Palmoplantar Keratoderma in *Slurp2*-Deficient Mice

Christopher M. Allan[1,6], Shiri Procaccia[1,6], Deanna Tran[1], Yiping Tu[1], Richard H. Barnes II[1], Mikael Larsson[1], Bernard B. Allan[2], Lorraine C. Young[1], Cynthia Hong[3,4], Peter Tontonoz[3,4], Loren G. Fong[1], Stephen G. Young[1,5] and Anne P. Beigneux[1]

SLURP1, a member of the lymphocyte antigen 6 protein family, is secreted by suprabasal keratinocytes. Mutations in *SLURP1* cause a palmoplantar keratoderma (PPK) known as *mal de Meleda*. SLURP2, another secreted lymphocyte antigen 6 protein, is encoded by a gene located ~20 kb downstream from *SLURP1*. SLURP2 is produced by suprabasal keratinocytes. To investigate the importance of SLURP2, we first examined *Slurp2* knockout mice in which exon 2–3 sequences had been replaced with *lacZ* and *neo* cassettes. *Slurp2*$^{-/-}$ mice exhibited hyperkeratosis on the volar surface of the paws (i.e., palmoplantar keratoderma), increased keratinocyte proliferation, and an accumulation of lipid droplets in the stratum corneum. They also exhibited reduced body weight and hind limb clasping. These phenotypes are similar to those of *Slurp1*$^{-/-}$ mice. To solidify a link between *Slurp2* deficiency and palmoplantar keratoderma and to be confident that the disease phenotypes in *Slurp2*$^{-/-}$ mice were not secondary to the effects of the *lacZ* and *neo* cassettes on *Slurp1* expression, we created a new line of *Slurp2* knockout mice (*Slurp2X*$^{-/-}$) in which *Slurp2* was inactivated with a simple nonsense mutation. *Slurp2X*$^{-/-}$ mice exhibited the same disease phenotypes. Thus, *Slurp2* deficiency and Slurp1 deficiencies cause the same disease phenotypes.

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INTRODUCTION

Clinical dermatologists are aware that *SLURP1* mutations cause a palmoplantar keratoderma (PPK) known as *mal de Meleda* (Eckl et al., 2003; Fischer et al., 2001; Marrakchi et al., 2003). *Mal de Meleda* patients have a thickened epidermis on the palms and soles, occasionally with pseudoainhum formation, but the skin elsewhere is normal or minimally affected. *Mal de Meleda* is a recessive syndrome, and every patient carries two mutant *SLURP1* alleles. Heterozygous carriers are free of disease.

SLURP1 is an 8.9-kDa protein of the lymphocyte antigen 6 (Ly6) family. The hallmark of this family is an Ly6 domain with 8–10 cysteines, all arranged in a characteristic spacing pattern and all disulfide-linked so as to create a three-fingered motif (Galat et al., 2008; Kieffer et al., 1994). The same structure is found in many secreted toxins in viper and cobra venom (Fry et al., 2003; Kini, 2002). Most Ly6 proteins in mammals are tethered to the plasma membrane by a glycosylphosphatidylinositol (GPI) anchor, but SLURP1 is an exception. SLURP1 is synthesized and secreted by keratinocytes (Favre et al., 2007), enters the plasma, and can be found in the urine (Andermann et al., 1999; Mastrangeli et al., 2003).

The function of SLURP1 in the skin is not well defined, although several studies have reported that it modulates acetylcholine signaling (Arredondo et al., 2005; Chernyavsky et al., 2010; Chimienti et al., 2003). SLURP1 is often presumed to bind to a cell-surface receptor on keratinocytes (Fischer et al., 2001), but thus far binding of SLURP1 to a specific keratinocyte protein has not been documented.

Inactivating *Slurp1* in mice (either by replacing exon 2 with *neo* and *lacZ* cassettes or by introducing a premature stop codon into exon 2) causes PPK (Adeyo et al., 2014). The PPK is obvious by ~6–8 weeks of age. *Slurp1* knockout mice also exhibit increased energy consumption and reduced body weight (Adeyo et al., 2014). The mechanism for those phenotypes is not clear, but they might be secondary to increased grooming (as a result of the PPK). *Slurp1*-deficient mice also exhibit hind limb clasping, a nonspecific neuromuscular phenotype (Dequen et al., 2010; Hayward et al., 2008; Lalonde and Strazielle, 2011). Again, the mechanism is unclear.

