or melanophagosomes but that they present, similarly to melanosomes in melanocytes, features of nonacidic, nondegradative organelles. Overall, these observations suggest that melanocore clusters do not correspond to autophagic organelles but represent reservoirs or protective structures for melanosome integrity and function. These results open avenues for understanding the basis of skin pigmentation in different skin color phenotypes.

INTRODUCTION

Melanosomes are organelles where melanin pigment is synthesized within skin melanocytes, in retinal pigment epithelium and choroidal melanocytes. They share features with lysosomes such as the presence of lysosomal membrane proteins and hydrolases; however, they are a distinct lineage of organelles originating from the endocytic pathway and are not accessible to endocytic tracers. Further, they have a higher pH that is optimal for melanin synthesis (Raposo et al., 2001). On this basis, they are classified as lysosome-related organelles (Marks et al., 2013). In the epidermis, at least three mechanisms were proposed for the transfer of melanosomes into neighboring keratinocytes such as cytophagocytosis, exocytosis/endocytosis, or transfer via filopodia (Scott et al., 2002; Wu and Hammer, 2014; Yamamoto and Bhawan, 1994). After transfer, melanosomes in keratinocytes are generally positioned around the nucleus, forming the so-called melanosome microparasol (Byers et al., 2003), which provides photoprotection against sun radiation DNA damage.

Abbreviation: ITA, individual typological angle

Received 9 January 2017; revised 11 September 2017; accepted 23 September 2017; accepted manuscript published online 18 October 2017; corrected proof published online 1 February 2018

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account the diversity of skin color, ITA value allows skin color to be objectively and rigorously described according to six groups on a pigmentation scale ranging from very light to intermediate to tan to brown to dark. Thus, the ITA-based classification offers advantages compared with the historical Fitzpatrick classification of pigmentation prototypes, which remains subjective because it is based on self-reported erythema sensitivity (i.e., sunburn) and tanning ability and is ill adapted to more pigmented populations, especially those of non-white ethnic origin (Pichon et al., 2010).

Additionally, we have explored the morphological and compositional features and nature of the melanosome clusters. We show that the clusters present in moderately and lightly pigmented skin consist of groups of melanocores surrounded by a single outer membrane. These clusters show features of lysosome-related organelles and are not autophagosomes or lysosomes, suggesting that they are not degradative organelles but rather melanosome reservoirs and photoprotective structures.

RESULTS AND DISCUSSION
Melanosomes are differently distributed in human skin depending on pigmentation level
A total of 13 human skin samples representative of the diversity of pigmentation levels were studied, from lightest to darkest (shown on macroscopic pictures and Fontana-Masson staining of skin paraffin sections in Figure 1a). Their pigmentation phenotypes were assessed with a spectrophotometer and classified according to their ITA. ITA values that are correlated to the melanin content (Figure 1b) allowed us to define three groups that include lightly pigmented skin (including very light and light skin, n = 4), moderately pigmented skin (including intermediate, tan, and brown skin, n = 6), and highly pigmented skin (dark skin, n = 3) (Figure 1, and see Supplementary Figure S1 online). Melanosome distribution, as seen by low magnification transmission electron microscopy in the whole epidermis, differs with the pigmentation level. Highly pigmented skin contains up to five times more melanosomes in the overall epidermis than lightly pigmented skin (Figure 1c and 1f, and see Supplementary Figure S2a online). Keratinocytes in the stratum basale contain most melanosomes (around 60%), especially in the supranuclear space. Numerous melanosomes can also be detected in keratinocytes of the stratum spinosum, stratum granulosum, and stratum corneum. In moderately pigmented skin, melanosomes are present in keratinocytes of the stratum basale but are less abundant than in highly pigmented skin (Figure 1d and 1f, and see Supplementary Figure S2a). However, they also represent 60% of the total melanosomes of the epidermis. Melanosomes are still visible in the upper layers (stratum spinosum, stratum granulosum, and stratum corneum) of moderately pigmented skin (Figure 1d and 1f, and see Supplementary Figure S2a). In lightly pigmented skin (Figure 1e and 1f, and see Supplementary Figure S2a), melanosomes are observed mostly in stratum basale (over 80% of melanosomes) and are barely found in stratum spinosum or stratum corneum. In very lightly pigmented skin, only the keratinocytes of the stratum basale display some rare melanosomes (data not shown).

