Basis for Enhanced Barrier Function of Pigmented Skin

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Humans with darkly pigmented skin display superior permeability barrier function in comparison with humans with lightly pigmented skin. The reduced pH of the stratum corneum (SC) of darkly pigmented skin could account for enhanced function, because acidifying lightly pigmented human SC resets barrier function to darkly pigmented levels. In SKH1 (nonpigmented) versus SKH2/J (pigmented) hairless mice, we evaluated how a pigment-dependent reduction in pH could influence epidermal barrier function. Permeability barrier homeostasis is enhanced in SKH2/J versus SKH1 mice, correlating with a reduced pH in the lower SC that colocalizes with the extrusion of melanin granules. Darkly pigmented human epidermis also shows substantial melanin extrusion in the outer epidermis. Both acute barrier disruption and topical basic pH challenges accelerate reacidification of SKH2/J (but not SKH1) SC, while inducing melanin extrusion. SKH2/J mice also display enhanced expression of the SC acidifying enzyme, secretory phospholipase A2f (sPLA2f). Enhanced barrier function of SKH2/J mice could be attributed to enhanced activity of two acidic pH-dependent, ceramide-generating enzymes, β-glucocerebrosidase and acidic sphingomyelinase, leading to accelerated maturation of SC lamellar bilayers. Finally, organotypic cultures of darkly pigmented human keratinocytes display enhanced barrier function in comparison with lightly pigmented cultures. Together, these results suggest that the superior barrier function of pigmented epidermis can be largely attributed to the pH-lowering impact of melanin persistence/extrusion and enhanced sPLA2f expression.

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INTRODUCTION

Because life in a terrestrial environment requires a highly competent permeability barrier, we proposed recently that interfollicular pigmentation evolved as a strategy to enhance epidermal barrier function in hominids dwelling in the UV-B-enriched, arid milieu of Sub-Saharan Africa (Elias *et al.*, 2009, Elias *et al.*, 2010). Although pigmentation provides additional functional advantages, such as thermal insulation, camouflage, and defense against photocarcinogenesis (Jablonski, 2006), the imperative to generate a competent permeability barrier is paramount. In support of this hypothesis, we showed that humans with darkly pigmented skin (type IV/V – Fitzpatrick scale) display superior barrier function in comparison with age-, gender-, and occupation-matched humans with lightly pigmented skin (Type I/II), independent of ethnicity (Reed *et al.*, 1995, Gunathilake *et al.*, 2009). Moreover, in a large, homogeneous Chinese population, epidermal barrier function was inferior in stable, noninflamed, depigmented (vitiliginous) skin in comparison with adjacent, pigmented skin sites in the same individuals (Liu *et al.*, 2010).

The lower pH of darkly pigmented human stratum corneum (SC) could account for enhanced barrier function (Gunathilake *et al.*, 2009), because acidification enhances epidermal structure and function by multiple mechanisms (e.g., (Mauro *et al.*, 1998; Hachem *et al.*, 2003)). Accordingly, acidification of the SC of lightly pigmented human subjects ''resets'' (improved) barrier function to levels found in darkly pigmented humans (Gunathilake *et al.*, 2009, Hachem *et al.*, 2010). Nevertheless, how pigmentation influences epidermal function remains uncertain. Melanocytes could regulate

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Abbreviations: Hr^{rh}, hairless mice gene; KC, keratinocyte; SC, stratum corneum; SG, stratum granulosum; sPLA2f, secretory phospholipase A2f; TMG, tetramethylguanidine; TYR, tyrosinase

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epidermal function (including acidification) by paracrine influences (Slominski and Paus, 1990, Slominski *et al.*, 1993), and/or by ''juxtacrine'' effects of melanocytes on neighboring keratinocytes (KC). Melanosomes are acidic organelles that enclose several proteins in the eumelanin synthetic pathway (Moellmann *et al.*, 1988; Schallreuter *et al.*, 2008), whose activities require a reduced pH (Chen *et al.*, 2002). Although the internal pH of melanosomes increases immediately before secretion (Ancans *et al.*, 2001), our studies showed that putative melanosomes in dendrites of darkly pigmented human melanocytes are more acidic than comparable vesicular structures in lightly pigmented subjects (Gunathilake *et al.*, 2009). Yet, how the subsequent transfer of melanosomes affects the pH of the epidermis *in vivo* remains unknown.

