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ORIGINAL ARTICLE

GPHR-Dependent Functions of the Golgi Apparatus Are Essential for the Formation of Lamellar Granules and the Skin Barrier

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The lumen of the Golgi apparatus is regulated to be weakly acidic, which is critical for its functions. The Golgi pH regulator (GPHR) is an anion channel essential for normal acidification of the Golgi apparatus, and is therefore required for its functions. The Golgi apparatus has been thought to be the origin of lamellar granules in the skin. To study the functional role(s) of GPHR in the skin, we established keratinocyte-specific *GPHR*-knockout mice using the Cre-*loxP* system. These mutant mice exhibited hypopigmented skin, hair loss, and scaliness. Histological examination of *GPHR*-knockout mice showed ballooning of the basal cells and follicular dysplasia. In addition, inflammatory cells were seen in the dermis. The expression of *trans*-Golgi network 46, a marker for lamellar bodies, and kallikrein 7, a protein within lamellar bodies, is diminished in *GPHR*-knockout mouse skin. Examination by electron microscopy revealed that keratinocytes produced aberrant lamellar bodies. The transepidermal water loss of these knockout mice was increased compared with wild-type mice. Moreover, expression of cathelicidin-related antimicrobial peptide (CRAMP) in the skin was diminished. These results suggest that GPHR is essential for the homeostasis of the epidermis including the formation of lamellar bodies and for the barrier function.

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INTRODUCTION

Organelles located in secretory and endocytotic pathways are known to acidify their lumens, and therefore they are called acidic organelles. Compromising the acidic environments of those organelles using compounds such as monensin, bafilomycin, and ammonium chloride, which do not specifically affect the Golgi apparatus, causes marked effects on trafficking, processing, and glycosylation of proteins and lipids (Weisz, 2003), although the mechanisms by which those processes are regulated by the acidic pH are largely unknown. Recently, we identified a new anion channel named Golgi pH regulator (GPHR) (Maeda *et al.*, 2008). GPHR functions as a counterion channel and is critical for Golgi acidification. The loss of GPHR function results in increased luminal pH, which in turn causes impaired transport, disrupted glycosylation, and abnormal Golgi morphology; thus, GPHR is indispensable for normal Golgi functions (Maeda *et al.*, 2008). As GPHR is localized in the Golgi, increased pH and impaired functions are observed in the Golgi selectively among acidic organelles (Maeda *et al.*, 2008). Lamellar granules include lipids, proteases, protease inhibitors, and proteins (Elias *et al.*, 1998; Madison, 2003; Ishida-Yamamoto *et al.*, 2004; Elias and Choi, 2005) that are needed to generate the skin barrier (Odland and Holbrook, 1981), and functional defects in these factors lead to impaired barrier formation. The origin of lamellar granules has been thought to be the *trans*-Golgi network (TGN; Elias *et al.*, 1998), but direct evidence for that has not been reported. If the origin of lamellar granules is the Golgi apparatus, impairing Golgi functions should result in the degeneration of lamellar granules. Here we show that skin-specific

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Abbreviations: CRAMP, cathelicidin-related antimicrobial peptide; GPHR, Golgi pH regulator; HPRT, hypoxanthine phosphorybosyl transferase; K, keratin; KLK7, kallikrein 7; PBS, phosphate-buffered saline; TEWL, transepidermal water loss; TGN, trans*-Golgi network*

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knockout of *GPHR* function markedly impairs the formation of lamellar granules, supporting the fact that they originate from the Golgi apparatus. The results further show that GPHR has a critical role in the skin barrier function, as well as in the development of other tissues.

RESULTS

Generation of skin-specific GPHR-knockout mice using the Cre-*loxP* system

We used the Cre-*loxP* system to disrupt the *GPHR* gene specifically in the skin. To generate the *GPHR*-targeting construct, one loxP site was inserted into intron 3 and the other *loxP* site was inserted into intron 5 of the *GPHR* gene (Figure 1a). A neomycin resistance gene was placed downstream of the first *loxP* site and was sandwiched with *FLP* sequences to delete the *Neo* gene after embryonic stem cell selection. Using this construct, we created *GPHR* flox mice. K5-Cre:$GPHR^{w/f}$ (contains *loxP* in one *GPHR* allele and K5-Cre transgene) mice and $GPHR^{f/f}$ (both *GPHR* alleles contain *loxP*) mice were mated to analyze the function of GPHR in the skin. First, we isolated keratinocytes from newborn mice to confirm whether the GPHR protein was deleted in the K5-cre:$GPHR^{f/f}$ keratinocytes. As expected, the expression of the GPHR protein was completely deficient in K5-Cre:$GPHR^{f/f}$ keratinocytes (Figure 1b). K5-Cre:$GPHR^{f/f}$ mice showed growth retardation and impaired development of their earlobes and external genitals in addition to the skin, and about half of them died within 1 month, most likely because of hydrolysis, but the precise reason is not yet known (Supplementary Figure S1a, S1b online). We characterized the stomach and esophagus of K5-Cre:$GPHR^{f/f}$ mice, but there was no abnormality that could have led to the mice not being able to eat (Supplementary Figure S1c, S1d, S1e, S1f online).

