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Loss of Gata6 causes dilation of the hair follicle canal and sebaceous duct

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Abstract

The uppermost aspect of the hair follicle, known as the infundibulum or hair canal, provides a passageway for hair shaft egress and sebum secretion. Recent studies have indicated that the infundibulum and sebaceous ducts are lined by molecularly distinct differentiated cells expressing markers including Keratin 79 and Gata6. Here, we ablated Gata6 from the skin, and observed dilation of both the hair canal and sebaceous ducts, independent of gender and hair cycle stage. Constitutive loss of Gata6 yielded only a mild delay in depilation-induced entry into anagen, while unperturbed mutant mice possessed overtly normal skin and hair. Furthermore, we noted that Keratin 79 and Gata6 expression and localization did not depend upon each other. Our findings implicate Gata6 in maintaining the upper hair follicle, and suggest that regulation of this transcription factor may be compromised in pathologies such as acne or infundibular cystic diseases that are characterized by abnormal expansion of this follicular domain.

Keywords

Gata6; K79; Krt79; skin pore; infundibulum; pilosebaceous unit; hair cycling

Background

The hair follicle canal serves a structural function, linking the surface epidermis with the mid-region of the follicle, while in the process providing an opening through which the hair shaft and sebum can exit (1). Although seemingly nondescript at first glance, this domain is colonized by a rich microflora (2, 3), is immunologically and molecularly distinct (4–8), and

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Author Contributions

J.B.S., A.N.V., and N.A.V. performed research. S.Y.W. performed research, wrote the manuscript and obtained funding.

Conflicts of Interest

The authors have no conflicts of interest to declare.

is perturbed in a variety of common skin diseases including acne, epidermoid cysts, hidradenitis suppurativa, keratosis pilaris and milia, among others (1, 9–11). Importantly, the unique structural features of the hair canal, also known as the infundibulum, likely modulate topical drug delivery through the skin (12, 13). Conversely, clogging of the hair canal can lead to enlarged follicular ostia or facial pores—a problem that remains a major focus of the multi-billion dollar cosmetics industry (14).

Studies in mice over the past few years have shown that the infundibulum is maintained by *Lrig1*⁺ stem cells located near the mid-section, or junctional zone region, of the hair follicle (7, 15). These stem cells also maintain the sebaceous glands, whose main function is to secrete sebum into the hair canal via the sebaceous duct (16, 17). Our previous studies have shown that the differentiated suprabasal cells that line both the hair canal and sebaceous duct can be identified by Keratin 79 (K79) (6). Gene expression experiments further showed that *Lrig1* and *K79* expression correlate with that of *Gata6*, which encodes a zinc finger transcription factor that plays multiple key roles during development (7, 18). Deletion of *Gata6* has been reported to stifle hair regeneration by causing replicative stress in fast-cycling matrix progenitor cells (19), and can also lead to a reduction in upper hair follicle cells that ordinarily express this protein (20). Morphological defects in the upper hair follicle, however, have not been described in these mutants. Here, we show that both constitutive and inducible deletion of *Gata6* cause expansion of the hair follicle infundibulum and sebaceous duct. Surprisingly, constitutive *Gata6* mutants did not exhibit major defects in hair growth, suggesting that other Gata factors may compensate.

Question Addressed

Does *Gata6* serve a role in maintaining the upper hair follicle?

Results

We began by assessing the localization of nuclear *Gata6* in telogen hair follicles in mice and observed substantial overlap with K79 expression in suprabasal cells of the infundibulum and sebaceous ducts (Figure 1A), confirming previous findings (20). While most *Lrig1*⁺ stem cells also exhibited nuclear *Gata6*, expression of *Lrig1* was stronger in basal cells, whereas *Gata6* was elevated in suprabasal cells (Figure 1A and S1). We further noted that nuclear *Gata6* was not detected in the epidermis or lower anagen follicle. This included early anagen, when matrix progenitors initially give rise to K79⁺ cells that form the companion layer (21), as well as later stages, when matrix cells surround the mesenchymal dermal papilla (Figure 1B). We confirmed these results using a second independent antibody against *Gata6* and similarly did not detect nuclear *Gata6* in matrix cells or their differentiated progeny (Figure S2). In human skin, nuclear *Gata6* was also localized to suprabasal cells in the upper hair follicle, but not in the lower anagen bulb (Figure 1C and S3).

To assess the function of *Gata6*, we generated mice expressing *Keratin 5* promoter-driven Cre recombinase coupled with homozygous floxed alleles of *Gata6* (*K5;Gata6*). *K5;Gata6* mice were born in the expected Mendelian ratios and did not exhibit overt phenotypes, with the exception of a single hindlimb supernumerary preaxial digit in ~50% of mutant animals

(Figure S4A), as previously reported (22). We confirmed loss of Gata6 from the upper hair follicle by immunohistochemistry, and noted that K79 and Lrig1 were both properly expressed in the absence of Gata6 (Figure 1A). Conversely, Gata6 expression was not dependent on K79 (Figure S4B). Finally, although nerves can regulate expression of stem cell markers such as Gli1 and Lgr6 in the skin (23, 24), we further noted that the domain of Gata6 expression was unchanged in denervated hair follicles (Figure S4C–D).

