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R164C mutation in FOXQ1 H3 domain affects formation of the hair medulla

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Abstract

A number of single gene mutations in laboratory mice produce hair follicle defects resulting in deformed hair shafts. The radiation induced (SB/LeJ-*Foxq1*^{sa}) satin mutant mice have a satin-like sheen to their hair and dilute coloration. This sheen is due to failure of the hair shafts to develop normal medullas, while the pigment dilution is due to the unrelated beige (lysosomal trafficking regulator, *Lyst*^{bg}) mutation. A new allelic mutation, *Foxq1*$^{sa\text{-}J}$, arose spontaneously on the albino (tyrosinase, *Tyr*^{c}) MRL/MpJ-*Fas*^{lpr} background. The *Foxq1*$^{sa\text{-}J}$ allele has a C to T transition at position 490. By contrast, the *Foxq1*^{sa} mutant allele was confirmed to be a 67 base pair deletion followed by two base changes (GA to AT). Morphologic changes were similar to those seen in *Hoxc13* transgenic and targeted mutant mice. This new allelic mutation provides yet another tool to investigate formation of the interior structures of hair shafts.

Keywords

Foxq1; satin; hair fiber; medulla; gene networks; mouse model

Background

The satin mutation is due to a deletion in the *Foxq1* gene (Hong, Noveroske et al. 2001). Asecond allele, *Foxq1*$^{sa\text{-}e1}$, arose in an ENU mutagenesis screen (Hong, Noveroske *et al.* 2001). A third mutation, satin-J (*Foxq1*$^{sa\text{-}J}$) arose spontaneously in the albino MRL/MpJ-*Fas*^{lpr} colony. All 3 fail to develop a hair shaft medulla. The *Foxq1*$^{sa\text{-}J}$ allele was found to be allelic with *Foxq1*^{sa} (Samples, Harris et al. 2002). We report here the phenotype and molecular defect of this new allelic mutation.

Questions Addressed

This study aimed to identify the molecular defect and contrast the phenotype of a new, spontaneous allelic mutation of the *Foxq1* gene (*Foxq1*$^{sa\text{-}J}$) to the commonly used satin allele.

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Author contributions are as follows: B.W., K.A.S, V.K., J.P.S. performed the research, B.W., J.P.S. designed the study, B.W., C.H.P, C.S.P, J.P.S. analyzed the data and wrote the paper.

Experimental Design

All studies were approved by The Jackson Laboratory Animal Care and Use Committee (ACUC). To define histologic lesions in the hair, 5 day old mice were used when hair follicles were in late anagen. Scanning electron microscopy (SEM), light and polarized light microscopy were performed on pelage hair samples from two each of SB/LeJ-*Foxq1*^{sa}/*Foxq1*^{sa}, MRL/MpJ-*Fas*^{lpr}, and MRL/MpJ-*Fas*^{lpr}-*Foxq1*$^{sa\text{-}J}$/*Foxq1*$^{sa\text{-}J}$ mice to compare medullary defects (Rice, Wong et al. 1999; Silva and Sundberg 2012).

Mutations in the *Foxq1* gene were identified by sequence comparison between MRL/MpJ-*Fas*^{lpr}-*Foxq1*$^{sa\text{-}J}$/*Foxq1*$^{sa\text{-}J}$ mutant mice and wildtype control mice.

Results

In the *Foxq1*$^{sa\text{-}J}$ ORF there was a novel C to T transition at position 490 (Supplemental Figure 1). This should result in an arginine (R) to cysteine (C) conversion at amino acid (aa) 164 (R164C). The previously reported 67 base deletion and downstream successive base changes(GA to AT) at positions 766 and 767 in the *Foxq1*^{sa} allele were also confirmed (Hong, Noveroske et al. 2001). The deletion causes a truncation of the protein after aa 128 affecting the transcriptional activity of the protein, but not its DNA binding capacity in contrast to the *Foxq1*$^{sa\text{-}J}$ allele.

The DNA binding domain of FOXQ1 consists of two wings (W1 and W2), three α-helices (H1, H2, and H3) and three β-strands (S1, S2, and S3), arranged in the following order, H1-S1-H2-H3-S2-W1-S3-W2 (Figure 1) (Clark, Halay et al. 1993; Gajiwala and Burley 2000). The *Foxq1*$^{sa\text{-}e1}$ and *Foxq1*$^{sa\text{-}J}$ mutations affect the C-terminal H1 and H3 domains, respectively (Figure 1). The resulting amino acid changes result in different protein polarity that is predicted to destabilize DNA-protein interactions (Figure 1). By contrast, the *Foxq1*^{sa} mutation occurs downstream from the DNA binding domain, which is predicted to interfere with transcriptional activation and/or repression activities (Qian and Costa 1995; Hong, Noveroske et al. 2001) (Figure 1). Thus, 3 mutations affecting different parts of the FOXQ1 protein lead to very similar phenotypes.

