HHS Public Access

Author manuscript

J Invest Dermatol. Author manuscript; available in PMC 2012 March 01.

Published in final edited form as:

J Invest Dermatol. 2011 September ; 131(9): 1936–1938. doi:10.1038/jid.2011.133.

Localization of the Novel Hair Shaft Protein VSIG8 in the Hair Follicle, Nail Unit and Oral Cavity

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To the Editor

The molecular bases of diseases of the epidermal appendages are gradually being elucidated as genes encoding their constituents and regulation are defined. A direct correspondence between altered protein sequence and aberrant hair shaft structure is evident in cases such as monilethrix (Schweizer, 2006). In others, gene defects lead to altered development and thus perturbed regulation of components. Identification of prominent constituents will speed recognition of genes whose defects contribute directly to aberrant structure or indirectly by exacerbating effects of deficiencies in other genes. Present work helps characterize the novel component V-set and immunoglobulin domain containing 8 (VSIG8) in hair shaft and nail plate to assist understanding its possible relation to disease states.

Protein components of hair shaft and nail plate were known for many years to consist largely of keratin intermediate filaments and keratin associated proteins. Other components, especially those subject to transglutaminase cross-linking, were difficult to identify. Using mass spectrometry-based shotgun proteomics, isolation of proteins is no longer necessary for their identification. Indeed, aggregates of dozens, even hundreds of proteins, are amenable to analysis. Such analysis confirmed that the woolly hair syndrome in one family is not due to a defective structural protein component but rather is a consequence of lipase H mutations (Shimomura *et al.*, 2009). Similarly, mouse strains can be distinguished by proteomic analysis of their pelage hair (Rice *et al.*, 2009). Our initial proteomic analysis of human hair identified a prominent protein component for which little information was available, VSIG8 (Lee *et al.*, 2006).

A specific anti-peptide rabbit antiserum was raised (Antibodies, Inc., Davis, CA) to a highly conserved unique peptide segment near the amino terminus of the predicted protein sequence (CSAVRINGDGQEVLYLAEGDNVRL, residues 20–42 with an additional amino terminal C permitting attachment to the KLH carrier protein) of VSIG8. A cDNA clone of the predicted coding sequence (NM_001013661.1) was prepared commercially (Origene

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Conflict of Interest: The authors state no conflict of interest.

Technologies, Rockville, MD), inserted into a mammalian expression vector, and used in transient transfections of HeLa and HEK293 cells as a positive control. Immunoblotting revealed a strongly immunoreactive band with mobility corresponding to ≈ 45 kDa, the predicted mass. Minor immunoreactive bands with mobilities similar to keratins were not seen when the antiserum was absorbed with protein solubilized from hair shaft and skin callus with SDS and dithioerythritol. Since VSIG8 protein is not solubilized in this way (Rice et al, 2010), absorption did not impair use of the antiserum in present work. The coding region was subjected to site directed mutagenesis (QuikChange kit, Stratagene, La Jolla, CA) converting either L at position 32 to M (mouse sequence) or Y at position 33 to F (rat sequence). Extracts of cultures transfected with all the constructs contained similarly immunoreactive bands.

To optimize the immunohistochemical (IHC) analysis, we tested mouse skin biopsies preserved in 5 fixatives. Fekete's solution (100 mL of 70% alcohol, 10 mL formalin, 5 mL glacial acetic acid) worked well, enabling us to use archival samples. For tissue distribution analysis, we recut blocks encompassing all major and minor organs from one male NON/ShiLtJ mouse at 12 months of age. VSIG8 protein expression was observed in the hair follicle at specific stages of the hair cycle, oral mucosa, and the nail matrix.

Expression in the hair follicle and shaft was found in the cuticle and cortex layers of the hair (Fig. 1a), where the labeling appeared intracellular and at the cell periphery (Fig. 1b). IHC analysis revealed that VSIG8 was located in the projections of the cortex between medullary cells (Fig. 1c,d), a poorly understood structure that is deficient in mice homozygous for the hair interior defect (*hid*) due to a mutation in *Soat1* (Wu *et al.*, 2010). To determine whether VSIG8 was expressed at all stages of the hair cycle or only in specific stages and anatomical sites, synchronized (wax stripped) hair follicles (Sundberg and Silva, 2011) were tested. Reactivity was restricted as we initially found in the hair follicle and hair shaft, and positive signals were limited to the late anagen and early catagen stages of the hair cycle (Fig. 1e–k).