These observations are consistent with previous studies (Konrad and Wolff, 1973; Szabo et al., 1969; Thong et al., 2003), although they mainly reported the distribution of melanosomes in the basal layer. Conversely, our data describe qualitatively and quantitatively the gradients of melanosomes from the basal layer to the stratum corneum in skin types representative of skin tone diversity.

The number, distribution, and size of clusters versus isolated melanosomes varies with skin pigmentation type. In lightly pigmented skin, the ratio of clusters versus isolated melanosomes is almost equal: 55% versus 45%, respectively, (Figure 2c–e, and see Supplementary Figure S2b), showing that melanosomes are not exclusively found as clusters but also appear isolated. Heterogeneity of the melanosome pattern can also be observed in moderately pigmented skin in which keratinocytes contain numerous clusters of melanosomes (Figure 2b). The mean content of clusters is 70% versus 30% of isolated melanosomes (Figure 2d, and see Supplementary Figure S2b).

Overall, the proportion of clusters versus single melanosomes decreases with increasing ITA for moderately and lightly pigmented skin: 80% for tan skin (ITA of 24°) versus 44% for very light skin (ITA of 66°) (Figure 2e). These results differ slightly from the findings of Thong et al. (2003), who reported a higher number of clusters (i.e., 84%) in white skin (Thong et al., 2003). This high level of clusters lets us hypothesize, on the basis of our ITA measurement, that the skin panel used in this previous study could correspond to moderately pigmented skin. The presence of both clusters and individual melanosomes in moderately pigmented skin is not a specificity of Asian skin, as suggested (Thong et al., 2003), but is related to the degree of skin pigmentation.

In highly pigmented skin, keratinocytes of the stratum basale display mainly isolated melanosomes (93%) and only some rare clusters of melanosomes (7%) that are evenly distributed within keratinocytes (Figure 2a and d).

The isolated melanosomes are found to be significantly smaller in moderately and lightly pigmented skin compared with those present in highly pigmented skin (Figure 2f), consistent with previous results (Alaluf et al., 2002; Thong et al., 2003). In highly pigmented skin, clustered small melanosomes are organelles whose size is comparable to large individual melanosomes present in highly pigmented skin. Therefore, it is tempting to conclude that the clusters of melanocores, present only in the basal layer of the epidermis, are able to reach a critical size for an optimal photoprotection, which is especially important for this proliferative cell layer. Likewise, the numerous clusters observed in moderately pigmented skin versus the low number present in lightly pigmented skin may be linked to the natural adaptation of the populations living in a higher UV light environment, whose skin requires more photoprotection (Jablonski and Chaplin, 2010).

Whatever the skin type, naked melanosomes deprived of their melanosomal membrane (melanocores), isolated or within clusters, are surrounded by a single outer membrane (see Supplementary Figure S3a and b online). The membrane contour is revealed by en bloc contrast that allows optimal visualization of membranes. The clustered melanocores are embedded in an electron-dense but heterogeneous granular...
Figure 1. Characteristics of pigmentation and melanosome distribution in skin from different color phenotypes. (a) Macroscopic and histological analysis of skin sample pigmentation: one representative example of skin from each color class and its corresponding Fontana-Masson–stained cross section are illustrated. Mean ITA values and corresponding color class of the collected skin samples are indicated. Scale bar = 50 μmol/L. (b) Significant correlation between melanin content (assessed by image analysis of Fontana-Masson–stained section) and ITA. Based on the ITA values, skin samples were clustered into three pigmentation groups: lightly pigmented (comprising very light and light skin, n = 4), moderately pigmented (including intermediate, tan, and brown skin; n = 6), and highly pigmented (dark skin, n = 3). (c) Observation of melanosome distribution in epidermis by electron microscopy from highly pigmented skin (ITA = -36.1°), (d) moderately pigmented skin (ITA = 19.6°), and (e) lightly pigmented skin (ITA = 57.4°). Circles indicate melanosomes in keratinocytes of Sp, Sg, Sc, or in Sb. Stars indicate pigmented keratinocytes in Sb of epidermis. Scale bar = 4 μm. (f) Histogram shows the number of melanosomes in keratinocytes in the different layers of epidermis in highly, moderately, and lightly pigmented skin. ITA values correspond to those of skin samples in c, d, and e. A total of 15–20 fields were analyzed per layer and per skin phenotype. Int., intermediate; ITA, individual typological angle; Sb, stratum basale; Sp, stratum spinosum; Sc, stratum corneum; Sg, stratum granulosum; Ss, stratum spinosum.
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matrix (inset of Figure 2c) previously described (Szabo et al., 1969). This single membrane-delimiting melanocore is not likely to originate directly from the transferred melanosome or from the plasma membrane of the melanocyte, because melanocores devoid of membranes are observed in the intercellular space (Tarađer et al., 2014).

