Gorab Is Required for Dermal Condensate Cells to Respond to Hedgehog Signals during Hair Follicle Morphogenesis

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GORAB is a golgin that localizes predominantly at the Golgi apparatus and physically interacts with small guanosine triphosphatases. GORAB is ubiquitously expressed in mammalian tissues, including the skin. However, the biological function of this golgin in skin is unknown. Here, we report that disrupting the expression of the *Gorab* gene in mice results in hair follicle morphogenesis defects that were characterized by impaired follicular keratinocyte differentiation. This hair follicle phenotype was associated with markedly suppressed hedgehog (Hh) signaling pathway in dermal condensates in vivo. *Gorab*-deficient dermal mesenchymal cells also displayed a significantly reduced capability to respond to Hh pathway activation in vitro. Furthermore, we found that the formation of the primary cilium, a cellular organelle that is essential for the Hh pathway, was impaired in mutant dermal condensate cells, suggesting that *Gorab* may be required for the Hh pathway through facilitating the formation of primary cilia. Thus, data obtained from this study provided insight into the biological functions of *Gorab* during embryonic morphogenesis of the skin in which Hh signaling and primary cilia exert important functions.

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INTRODUCTION

The Golgi apparatus is a cellular organelle essential for the posttranslational processing, sorting, and transport of proteins. These diverse functions of the Golgi are mediated by a host of Golgi-associated proteins. GORAB, also called SCY1-like 1-binding protein 1 (SCYL1BP1) or N-terminal kinase-like protein binding protein 1 (NTKL-BP1), contains coiled-coil motif and localizes predominantly at the trans-Golgi network (Liu et al., 2012). GORAB also interacts with RAB6 (Hennies et al., 2008), a small guanosine triphosphatase that is extensively involved in the secretory and endocytic pathways of intracellular trafficking (Stenmark, 2009). These properties qualify GORAB as a golgin. They also suggest that it functions in intracellular trafficking.

The *GORAB* gene is highly conserved from fly to human. Autosomal recessive mutations in the human *GORAB* gene cause geroderma osteodysplasticum or gerodermia osteodysplastica (GO; OMIM 231070) (Hennies et al., 2008), a congenital condition characterized by wrinkly skin and osteoporosis. Currently, the molecular mechanism underlying the pathogenesis of GO is unclear. However, the association of *GORAB* with the congenital phenotypes in GO strongly suggests that GORAB may play important functions in embryonic morphogenesis.

In mice, the morphogenesis of the skin initiates when cells of the surface ectoderm commit to an epidermal fate (Koster and Roop, 2007). It is followed by a series of epidermal stratification and differentiation programs regulated by transcriptional factors, notably TRP63 (p63) (Koster and Roop, 2007). Epidermal differentiation results in the formation of the suprabasal and spinous layers of the epidermis between E14.5 and E16.5, and ultimately the cornified cell envelope and epidermal barrier. Formation of the hair follicle is initiated by hair follicle induction, the formation of the hair placodes or stage 1 hair follicles, at approximately at E14.5 (Schneider et al., 2009). It is followed by organogenesis and cytodifferentiation, through which stage 2 hair germs mature into stage 5 hair pegs. During this process, the KRT14-positive outer root sheath starts to differentiate and gives rise to the KRT75-positive companion layer (Schweizer et al., 2007). Further differentiation of embryonic hair follicles results in formation of the inner root sheath, the hair follicle cortex, and ultimately the hair shaft (Paus et al., 1999).

Throughout the morphogenesis of hair follicles, extensive mesenchymal-epithelial interactions occur, in which dermal condensate or dermal papilla cells play instructive roles

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Abbreviations: GO, gerodermia osteodysplastica; Hh, hedgehog; RT-PCR, reverse transcriptase-PCR; SAG, smoothened agonist; SHH, sonic hedgehog

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(Botchkarev and Paus, 2003). Canonical Wnt and Hedgehog (Hh) signaling are among the best characterized molecular signaling pathways during hair follicle morphogenesis (Millar, 2002; Schmidt-Ullrich and Paus, 2005; Yang and Cotsarelis, 2010). Wnt signaling is believed to be the "first dermal signal" (Hardy, 1992) and essential for hair follicle induction (Andl et al., 2002; Gat et al., 1998). Hh signaling is required for cytodifferentiation of follicular keratinocytes (Chiang et al., 1999; Mill et al., 2003; St-Jacques et al., 1998; Woo et al., 2012). During skin morphogenesis, sonic hedgehog (SHH), the predominant Hh ligand in the skin, is produced by follicular keratinocytes. However, it is able to regulate Hh signaling in follicular keratinocyte and dermal papilla cells (St-Jacques et al., 1998).

The primary cilium, or nonmotile cilium, is a singular hair-like structure that protrudes from the surface of most mammalian cell types (Goetz and Anderson, 2010; Singla and Reiter, 2006), including epidermal keratinocytes and dermal papilla cells. One of the best understood functions of primary cilia during tissue morphogenesis is the processing of Hh signals. Abnormal cilia formation and function result in impaired Hh signaling and contribute to the development of ciliopathies (Badano et al., 2006; Hildebrandt et al., 2011; Tobin and Beales, 2009). Disrupting primary cilia formation during skin morphogenesis can result in severely impaired Hh signaling and hair follicle formation (Chen et al., 2015; Croyle et al., 2011; Dai et al., 2011; 2013; Ezratty et al., 2011; Lehman et al., 2009), suggesting that primary cilia are essential for proper Hh signaling during hair follicle morphogenesis.

