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Cytoplasmic RNA quality control failure engages mTORC1-mediated autoinflammatory disease

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Inborn errors of nucleic acid metabolism often cause aberrant activation of nucleic acid sensing pathways, leading to autoimmune or autoinflammatory diseases. The SKIV2L RNA exosome is cytoplasmic RNA degradation machinery that was thought to be essential for preventing the self-RNA-mediated interferon (IFN) response. Here, we demonstrate the physiological function of SKIV2L in mammals. We found that Skiv2l deficiency in mice disrupted epidermal and T cell homeostasis in a cell-intrinsic manner independently of IFN. Skiv2l-deficient mice developed skin inflammation and hair abnormality, which were also observed in a Skiv2l-deficient patient. Epidermis-specific deletion of Skiv2l caused hyperproliferation of keratinocytes and disrupted epidermal stratification, leading to impaired skin barrier with no appreciable IFN activation. Moreover, Skiv2l-deficient T cells were chronically hyperactivated and these T cells attacked lesional skin as well as hair follicles. Mechanistically, SKIV2L loss activated the mTORC1 pathway in both keratinocytes and T cells. Both systemic and topical rapamycin treatment of Skiv2l-deficient mice ameliorated epidermal hyperplasia and skin inflammation. Together, we demonstrate that mTORC1, a classical nutrient sensor, also senses cytoplasmic RNA quality control failure and drives autoinflammatory disease. We also propose SKIV2L-associated trichohepatoenteric syndrome (THES) as a new mTORopathy for which sirolimus may be a promising therapy.

Introduction

Recognition of microbial nucleic acids by the innate immune system and activation of the type I interferon (IFN) response are fundamental mechanisms of host defense against infection. Inappropriate activation of IFN due to inborn errors of nucleic acid metabolizing enzymes can also drive a variety of autoimmune and autoinflammatory diseases (1). One classical example is TREX1/DNaseIII deficiency, which causes accumulation of self-DNA in the cytosol and activation of the DNA-sensing cGAS/STING/IFN pathway. TREXI mutations are associated with clinical diseases such as Aicardi-Goutières syndrome and systemic lupus erythematosus (2). Similarly, the accumulation of self-RNA activates the RIG-I-mediated IFN response. The SKIV2L RNA exosome has been shown to degrade self-RNAs in cells undergoing ER stress that produce IRE1-cleaved immunostimulatory self-RNA fragments (3). However, physiological functions of the SKIV2L RNA exosome at homeostasis and in vivo remain unknown.

The RNA exosome is an evolutionarily conserved intracellular 3‘ to 5‘ RNA degradation complex in eukaryotes, and it is involved in RNA processing, maturation, surveillance, and turnover (4). Mammalian RNA exosomes contain shared catalytic subunits as well as distinct cofactor complexes that bring in different RNA substrates (5). The cofactor for the cytoplasmic RNA exosome is the super-killer (SKI) complex, which contains a Ski2-like RNA helicase (SKIV2L), tetratricopeptide repeat domain 37 (TTC37), and WD repeat domain 61 (WDR61; ref. 6). SKIV2L is an RNA helicase that unwinds RNA substrates and threads them through the RNA exosome for degradation, while TTC37 and WDR61 contribute to the structure and activity of the SKI complex. The SKI complex directly associates with the 80S ribosome and extracts mRNA from stalled ribosomes for degradation as part of ribosome-associated mRNA surveillance (7, 8). Mutations in either the SKIV2L or TTC37 gene are associated with a rare inherited autosomal recessive disorder, trichohepatoenteric syndrome (THES) (OMIM 222470 THES1, 614602 THES2; refs. 9, 10). THES is clinically characterized by intrauterine growth retardation, intractable diarrhea, primary immunodeficiency, liver diseases, and skin and hair abnormalities (11). However, the molecular mechanism of THES remains unknown and no targeted treatment is currently available.