Analyses of skin-specific GPHR-knockout mice

The appearance of K5-Cre:$GPHR^{f/f}$ mice is shown in Figure 2. There was no significant difference between K5-Cre:$GPHR^{f/f}$ and wild-type neonatal mice immediately after birth (Supplementary Figure S2a online). However, hypopigmention and scaliness became apparent at about 4 days and 1 week after birth, respectively (Figure 2a). Skin inflammation with a scaly appearance gradually developed and was associated with hair loss (Figure 2b). A histological examination of the newborn K5-Cre:$GPHR^{f/f}$ mice showed ballooning of the basal cells and follicular dysplasia (Figure 3a and b). These histological findings were almost the same until 1 week after birth (Figure 3c–f). In addition, inflammatory cells containing melanin and an enlargement of the sebaceous glands were seen in the dermis at 1 month after birth (Figure 3e and f).

A panel of antibodies recognizing proteins expressed at defined stages of epidermal differentiation was used to examine whether the *GPHR* deficiency affects keratinocyte maturation. We used those antibodies to detect keratin (K) 14, K10, K6, involucrin, loricrin, and filaggrin (Figure 4a and b, Supplementary S2b–S2m online). K6 expression, a marker of abnormal differentiation, was observed (Supplementary Figure S2j, S2k online) but filaggrin expression was reduced in *GPHR*-deficient skin (Figure 4a and b). The level of filaggrin mRNA was also reduced in *GPHR*-deficient skin (Supplementary Figure S2n online). There was no significant difference in staining with other antibodies recognizing skin differentiation markers between the wild-type and K5-Cre:$GPHR^{f/f}$ newborn mice (Supplementary Figure S2b–i online). Ki-67 expression patterns were also similar (Supplementary

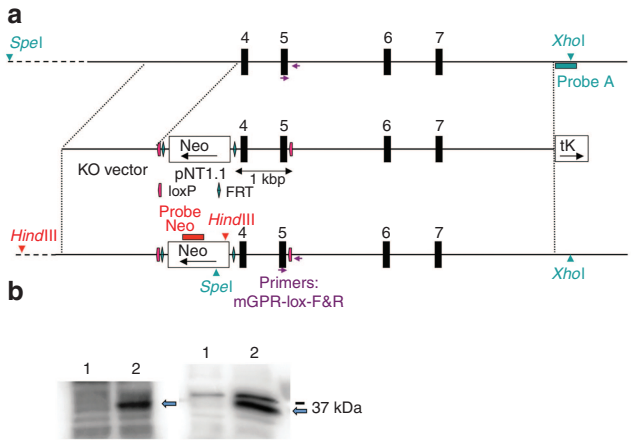

Figure 1. Keratinocyte-specific disruption of *GPHR* gene using the Cre-*loxP* system. (**a**) Targeted insertion of *loxP* sites into the *GPHR* gene. Part of the wild-type *GPHR* locus showing the positions of exons 4–7, the targeting construct, and the *GPHR* allele containing the introduced *loxP* sites. (**b**) Golgi pH regulator (GPHR) protein levels were assessed by immunoprecipitation, followed by western blot analysis of GPHR from K5-Cre:$GPHR^{f/f}$ (lane 1) and from wild-type (lane 2) mice keratinocytes. Arrow indicates the GPHR protein band. KO vector, knockout vector.

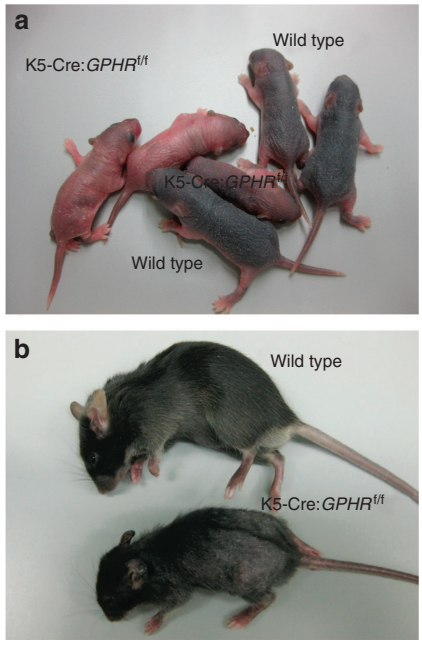

Figure 2. Macroscopic analyses of K5-Cre:$GPHR^{f/f}$ mice. Appearance of hypopigmention and scaliness in K5-Cre:$GPHR^{f/f}$ mice. (**a**) Five days after birth; (**b**) 1 month after birth.

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