Three-dimensional organization of isolated and clustered melanocores

Electron tomography of isolated and clustered melanocores was used to determine their organization at the subcellular level in the tissue (Daniele et al., 2014; Hurbain et al., 2008). The clusters of melanocores often show an irregular shape as shown by three-dimensional reconstruction (see Supplementary Movie S1a and b online), and the membrane of the cluster follows the shape and position of the melanocores organized in a network.

The melanocores in clusters show well-delimited contours (see Supplementary Figure S4b and d online) but also fuzzy borders (see Supplementary Figure S4a and c) that make it difficult to distinguish the single melanocores. This suggests that melanin deposits contained in the melanocores within the clusters are not as tightly packed as in isolated melanosomes. Especially in lightly and moderately pigmented skin, the less dense border suggests that pigment may detach from the melanosome fibrillar matrix that normally consists of a physiological amyloid generated by the protein PMEL (Berson et al., 2001; Theos et al., 2005). Because the nature of the granular matrix remains unsolved, we suggest that it could originate from such melanin detachment. Conversely, in isolated melanosomes the melanin forms a dense core delimited by the membrane (see Supplementary Figure S4e–h).

Tomography also showed that melanosomes are in close contact with rough and smooth endoplasmic reticulum and in the perinuclear area with mitochondria (Figure 2g–l). Tubular structures in contact with isolated melanosomes in highly pigmented skin (Figure 2g, inset) or with clusters of melanosomes in moderately and lightly pigmented skin (Figure 2i, inset) are also observed. These short tubules contain granulous material (Figure 2i, inset) and may correspond to endosomal tubules reported to be in close contact with melanosomes, which play key roles in melanosome biogenesis and maintenance (Delevoye et al., 2009). It cannot be excluded that apposed tubules may facilitate the degradation of structural proteins in which melanin is embedded. However, such tubules when emerging from the melanosome could also be proposed to participate in melanosome motility by remodeling the limiting membrane and its contents (Dennis et al., 2016). This suggests that upon transfer to keratinocytes, melanosome biogenesis and homeostasis are likely to continue to maintain melanosome function. The rich organellar environment around melanosomes suggests that, as in melanocytes, melanosomes in keratinocytes depict inter-organellar contact (Daniele et al., 2014). Such contacts may ensure different functions as, for example, lipid or calcium homeostasis that could be essential for melanosome integrity.

