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A hypomorphic *Egfr* allele does not ameliorate the palmoplantar keratoderma caused by SLURP1 deficiency

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Abstract

Mutations in *SLURP1*, a secreted protein of keratinocytes, cause a palmoplantar keratoderma (PPK) known as *mal de Meleda*. *Slurp1* deficiency in mice faithfully recapitulates the human disease, with increased keratinocyte proliferation and thickening of the epidermis on the volar surface of the paws. There has long been speculation that SLURP1 serves as a ligand for a receptor that regulates keratinocyte growth and differentiation. We were intrigued that mutations leading to increased signaling through the epidermal growth factor receptor (EGFR) cause PPK. Here, we sought to determine whether reducing EGFR signaling would ameliorate the PPK associated with SLURP1 deficiency. To address this issue, we bred *Slurp1*-deficient mice that were homozygous for a hypomorphic *Egfr* allele. The hypomorphic *Egfr* allele, which leads to reduced EGFR signaling in keratinocytes, did not ameliorate the PPK elicited by SLURP1 deficiency, suggesting that SLURP1 deficiency causes PPK independently (or downstream) from the EGFR pathway.

A variety of missense and nonsense mutations in *SLURP1* cause a palmoplantar keratoderma (PPK) known as *mal de Meleda* (1). *Slurp1* deficiency in mice recapitulates the PPK phenotype in humans, with marked thickening of the epidermis on the volar surface of paws (2). *Slurp1*-deficient mice (*Slurp1*$^{-/-}$) also exhibit hind-limb clasping and reduced body weight; those phenotypes are not fully understood but most likely are secondary to the PPK (2).

PPK in humans can be caused by genetic defects in many different proteins, including crucial structural proteins of keratinocytes (*e.g.*, desmosomal proteins). The PPK of *mal de Meleda* is unusual in that it is caused by a defect in a protein (SLURP1) that is secreted by suprabasal keratinocytes (1). The discovery that defects in a *secreted* protein could elicit

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PPK immediately prompted speculation that SLURP1 might be a ligand for a receptor on the surface of keratinocytes (1).

SLURP1 is a member of the Ly6 family of proteins, which contain a cysteine-rich three-fingered domain closely resembling the structure of toxins from cobras and vipers. Many of the snake toxins bind neuromuscular acetylcholine receptors with extremely high affinity. The level of amino acid identity between SLURP1 and the snake toxins is very low, but the similarities in the structures of SLURP1 and the snake toxins led to speculation that *mal de Meleda* might be caused by perturbed acetylcholine signaling in the skin (1). Thus far, however, no group has shown that SLURP1 protein binds specifically to acetylcholine receptors in keratinocytes.

SLURP1 deficiency in mice causes dramatically increased proliferation of basal keratinocytes (2), suggesting that an absence of SLURP1 might "release the brakes" on keratinocyte proliferation. That observation prompted us to wonder whether the epidermal pathology in *Slurp1*$^{-/-}$ mice might be caused by increased EGFR signaling—either in a direct fashion or indirectly *via* other signaling pathways. Our interest in this concept stemmed from reports linking perturbed EGFR signaling to increased keratinocyte proliferation and PPK. First, iRHOM2 missense mutations can increase ADAM17-mediated shedding of EGFR ligands, resulting in PPK and increased susceptibility to esophageal cancer (Howel-Evans syndrome) (3). Second, Olmsted syndrome (characterized by PPK and periorificial plaques) is caused by gain-of-function mutations in TRPV3, an ion channel that regulates EGFR signaling. Treatment of an Olmsted syndrome patient with an EGFR inhibitor (erlotinib) ameliorated the PPK (4). Third, SLURP1 deficiency in mice leads to upregulated expression of keratins 6 and 16 (5), and treatment of keratinocytes with EGFR ligands increases the expression of those same keratins (6). Finally, a deficiency in ERRFI1 (also called mitogen-inducible gene 6) increases EGFR signaling and leads to epidermal thickening (7). The hyperkeratosis in *Errfi1*$^{-/-}$ mice was rescued by homozygosity for a hypomorphic *Egfr* allele (*Egfr*^{wa2}) (7). Homozygosity for the allele dramatically reduces EGFR activity, as judged by the level of autophosphorylation (8). *Egfr*$^{wa2/wa2}$ are readily identified by wavy hair but do not have hyperkeratosis.

