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Regulation of Melanosome Transfer to and Distribution in Keratinocytes

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Skin pigmentation, also known as complexion coloration, results from the biosynthesis of melanin within the melanocytes of the Stratum basalum and the subsequent transfer, translocation, and degradation of this pigment to, in, and by the neighboring keratinocytes respectively. Melanins are produced and retained in melanosomes synthesized in the cell body that are translocated along the dendrites using microtubules via motor proteins. Melanosomes are eventually captured and retained at the tips of dendrites by attachment to the peripherally localized actin. Melanosomes reaching the dendritic tips are transferred to keratinocytes, primarily via phagocytosis of released melanosomes by keratinocytes. Molecules responsible for cell/cell recognition and interaction that regulate transfer are being identified. Some of these putative mediators appear to be affected by ultraviolet radiation. After the keratinocytes receive melanosomes, the granules are distributed individually or as clusters in dark versus light skin respectively. These melanosomes are then aggregated over the nucleus for photoprotection of keratinocyte DNA and eventually degraded.

Key words: Melanocyte, complexion, melanogenesis, keratinocyte, melanosome transfer

Melanosome Transfer along Dendrites

Pigmented melanosome generated within the cell body of the melanocytes are translocated down the dendrites for transfer to neighboring keratinocytes. Cytoskeletal elements facilitate this movement of melanosome. Movement of melanosomes along dendrites in mammalian melanocytes occurs bi-directionally along microtubules as recently demonstrated by Hammer et al. [1]. Using time-lapse photography, bi-directional movement of melanosomes from the cell center to the periphery and back again was visualized. Motor proteins are responsible for this movement. Kinesin propels the melanosomes towards the plus ends of the filaments [2] whereas dynesin putatively moves the melanosomes in the opposite direction [3]. Melanosomes reaching the tips of dendrites are captured on actin by myosin Va to prevent their centripetal re-trafficking [4, 5]. The capture of melanosomes in the dendritic tips relies on Rab 27a, a member of the family of Rab GTPases involved in vesicular movement/fusion [6]. Rab 27a recruits myosin onto the melanosome surface [7, 8]. In addition, melanophilin, a member of the RAB effector family, regulates melanosome movement along dendrites [9] and is necessary for the association of myosin-Va with the melanosome [10, 11]. It was proposed from these studies that a molecular complex of at least Rab27a, Myo-5, and Mlph, and possible other proteins, facilitate the transport of melanosomes along the dendrites and their ultimate capture at the distal ends. The summation of these forces results in the melanosomes reaching and being retained in the tips of the dendrites, positioned for transfer.

Several human diseases have recently been identified that result in disrupted translocation/capture of melanosomes in the dendritic tip. The form of Griscelli Syndrome combining partial albinism with severe immunodeficiency results from mutations in *RAB27A* [12], while the form of Griscelli Syndrome associated with neurological disease results from mutations in *MYO5A* [13, 14].

Transfer of Melanosomes from the Melanocyte to the Keratinocyte

Melanosomes captured and aggregated in the dendritic tips are then extruded and incorporated into neighboring keratinocytes. The process of transfer has not been delineated clearly. Various mechanisms have been proposed (reviewed in Jimbow & Sugiyama [15]) that consist of (i) the release of melanosomes by the melanocytes and the subsequent endocytosis of the released granules by the keratinocyte; (ii) the keratinocyte engulfing the dendritic tips of the melanocytes by active phagocytosis and incorporating portions of the melanocytes within them [i.e., cytophagocytosis] [16]; (iii) the active transfer or

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injection of melanosomes by the melanocyte directly into keratinocytes; and (iv) development of a continuous pore developing between the plasma membrane of the melanocytes and the keratinocytes through which the melanosomes are passed. Recently, experiments by Hearing et al. demonstrated that transfer of melanosomes to keratinocytes occurs predominantly via the first postulated mechanism, i.e., phagocytosis of released melanosomes by keratinocytes [17].

It has been hypothesized that plasma membrane molecules of both the melanocyte and the keratinocyte must exist to facilitate the recognition, the physical interaction, and the subsequent transfer of material between these two cell types. Plasma membrane glycoproteins and lectins are categories of putative mediators. Kieda et al demonstrated that various lectins or neoglycoproteins could inhibit melanosome transfer between human melanoma cells and squamous cell carcinoma-derived keratinocytes in co-culture [18, 19]. We have recently developed an *in vitro* model in which melanosome transfer between normal human skin-derived melanocytes and keratinocytes in co-culture can be monitored and quantitated [20]. In this model, melanocytes were pre-labeled with a fluorescent dye, succinimidyl ester of carboxy fluorescein diacetate, and in subsequent co-cultures with keratinocytes the transfer of dye was monitored by confocal microscopy and quantitated by flow cytometry. The transfer of melanosomes was subsequently confirmed by electron microscopy. With this model system, we confirmed the observations of Keida et al [18, 19] that various lectins and neoglycoproteins could inhibit the transfer of melanosomes to keratinocytes. Recently, niacinamide, the physiologically active amide of niacin known as vitamin B3 that exhibits skin-lightening effects, also inhibits melanosome transfer in our model system [21].