The nail unit is a highly specialized structure with a variety of functions (Fleckman, 2005). While numerous genes involved with normal hair follicle and shaft development are also involved in the nail (De Berker *et al.*, 2000; Kitahara and Ogawa, 1997), much remains unknown. We found that VSIG8 was not limited to the hair shaft and precortex of the hair follicle bulb but also was found in the superficial layers of the nail matrix (Fig. 1l,m).

mRNA encoding VSIG8 protein was previously detected at vanishingly low levels in a variety of major organs using cDNA prepared for commercial tissue blots (Rice *et al.*, 2010). Lack of expression outside the hair follicle and nail unit is largely parallel to mRNA measurements in the mouse, where appreciable levels were detected only in snout and tongue epidermis among 61 tissues surveyed from C57BL/6 mice at 8 weeks of age (Su *et al.*, 2004). Strong IHC labeling was found in the superficial layers of interpapillary epithelium of the dorsal tongue (Fig. 1n), the base of the tongue (Fig. 1o) and in gingival epithelium adjacent to the tooth (Fig. 1p).

In extracts of mouse and rat tissues, dorsal tongue, buccal, and esophageal epithelia were clearly immunoreactive, yielding a single band of ≈ 45 kDa, matching the mobility of the

expressed coding region in transfected cultures (Fig 2). The distribution is reminiscent of certain keratins in dorsal tongue epithelium in mouse and human that are also found in hair and esophageal epithelium (Dhouailly *et al.*, 1989). These findings raise the possibility that VSIG8 also has an important role in proper epithelial differentiation and function in the upper alimentary tract. The gene is reportedly expressed at low but genotype-dependent levels in mouse midbrain (Kozell *et al*., 2009). To elucidate possible roles of VSIG8 in normal function and disease, particularly in the integument, hundreds of mouse models for specific human diseases provide invaluable tools for future work (Sundberg and King, 1996; Plikus *et al.*, 2007).

Acknowledgments

We thank Qin Qin, Hua-Sheng Tsai, Tuan-Yi Hsu, Jarred Caldwell, Noreene Shibata, Olga Gulyaeva and Kathleen A. Silva for expert technical assistance. This work was supported by NIH grants 2 P42 ES04699 (RHR) and AR056635 (JPS).

References

De Berker D, Wojnarowska F, Sviland L, et al. Keratin expression in the normal nail unit: markers of regional differentiation. Br J Dermatol. 2000; 142:89–96. [PubMed: 10651700]

Dhouailly D, Xu C, Manabe M, et al. Expression of hair-related keratins in a soft epithelium: subpopulations of human and mouse dorsal tongue keratinocytes express keratin markers for hair-, skin- and esophageal-types of differentiation. Exp Cell Res. 1989; 181:141–158. [PubMed: 2465162]

Fleckman, P. Structure and function of the nail unit. In: Scher, RK.; Daniel, CRI., editors. Nails Diagnosis, Therapy, Surgery. 3. Elsevier Saunders; Philadelphia: 2005. p. 13-25.

Kitahara T, Ogawa H. Cellular features of differentiation in the nail. Microsc Res Tech. 1997; 38:436–443. [PubMed: 9297693]

Kozell LB, Walter NAR, Milner LC, et al. Mapping a barbiturate withdrawal locus to a 0.44 Mb interval and analysis of a novel null mutant identifies a role for Kcnj9 (GIRK3) in withdrawal from pentobarbital, zolpidem and ethanol. J Neurosci. 2009; 29:11662–11673. [PubMed: 19759313]

Lee YJ, Rice RH, Lee YM. Proteome analysis of human hair shaft: From protein identification to posttranslational modification. Molec Cell Proteom. 2006; 5:789–800.

Plikus, MV.; Sundberg, JP.; Chuong, C-M. Mouse skin ectodermal organs. In: Fox, JG.; Barthold, SW.; Davisson, MT., et al., editors. The Mouse in Biomedical Research. 2. Vol. 3. Academic Press; Burlington, MA: 2007. p. 691-730.

