A mechanistic target of rapamycin complex 1/2 (mTORC1)/V-Akt murine thymoma viral oncogene homolog 1 (AKT1)/cathepsin H axis controls filaggrin expression and processing in skin, a novel mechanism for skin barrier disruption in patients with atopic dermatitis

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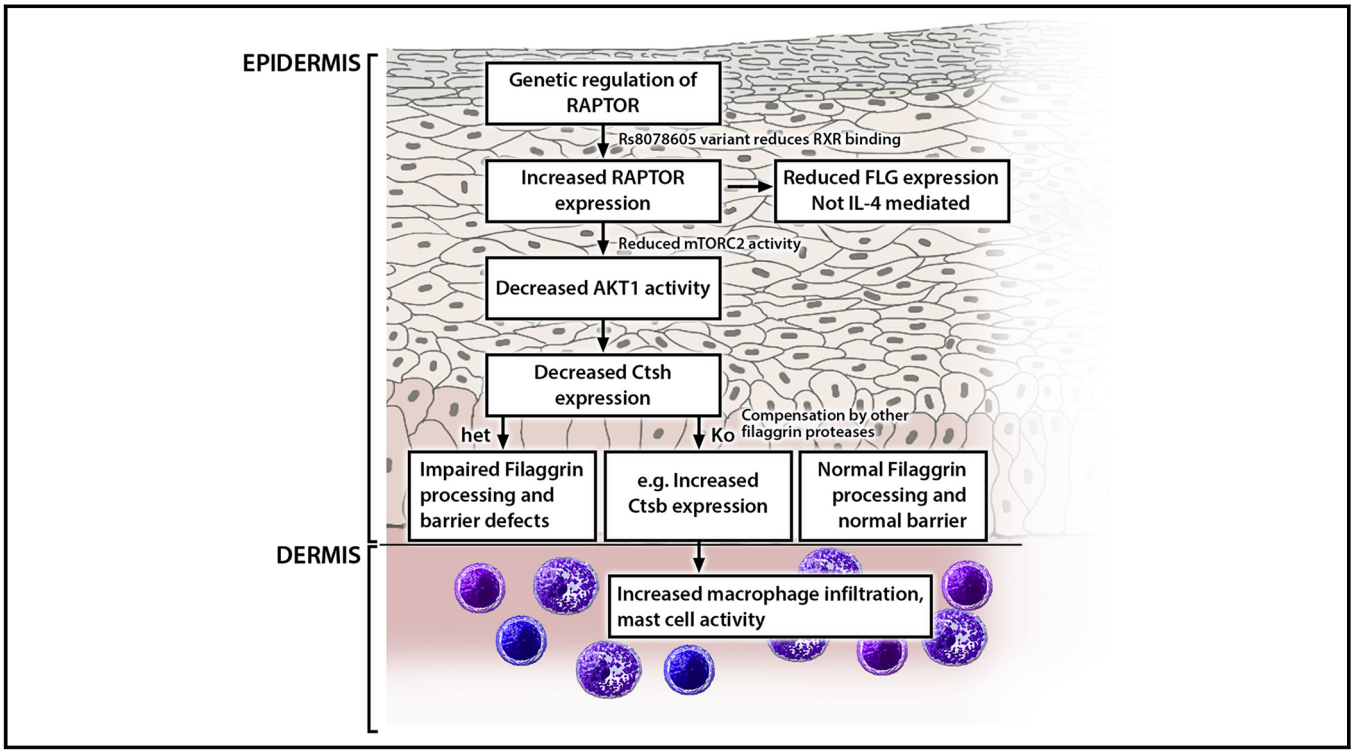
GRAPHICAL ABSTRACT



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Background: Filaggrin, which is encoded by the filaggrin gene *(FLG)*, is an important component of the skin's barrier to the external environment, and genetic defects in *FLG* strongly associate with atopic dermatitis (AD). However, not all patients with AD have *FLG* mutations.

Objective: We hypothesized that these patients might possess other defects in filaggrin expression and processing contributing to barrier disruption and AD, and therefore we present novel therapeutic targets for this disease.

Results: We describe the relationship between the mechanistic target of rapamycin complex 1/2 protein subunit regulatory associated protein of the MTOR complex 1 (RAPTOR), the serine/threonine kinase V-Akt murine thymoma viral oncogene homolog 1 (AKT1), and the protease cathepsin H (CTSH), for which we establish a role in filaggrin expression and processing. Increased RAPTOR levels correlated with decreased filaggrin expression in patients with AD. In keratinocyte cell cultures RAPTOR upregulation or AKT1 short hairpin RNA knockdown reduced expression of the protease CTSH. Skin of CTSH-deficient mice and CTSH short hairpin RNA knockdown keratinocytes showed reduced filaggrin processing, and the mouse had both impaired skin barrier function and a mild proinflammatory phenotype.

Conclusion: Our findings highlight a novel and potentially treatable signaling axis controlling filaggrin expression and processing that is defective in patients with AD. (J Allergy Clin Immunol 2017;139:1228-41.)

