ResTORing barrier function in the skin

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The primary functions of the epidermis include water retention, thermal and pH homeostasis, and protection against entry of pathogenic microbes or toxic substances into the body.[1,2] Appropriate epidermal barrier formation relies on progressive differentiation of keratinocytes from the proliferating cells within the basal cell layer to the terminally differentiated cornified layer or stratum corneum.[2] The stratum corneum is composed of keratin macrofibrils and cross-linked cornified envelopes encased in lipid bilayers. Filaggrin (FLG) and lipids produced by epidermal granular cells control the assembly of a lipid-keratin matrix in forming this semipermeable barrier. Defects in the integrity of the epidermal barrier can result in a variety of inflammatory skin disorders, including atopic dermatitis (AD) and ichthyosis.

Ichthyosis is characterized by the presence of excessive amounts of dry, scaly, and thick skin surface. The cause of the ichthyosis is attributed to the complex interplay between keratinocyte differentiation and metabolic dysregulation. Genetic and clinical evidence indicates that defective expression and function of genes involved in FLG processing and lipid synthesis impair epidermal barrier acquisition and drive the onset of ichthyosis,[1] resulting in water loss, entry of infectious microbes, and infiltration of inflammatory lymphocytes. From a developmental perspective, it has been proposed that balancing keratinocyte

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proliferation and differentiation is crucial for shaping epidermal barrier formation and function. Yet how keratinocytes coordinate their differentiation and lipid metabolism in formation and maintenance of the epidermal barrier remains elusive.

As with most allergic diseases, AD is a multifactorial disease, and genetics plays a significant role in disease progression. Because AD is a T_H2-biased disease, many predisposing genes are linked to immune responses.[3,4] Recent studies have also identified the critical role of fatty acid synthesis in epidermal barrier formation and homeostasis.[5,6] One of the best known risk factor genes, *FLG*, encodes an epidermal differentiation protein that is critical in forming the lipid-keratin matrix and epidermal integrity.[3,4] Mutations in the *FLG* gene can cause ichthyosis vulgaris and are a strong risk factor for AD.[3,4] However, *FLG* mutations do not account for all AD cases, and not every carrier of an *FLG* mutation has AD. Therefore the underlying molecular mechanisms that regulate epidermal barrier function remain incompletely defined.

The mechanistic target of rapamycin (mTOR) is an evolutionarily conserved serine-threonine kinase that functions through 2 multiprotein complexes,[7] namely mechanistic target of rapamycin complex (mTORC) 1 and mTORC2, characterized by the obligate proteins regulatory-associated protein of mTOR (Raptor) and rapamycin-insensitive companion of mTOR (Rictor), respectively. As a central node in cellular metabolism and cell growth, these mTOR complexes dictate the cell fate decisions and functions of a variety of cells through coupling proliferation and differentiation in response to microenvironmental cues. In contrast to extensive studies of mTOR complexes in T cells,[8] it is only now beginning to be understood how mTOR signaling regulates epidermal barrier formation. Recent studies have revealed that mTORC1 is crucial for keratinocyte proliferation and the early epidermal differentiation program.[9] In this issue of the *Journal of Allergy and Clinical Immunology*, Ding et al[10] show that mTORC2 signaling controls the terminal differentiation and function of keratinocytes in the late stage of epidermal barrier formation (Fig 1).

Previous studies by Ding et al[10] demonstrated that mTOR signaling is indispensable for epidermal barrier formation,[9] although with differential roles defined for mTORC1 and mTORC2. By crossing mice carrying loxP-flanked target alleles (*Mtor*$^{fl/fl}$, *Rptor*$^{fl/fl}$, or *Rictor*$^{fl/fl}$) with human keratin 14 Cre recombinase transgenic mice, Ding et al[10] were able to respectively abolish complete mTOR signaling, mTORC1 activation, or mTORC2 activation specifically in the epidermis.[9] Deletion of *Mtor* in mouse epidermis (mTOR^{EKO}) resulted in newborns with defective epidermal barrier function that succumb to death shortly after birth.[9] They also demonstrated that mTORC1 and mTORC2 have distinct functions in maintaining healthy epidermal differentiation and formation. Newborn mice without Raptor displayed a similar phenotype as the mTOR^{EKO} mice. However, mice with deletion of Rictor in the epidermis (Ric^{EKO}) were able to survive despite abnormal skin development.[9]

In the current study Ding et al[10] follow up on their initial findings with more detailed experiments investigating the precise mechanism by which mTORC2 regulates epidermal barrier formation. Using Ric^{EKO} mice, Ding et al[10] demonstrate that disruption of mTORC2 function alters normal epidermal formation. Ric^{EKO} mice have an ichthyosis-like phenotype at birth with reduced epidermal thickness and enhanced transepidermal water loss, which is similar to what is observed in human patients.[4] By using RNA sequencing of the epidermis from wild-type and Ric^{EKO} E19.5 embryos, they reveal that many genes involved in keratinization, wound repair, and keratinocyte differentiation are upregulated in the mutants, whereas immune-regulating and lipid metabolism genes are downregulated. These findings suggest that dysfunctional mTORC2 promotes compensatory repair pathways and that lipid metabolism might be critical in epidermal formation.

To begin to address the role of lipid metabolism in regulating epidermal development, Ding et al[10] measured lipid content in the epidermis and showed that these are decreased and that there are altered lipid contents in several layers of the epidermis. Furthermore, they showed that mice with Rictor-deficient epidermis have altered proteolytic activity, resulting in lower amounts of FLG monomers

despite comparable levels of *FLG* mRNA and profilaggrin protein with control subjects. A direct link between mTORC2 function and FLG processing is established through complementation of the FLG-processing defect in Ric^{EKO} primary keratinocyte culture after Akt-Ser473 phosphorylation. Ric^{EKO} mice also demonstrated altered immune cell profiles, including more $CD4^+$ T cells and fewer γδ T cells in the epidermis. With defective mTORC2 function and inappropriate immune cell composition, Ric^{EKO} mice respond poorly to the hapten dinitrofluorobenzene. They also upregulate stress and proinflammatory genes in their ear skin in response to dinitrofluorobenzene.

