Skin Barrier Development Depends on CGI-58 Protein Expression during Late-Stage Keratinocyte Differentiation

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Adipose triglyceride lipase (ATGL) and its coactivator comparative gene identification-58 (CGI-58) are limiting in cellular triglyceride catabolism. Although ATGL deficiency is compatible with normal skin development, mice globally lacking CGI-58 die postnatally and exhibit a severe epidermal permeability barrier defect, which may originate from epidermal and/or peripheral changes in lipid and energy metabolism. Here, we show that epidermis-specific disruption of CGI-58 is sufficient to provoke a defect in the formation of a functional corneocyte lipid envelope linked to impaired ω-O-acylceramide synthesis. As a result, epidermis-specific CGI-58-deficient mice show severe skin dysfunction, arguing for a tissue autonomous cause of disease development. Defective skin permeability barrier formation in global CGI-58-deficient mice could be reversed via transgenic restoration of CGI-58 expression in differentiated but not basal keratinocytes suggesting that CGI-58 is essential for lipid metabolism in suprabasal epidermal layers. The compatibility of ATGL deficiency with normal epidermal function indicated that CGI-58 may stimulate an epidermal triglyceride lipase beyond ATGL required for the adequate provision of fatty acids as a substrate for ω-O-acylceramide synthesis. Pharmacological inhibition of ATGL enzyme activity similarly reduced triglyceride-hydrolytic activities in wild-type and CGI-58 overexpressing epidermis implicating that CGI-58 participates in ω-O-acylceramide biogenesis independent of its role as a coactivator of epidermal triglyceride catabolism.

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INTRODUCTION

Humans carrying mutant alleles of the lipolytic coactivator comparative gene identification-58 (CGI-58), also designated as α/β-hydrolase domain-containing 5, develop neutral lipid storage disease with ichthyosis (NLSDI) (Lefèvre et al., 2001; Schweiger et al., 2009). In mice, the phenotype of CGI-58 deficiency is even more severe leading to premature lethality soon after birth due to a defect in the transepidermal barrier function of the skin (Radner et al., 2010, 2011). CGI-58 is a cofactor required for the stimulation of the enzymatic activity of adipose triglyceride lipase (ATGL), the rate-limiting enzyme in the catabolism of intracellular triglyceride (TG) deposits in most if not all organs of the body (Lass et al., 2006, 2011; Zierler et al., 2014). Remarkably, humans and mice harboring mutant *ATGL* alleles show normal skin development and function indicating that CGI-58 possesses an ATGL-independent role in the skin. The fact that CGI-58 is critically required for the ATGL-mediated TG catabolism in multiple organs of the body including liver, muscle, and adipose raised the question whether the skin barrier defect and postnatal lethality of mice globally lacking CGI-58 (*Cgi-58*$^{-/-}$) solely originates from the lack of epidermal CGI-58 or from changes in whole-body TG and energy homeostasis.

To investigate the epidermis-specific role of CGI-58 in skin development, we disrupted CGI-58 expression exclusively in keratinocytes and examined the consequences on epidermal barrier formation and systemic lipid and energy homeostasis. Moreover, we generated CGI-58-deficient mice solely expressing CGI-58 in an early or late stage of keratinocyte differentiation to unravel the temporal and spatial role of CGI-58 in epidermal development. Finally, we examined the role of CGI-58 in ATGL-dependent and -independent

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Abbreviations: ATGL, adipose triglyceride lipase; CE, cornified envelope; CGI-58, comparative gene identification-58; CLE, corneocyte lipid envelope; En, embryonic day n; FA, fatty acid; FLG, filaggrin; IVL, involucrin; Kn, keratin n; NLSDI, neutral lipid storage disease with ichthyosis; ω-O-AcylCer, ω-O-acylceramide; ω-OH-Cers, ω-hydroxy-ceramides; SC, stratum corneum; TEM, transmission electron microscopy; TEWL, transepidermal water loss; TG, triglyceride; WT, wild type

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epidermal TG catabolism to address whether CGI-58 may additionally activate a yet unknown epidermal TG lipase required for efficient fatty acid (FA) supply as a substrate for the synthesis of complex epidermal lipids.

RESULTS

Epidermal CGI-58 is a prerequisite for functional skin barrier formation

To generate mice lacking CGI-58 exclusively in the epidermis, we bred mice expressing the *Cre* recombinase transgene under the control of the epidermis-specific human keratin 14 (*K14*) promoter (Vasioukhin et al., 1999) with mice homozygous for the *Cgi-58-floxed* allele (*Cgi-58*$^{flox/flox}$) (Zierler et al., 2013). Very similar to mice globally lacking CGI-58, *Cgi-58*$^{flox/flox}$ mice heterozygous for the *K14-Cre* recombinase transgene (*Cgi-58*$^{epid-/-}$) died within 12 hours after birth and exhibited a glossy tight skin (Figure 1a). Western blotting experiments revealed that CGI-58 protein expression was not detectable in the epidermis of *Cgi-58*$^{epid-/-}$ mice, mildly reduced in the dermis, and unchanged in liver, lung, and heart when compared with levels in *Cgi-58*$^{flox/flox}$ controls (Supplementary Figure S1 online). Reduced CGI-58 protein expression in the tongue is in accordance with K14 expression in tongue epithelial cells (Vasioukhin et al., 1999). Morphologically, newborn *Cgi-58*$^{epid-/-}$ mice were smaller and wet weight was reduced (−25%) compared with *Cgi-58*$^{flox/flox}$ littermates (Figure 1b). Plasma energy substrates including FA, glycerol, TG, and glucose were markedly decreased in *Cgi-58*$^{epid-/-}$ mice (ranging from −47% to −86%) compared with controls (Table 1), which may derive from the absence of suckling in newborn *Cgi-58*$^{epid-/-}$ mice.