In mammals, SLURP1 is not the only secreted Ly6 protein. SLURP2, an ~8-kDa Ly6 protein, is synthesized and secreted by keratinocytes. Studies of human skin with a human SLURP2–specific monoclonal antibody revealed that

[1]Department of Medicine, Divisions of Cardiology and Dermatology, David Geffen School of Medicine, University of California, Los Angeles, California, USA; [2]Department of Molecular Biology, Genentech, South San Francisco, California, USA; [3]Department of Pathology and Laboratory Medicine, David Geffen School of Medicine, University of California, Los Angeles, California, USA; [4]Howard Hughes Medical Institute, University of California, Los Angeles, California, USA; and [5]Department of Human Genetics, David Geffen School of Medicine, University of California, Los Angeles, California, USA

[6]These authors contributed equally to this work

Correspondence: Anne P. Beigneux, 650 Charles E. Young Dr. South, Los Angeles, California 90095 USA. E-mail: abeigneux@mednet.ucla.edu or Stephen G. Young, 650 Charles E. Young Dr. South, Los Angeles, California 90095 USA. E-mail: sgyoung@mednet.ucla.edu or Loren G. Fong, 650 Charles E. Young Dr. South, Los Angeles, California 90095 USA. E-mail: lfong@mednet.ucla.edu

Abbreviations: GPI, glycosylphosphatidylinositol; GPIHBP1, glycosylphosphatidylinositol-anchored high-density lipoprotein binding protein 1; Ly6, lymphocyte antigen 6; PPK, palmoplantar keratoderma

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SLURP2, like SLURP1, is made by suprabasal keratinocytes (Arredondo et al., 2006). SLURP2 was initially identified as a cDNA that is up-regulated in psoriasis vulgaris (Tsuji et al., 2003). The gene for SLURP2 is immediately upstream from *LYNX1* and 21.9 kb downstream from *SLURP1*; *SLURP2* is ~443 kb upstream from the gene for glycosylphosphatidylinositol-anchored high-density lipoprotein binding protein 1 (GPIHBP1), a GPI-anchored Ly6 protein that shuttles lipoprotein lipase to the capillary lumen (Beigneux et al., 2007; Davies et al., 2010; Goulbourne et al., 2014). The function of SLURP2 is unclear, but one article proposed that SLURP2 modulates acetylcholine signaling (Arredondo et al., 2006). Similar to the situation with SLURP1, specific interactions between SLURP2 and any keratinocyte protein have not yet been identified.

No insights into the possible consequences of *SLURP2* deficiency have been developed. One could easily imagine that the consequences of SLURP2 and SLURP1 deficiency might be similar, given that both are members of the Ly6 family and both are secreted by keratinocytes. However, one could be skeptical about that possibility given that different Ly6 family members can play highly diverse functions in mammalian biology (Galat et al., 2008). Moreover, the level of amino acid sequence identity between SLURP1 and SLURP2 is extremely low—only 17% after the 10 cysteines of the Ly6 domain are excluded from consideration (see Supplementary Figure S1 online). The level of sequence identity between the Ly6 domains of SLURP2 and GPIHBP1 (the LPL transporter) is 21%.

To define the in vivo functional relevance of SLURP2 in mammals and to determine whether SLURP2 might be relevant to skin disease, we characterized two independent lines of *Slurp2* knockout mice.

RESULTS

We first examined *Slurp2* knockout mice ($Slurp2^{-/-}$) that were created by replacing an exon 2–3 fragment with *neo* and *lacZ* cassettes (see Supplementary Figure S2 online). As expected, *Slurp2* transcripts were half-normal in heterozygotes and absent in homozygotes (see Figure 3). We attempted to visualize mouse SLURP2 in the skin of wild-type mice by western blotting and immunohistochemistry with our rabbit antiserum against a mouse SLURP2 peptide, but we were unable to detect a specific signal.