We next assessed hair cycling by shaving *K5;Gata6* and littermate control animals at ~3.5 weeks of age to directly observe synchronized entry into anagen. In 10 gender-matched cohorts, we did not observe any major changes in hair cycling kinetics between mutant and control animals (Figure S5A). To determine whether Gata6 affects experimentally-induced hair regeneration, we depilated adult *K5;Gata6* and littermate control animals at 8 weeks of age. In 5 gender-matched cohorts, we observed that mutant males displayed a mild 2–7 day delay in anagen re-entry, whereas mutant females did not differ from controls (Figure 1D and Figure S5B). All mice, irrespective of genotype, eventually regenerated and maintained a full coat of hair up to 20 weeks of age (Figure 1E). Histological analysis further revealed that untreated *K5;Gata6* mutants properly entered postnatal anagen at 4 weeks of age (Figure 1F), while an independent cohort of 5 completely unperturbed *K5;Gata6* mutants also did not exhibit overt hair phenotypes between 18–45 weeks of age (Figure S5C).

Although indistinguishable by eye, *K5;Gata6* mice possessed microscopic structural defects in the upper hair follicle. We observed that both the sebaceous duct and infundibulum were dilated, with hair canals resembling early cystic lesions containing keratotic material (Figure 1A, 2A–B). These aberrant domains expressed markers of epidermal and infundibular differentiation (Figure S6), did not upregulate Gata3 (Figure S7), and were not associated with increased skin inflammation (Figure S8). In control animals, hair canal and sebaceous duct size did not vary by gender but increased slightly during anagen (Figure 2C–D); nonetheless, sub-group analysis revealed that the differences seen in *K5;Gata6* mice largely persisted even when controlling for gender and overall hair cycle stage (Figure S9). In immature 4 week old animals, *K5;Gata6* mutants already exhibited enlarged sebaceous ducts (Figure 2E). Finally, we generated mice harboring tamoxifen-inducible *Lrig1* promoter-driven Cre recombinase coupled with *Gata6* floxed alleles (*Lrig1;Gata6* mice) to induce deletion in adults. As before, we confirmed loss of Gata6 (Figure S1), and observed that *Lrig1;Gata6* mice recapitulated the appearance of dilated hair canal and sebaceous ducts, ~13 weeks after deletion (Figure 2F). Altogether, these findings support a role for Gata6 in maintaining proper upper hair follicle morphology.

Conclusions and Discussion

Our findings differ from those of Wang et al., who previously reported that nuclear Gata6 is present in the epidermis, infundibulum, matrix and inner root sheath, and plays a crucial role during anagen (19). Recent gene expression studies have also shown that *Gata6* mRNA is expressed in hair follicle stem cells and matrix progenitors (25, 26). Although these discrepancies in *Gata6* mRNA and protein localization are difficult to reconcile, it should be noted that our hair cycle studies utilized mice where *Gata6* was constitutively deleted from the skin, whereas Wang et al., inducibly and acutely deleted *Gata6*. Thus, it is conceivable

that chronic loss of *Gata6* enabled other Gata factors to compensate during hair growth, yielding a milder phenotype. Indeed, Gata4 can play overlapping roles with Gata6 during pancreas and cardiovascular development (27–29). At the same time, the lack of a major hair cycling defect in *Gata6* mutants is also consistent with our observation that Gata6 protein is not readily detected in the lower anagen follicle.

As the mechanism underlying hair canal dilation in *Gata6* mutants remains unclear, we cannot formally rule out the possibility that loss of Gata6 affects the structural integrity of the upper follicle, possibly leading to histological artifacts. However, consistent with our findings, loss of *Gata6* has previously been associated with cyst formation in other organs such as the pancreas and ovary (30, 31). In the skin, the dilated hair canals are reminiscent of early utriculi-like structures that appear in hairless (hr) mice or when Notch signaling is suppressed in *Lrig1*⁺ stem cells (6, 32). Disruption of Notch pathway components causes cyst formation in the hair follicle resembling nevus comedonicus (33), while mutations in γ -secretase, which activates Notch, have been associated with hidradenitis suppurativa (34). Whether a functional link exists between Notch and Gata6 is tantalizing but currently remains unclear and will require further investigation.

Experimental Design

Mouse strains included *Gata6*^{tm2.1Sad/J}, *K79*^{tm2a}, *Tg(KRT5-cre)*^{5132Jl} and *Lrig1*^{tm1.1(cre/ERT2)Rjc/J} (21, 35–37). Gata6 antibodies were from Cell Signaling (D61E4) or provided by Dr. Xiang-Xi Xu (38). Denervation was described previously (39). For additional details, see Supplementary Materials.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Gata6 localization and effects on hair cycling.

(A) Localization of nuclear Gata6 (green) in control (top) or *K5;Gata6* mutant skin (bottom). Arrows, Gata6 in upper follicle. Arrowheads, dilated sebaceous ducts. Magnified views also shown. (B) Lack of Gata6 (green) in early- (left) or mid-anagen (right) control follicles. On left, K79 (red) identifies early companion layer (arrow) (21). (C) Nuclear Gata6 (green, arrow) in upper domain of human hair follicle. Magnified view on right. (D) Control (left) and *K5;Gata6* male mice (right), 2 weeks post-depilation. (E) The same mice at ~20 weeks of age. (F) Histology of untreated control and *K5;Gata6* anagen skin at 4 weeks of age. Scale bars, 50 μ m.

Figure 2. Loss of *Gata6* causes upper hair follicle expansion.

(A) Dorsal skin from control (left) or *K5;Gata6* (right) littermate mice at ~20 weeks of age. Arrowheads, dilated hair canals, magnified in right panel. (B) Hair canal (HC) or sebaceous duct (SD) area in control or *K5;Gata6* mice at ~20 weeks of age. (C) HC and SD area in control mice at ~20 weeks of age, sub-divided by gender or hair cycle stage (D). (E) HC and SD area in *K5;Gata6* and control mice at 4 weeks of age. (F) Consecutive sections from *Lrig1;Gata6* follicle, 13 weeks post-tamoxifen. *, $p < 0.05$; ***, $p < 0.001$. Scale bars, 50 μm .