Collectively, Ding et al[10] demonstrated a distinctive role of mTORC2 in regulating intact skin barrier function. The animal model they chose parallels several hallmarks in patients with AD, including altered skin morphology and disrupted immune cell composition, which suggest that their findings can be potentially translated to human studies.

Among the 2 complexes of mTOR, mTORC1 has been studied more extensively because of its responsiveness to acute treatment of rapamycin.[7] However, more and more effort has been devoted to investigating the importance of mTORC2 in cellular regulation and disease progression. Ding et al[10] conducted the first study to demonstrate that mTORC2 enforces the AKT-dependent FLG processing and orchestrates *de novo* lipid synthesis in keratinocytes. These processes shape the immune cell composition in the skin at steady state and its immune responses to allergens. These results indicate that dysfunctional mTORC2 could be another risk factor for skin diseases. Hence carriers of mutations in mTORC2 could have greater risk of skin disease. Certain cancer treatments also inhibit mTOR activities, which might compromise patients' skin integrity.[7] Combined therapy should be designed carefully to manage this potential side effect. Further studies are warranted to examine how mTORC2-dependent signaling regulates lipid metabolism. Moreover, the relevant ligands that activate mTORC2-dependent signaling have not been defined, and whether other environmental signals, such as the epidermal microbiome, detergents, or particulate matter and pollution, play a role in activating mTOR has not been

tested. mTORC2 regulates many interacting pathways that are druggable and could serve as potentially novel therapeutic targets to re-establish epidermal barrier integrity and improve the treatment for common skin disorders.[7] Hence identifying the roles of mTORC2-regulated metabolic networks in skin disease development has become the next pressing question.

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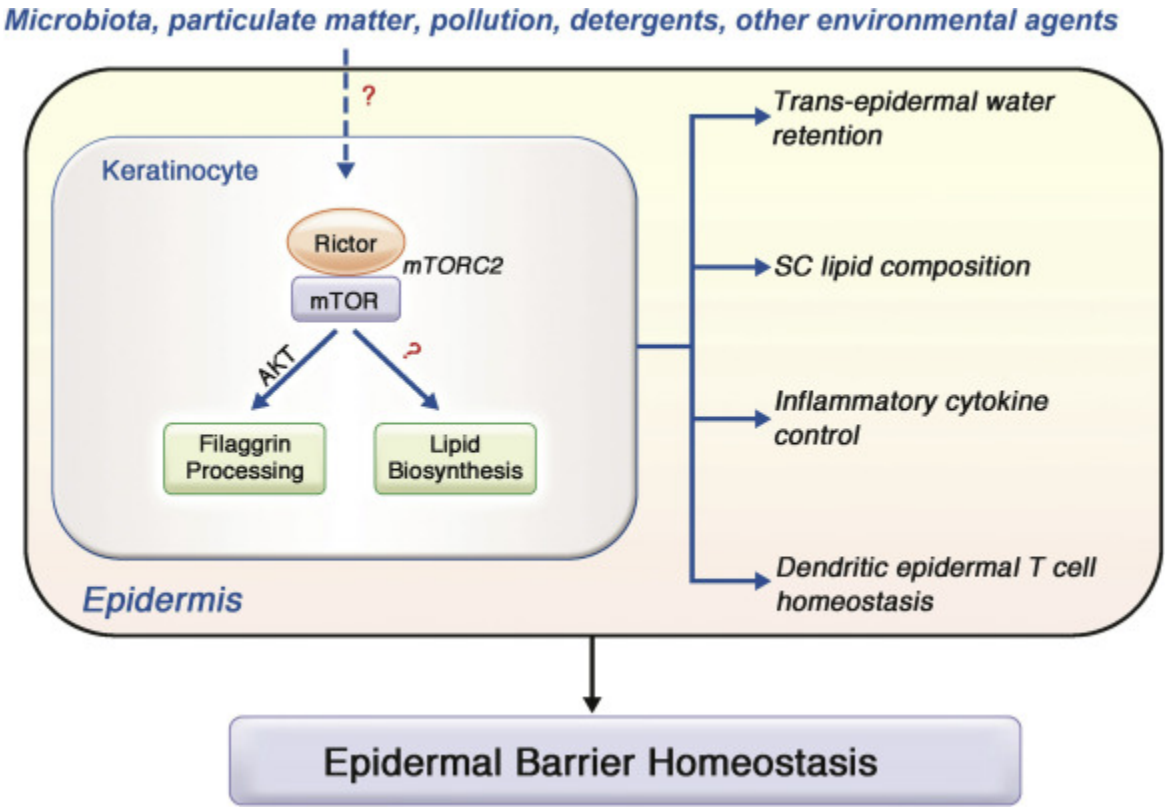

Fig 1. Keratinocyte mTORC2 couples FLG processing and lipid biosynthesis to maintain epidermal barrier homeostasis. In response to macroenvironmental and microenvironmental cues, keratinocytes activate Rictor-dependent mTORC2 signaling, which promotes FLG processing and lipid biosynthesis. Although AKT is downstream of mTORC2 in promoting FLG processing, the molecular mechanism linking mTORC2 and lipid biosynthesis remains elusive. Both of these pathways are critical in controlling inflammation and barrier function. *SC*, Subcutaneous.