$Slurp2^{-/-}$ mice appeared normal at birth and at weaning, but hyperkeratosis on the volar surface of the paws (i.e., PPK) was invariably present by 6–8 weeks of age (Figure 1a). Grossly, the PPK in $Slurp2^{-/-}$ mice was indistinguishable from that in $Slurp1^{-/-}$ mice (Adeyo et al., 2014). On hematoxylin and eosin–stained sections, the epidermis of the paw in $Slurp2^{-/-}$ mice exhibited hyperkeratosis, and the stratum granulosum was poorly demarcated (Figure 1b). The stratum corneum contained many tiny lipid droplets, as judged by hematoxylin and eosin and BODIPY (ThermoFisher Scientific, Waltham, MA) staining (Figure 1c and d). There was no inflammation in the dermis or epidermis (confirmed by a UCLA dermatopathologist, Dr. Peter G. Sarantopoulos). Also, we did not observe consistently higher cytokine transcripts in the paw skin of $Slurp2X^{-/-}$ mice, whereas the expression of each of the cytokines was increased in the skin of $Apoe^{-/-}Lxra^{-/-}$ mice (in which cholesterol accumulation in the skin is accompanied by histologic evidence of inflammation) (see Supplementary Figure S3 online) (Bradley et al., 2007).

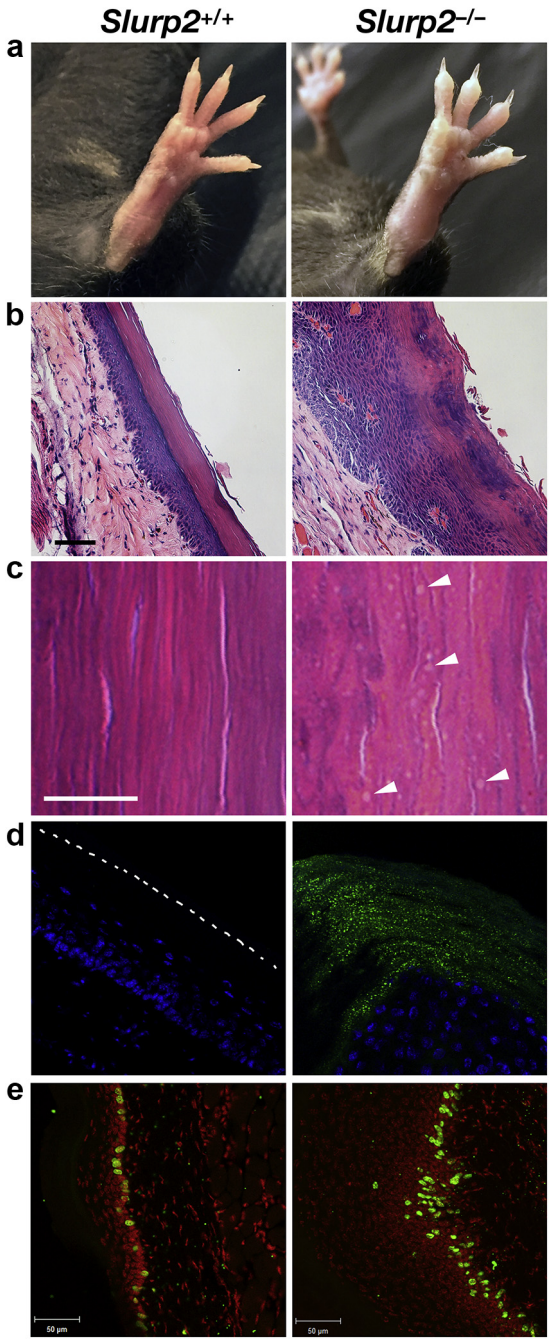

Figure 1. Palmoplantar keratoderma in *Slurp2*$^{-/-}$ mice. (**a**) Paws from wild-type and *Slurp2*$^{-/-}$ mice. The epidermis of the entire paw was thick, but the palmoplantar keratoderma (PPK) was quite evident at 6–8 weeks of age by the bulbous appearance of the tips of the digits. (**b**) Hematoxylin and eosin–stained sections showing hyperkeratosis in *Slurp2*$^{-/-}$ paw skin. Bar = 50 µm. (**c**) Numerous small lipid droplets in the stratum corneum of *Slurp2*$^{-/-}$ mice (arrowheads). Bar = 10 µm. (**d**) BODIPY 493/503 staining showing tiny lipid droplets (green) in the stratum corneum of *Slurp2*$^{-/-}$ paw skin. DNA was stained with 4′,6-diamidino-2-phenylindole (blue). In the left panel (wild-type mouse), the stratum corneum is above the white line. (**e**) Increased BrdU incorporation (green) into the paw skin of *Slurp2*$^{-/-}$ mice. DNA was stained with 4′,6-diamidino-2-phenylindole (red). Bar = 50 µm.