Figure 2. Distribution and characterization of isolated and clustered melanocores in skin from different color phenotypes. (a) Keratinocytes of the stratum basale of highly, (b) moderately, and (c) lightly pigmented skin. Melanosomes in highly pigmented skin are essentially isolated (arrows in a, b, and c and inset of a, b, c). In intermediate and lightly pigmented skin, clusters (arrowheads in b and c and insets of b and c) but also isolated melanosomes can be found. (d, e) Statistics show percentage of isolated melanosomes and clusters in the different cell types and (f) the surface area of isolated melanosomes and clusters of melanosomes. A total of 100–900 individual structures were counted or measured for each skin sample, depending on pigmentation status. (e) In histogram, the percentage of clusters and isolated melanosomes are shown for each individual skin according to the ITA. Tomography: three-dimensional structure of melanosomes in relation to surrounding organelles in keratinocytes of the stratum basale from (g) highly, (h) moderately, and (i) lightly pigmented skin. Insets of g and h show tubules (arrows) in contact with the melanosomes. (h, j, l) Models of isolated melanosomes or clusters. Membranes of melanosomes or clusters are represented in red, melanin in black, endosomal tubules or endoplasmic reticulum in white, ribosomes in blue, and mitochondria in yellow. Scale bar in a–c = 1 μm and 400 nm in insets. Scale bar in g, k, and i = 200 nm. Scale bar in h, j, and l = 100 nm. H, highly pigmented skin; ITA, individual typological angle; L, lightly pigmented skin; M (in e), moderately pigmented skin; M (in k), mitochondria; rER (in i), rough endoplasmic reticulum.
lightly pigmented skin, but its distribution was not detailed. Therefore, these observations indicate that clusters of melanosomes and isolated melanosomes do not correspond to autolysosomes/melanophagosomes (see Supplementary Figure S6 and S7). Of interest, labeling for CD63 is observed in isolated melanosomes and clusters in keratinocytes (Figure 4, and see Supplementary Figure S5). The CD63 labeling at the melanosomal and cluster membrane is less dense compared with the labeling observed in melanosomes within melanocytes. Nevertheless, it is significant given the efficiency of labeling in ultrathin cryosections. Furthermore, it put forward the hypothesis that melanosomes transferred to keratinocytes maintain features of lysosome-related organelles, because this tetraspanin is associated with lysosome-related organelles of specialized cell types and is not a lysosomal marker per se (Marks et al., 2013). The non-autophagosomal, non-degradative features of melano cores clusters are reinforced by the observation that the weak base DAMP detected with an anti-DNP [2,4-dinitrophénol] antibody (Anderson et al., 1984) accumulated in endo-/lysosomal structures but was barely detected in the transferred melanosomes and clusters (see Supplementary Figure S8 online). The data together indicate that the clusters show the same features, as defined for melanosomes in particular in skin melanocytes (lysosomal membrane proteins, CD63, pH higher than in lysosomes), but they do not contain lysosomal hydrolases (Raposo et al., 2001). Because the clusters do not correspond to autophagic structures, do not contain LC3, and are not acidic, we hypothesize that they likely correspond to reservoirs of melanin pigment that are maintained in the basal layer of the epidermis.

How are the clusters formed? In cell models and in vivo in skin, it has been observed that transferred melanosomes do not carry proteins present in the limiting membrane of the melanosome in melanocytes, as for example in TYRP-1 (Tarafder et al., 2014). These results put forward a model of melanosome transfer by exocytosis and endocytosis of melanosomes that then form clusters, once uptaken (Tarafder et al., 2014). In

**Figure 3. Distribution of Lamp1 in melanocytes and keratinocytes of the basal layer.** Ultrathin cryosections of different skin phenotypes were immunogold labeled for Lamp1 (PAG10). Scale bar = 200 nm. Relative distribution of Lamp1 apposed on melanosome membrane (arrow) in keratinocyte and melanocyte in the basal layer of highly, moderately, and lightly pigmented skin. HPS, highly pigmented skin; LPS, lightly pigmented skin; MPS, moderately pigmented skin.

![Figure 3](image-url)
this study, in human skin samples and independent of the pigmentation level, we often observed single melanocores in the extracellular space between melanocytes and keratinocytes (data not shown), reinforcing these recent studies. However, one cannot exclude that the different modes of transfer are certainly not mutually exclusive and may depend on the model systems used and region of the skin.

These results are summarized in two models addressing (i) the distribution of melanosomes in skin from different pigmentation phenotypes (see Supplementary Figure S9 online) and (ii) the transfer and organization of melanocores in keratinocytes (Figure 5). Based on the findings, questions and hypotheses regarding the role and formation of the clusters can be raised.

Melanosome disintegration has been viewed as a progressive process depending on the skin type (Ebanks et al., 2011, 2013). In vitro, keratinocytes from light skin show accelerated loss of melanosomes compared with keratinocytes from dark skin. Our observations suggest that clusters are not degradative or autophagic organelles, as previously proposed (Ebanks et al., 2013; Murase et al., 2013; Szabo et al., 1969). Because they do not appear to correspond to degradative acidic structures, the clusters may be photoprotective or reservoir structures that could be quickly mobilized under a UV stress. This hypothesis is supported by the presence of mitochondria and endoplasmic reticulum in close contact with the clusters. Such contacts could contribute to maintain the homeostasis of the clusters, in a