Rice RH, Rocke DM, Tsai H-S, et al. Distinguishing mouse strains by proteomic analysis of pelage hair. J Invest Dermatol. 2009; 129:2120–2125. [PubMed: 19295613]

Rice RH, Xia Y, Alvarado RJ, et al. Proteomic analysis of human nail plate. J Proteome Res. 2010; 9:6752–6758. [PubMed: 20939611]

Schweizer J. More than one gene involved in monilethrix: intracellular but also extracellular players. J Invest Dermatol. 2006; 126:1216–1219. [PubMed: 16702971]

Shimomura Y, Wajid M, Zlotogorskic A, et al. Founder mutations in the lipase H (LIPH) gene in families with autosomal recessive woolly hair/hypotrichosis. J Invest Dermatol. 2009; 129:1927–1934. [PubMed: 19262606]

Su A, Wiltshire T, Batalov S, et al. A gene atlas of the mouse and human protein-encoding transcriptomes. Proc Natl Acad Sci USA. 2004; 101:6062–6067. [PubMed: 15075390]

Sundberg JP, King LJ. Mouse mutations as animal models and biomedical tools for dermatological research. J Invest Dermatol. 1996; 106:368–376. [PubMed: 8601744]

Sundberg JP, Silva KA. What color is the skin of a mouse? Vet Pathol. 2011 in press.

Wu B, Potter CS, Silva KA, et al. Mutations in sterol O-acyltransferase 1 (Soat1) result in hair interior defects in AKR/J mice. J Invest Dermatol. 2010; 130:2666–2668. [PubMed: 20574437]

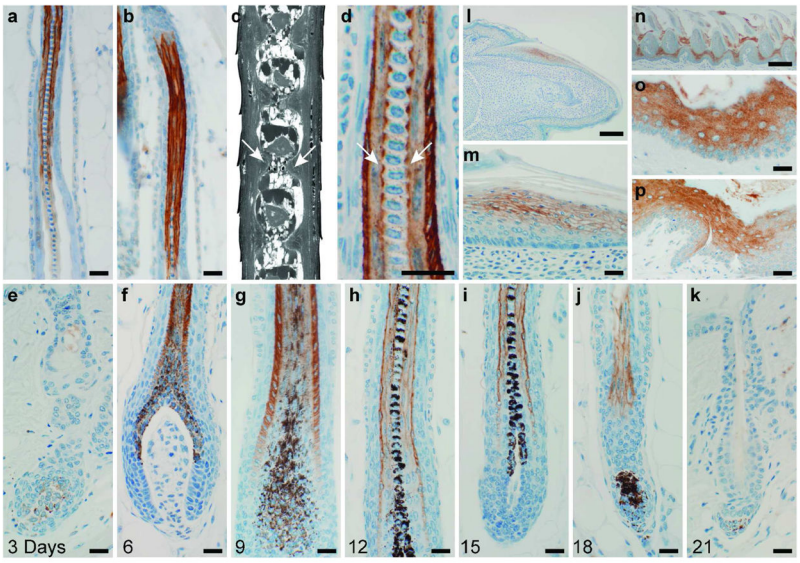

Figure 1.

Immunoreactivity of VSIG8 antiserum. In BALB/cByJ+/+ albino mouse hair follicles. Immunoreactivity was limited to cuticle and cortex layers of the hair shaft, while the medulla was not labeled (a). Immunostaining was evident intracellularly and at the periphery of cuticle cells (b). In the transmission electron micrograph of hairs from FVB/NJ mice, note prominent projections from the cortex into the medulla (white arrows) (c). In immunohistochemical analysis of BALB/cByJ hair fibers (late anagen follicles), VSIG8 antiserum labels these cortical projections intensely (white arrows) (d). Immunoreactivity is shown through the hair cycle in representative hair follicle vertical sections from C3H/HeJ +/+ mice (e–k). Skin samples were collected for analysis at 3 day intervals after synchronization by wax stripping. Black melanin pigment is easily differentiated from the brown diaminobenzidine label. The cuticle of the hair shaft and cortex are labeled. Samples at days 0 (not shown) and 3 revealed no immunoreactivity, those at days 6–18 were immunopositive (late anagen and early catagen), and those at days 21 and 24 (not shown) were immunonegative. VSIG8 expression in the superficial layers of nail matrix is shown in a newborn C57BL/6J mouse (l), enlarged below (m). VSIG8 immunoreactivity in the oral cavity was evident in superficial layers of the interpapillary epithelium of the dorsal tongue (n), at the base of the tongue (o), and in gingival epithelium adjacent to the tooth (p). For these studies, wildtype mice of 6 strains were used (9 C57BL/6J, 3 NON/ShiLtJ, 1 DBA/1LacJ, 1 C3H/HeJ, and 1 CBA/J), plus 2 female C3H/HeJ mice at each time point beginning at 70 days of age (24 mice) were used for wax stripping. No variation in expression patterns was observed. Scale bars: 2 μm (a,b,e–k,m,o,p) and 10 μm (l,n).

