Localization of 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 being 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 the effects of deficiencies in other genes. The present work helps characterize the component V-set and immunoglobulin domain containing 8 (VSIG8) in hair shaft and nail plate to assist in 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 antipeptide rabbit antiserum was raised (Antibodies, 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 complementary DNA clone of the predicted coding sequence (NM_001013661.1) was prepared commercially (Origene Technologies, Rockville, MD), inserted into a mammalian expression vector, and used in transient transfections of HeLa and human embryonic kidney 293 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 observed when the antiserum was absorbed with protein solubilized from hair shaft and skin callus with SDS and dithioerythritol. As VSIG8 protein is not solubilized in this way (Rice *et al*, 2010), absorption did not impair the use of antiserum in this study. 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 biopsy specimens preserved in five fixatives. Fekete's solution (100 ml of 70% alcohol, 10 ml formalin, and 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, the oral mucosa, and the nail matrix.

Expression in the hair follicle and shaft was found in the cuticle and cortex layers of the hair (Figure 1a), where the labeling appeared intracellular and at the cell periphery (Figure 1b). IHC analysis revealed that VSIG8 was located in the projections of the cortex between medullary cells (Figure 1c and d), a poorly understood structure that is deficient in mice homozygous for the hair interior defect 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, in press) 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 (Figure 1e–k).

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

Messenger RNA encoding VSIG8 protein was detected previously at

Abbreviations: IHC, immunohistochemical; VSIG8, V-set and immunoglobulin domain containing 8