Here, we report that Skiv2l deficiency causes skin-specific autoinflammation without triggering innate IFN signaling. Loss of SKIV2L disrupts both epidermal and T cell homeostasis in a cell-intrinsic manner through the mTORC1 pathway, which can be targeted to ameliorate disease pathology associated with Skiv2l deficiency.
Results

Loss of SKIV2L causes epidermal hyperplasia and impairs skin barrier integrity. Germline whole-body deletion of Skiv2l resulted in early embryonic lethality before E13.5 (Supplemental Figure 1; supplemental material available online with this article; https://doi.org/10.1172/JCI146176DS1). We therefore generated a loxp-flanked Skiv2l conditional allele and bred Skiv2lfl/fl mice with UBC-Cre/ERT2 mice to generate Skiv2lfl/flUBC-Cre/ERT2 mice, which allow postnatal whole-body deletion of Skiv2l by tamoxifen administration at 4 weeks of age (Figure 1A, we call this iSkiv2l½ throughout). iSkiv2l½ mice have reduced body weight compared with Skiv2lfl/fl littermate controls (Supplemental Figure 2A). iSkiv2l½ mice developed phenomonal skin lesions and hair loss 4 weeks after tamoxifen injection (at 8 weeks of age, Figure 1B and Supplemental Figure 2B). Skin redness, scaly plaques, and hair abnormalities were first observed in the skin around the eyes, nose, and neck as early as 2 weeks after induction of Skiv2l deletion (Supplemental Figure 2C). By 8 weeks after tamoxifen administration, iSkiv2l½ mice lost most of their body hair, then regrew thin hypo pigmented hairs (Figure 1B). Histopathological examination of iSkiv2l½ dorsal skin revealed striking thickening of the epidermis (epidermal hyperplasia) and massive immune infiltrates in the dermis (Figure 1C). We also observed dystrophic anagen hair follicles (HFs) in iSkiv2l½ skin (Supplemental Figure 2D). Surprisingly, we did not observe significant inflammation in duodenum, colon, liver, or kidney of iSkiv2l½ mice, nor any changes in cytokines and chemokines in the serum compared with Skiv2lfl/fl littermate controls (Supplemental Figure 2, E and F), suggesting that the inflammation is restricted to the skin even though the Skiv2l gene is deleted in the whole body.

The prominent epidermal hyperplasia in the iSkiv2l½ mouse skin prompted us to examine keratinocyte proliferation and differentiation. Normally, keratinocytes in the epidermis continuously differentiate from a proliferative state at the basal layer to a nondividing state at the cornified layer at the top of the skin. This process is tightly regulated to ensure epidermal barrier integrity (Figure 1D). Fluorescence immunohistochemistry analysis of proliferation marker Ki67 showed more proliferating keratinocytes at the basal layer in iSkiv2l½ epidermis compared with that in Skiv2lfl/fl controls (Figure 1E). Cell cycle analysis of keratinocytes by flow cytometry also revealed increased mitotic cells in iSkiv2l½ epidermis compared with Skiv2lfl/fl control (Figure 1F). Basal keratinocyte markers keratin 14 (K14) and keratin 5 (K5) as well as suprabasal postmitotic marker keratin 10 (K10) all showed massive expansion in iSkiv2l½ mice (Figure 1G), suggesting dysregulation of epidermal stratification. Functionally, iSkiv2l½ mouse skin displayed impaired epidermal permeability as evidenced by the penetration of toluidine blue dye through the lesional skin (Figure 1H), as well as increased transdermal water loss (Figure 1I).

**SKIV2L acts cell-intrinsically in keratinocytes.** We next analyzed the role of SKIV2L in keratinocytes in 2 additional knockout mouse models. First, we performed local deletion of Skiv2l by topically applying 4-hydroxytamoxifen (4-OHT, the active metabolite of tamoxifen) on the right dorsal flank of the Skiv2lfl/flUBC-Cre/ERT2 mice. We also applied vehicle (DMSO) on the left side of the same mouse as a control (Figure 2A). Topical treatment with 4-OHT induced epidermal thickening and immune infiltration similar to pathologies observed in iSkiv2l½ mice (Figure 2B). No histological abnormality was observed in the vehicle-treated contralateral skin (Figure 2B). In addition, we observed a clear boundary of skin epidermal thickening only in the 4-OHT-treated area but not in the adjacent nontreated area (Figure 2C). We also observed lymphadenopathy of inguinal lymph nodes (LNs) from 4-OHT topical-treated but not vehicle-treated skin (Figure 2D). These data suggest that inducible Skiv2l gene deletion locally in the adult mouse skin is sufficient to cause tissue pathology.