A major regulator of melanosome transfer is the Protease-activated receptor-2 (PAR-2), a seven transmembrane G-protein-coupled receptor on the plasma membrane that is cleaved extracellularly by serine proteases resulting in self-activation [22]. PAR-2 receptors are on keratinocytes [23] and their activation results in increased phagocytic activity of keratinocytes towards melanosomes [24]. Seiberg et al demonstrated that upregulation of PAR-2 activity with a synthetic peptide (SLIGRL) or the downregulation with trypsin inhibitors resulted in darkening or lightening respectively of *in vitro* epidermal equivalents, as well as in pigmented skin of Yucatan swine and in human skin transplanted onto SCID mice [23-25].

Translocation, Distribution, and Degradation of Melanosomes by the Keratinocyte

Once melanosomes are transferred into the recipient keratinocytes they are selectively and predominantly translocated to the apical pole of the keratinocyte where they can effectively absorb incident ultraviolet light and protect the underlying nucleus from mutagenic damage. Byers and Maheshwary [26] have reported that dynein co-localizes with phagocytosed melanosomal aggregates throughout the cytoplasm, predominantly at the microtubule-organizing center in keratinocytes.

The distribution of recipient melanosomes within the keratinocytes varies according to complexion coloration [27, 28]. In dark skinned individuals, melanosomes are approximately 0.8 microns in diameter and are maintained as individual organelles throughout the cytosol of the keratinocyte. In contrast, in light skinned individuals melanosomes are significantly smaller that 0.8 microns and are aggregated into membrane bound clusters of four to eight organelles. It is uncertain whether these distinct distribution patterns are determined by factors within the transferred melanosome or is innate to the recipient keratinocytes. In a recent study, melanosome distribution in keratinocytes of an *in vitro* skin reconstruction model utilizing combinations of keratinocytes and melanocytes from different complexion colorations was assessed and the investigators concluded that the distribution pattern of recipient melanosomes was dictated by the type of donor melanocyte [29]. In contrast, we have recently demonstrated in melanocyte/keratinocyte co-cultures that the distribution pattern of transferred melanosomes is regulated by the skin type from which the recipient keratinocyte was derived and unrelated to melanosome size [30].

Effect of Ultraviolet Irradiation

Ultraviolet irradiation (UVR) of the skin results in increased cutaneous pigmentation by immediate pigment darkening (IPD) and a delayed pigment darkening (DPD) mechanisms 9for review see Gilchrest et al. [31]0. The primary consequence of UVR resulting in DPD occurs via the upregulation of melanin synthesis within the melanocytes of the irradiated skin and the subsequent increase in melanin transfer to and content of the epidermal keratinocytes [32]. In contrast, the relatively transient IPD appears to result from rapid changes in orientation of cytoskeletal elements, translocation of the melanosomes down dendrites, transport of melanosomes to the keratinocytes, and altered distribution of melanosomes within the keratinocytes [33]. Therefore, one of the mechanisms responsible for UVR pigmentation of the skin is an increase in the immediate and/or delayed transfer of melanosomes to neighboring keratinocytes. UVR has been demonstrated to increase melanocyte dendricity in the skin [34], possibly mediated by endothelin-1 released by irradiated keratinocytes [35].

Mediators of melanosome transfer from the melanocyte to the keratinocyte are also affected by UVR. Expression of lectin binding receptors on melanoma cells is up-regulated by UVR [36]. The function of PAR-2, responsible for mediating melanosome transfer by regulating keratinocyte phagocytosis, also appears to be influenced by UVR. It has previously been demonstrated that inhibition of PAR-2 prevented UVR induced pigmentation [24]. Recently, Scott et al demonstrated that expression of PAR-2, generally restricted to the basal epithelial layers, is dramatically upregulated throughout the epidermis upon UV irradiation in skin types II and III and delayed in skin type I [37]. In addition, cultured keratinocytes demonstrated an increase in PAR-2 cleavage activity after UVR [37]. Finally, UVR increased the phagocytic activity of cultured murine keratinocytes [17].

Conclusion

Skin pigmentation/complexion coloration is regulated by the constitutive amount and type of melanin synthesized by the melanocyte, the facultative regulation of this melanin synthesis, and the transfer and maintenance of melanosomes in the keratinocytes. To date a few regulators of this latter process have been identified or eluded to. Further experimentation is needed to more accurately define the molecular/cellular process of melanosome transfer from the epidermal keratinocyte.

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