To gain insight into the molecular functions of GORAB during skin morphogenesis, we engineered a mouse model in which the expression of the *Gorab* gene was disrupted. Striking hair follicle morphogenesis defects were observed in homozygous *Gorab* mutants. Further examination associated these phenotypes with disrupted Hh signaling and impaired primary cilia formation in dermal condensate cells. Data obtained from this study underscore the role of golgins in orchestrating molecular signaling during embryonic morphogenesis.

RESULTS

Gorab is expressed in embryonic skin

Gorab is highly expressed in the skin as determined by quantitative reverse transcriptase (RT)-PCR (Hennies et al., 2008). To further define its expression pattern in embryonic mouse skin, we performed in situ hybridization and observed that *Gorab* is ubiquitously expressed in basal epidermal keratinocytes, follicular keratinocytes, dermal fibroblasts, and dermal papilla cells (Figure 1a).

Generation of *Gorab*-deficient mouse model

To gain insight into the biological function of GORAB, we generated a mutant mouse model from ES cell clone XG183 (BayGenomics Consortium, San Francisco, CA), in which a gene-trap vector (SA β-geo) was inserted into intron 1 of the *Gorab* locus (Figure 1b). This trap vector is expected to interfere with splicing, resulting in a fusion transcript composed of exon 1 of *Gorab* and β-geo (Figure 1b). Genotyping with intron-F and intron-R primers and direct genomic DNA sequencing confirmed that the β-geo cassette was inserted into intron 1 (Figure 1c and Supplementary Figure S1 online). In situ hybridization and western blotting with a polyclonal GORAB antibody against 1–264 amino acid of human GORAB demonstrated that *Gorab* transcripts and protein were absent from homozygous mutants (Figure 1a and d). Thus, this mutant model is regarded as a null allele of *Gorab*, hereafter referred to as $Gorab^{-/-}$.

Homozygous mutants ($Gorab^{-/-}$) were obtained by crossing heterozygous mutants ($Gorab^{+/-}$). Wild-type ($Gorab^{+/+}$), heterozygous ($Gorab^{+/-}$), and homozygous ($Gorab^{-/-}$) were obtained at normal mendelian ratios at birth. The size of $Gorab^{-/-}$ pups appeared comparable with that of $Gorab^{+/+}$ or $Gorab^{+/-}$ littermates (Figure 1e). However, $Gorab^{-/-}$ mutants displayed hunched backs and craniofacial deformities (Figure 1e). In addition, $Gorab^{-/-}$ mutants started to gasp for air within minutes of birth and dying. These phenotypes suggested that $Gorab^{-/-}$ mutants may have morphogenesis defects in the musculoskeletal and respiratory systems.

Gorab is indispensable for hair follicle morphogenesis

This investigation is focused on the skin. Skins of newborn $Gorab^{-/-}$ pups appeared edematous but otherwise unremarkable (Figure 1e). Histologic examination of the embryonic skin of $Gorab^{-/-}$ mutants revealed normal architecture of the epidermis (Figure 1f). In addition, immunofluorescence examination of early and late epidermal differentiation markers, keratin 1 (KRT1) and loricrin (LOR), in E18.5 skins demonstrated comparable epidermal differentiation profiles in control and $Gorab^{-/-}$ mutants (see Supplementary Figure S2 online). These observations suggested that *Gorab* may not play a significant role during formation of the epidermis.

Skins of E15.5 control and $Gorab^{-/-}$ mutants contained comparable numbers of hair germs. In contrast, E18.5 mutant skins contained significantly reduced numbers of hair follicles (Figure 1f and g). In addition, hair follicles in the mutant skin appeared less developed, suggesting that progression of hair follicle morphogenesis is impaired. To follow postnatal hair follicle development, skin biopsies from E18.5 embryos were transplanted onto nude mice. Three weeks later, control transplants developed abundant hair, whereas $Gorab^{-/-}$ transplants developed barely any hair (Figure 1h). Histology of transplants showed that hair defects in $Gorab^{-/-}$ transplants were associated with the lack of morphologically normal hair follicles (Figure 1i). However, hair follicle-like remnants in the mutant transplants indicated that hair follicles had nevertheless undergone significant development before degeneration (Figure 1i, asterisk). TUNEL staining revealed that apoptotic cells were restricted to the orifices of hair follicles in control skin transplant, whereas the number of apoptotic cells not only increased in $Gorab^{-/-}$ transplants but also dispersed into the dermal mesenchyme (Figure 1j), indicating a mechanism through which hair follicle remnants were cleared from postnatal skin. Thus, data demonstrated that *Gorab* is indispensable for the morphogenesis of all types of hair follicles of dorsal skin.

To further evaluate hair follicle induction, the formation of hair germs in E15.5 skins was examined by