Second, we crossed Skiv2lfl/fl with K14-Cre mice and generated keratinocyte-specific Skiv2l knockout mice (constitutive Cre expression driven by K14 promoter). Keratinocyte-specific depletion of SKIV2L protein was confirmed by Western blot using Skiv2lfl/flK14-Cre PO pup epidermis (Figure 3A and Supplemental Figure 3A). Skiv2lfl/flK14-Cre mice and littermates were born at expected Mendelian ratio (Figure 3B). However, only Skiv2lfl/flK14-Cre newborn pups showed abnormal ‘glassy’ skin and died within 24 hours after birth with 100% penetrance (Figure 3C). Although PO pups have no body hair, we found that Skiv2lfl/flK14-Cre pups lacked whiskers or developed distorted thin and fine whiskers (Figure 3D), suggesting defective hair morphogenesis. Histological analysis revealed thickening of Skiv2lfl/flK14-Cre epidermis, particularly in the spinous layer, while no apparent immune infiltration was observed in the dermis (Figure 3E). Skiv2lfl/flK14-Cre epidermis contained increased proliferating basal keratinocytes compared with controls, as evidenced by positive Ki67 staining and cell-cycle analysis by flow cytometry (Figure 3, F and G). Fluorescence immunohistochemistry analysis further revealed substantial expansion of keratinocytes expressing basal K14 and K5 as well as suprabasal K10 in Skiv2lfl/flK14-Cre compared with Skiv2lfl/fl epidermis (Figure 3H). Toluidine blue exclusion assay revealed impaired barrier integrity of Skiv2lfl/flK14-Cre mouse skin (Figure 3I). Together, these results suggest that the SKIV2L RNA exosome is lost in keratinocytes (Supplemental Figure 4C). We further generated myeloid-specific Skiv2l knockout mice Skiv2lfl/flLysM-Cre (Supplemental Figure 5, A–D). Skiv2lfl/flLysM-Cre mice were born at the expected Mendelian ratio (data not shown) and exhibited normal skin and hair with no signs of skin inflammation or any immunopathology (Supplemental Figure 5, E and F). Cytokines and chemokines in the serum of Skiv2lfl/flLysM-Cre mice were also indistinguishable from those of Skiv2lfl/fl controls (Supplemental Figure 5G). Together, these data suggest that Skiv2l knockout does not activate IFN signaling in vivo. We next assessed whether Skiv2l knockout activates IFN signaling in vivo. In disease-affected Skiv2lfl/flK14-Cre PO pup epidermis, we did not detect any increase in the expression of IFN-stimulated genes (ISGs) compared with littermate controls (Supplemental Figure 3B). We also isolated primary keratinocytes from uninduced Skiv2lfl/flUBC-Cre/ERT2 and Skiv2lfl/fl neonates, then induced Skiv2l deletion ex vivo by treating cell culture with 4-OHT (Supplemental Figure 4, A and B). We did not observe significant changes in the expression of IFN genes, ISGs, or inflammatory genes, indicating the lack of cell-intrinsic IFN signaling when Skiv2l function is lost in keratinocytes (Supplemental Figure 4C). We further generated myeloid-specific Skiv2l knockout mice Skiv2lfl/flLysM-Cre (Supplemental Figure 5, A–D). Skiv2lfl/flLysM-Cre mice were born at the expected Mendelian ratio (data not shown) and exhibited normal skin and hair with no signs of skin inflammation or any immunopathology (Supplemental Figure 5, E and F). Cytokines and chemokines in the serum of Skiv2lfl/flLysM-Cre mice were also indistinguishable from those of Skiv2lfl/fl controls (Supplemental Figure 5G). Together, these data suggest that Skiv2l knockout does not activate IFN signaling in vivo.