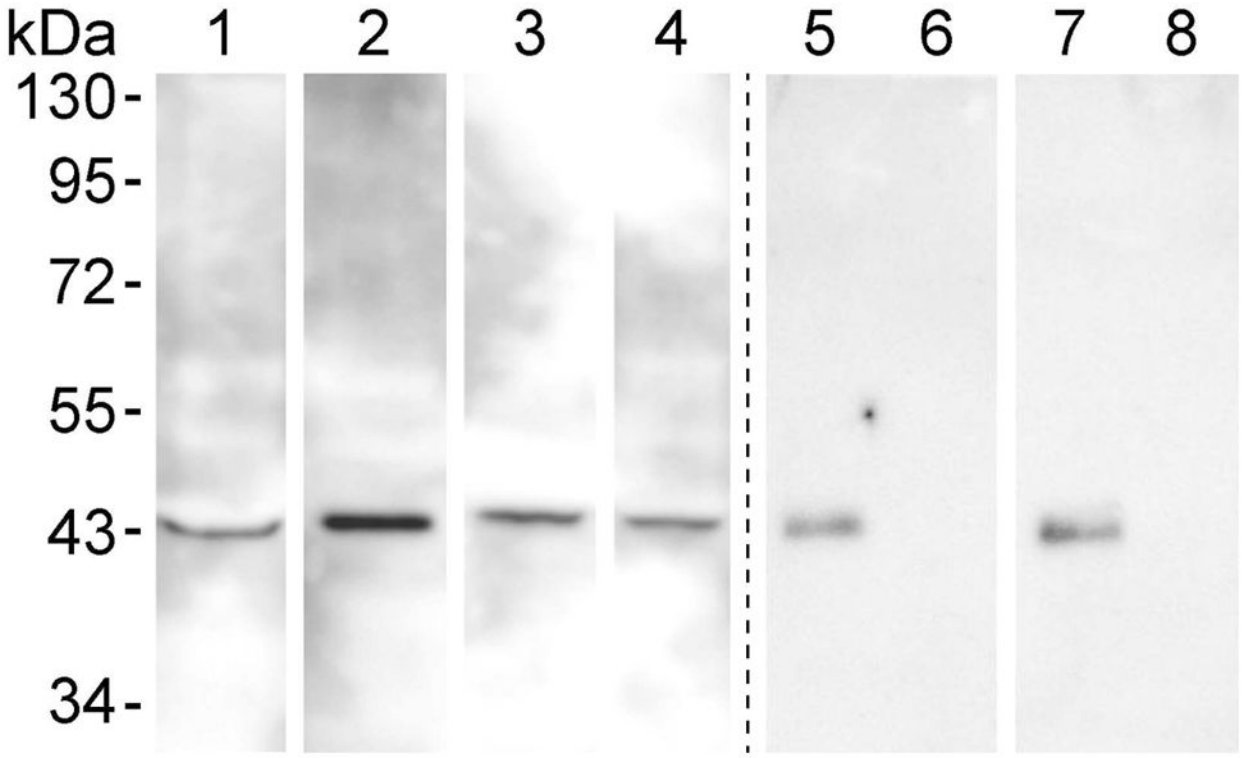

Figure 2.

Immunoblotting of tissue extracts and transfected coding region. Extracts of rat esophagus (lane 1) and tongue (lane 2) and mouse tongue (lane 3) and esophagus (4) gave single bands of ≈ 45 kDa with mobility matching that expressed by the human VSIG8 coding sequence (lane 5 and 7) upon transient transfection of HeLa (or HEK293, not shown) cultures. Lanes 6 and 8 show no bands in parallel mock transfected cultures. Lanes 5 and 6 were blotted with absorbed antiserum, while 7 and 8 were blotted with unabsorbed antiserum.