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**Figure 4. Distribution of CD63 in melanocytes and keratinocytes of the basal layer.** Ultrathin cryosections of different skin phenotypes were immunogold labeled for CD63 (PAG10). Scale bar = 200 nm. Relative distribution of CD63 apposed on melanosome membrane (arrow) in \( \text{a, c, e} \) the keratinocyte and \( \text{b, d, f} \) the dendrite of a melanocyte in the basal layer of \( \text{a, b} \) highly, \( \text{c, d} \) moderately, and \( \text{e, f} \) lightly pigmented skin. HPS, highly pigmented skin; LPS, lightly pigmented skin; MPS, moderately pigmented skin.
similar manner as described for isolated melanosomes in melanocytes (Daniele et al., 2014; Delevoye et al., 2009; Hurbain et al., 2008). The function of the clusters in the basal layers deserves further investigation. It is tempting to hypothesize that expanding the surface of melanosomes by clustering them may increase their UV absorbing/filtering efficiency compared with the small isolated melanosomes present in moderately and lightly pigmented skin. Moreover, the membrane-surrounding clusters or isolated melanosomes are potentially equipped with effector molecules that could facilitate their dynamics toward the nucleus and their capping (Byers et al., 2003) to ensure photoprotection.

By enlightening melanosome structure and fate in keratinocytes, these results open avenues to (i) understand the basis of skin pigmentation in the differently colored phenotypes, (ii) unveil natural photoprotection mechanisms, and (iii) develop strategies to modulate pigmentation in health or disease.

MATERIALS AND METHODS

Skin samples
A total of 13 normal skin samples were obtained from surgical leftover residues of breast or abdominal reduction from healthy women (mean age = 29.6 ± 7.9 years). Written informed consent was obtained in accordance with the Helsinki Declaration and with article L.1243-4 of the French Public Health Code. Given its special nature, surgical residue is subject to specific legislation included in the French Code of Public Health (anonymity, gratuity, sanitary/safety rules, etc.). This legislation does not require prior authorization by an ethics committee for sampling or use of surgical waste (see http://www.ethique.sorbonne-paris-cite.fr/nq=node/1767).

Colorimetric measurements of skin samples and ITA determination
Colorimetric measurement was performed on fresh unfixed tissue no later than 24 hours after skin collection. A Mercury 2000 spectrophotometer (Datacolor, Montreuil, France) was used to measure the L* and b* parameters of the standard CIE L*a*b* color space (CIELAB 1976 system from the skin samples). L* expressed the luminance or level of grey from black (value = 0) to white (value = 100). The b* parameter indicates the balance of chrominance between yellow (b* positive) and blue (b* negative) of the skin sample. The ITA in degrees was determined according to the formula $\text{ITA} = \arctan \left( \frac{L^* - 50}{b^*} \right) \times \frac{180}{\pi}$. ITA values taking account of the L* and b* values allow skin color types to be classified into six different groups as previously described (Chardon et al., 1991; Del Bino et al., 2013): very light > 28° > light > 25° > intermediate > 22° > tan > 18° > brown > 15° > dark.

Melanin detection and quantification on skin sections
Pieces of skin were fixed in formaldehyde and embedded in paraffin, and 5-μm paraffin skin sections were stained with Fontana-Masson to show melanin pigments (Nuclear Fast red counterstaining). Melanin content was quantified by image analysis using Histolab software (Microvision Instruments, Evry, France). After determination of a threshold of detection of Fontana-Masson–stained melanin pixels, the area occupied by melanin pixels was measured in the epidermis surface over a defined length of dermal-epidermal junction. Melanin content was estimated by the ratio of the melanin pixel area relative to a standardized length of the dermal-epidermal junction. Melanin content quantification for each skin sample, donor age, ITA, and skin color type are given in Supplementary Figure S1. The relationship between ITA and melanin content determined by analyzing the Fontana-Masson–stained section by the square of correlation coefficient ($r^2$).

Fixation
Tissues were fixed for conventional electron microscopy with 2% paraformaldehyde, 2.5% glutaraldehyde, 0.06% calcium chloride (CaCl2), 0.1 mol/L cacodylate buffer, pH 7.2, at 4°C for 24 hours. Postfixation was performed in 1% osmium tetroxide containing 1.5% potassium ferrocyanide. In some cases, tissue was stained en bloc by a further incubation of the tissue in 1% tannic acid in 0.1 mol/L cacodylate buffer, pH 7.2, for 1 hour at room temperature, followed by a second incubation in 2% osmium tetroxide for 40 minutes at room temperature. For immunolabeling, tissues were

Figure 5. Model of intercellular melanosome transfer and organization of melanosomes in human skin. Single melanosomes surrounded by a membrane are present in the melanocyte dendrite (d). Melanocores, without delimiting membrane, are found in the intercellular space. In keratinocytes (K), melanosomes are isolated or grouped as melanocores within clusters delimited by a single membrane (mb). In clusters, melanocores are embedded within a heterogeneous granular matrix. Isolated melanosomes display a well-delimited contour. Clustered and isolated melanosomes are in close contact with rough (rer) and smooth endoplasmic reticulum (ser) and/or endosomal tubules and also with mitochondria in the perinuclear area.
fixed either with 0.125% glutaraldehyde and 2% paraformaldehyde or with only 2% paraformaldehyde in 0.1 mol/L phosphate buffer.