Figure 1. Loss of epidermal homeostasis and skin barrier integrity in postnatal inducible whole-body Skiv2l knockout mice. (A) A schematic diagram showing experimental design for generation of postnatal tamoxifen-inducible whole-body Skiv2l knockout mice (iSkiv2l<sup>−/−</sup>) by intraperitoneal tamoxifen injection in Skiv2l<sup>fl/fl</sup>UBC-cre/ERT2 mice. (B) Skin lesion and hair loss of iSkiv2l<sup>−/−</sup> mice at indicated ages. (C) H&E staining of iSkiv2l<sup>−/−</sup> mouse dorsal skin and Skiv2l<sup>fl/fl</sup> littermate controls (3 months old). White arrows, enlarged keratinocytes with loose chromatin. Quantification of epidermal thickness of iSkiv2l<sup>−/−</sup> and Skiv2l<sup>fl/fl</sup> is shown on the right bar graph. n = 5 mice per genotype. Unpaired 2-sided Student’s t test, ***P < 0.001. (D) A schematic diagram showing epidermal layers. (E) Fluorescence immunohistochemistry analysis of proliferation marker Ki67 (green signaling in the nucleus) of iSkiv2l<sup>−/−</sup> mouse dorsal skin and Skiv2l<sup>fl/fl</sup> littermate controls (3 months old). Nuclei were counterstained with DAPI (blue). White arrows, Ki67 positive cells. Asterisk indicates nonspecific staining of stratum corneum of epidermis. Isotype IgG was used as a negative control. Dashed line, epidermal-dermal junction. Scale bar: 50 μm. (F) Cell-cycle analysis of keratinocytes isolated from iSkiv2l<sup>−/−</sup> mice and Skiv2l<sup>fl/fl</sup> littermate controls (3 months old). Statistical analysis of cell-cycle distributions are shown on the right. n = 4 per genotype. Unpaired 2-sided Student’s t test, **P < 0.01, ***P < 0.001. (G) Fluorescence immunohistochemistry analysis of keratinocyte differentiation markers K14, K5, and K10 in iSkiv2l<sup>−/−</sup> mouse dorsal skin and Skiv2l<sup>fl/fl</sup> littermate controls (3 months old). Nucleus was stained with DAPI (blue). K14, keratin 14; K5, keratin 5; K10, keratin 10. Dashed line, epidermal-dermal junction. Scale bar: 50 μm. (H) Representative Toluidine blue staining of depilated iSkiv2l<sup>−/−</sup> mice and Skiv2l<sup>fl/fl</sup> littermate controls. (I) TEWL of iSkiv2l<sup>−/−</sup> mice (n = 20) and Skiv2l<sup>fl/fl</sup> littermate controls (n = 11). Unpaired 2-sided Student’s t test, ***P < 0.001.
pathway genes in data set showed broad elevation in the expression of the mTORC1ylation of S6 ribosome protein and 4E-BP1, 2 downstream effectors in a cohort of mice (Figure 4E and Supplemental Figure 6G). We further confirmed by qRT-PCR analysis using an additional cohort of mice (Figure 4F and Supplemental Figure 7D), consistent with the notion that mTORC1 controls mammalian cell size by regulating protein translation (12). These data further support mTORC1 activation in Skiv2l-deficient cells in vivo.