The skin of *Cgi-58*$^{epid-/-}$ mice exhibited intense penetration of a toluidine blue solution characteristic for a severe defect in the epidermal water permeability barrier (Figure 1c). Histological analyses of skin sections by light or transmission electron microscopy (TEM) revealed orthohyperkeratosis with a strongly condensed *stratum corneum* (SC) and a relatively thin layer of granular keratinocytes (Figure 1d and e). Consistent with this ichthyosiform skin phenotype, degradation of corneodesmosomes was decelerated in *Cgi-58*$^{epid-/-}$ SC indicative of an abnormal desquamation. In fact, TEM revealed retained nonperipheral corneodesmosomes up to the upper SC layers of *Cgi-58*$^{epid-/-}$ epidermis (Figure 1f), which are normally degraded during corneocyte maturation (Ishida-Yamamoto and Igawa 2014). Correspondingly, desmoglein 1, a major extracellular component of corneodesmosomes, was undetectable in the SC of control mice but preserved throughout the SC of *Cgi-58*$^{epid-/-}$ mice (Figure 1g).

Next, the expression and distribution of proteins representative for basal or hyperproliferative keratinocytes were examined by immunohistochemistry of skin sections. K6 was massively stained in keratinocytes of the upper *stratum spinosum* and *stratum granulosum* throughout the interfollicular epidermis of *Cgi-58*$^{epid-/-}$ mice (Figure 1h, upper panel) compared with the unique localization of K6 to hair follicles in *Cgi-58*$^{flox/flox}$ controls. K14 was exclusively present in the basal and lower spinous epidermal layers of controls, whereas the protein was more diffusely distributed reaching keratinocytes of the upper *stratum spinosum* and *stratum granulosum* (Figure 1h, middle panel) in *Cgi-58*$^{epid-/-}$ mice. Expression of filaggrin (FLG), a marker for terminally differentiated keratinocytes (Sandilands et al., 2009), was reduced in *Cgi-58*$^{epid-/-}$ epidermis compared with controls (Figure 1h, lower panel), indicating a delay in keratinocyte differentiation. The latter was in line with TEM findings, revealing smaller proFLG containing F-granules in *Cgi-58*$^{epid-/-}$ granular keratinocytes (Figure 1e). Western blot analysis of FLG protein expression further showed insufficient proFLG processing with increased abundance of multimeric, nonprocessed FLG units at the expense of active, monomeric FLG in *Cgi-58*$^{epid-/-}$ compared with *Cgi-58*$^{flox/flox}$ epidermis (Figure 1i). In accordance with delayed keratinocyte differentiation, protein expression of loricrin, the main constituent of the cornified envelope (CE) expressed late during cornification (Candi et al., 2005), was strongly decreased in epidermal extracts of *Cgi-58*$^{epid-/-}$ mice (Figure 1j, upper panel). In contrast, protein expression of involucrin (IVL), which is an early marker in CE formation, was only marginally reduced (Figure 1j, lower panel). Taken together, these findings demonstrate that similar to global CGI-58 deficiency, epidermis-specific deletion of CGI-58 causes a defect in the epidermal permeability barrier that is linked to abnormal desquamation and delayed keratinocyte differentiation. Thus, tissue autonomous alterations in epidermal keratinocyte metabolism are causative for defective epidermal development in global CGI-58-deficient mice.

CGI-58 is essential for embryonic skin barrier formation

Cgi-58$^{epid-/-}$ (and *Cgi-58*$^{-/-}$) mice show severe ichthyosis already at birth indicating that CGI-58 is critically required for prenatal skin barrier formation. Thus, we explored the role of CGI-58 in embryonic skin development using both an inward skin permeability assay that reveals first stages in skin barrier formation (Hardman et al., 1998) and a gravimetric measurement of the transepidermal water loss (TEWL) (Hanley et al., 1996; Nolte et al., 1993). The epidermal barrier forms late in embryogenesis starting at embryonic day (E) 16.5 of gestational age (Hardman et al., 1998), which is in accordance with the intensive toluidine blue staining of *Cgi-58*$^{flox/flox}$ and *Cgi-58*$^{epid-/-}$ embryos at E15.5 (Figure 2a). At E16.5, both *Cgi-58*$^{flox/flox}$ and *Cgi-58*$^{epid-/-}$ mice showed a reduction in dorsal toluidine blue staining indicating the initiation of barrier acquisition. At this stage, the extent of TEWL was comparable between *Cgi-58*$^{flox/flox}$ and *Cgi-58*$^{epid-/-}$ skin explants (Figure 2b) despite the apparent lack of CGI-58 in the epidermis (Figure 2c). Barrier function was almost complete at E17.5 in *Cgi-58*$^{flox/flox}$ control mice as evident from marginal toluidine blue staining and a drastic reduction in TEWL values (−88%). Curiously, *Cgi-58*$^{epid-/-}$ mice also established a pronounced toluidine blue-resistant barrier at E17.5 (Figure 2a) and exhibited functional proFLG processing (Figure 2d), indicative of proper keratinocyte differentiation at this stage. In line, TEWL levels markedly decreased in E17.5 *Cgi-58*$^{epid-/-}$ skin explants (−60%), yet to a lesser extent compared with *Cgi-58*$^{flox/flox}$ controls (Figure 2b). Concomitant with a marked increase in covalently bound ω-hydroxy-ceramides (ω-OH-Cer) (Figure 2e), TEWL decreased to the level of neonates in E18.5 *Cgi-58*$^{flox/flox}$ embryos (Figure 2b) indicating full barrier competence.