The fact that the hyperkeratosis in *Errfi1*$^{-/-}$ mice was rescued by the *Egfr*^{wa2} allele prompted us to consider whether the *Egfr*^{wa2} allele might also rescue—or at least ameliorate—the PPK caused by *Slurp1* deficiency. To address this issue, we intercrossed *Slurp1*$^{-/-}$ mice (2) and *Egfr*$^{wa2/wa2}$ mice (purchased from The Jackson Laboratory) (10), generating *Slurp1*$^{+/-}$;*Egfr*$^{+/wa2}$ mice, which were then intercrossed to create four groups of littermate mice (*Slurp1*$^{+/+}$;*Egfr*$^{+/+}$, *Slurp1*$^{+/+}$;*Egfr*$^{wa2/wa2}$, *Slurp1*$^{-/-}$;*Egfr*$^{+/+}$, *Slurp1*$^{-/-}$;*Egfr*$^{wa2/wa2}$). Mice were genotyped by PCR (Fig. S1). All mice were 16-week old males in the C57BL/6J background; studies were approved by UCLA's Animal Research Committee. Examination of those mice (n = 8/group) revealed PPK in *Slurp1*$^{-/-}$;*Egfr*$^{+/+}$ and *Slurp1*$^{-/-}$;*Egfr*$^{wa2/wa2}$ mice, but not in *Slurp1*$^{+/+}$;*Egfr*$^{+/+}$ or *Slurp1*$^{+/+}$;*Egfr*$^{wa2/wa2}$ mice (Fig. 1). Also, the *Slurp1*$^{-/-}$;*Egfr*$^{+/+}$ and *Slurp1*$^{-/-}$;*Egfr*$^{wa2/wa2}$ mice, but not the other mice, exhibited hind-limb clasping (invariably apparent when the PPK was obvious, at 8–10 weeks of age). The fur was "waved" in both *Slurp1*$^{+/+}$;*Egfr*$^{wa2/wa2}$ and *Slurp1*$^{-/-}$;*Egfr*$^{wa2/wa2}$ mice (Fig. 1). Body weight is invariably reduced in *Slurp1* deficiency (2). In the current study, we

confirmed that finding and showed that this phenotype was not ameliorated by homozygosity for the *Egfr*^{wa2} allele (Fig. S2).

To explore the possibility that homozygosity for the *Egfr*^{wa2} allele might ameliorate the PPK caused by *Slurp1* deficiency, we quantified the thickness of the stratum corneum on sections of the hind paw skin (n = 6/group) (Fig. 2A–B). There was no significant difference in thickness of the stratum corneum in the distal, middle, or proximal paw skin in *Slurp1*$^{-/-}$;*Egfr*$^{+/+}$ and *Slurp1*$^{-/-}$;*Egfr*$^{wa2/wa2}$ mice, but the paw skin was much thicker in those mice than in *Slurp1*$^{+/+}$;*Egfr*$^{+/+}$ or *Slurp1*$^{+/+}$;*Egfr*$^{wa2/wa2}$ mice ($p < 0.0001$) (Fig. 2B). We previously reported that expression levels of *Krt16* and *Lce3a* were increased in the paw skin of *Slurp1*$^{-/-}$ mice, while levels of *Krt24* and *Lce1m* expression were reduced (5). In the current study, we confirmed those findings, and we found no evidence that those gene-expression abnormalities were mitigated by homozygosity for the *Egfr*^{wa2} allele (Fig. 2C).

At the levels of gross morphology, morphometric analyses of epidermal thickness, and gene-expression perturbations, we found no evidence that the hypomorphic *Egfr*^{wa2} allele ameliorated the PPK caused by SLURP1 deficiency. Our findings stand in contrast to the ability of the *Egfr*^{wa2} allele to reverse the hyperkeratosis in *Errfi1*$^{-/-}$ mice. Our results suggest that SLURP1 deficiency causes PPK independently (or downstream) from the EGFR signaling pathway.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. PPK, hair, and hind-limb clasping phenotypes in *Slurp1*$^{+/+}$;*Egfr*$^{+/+}$, *Slurp1*$^{+/+}$;*Egfr*$^{wa2/wa2}$, *Slurp1*$^{-/-}$;*Egfr*$^{+/+}$, and *Slurp1*$^{-/-}$;*Egfr*$^{wa2/wa2}$ mice

PPK and hind-limb clasping were invariably present in *Slurp1*-deficient mice; "wavy hair" was invariably present in mice homozygous for the *Egfr*^{wa2} allele.