Figure 2. Topical inducible deletion of Skiv2l in epidermis results in localized inflammation in the skin. (A) A schematic diagram showing experimental design for Skiv2l deletion in mouse skin by topical application of 4-OHT at 4 weeks old for 5 consecutive days. DMSO was applied on the contralateral skin as vehicle control. (B) H&E staining of 4-OHT– or DMSO–applied skin of Skiv2lfl/fl K14-Cre mice and Skiv2lfl/fl littermates 3 weeks after treatment. (C) Long section including both 4-OHT–treated and adjacent untreated skin of Skiv2lfl/fl/UBC-Cre/ERT2 mice. Scale bar: 200 μm. (D) Skin-draining inguinal lymph node of 4-OHT– or DMSO–treated skin of Skiv2lfl/fl/UBC-Cre/ERT2 mice and Skiv2lfl/fl littermate controls.

Interestingly, several metabolic pathways (e.g., lipid and glucose metabolism) were also significantly enriched in Skiv2lfl/fl/UBC-Cre/ERT2 epidermis compared with controls (Figure 4C). This prompted us to examine the mTOR pathway, which is the master regulator of cellular metabolism. Gene set enrichment analysis (GSEA) using hallmark gene sets revealed enrichment of mTORC1 signaling pathway in Skiv2lfl/fl/UBC-Cre/ERT2 samples (Figure 4D). The RNA-seq data set showed broad elevation in the expression of the mTORC1 pathway genes in Skiv2lfl/fl/UBC-Cre/ERT2 dermis compared with controls, which we further confirmed by qRT-PCR analysis using an additional cohort of mice (Figure 4E and Supplemental Figure 6G). We next examined mTORC1 activity by directly measuring phosphorylation of S6 ribosome protein and 4E-BP1, 2 downstream effectors of the mTORC1 pathway. Both p-S6 and p-4E-BP1 were drastically increased in Skiv2lfl/fl/UBC-Cre and iSkiv2lfl/fl mice, quantified as signaling intensity per cell, compared with littermate controls (Figure 4, F and G and Supplemental Figure 7, A and B). These data suggest that Skiv2l deficiency activates mTORC1 in keratinocytes.

The SKI complex interacts with the ribosome to extract mRNA from stalled ribosomes for degradation (7, 8). We hypothesized that loss of SKIV2L may impair ribosome recycling on certain mRNAs, which could activate mTORC1 and global protein synthesis. To measure global protein synthesis in vivo, we adapted a pulse-labeling assay using O-propargyl-puromycin (OP-Puro) that can be injected in mice and labels nascent polypeptides in cells. Then, we labeled OP-Puro fluorescently by click chemistry and quantitated protein synthesis in single cells by flow cytometry. iSkiv2l−/− keratinocytes showed a significant increase in OP-Puro incorporation, suggesting increased global protein synthesis (Supplemental Figure 7C). We also found that Skiv2l-deficient keratinocytes were substantially larger than control cells (Figure 4H and Supplemental Figure 7D), consistent with the notion that mTORC1 controls mammalian cell size by regulating protein translation (12). These data further support mTORC1 activation in Skiv2l-deficient cells in vivo.

Aberrant activation of the mTOR pathway in Skiv2l-deficient keratinocytes. To explore the underlying mechanism that drives epidermal hyperplasia in Skiv2l-deficient mice, we performed whole transcriptome RNA sequencing analysis comparing Skiv2lfl/fl K14-Cre versus Skiv2lfl/fl (includes Skiv2lfl/fl, Skiv2lfl/+ , and Skiv2lfl/+) PO mouse epidermis. Differentially expressed genes (DEGs) enriched in Skiv2lfl/fl K14-Cre samples include keratinization-related genes (Krt), keratinocyte damage-associated molecular patterns (DAMPs) genes, and genes associated with inflammatory skin diseases (e.g., psoriasis, atopic dermatitis) (Figure 4A and Supplemental Figure 6, A–C). qRT-PCR analysis of an additional cohort of mice confirmed dysregulated gene expression in Skiv2lfl/fl K14-Cre epidermis compared with controls (Supplemental Figure 6, D–F). Further pathway analysis revealed enrichment of keratinization and cornification pathways, consistent with the epidermal pathology (Figure 4, B and C).

Interestingly, several metabolic pathways (e.g., lipid and glucose metabolism) were also significantly enriched in Skiv2lfl/fl/UBC-Cre epidermis compared with controls (Figure 4C). This prompted us to examine the mTOR pathway, which is the master regulator of cellular metabolism. Gene set enrichment analysis (GSEA) using hallmark gene sets revealed enrichment of mTORC1 signaling pathway in Skiv2lfl/fl/UBC-Cre samples (Figure 4D). The RNA-seq data set showed broad elevation in the expression of the mTORC1 pathway genes in Skiv2lfl/fl/UBC-Cre dermis compared with controls, which we further confirmed by qRT-PCR analysis using an additional cohort of mice (Figure 4E and Supplemental Figure 6G). We next examined mTORC1 activity by directly measuring phosphorylation of S6 ribosome protein and 4E-BP1, 2 downstream effectors of the mTORC1 pathway. Both p-S6 and p-4E-BP1 were drastically increased in Skiv2lfl/fl/UBC-Cre and iSkiv2lfl/fl mice, quantified as signaling intensity per cell, compared with littermate controls (Figure 4, F and G and Supplemental Figure 7, A and B). These data suggest that Skiv2l deficiency activates mTORC1 in keratinocytes.