We explored this issue here in two closely related mouse strains (SKH2/J-Hr^{rh}/Hr^{rh} (hairless pigmented inbred) and Crl:SKH1 (outbred hairless and nonpigmented)) that exhibit defined differences in both the extent and localization of pigmentation. Permeability barrier homeostasis is superior in SKH2/J mice, which correlates with a reduced pH in the lower SC in these mice. Not only the persistence of engulfed melanosomes but also their subsequent delayed degradation and extrusion into the outer epidermis of SKH2/J mice (and darkly pigmented human skin) correlates with acidification of these sites, as well as accelerated processing of secreted lamellar body–derived lipids into mature lamellar bilayers. Moreover, experimental maneuvers that increase the pH of the SC provoke more rapid reacidification of the SC, and accelerate melanin granule extrusion, in SKH2/J mice. Finally, we showed that melanized KCs display superior barrier function in comparison with lightly pigmented KCs in organotypic human KCs. Together, these results show that pigmentation enhances barrier function by a hitherto unrecognized, juxtacrine (acidifying) cellular mechanism.

RESULTS

Distinctive differences in melanocyte and melanin localization in SKH1 and SKH2/J epidermis

Adult SKH1 mouse skin appears nonpigmented (Figure 1a), and it lacks Fontana–Masson-positive melanin staining in the epidermis (Figure 1b), as well as an absence of the melanocyte marker Mel-5 (not shown). Nevertheless, neonatal SKH1 skin contains dendritic cells, identified as melanoblasts by western blotting and by immunohistochemical staining for tyrosinase-related protein and dopachrome tautomerase (Figure 1d), and melanocytes could be cultured from neonatal SKH1 skin (Supplementary Figure S1 online). In contrast, both Mel-5-positive melanocytes and abundant melanin are present in SKH2/J mouse epidermis (Figure 1c), where they localize solely to the interfollicular epidermis—neither melanocytes nor melanin could be detected below the follicular infundibulum of SKH2/J mice (Figure 1c). On the basis of this background information, we deployed these two closely related hairless mouse models to assess the impact of epidermal pigmentation on a variety of cutaneous functions, as well as to address potential cellular and metabolic mechanisms that could account for the putative, pigmentation-induced enhancement of epidermal barrier function.

Pigmentation positively impacts barrier function by acidifying the outer epidermis

We first assessed the role of pigmentation in regulating epidermal structure and function by quantifying differences

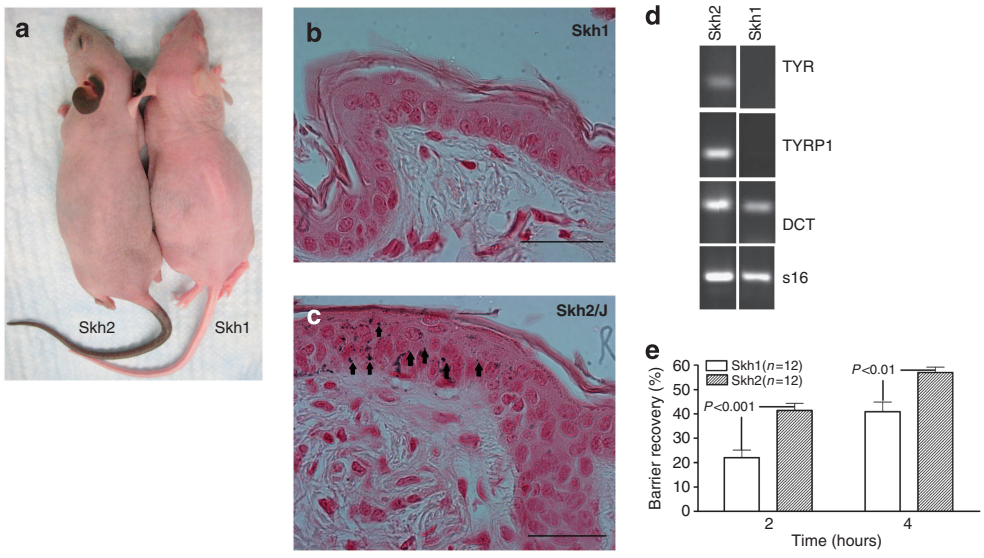

Figure 1. Localization of melanocytes and pigmentation differs in Skh1 versus Skh2/J epidermis. (**a**) Appearance of SKH2/J and SKH1 mice. Note intense pigmentation of face, ears, and tail and moderate pigmentation of shoulders and flanks in SKH2/J mice. (**b**, **c**) Note the absence of Fontana–Masson staining for melanin in adult SKH1, with moderate staining for melanin in the interfollicular epidermis of SKH2/J mice (**c**, arrows). (**d**) Adult SKH2/J mouse skin, but not SKH1 skin, expresses the melanocyte differentiation markers, tyrosinase (TYR) and tyrosinase-related protein 1 (TRYP1). Both mouse strains express the melanoblast differentiation marker dopachrome tautomerase (DCT), also known as tyrosinase-related protein 2. (**e**) After a comparable extent of acute barrier disruption by sequential tape stripping, recovery kinetics accelerate significantly in SKH2/J versus SKH1 mice. All shown data reflect mean ± SEM. Scale bar = 10 μm.