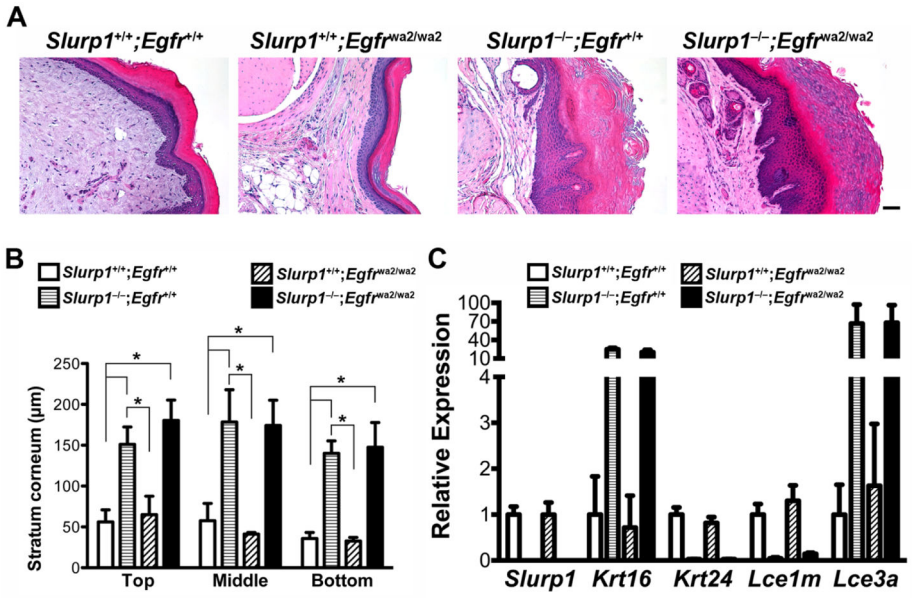

Figure 2. Impact of the *Egfr*^{wa2} allele on the PPK in *Slurp1*-deficient mice

The epidermis was harvested from the volar surface of the hind paw and examined at three levels in the paw skin [Top (distal), Middle, Bottom (proximal)]. (**A**) Hematoxylin and eosin–stained sections. Scale bar, 50 μm. (**B**) Measurements of stratum corneum thickness (n = 6 mice/group). The stratum corneum was thicker in *Slurp1*$^{-/-}$;*Egfr*$^{+/+}$ and *Slurp1*$^{-/-}$;*Egfr*$^{wa2/wa2}$ mice than in wild-type (*Slurp1*$^{+/+}$;*Egfr*$^{+/+}$) or *Slurp1*$^{+/+}$;*Egfr*$^{wa2/wa2}$ mice (*$p < 0.0001$), but there was no difference in paw skin thickness between *Slurp1*$^{-/-}$;*Egfr*$^{+/+}$ and *Slurp1*$^{-/-}$;*Egfr*$^{wa2/wa2}$ mice. (**C**). Gene expression as judged by qRT-PCR (n = 6 mice/group). Gene expression was normalized to cyclophilin A and compared to expression levels in wild-type mice (set at 1.0). The expression of five genes (*Slurp1*, *Krt16, Lce3a, Krt24*, *Lce1m*) was significantly perturbed in *Slurp1*$^{-/-}$;*Egfr*$^{+/+}$ and *Slurp1*$^{-/-}$;*Egfr*$^{wa2/wa2}$ mice compared to *Slurp1*$^{+/+}$;*Egfr*$^{+/+}$ or *Slurp1*$^{+/+}$;*Egfr*$^{wa2/wa2}$ mice, but there were no significant differences in the expression of those genes in *Slurp1*$^{-/-}$;*Egfr*$^{+/+}$ and *Slurp1*$^{-/-}$;*Egfr*$^{wa2/wa2}$ mice.