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Skiv2l deficiency disrupts T cell homeostasis. Besides epidermal hyperplasia, we also observed immune cell infiltrates, particularly T cells, in iSkiv2l−/− mouse dermis and hair follicles (Supplemental Figure 8, A and B). iSkiv2l−/− mice exhibited lymphadenopathy and an increase in cellularity of skin-draining lymph nodes (Supplemental Figure 8C). The lymph node in iSkiv2l−/− mice exhibits hyperplasia with a larger number of follicles in the paracortex, suggesting an expansion of T cell clusters (Supplemental Figure 8D).

We next examined whether T cell immune homeostasis is perturbed in iSkiv2l−/− mice. In the spleen, the number of CD3+ T cells and the CD4/CD8 ratio in iSkiv2l−/− mice are normal (Supplemental Figure 8, E and F). However, iSkiv2l−/− mice showed significantly increased effector memory (CD62L−/CD44+) and central memory (CD62L+CD44−) CD4+ and CD8+ T cells with a corresponding reduction in naive (CD62L+CD44−) T cells in the spleen (Figure 5A and Supplemental Figure 9, A and B), indicating loss of quiescence in Skiv2l-deficient T cells. We further evaluated in vivo proliferation of T cells using a bromodeoxyuridine (BrdU) incorporation assay. iSkiv2l−/− mice had more Brdu-incorporated T cells than controls under steady-state conditions (Figure 5B). After anti-CD3/CD28 stimulation, expression levels of T cell activation markers CD25 and CD69 were much higher in iSkiv2l−/− compared with control T cells (Figure 5C and Supplemental Figure 9C). iSkiv2l−/− T cells also proliferated more rapidly and produced more...
became more proliferative compared with controls (Supplemental Figure 10, B–D). These results suggest an intrinsic role for SKIV2L in maintaining T cell homeostasis.

We also observed increased p-S6 staining in both CD8^+ and CD4^+ T cells of iSkiv2l^–/– mice compared with controls (Figure 5E). Western blot analysis of sorted splenic T cells further revealed increased IFN-γ and granzyme B than controls (Figure 5D and Supplemental Figure 9, D–F). To further confirm a cell-intrinsic role of Skiv2l deficiency in T cells, we isolated naive CD4^+ T cells that have not yet encountered antigen and stimulated them ex vivo with anti-CD3/CD28 antibodies (Supplemental Figure 10A). Skiv2l-deficient CD4^+ T cells expressed higher levels of CD25 and CD69 and became more proliferative compared with controls (Supplemental Figure 10, B–D). These results suggest an intrinsic role for SKIV2L in maintaining T cell homeostasis.

