The lipoproteinase inhibitor ML355 prevents covalent addition of the corneocyte lipid envelope in a novel preclinical model of congenital ichthyosis

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Epidermal lipoproteinase (LOX) deficiency is a major cause of autosomal recessive congenital ichthyosis (ARCI) and also drives cornification abnormalities. To date, LOX inhibitors were not tested in preclinical models. In this study, we evaluated the pharmacological model of epidermal LOX deficiency using the commercially available platelet 12S-LOX inhibitor ML355. ML355 inhibited methyl arachidonate LOX activity in differentiated human foreskin keratinocytes (HFK) lysates in a dose-dependent and noncompetitive manner, with an IC50 of ~30 μM and >75% inhibition at 100 μM. ML355 did not compromise keratinocyte viability (assessed by trypan blue exclusion in HFK), but caused dose-dependent barrier dysfunction in human epidermal equivalent (HE) cultures (assessed by transepithelial electrical resistance; TEP: Protein-bound ceramides and omega-hydroxy fatty acids (analyzed by thin-layer chromatography) were reduced by ML355, while free lipids were relatively preserved. The histopathology and ultrastructure of ML355-treated HEs were characterized by hyperkeratosis, cytoplasmic lipid droplet accumulation, and defective lamellar lipid processing, despite normal lamellar body structure and no overt signs of cellular toxicity. Lipid membranes were present surrounding the corneocyte protein envelope in ML355-treated HEs, but unlike the corneocyte lipid envelopes (CLE) in control HEs, these could be removed by organic solvent treatment, indicating that ML355 inhibited covalent addition of the CLE but not the delivery of CLE lipids.

These results demonstrate that ML355 inhibits keratinocyte LOX activity and causes an ichthyosis-like phenotype in HE cultures. ML355 should be a useful model to test treatments for ARCI and other diseases associated with epidermal LOX deficiency.

Loss of EPHA2 represses GATA-3 function and causes a terminal differentiation defect

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Loss of EPHA2 activity results in a keratinocyte differentiation defect. Using the commercially available platelet 12S-LOX inhibitor ML355. ML355 inhibited methyl arachidonate LOX activity in differentiated human foreskin keratinocytes (HFK) lysates in a dose-dependent and noncompetitive manner, with an IC50 of ~30 μM and >75% inhibition at 100 μM. ML355 did not compromise keratinocyte viability (assessed by trypan blue exclusion in HFK), but caused dose-dependent barrier dysfunction in human epidermal equivalent (HE) cultures (assessed by transepithelial electrical resistance; TEP: Protein-bound ceramides and omega-hydroxy fatty acids (analyzed by thin-layer chromatography) were reduced by ML355, while free lipids were relatively preserved. The histopathology and ultrastructure of ML355-treated HEs were characterized by hyperkeratosis, cytoplasmic lipid droplet accumulation, and defective lamellar lipid processing, despite normal lamellar body structure and no overt signs of cellular toxicity. Lipid membranes were present surrounding the corneocyte protein envelope in ML355-treated HEs, but unlike the corneocyte lipid envelopes (CLE) in control HEs, these could be removed by organic solvent treatment, indicating that ML355 inhibited covalent addition of the CLE but not the delivery of CLE lipids. These results demonstrate that ML355 inhibits keratinocyte LOX activity and causes an ichthyosis-like phenotype in HE cultures. ML355 should be a useful model to test treatments for ARCI and other diseases associated with epidermal LOX deficiency.

IRAK2 promotes abnormal epidermal differentiation during inflammatory states to facilitate and amplify immune responses in skin

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Mature keratinocyte diseases of the skin including atopic dermatitis (AD) and psoriasis are characterized by altered epidermal differentiation, but the mechanisms for these changes have remained unclear, in particular given their diverse immunologic pathogenesis. Here we show that IRAK2-deficient mouse keratinocytes exhibit a normal, protective response to keratinocyte differentiation. Using 3D epidermal raft models, we demonstrate that IRAK2 is responsible for epidermal thickening (acanthosis) and promotion of epidermal immune responses. Lastly, through RNA-sequencing and single cell RNA-sequencing analyses we show that the transcription factor ZNF750 is a critical downstream mediator of this role of IRAK2, influencing both epidermal and pro-inflammatory processes. Taken together, these results suggest that IRAK2 plays a common critical role in promoting altered epidermal differentiation that is responsible for the feed-forward amplification of inflammatory responses to otherwise diverse etiologies in skin. This work identifies IRAK2 as a novel potential therapeutic target for inflammatory skin diseases including AD and psoriasis, which likely broadens to other chronic inflammatory diseases involving epidermal hyperplasia.