Key words: *Atopic dermatitis, skin barrier, filaggrin, regulatory associated protein of the MTOR complex 1, protease*

Abbreviations used

AD:	Atopic dermatitis
AKT1:	V-Akt murine thymoma viral oncogene homolog 1
CTSH:	Cathepsin H
EM:	Electron microscopy
FLG:	Filaggrin gene
GAPDH:	Glyceraldehyde-3-phosphae dehydrogenase
mTORC1/2:	Mechanistic target of rapamycin complex 1/2
RAPTOR:	Regulatory associated protein of the MTOR complex 1
REK:	Rat epidermal keratinocyte
RXRα:	Retinoid-X receptor α
shRNA:	Short hairpin RNA
SNP:	Single nucleotide polymorphism
WT:	Wild-type

Atopic dermatitis (AD) is a common disease in which the skin is sensitive to allergens and irritants, resulting in an immune response characterized by redness and scaling. Current evidence suggests that the primary cause of disease development in the majority of patients with AD is a defective skin barrier.[1,2] There is a strong genetic component to AD associated with skin barrier dysfunction.[3] One important protein is the epidermal structural protein filaggrin. Null mutations in the filaggrin gene *(FLG)* are responsible for the common inherited dry skin condition ichthyosis vulgaris and are a major predisposing factor for AD.[4,5] However only approximately 40% of patients with AD in the United Kingdom and around 10% of patients with AD in the rest of the world have filaggrin mutations,[6,7] and conversely, not all persons with filaggrin mutations have AD,[8] suggesting that other mechanisms might contribute to filaggrin expression and processing defects and hence to the barrier defect observed in patients with AD.

Profilaggrin to filaggrin processing is complex, requiring dephosphorylation and numerous proteolytic events; several proteases have been identified that cleave profilaggrin at specific sites, releasing the filaggrin monomers and both the N- and C-termini.[9] Proteases, such as elastase 2, aspartic peptidase, retroviral-like 1 (SASPase), and matriptase, are reported to be involved in profilaggrin to filaggrin processing.[10-13] There are also reports of aspartic- and cysteine-type cathepsin proteases playing a role in this process.[14-16] AKT1 is required for correct formation of the cornified envelope.[18] AKT1 activity in the epidermis is increased by treatment with the mechanistic target of rapamycin complex 1/2 (mTORC1; regulatory associated protein of the MTOR complex 1 [RAPTOR]) inhibitor rapamycin,[18] suggesting a role of RAPTOR in modulating AKT1 activity. Therefore we hypothesized that AKT1 activity might be reduced in AD skin, leading to alteration in protease expression, reduced filaggrin expression and processing, and skin barrier disruption.

Using a combination of keratinocyte short hairpin RNA (shRNA) knockdown models, human clinical samples, and mouse knockouts, we show that increased RAPTOR expression correlates with reduced filaggrin expression in the skin of atopic subjects, being most apparent in those with *FLG* compound heterozygous mutations. RAPTOR overexpression in keratinocytes reduced filaggrin expression, loss of AKT1 activity and filaggrin, and loss of cathepsin H (CTSH). CTSH-deficient mice have reduced filaggrin processing, subtle barrier defects, and an increase in proinflammatory molecules associated with increased macrophage infiltration of the skin and increased mast cell degranulation. Taken together, this provides strong evidence that RAPTOR levels and AKT1 signaling are important in modulating filaggrin levels and the immune environment in patients with AD.

METHODS

Animals

Ctsh knockout and heterozygote mice were generated, as previously described,[19] and backcrossed onto the C57BL/6J background for eight generations. $Ctsh^{-/-}$ and $Ctsh^{+/-}$ mice and wild-type (WT) littermate control animals were bred under specific pathogen-free conditions in accordance with the German law for Animal Protection (Tierschutzgesetz), as published on May 25, 1998. Three-day-old (neonate) mice were obtained from 5 litters, and 6-month-old (adult) mice were obtained from 2 separate litters. A maximum of 5 WT, 8 $Ctsh^{+/-}$, and 10 $Ctsh^{-/-}$ neonatal mice and 3 adult mice of each phenotype were used in all analyses, and blinding was not used in the assessment of mouse skin.

Short hairpin RNA knockdown, cell and organotypic culture, and mouse tissue

Four shRNA plasmids (Qiagen, Hilden, Germany) were used to knock down Akt1 expression (shRNA1: GCACCGCTTCTTTGCCAACAT, shRNA2: AAGGCACAGGTCGCTACTAT, shRNA3: GAGGCCCAA CACCTTCATCAT, and shRNA4: GCTGTTCGAGCTCATCCTAAT), and of these, 1 and 3 were used for further experiments. Ctsh knockdown was successfully achieved by means of transient transfection with 2 shRNA plasmids (shRNA1: CAAGAATGGTCAGTGCAAATT and shRNA3: CTA GAGTCAGCTGTGGCTATT). The following scrambled control was used: GGAATCTCATTCGATGCATAC. Akt1 and Ctsh shRNA knockdown plasmids were transfected into REK cells[17] by using lipofectamine (Invitrogen, Carlsbad, Calif), according to the manufacturer's instructions.