The *Foxq1*^{sa} and *Foxq1*$^{sa\text{-}J}$ mutant mice have a satiny hair sheen due to refraction of light through the defective hair shafts (Figure 2a–c). No structural abnormalities were noted on the external surface of the mutant hair shafts by scanning electron microscopy (Figure 2d–f). Light microscopy reveals normal medullary septation and septulation patterns in wildtype mice (Figure 2h, k), but loss of these patterns in *Foxq1*^{sa} and *Foxq1*$^{sa\text{-}J}$ mutant mice (Figure 2g, i, j, l). Differences between the hairs of these two allelic mutations are limited to pigment clumping resulting from the *Lyst*^{bg} mutation in *Foxq1*^{sa} mice (Figure 2g, j, m; arrowheads) and the *Tyr*^{c} mutation in the *Foxq1*$^{sa\text{-}J}$ mouse.

Histological examination of hair follicles revealed that the normally well-organized, compressed cells of the premedulla and medulla (Figure 2n) formed in a disorganized manner in satin mutant mice. Rather than the nuclei being flattened parallel to the epidermis the mutant hair medulla nuclei were elongated perpendicular to the epidermis (Figure 2m, o). By transmission electron microscopy, normal septation and septulation patterns of hair shafts from the control mice (Figure 2q, t) were contrasted with the loss of these structures in the two satin allelic mutations (Figure 2p, s, r, u). Wild-type and heterozygous *Foxq1*$^{sa\text{-}J}$ hairs were clinically normal with well formed hair medullas. Longitudinal sections revealed regularly patterned, rectangular cells in the medulla with clear spaces representing contraction artifact (data not shown). Both the *Foxq1*^{sa} (Figure 2p, s) and *Foxq1*$^{sa\text{-}J}$ (Figure 2r, u) hairs had cells in the medulla that were poorly preserved. In control mice these cells were formed regular, rectangular structures. By contrast, both satin mutant mice had medulla

cells that lost this regular rectangular medullary cell pattern; instead, the cells were elongated and filled the cavity (Figure 2p, s).

Conclusion

A vast array of signaling pathways operate in the developing hair follicle, whose coordination is essential for proper hair formation (Botchkarev 2003; Botchkarev 2003; Botchkarev and Paus 2003). Yet, the molecular mechanisms governing these processes are still largely unknown. We report here a new publicly available, spontaneous mutant (*Foxq1*$^{sa\text{-}J}$) that results in similar hair sheen and medullation defects as the *Foxq1*^{sa} allele (Hong, Noveroske et al. 2001).

Foxq1 is expressed in the upper bulb of the anagen hair follicle (Potter, Peterson et al. 2006), a region in which keratinocytes undergo patterns of differentiation to form the respective elements of the hair shaft and follicle (Legue and Nicolas 2005). *Foxq1* is one of many genes whose mutations in mice are linked to hair shaft defects including *Foxn1*^{nu} (Nehls, Pfeifer et al. 1994), *Hoxc13* (Godwin and Capecchi 1998), and *Dsg4* (Kljuic, Bazzi et al. 2003). Both *Foxq1* and *Foxn1* are transcription factors and targets of HOXC13 regulation (Potter, Peterson et al. 2006; Potter, Pruett et al. 2011). In light of these mutations, we hypothesize that these genes work in a coordinate manner to direct development and cycling of the hair shaft.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

Foxq1	forkhead box Q1 gene
***Foxq1*^{sa}**	satin allele
***Foxq1*$^{sa\text{-}J}$**	satin-J allele
***Foxq1*$^{sa\text{-}e1}$**	satin-e1 allelic mutation
SEM	scanning electron microscopy
TEM	transmission electron microscopy

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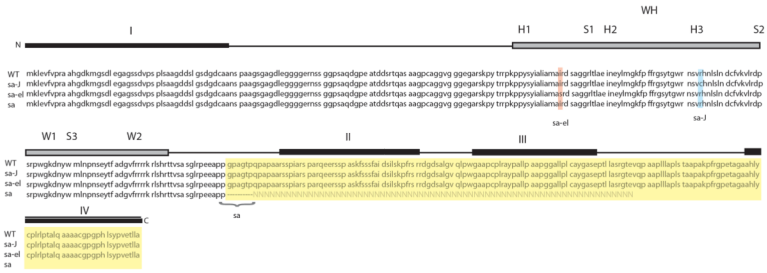

Figure 1.

A graphical representation of the FOXQ1 amino acid sequence indicates the mutations associated with each of the *Foxq1* alleles and the domains affected by each. The *sa-el* allele results in a serine (S) replacement of the normal isoleucine (I), while the new *sa-J* allele replaces an arginine (R) with a cysteine (C). Both mutations occur in the winged helix (WH) domain of the protein theoretically affecting its DNA binding ability. The *sa* allele 67bp deletion causes a frameshift mutation (N) and premature truncation. This mutation occurs outside of the WH domain but does mutate domains II, III, and IV probably affecting the ability of the protein to transcriptionally activate or repress targets (Hong, Noveroske et al. 2001).