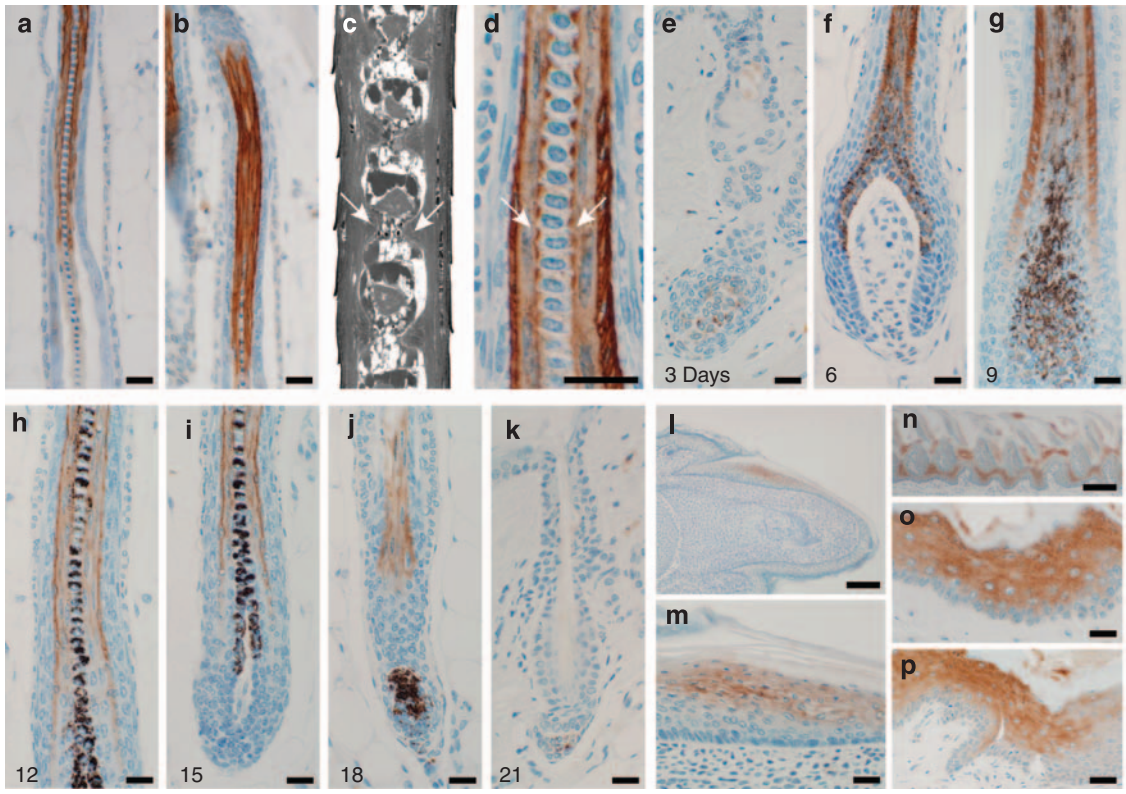

Figure 1. Immunoreactivity of VSIG8 antiserum in BALB/cByJ +/+ albino mouse hair follicles. (**a**) Immunoreactivity was limited to cuticle and cortex layers of the hair shaft, whereas the medulla was not labeled. (**b**) Immunostaining was evident intracellularly and at the periphery of cuticle cells. (**c**) In the transmission electron micrograph of hairs from FVB/NJ mice, note the prominent projections from the cortex into the medulla (white arrows). (**d**) In immunohistochemical analysis of BALB/cByJ hair fibers (late anagen follicles), VSIG8 antiserum labels these cortical projections intensely (white arrows). (**e–k**) Immunoreactivity is shown through the hair cycle in representative hair follicle vertical sections from C3H/HeJ +/+ mice. 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 (**l**) in a newborn C57BL/6J mouse, (**m**) enlarged below. (**n**) VSIG8 immunoreactivity in the oral cavity was evident in superficial layers of the interpapillary epithelium of the dorsal tongue, (**o**) at the base of the tongue, and (**p**) in the gingival epithelium adjacent to the tooth. For these studies, wild-type mice of six strains were used (nine C57BL/6J, three NON/ShiLtJ, one DBA/1LacJ, one C3H/HeJ, and one CBA/J); in addition, two 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. (**a, b, e–k, m, o, p**) Bars = 2 μm and (**l, n**) bars = 10 μm.

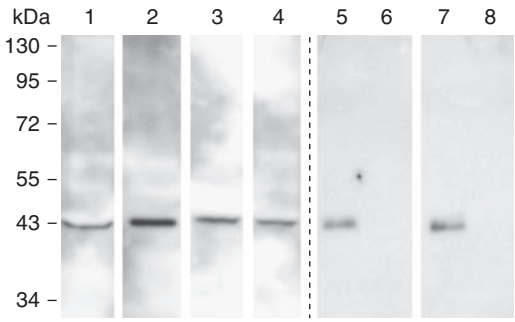

Figure 2. Immunoblotting of tissue extracts and transfected coding region. Extracts of rat esophagus (lane 1), tongue (lane 2), mouse tongue (lane 3), and esophagus (lane 4) yielded single bands of ≈45 kDa with mobility matching that was expressed by the human VSIG8 coding sequence (lanes 5 and 7) upon transient transfection of HeLa (or human embryonic kidney 293, not shown) cultures. Lanes 6 and 8 show no bands in parallel mock-transfected cultures. Lanes 5 and 6 were blotted with absorbed antiserum, whereas lanes 7 and 8 were blotted with unabsorbed antiserum.

vanishingly low levels in a variety of major organs using complementary DNA prepared for commercial tissue blots (Rice *et al.*, 2010). Lack of expression outside the hair follicle and nail unit is largely parallel to messenger RNA 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 the interpapillary epithelium of the dorsal tongue (Figure 1n), the base of the tongue (Figure 1o), and in the gingival epithelium adjacent to the tooth (Figure 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 (Figure 2). The distribution is reminiscent of certain keratins in the 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 the 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 studies (Sundberg and King, 1996; Plikus *et al.*, 2007).

CONFLICT OF INTEREST

The authors state no conflict of interest.

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Full-Thickness Human Skin Models for Congenital Ichthyosis and Related Keratinization Disorders

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TO THE EDITOR

In recent years, a variety of reconstructed human epidermis models and full-thickness human skin models, e.g., EpiDerm FT (MatTek, Ashland, MA) and Phenion Full Thickness Skin Model (Henkel, Düsseldorf, Germany), were developed. Epidermal barrier function of artificial skin, as demonstrated for instance by standardized penetration assays, was shown to be highly similar to human skin (Schäfer-Korting *et al.*, 2008; Ackermann *et al.*, 2010). These results identified reconstructed human epidermis and full-thickness skin models as effective models for the study of normal skin. Three-dimensional artificial skin models for disorders of keratinization such as psoriasis (Tjabringa *et al.*, 2008), ichthyosis vulgaris and atopic dermatitis (Mildner *et al.*, 2010), and Harlequin ichthyosis (Thomas *et al.*, 2009), however, were used only rarely and not characterized in detail. Moreover, an artificial rat skin model with *Tgm1* deficiency (O'Shaughnessy *et al.*, 2010) and a humanized mouse model for pachyonychia congenita based on engraftment of skin equivalents (García *et al.*, 2011) were described recently. For disorders affecting the dermis and/or dermal–epidermal junction zone, specific models for xeroderma pigmentosum (Bernerd *et al.*, 2005) and dystrophic epidermolysis bullosa (Gache *et al.*, 2004) were presented.

To develop organotypic systems that can be easily adapted to several disorders of keratinization, we have established and characterized full-thickness human skin models for autosomal recessive congenital ichthyosis (ARCI) in this study. ARCI can be caused

Abbreviations: ARCI, autosomal recessive congenital ichthyosis; SC, stratum corneum