Electron microscopy

Conventional electron microscopy analysis of human skin epidermis tissues were processed for EPON resin embedding and ultrathin sections and then contrasted with uranyl acetate and lead citrate with the exception of tissue stained en bloc with tannic acid. Protein A-10nm gold conjugate was used for ultracytomyctrometry and immunogold labelling. Tissue blocs were processed as reported (Raposo et al., 2001). All samples were analyzed with a Tecnai Spirit electron microscope (Thermo Fisher Scientific, Eindhoven Netherlands) and digital acquisitions were made with a numeric camera (Quemesa; EMSIS, Münster, Germany). The following antibodies were used for immunolabeling: CD63, mouse anti-human (Abcam, Cambridge, UK), Lamp1, rabbit anti-human (Pharmingen, San Diego, CA), cathepsin V, goat anti-human (R&D Systems, Minneapolis, MN) and LC3A, rabbit anti-human (Abgent, San Diego, CA).

Electron tomography

Epidermis was fixed chemically with 0.125% glutaraldehyde and 2% paraformaldehyde for 1 hour at 4°C and then cut with a razor blade in 200- to 300-μm—thick slices. High pressure freezing was done as described previously (Hurbain et al., 2008). Hexadecane served as a cryoprotectant. Tomographic acquisitions were made from 300-nm—thick sections, and three dimensional reconstructions were performed as described (Heiligenstein et al., 2014).

Image analysis and quantification

Areas and percentage of melanosomes and clusters were evaluated on randomly selected cell profiles from three different individuals per phenotype group (highly, moderately, and lightly pigmented skin) within keratinocytes of the stratum basale using iTEM software (Soft Imaging System, EMSIS, Germany). The definition of the distinct compartments was based on their morphology. The mean area of each group was deduced, and significance of phenotype-related alterations was determined using Student test, in which a P value less than 0.05 was considered statistically highly significant. The size of measured structures was expressed in μm² × 10⁻². Depending on the skin’s pigmentation status, 100 to 900 individual structures were counted or measured for each skin.

For evaluation of melanosomes per area in all epidermal layers, 150—20 images were acquired per layer randomly on one skin per phenotype group, and 700—2,500 μm² were evaluated. The number of melanosomes was expressed as number per 100 μm². For evaluation of distribution of LAMP1 and CD63 in keratinocytes and melanocytes in the three different skin phenotypes (highly, moderately, and lightly pigmented), 25 images were randomly acquired on one skin per phenotype. Overall, 125 melanosomes were evaluated in keratinocytes in lightly pigmented skin, whereas 1,300 melanosomes were counted in highly pigmented skin, and 40—100 melanosomes were evaluated in dendrites of melanocytes. Gold particles associated with melanosomes were counted to obtain an average and the standard deviation.

CONFLICT OF INTEREST

PS, EB, CM, FB, and CD are full employees of L’Oreal Research and Innovation.

ACKNOWLEDGMENTS

We are grateful to Cedric Delevoye and Guillaume van Niel for fruitful discussions and Allison de Horsey (St. George’s School, Newport, Rhode Island) for reading the manuscript. This work was supported by the French National Research Agency through the Investments for the Future program (France-BiImaging, ANR-10-INSB-04). We acknowledge the PICT-IIBSA, member of the France-BiImaging national research infrastructure, supported by the CellTisPhyBio Labex (no. ANR-10-LBX-0030) part of the Initiative d’Excellence Paris Lettres (no. ANR-10-IDEX-0001-02 PSL). Research in our group is supported by the Fondation pour la Recherche Médicale (Equipes FRM), Association de Recherche pour le Cancer (ARC), Indian French cooperation (CEFIPRA), and L’Oréal Research and Innovation.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2017.09.039.

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