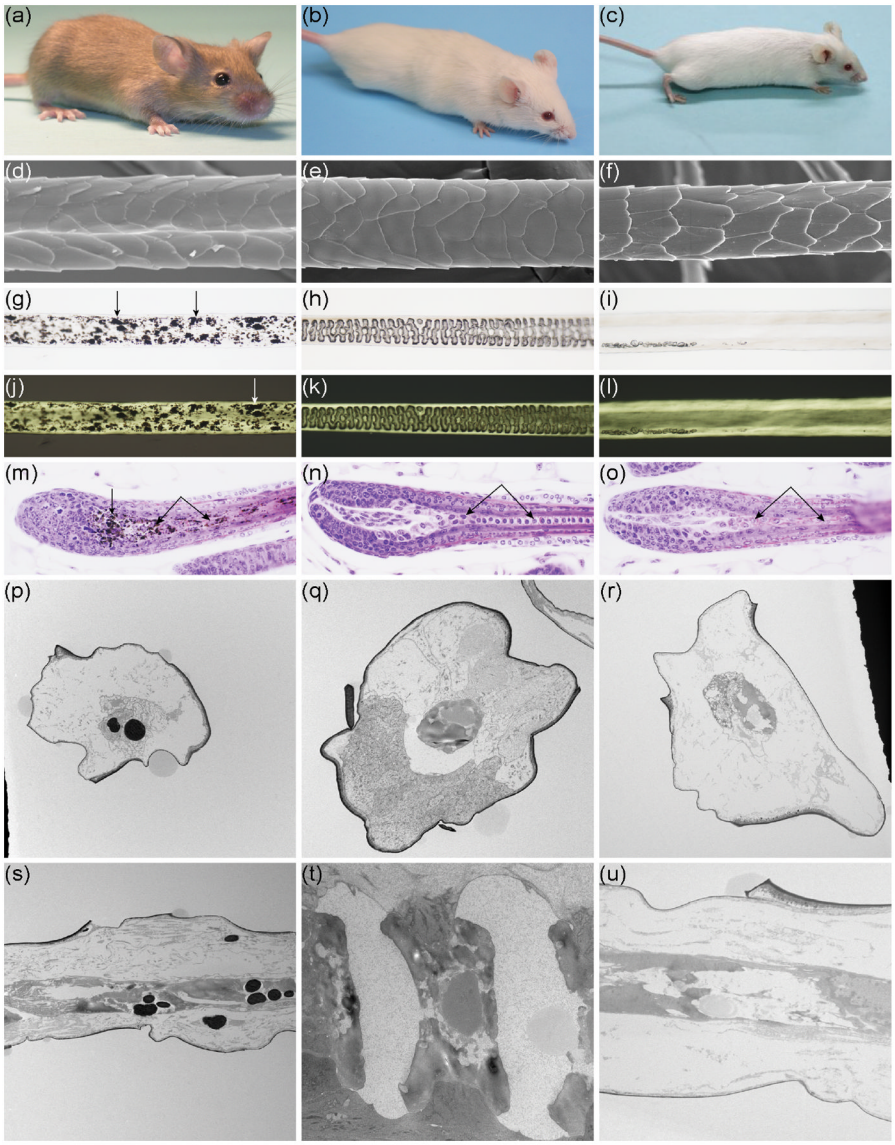

Figure 2.

The satin (SB/LeJ-*Foxq1*^{sa}/*Foxq1*^{sa}; a) and satin-J (MRL/MpJ-*Fas*^{lpr}-*Foxq1*$^{sa\text{-}J}$/*Foxq1*$^{sa\text{-}J}$; c) had hair with a “satiny sheen” compared with the wildtype control for the satin-J (MRL/MpJ-*Fas*^{lpr}-+/+; b). No defects in the cuticle were evident by scanning electron microscopy (d–f) but the medulla did not form properly in either of the satin allelic mutations as evidenced by both white (g–i) and polarized light (j–l) microscopy. Histologically, normally cells in the premedulla form an organized, slightly compressed line of cells (arrows, n). By contrast, in both satin mutants the premedulla formed irregular, elongated cells in the developing medulla (arrows, m, o). Pigment clumping, due to the beige (*Lyst*^{bg}) mutation (m), makes visualization of the developing hair shaft difficult, revealing the advantage of the satin-J allele on an albino background. The satin (p and s) SB/LeJ - *Foxq1*^{sa}/*Foxq1*^{sa}, *Lyst*^{bg}/*Lyst*^{bg}) and satin-J (r and u, MRL/MpJ-*Fas*^{lpr}-*Foxq1*$^{sa\text{-}J}$/*Foxq1*$^{sa\text{-}J}$) mutant mice exhibited loss of cellular detail and architecture within the hair fiber medulla. No spaces were present between the abnormal cells. By contrast, control hairs (q and t MRL/MpJ-*Fas*^{lpr}-+/+) had distinct, longitudinally flattened cells within the medulla with a prominent empty space between each cell.