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Figure 4. Aberrant activation of the mTORC1 pathway in keratinocytes of germline keratinocyte-specific Skiv2l knockout mice. (A) MA plot comparing gene expression of Skiv2l<sup>ctl</sup> and Skiv2l<sup>fl/fl</sup>K14-Cre (germline keratinocyte-specific Skiv2l knockout) P0 epidermis (n = 2 mice per genotype). Data are shown as average gene expression (log<sub>2</sub>avg FPKM) on the y axis and log<sub>2</sub>fold change (Skiv2l<sup>fl/fl</sup>K14-Cre [KO] versus Skiv2l<sup>ctl</sup>) on the x axis, with direction of enrichment as indicated. Red dots, keratinization-related genes; orange squares, keratinocyte-associated DAMPs genes; purple triangles, inflammatory skin disease related genes. (B) Heatmap of DEGs of Skiv2l<sup>ctl</sup> versus Skiv2l<sup>fl/fl</sup>K14-Cre. n = 2 mice per genotype. (C) Reactome pathway analysis of DEGs of Skiv2l<sup>ctl</sup> versus Skiv2l<sup>fl/fl</sup>K14-Cre. Dashed line showing adjusted P value as 0.05. Pathways without statistical significance (adj. P value > 0.05) are shown in gray. Normalized enrichment score (NES) is shown with color bar (red, enriched in Skiv2l<sup>ctl</sup>; green, enriched in Skiv2l<sup>fl/fl</sup>K14-Cre). Circle size indicates number of genes in a gene set. (D) CSEA of Skiv2l<sup>ctl</sup> and Skiv2l<sup>fl/fl</sup>K14-Cre P0 epidermis RNA-seq data set. CSEA plot of hallmark mTORC1 signaling. (E) A heatmap showing gene expression of the mTORC1 pathway in Skiv2l<sup>ctl</sup> and Skiv2l<sup>fl/fl</sup>K14-Cre P0 epidermis. n = 2 mice per genotype. (F and G) Fluorescence immunohistochemistry analysis of p-S6 ribosomal protein (S235/236) and p-4E-BP1 (T37/46) in Skiv2l<sup>ctl</sup> and Skiv2l<sup>fl/fl</sup>K14-Cre P0 pup skin tissues (F). Dashed line, epidermal-dermal junction. Scale bar: 50 μm. Quantification of p-S6 or p-4E-BP1 fluorescence intensity per cell (>50 cells each genotype) is shown in (G). Unpaired 2-sided Student’s t test, ***P < 0.001. (H) Representative images showing enlarged keratinocyte cell size in Skiv2l<sup>fl/fl</sup>K14-Cre P0 pup skin. Scale bar: 20 μm. The K14 immunofluorescence images of Skiv2l<sup>ctl</sup> and iSkiv2l<sup.–/–</sup> epidermis are crops of images shown in Figure 3H.
increased phosphorylation of mTORC1 substrates S6K and 4E-BP-1, while phosphorylation of mTORC2 downstream targets FoxO1 and FoxO3a was reduced (Supplemental Figure 11, A–C). iSkiv2l−/− CD8+ and CD4+ T cells are also larger in size than control T cells (Figure 5F). Together, these data suggest that Skiv2l deficiency activates mTORC1 and induces T cell hyperactivation.

mTORC1 inhibitor rapamycin ameliorates iSkiv2l−/− disease pathology. We next assessed whether targeting mTORC1 can reduce disease pathology associated with Skiv2l deficiency (Figure 6A). Remarkably, systemic treatment of iSkiv2l−/− mice with rapamycin (by intraperitoneal injection) led to significantly reduced skin lesion and hair loss (Figure 6, B and C). Histopathology analysis revealed that rapamycin dramatically ameliorated epidermal hyperplasia in iSkiv2l−/− mice (Figure 6D). We also observed reduced p-S6 staining and fewer Ki67+ cells within the skin of rapamycin-treated compared with vehicle-treated iSkiv2l−/− mice.
Histopathologic analysis of a lesional skin biopsy identified interface dermatitis with lymphocyte infiltration in the dermis (Figure 7C). RNA-seq analysis of PBMCs from the patient with THES2 did not identify elevated ISG expression when compared with healthy controls (Figure 7D and Supplemental Figure 13A). Further immunostaining revealed substantially increased p-S6 in epidermis from the patient with THES2, consistent with activated mTORC1 pathway (Figure 7E). We also noted an interesting difference between THES2 and an unrelated case of atopic dermatitis (AD). p-S6 staining in THES2 is stronger toward the basal layer of the expanded epidermis whereas p-S6 staining in AD is lacking at the basal layer. Further, we observed a moderate increase in MX1 expression in skin tissue from the patient with THES2 (Supplemental Figure 13B). The findings from the patient with THES2 match those from mice and together they suggest that loss of SKIV2L activates mTORC1 signaling, which induces skin auto-inflammation (Figure 7F).

Discussion

Inborn errors in RNA metabolism have been associated with human autoimmune diseases. For instance, Aicardi-Goutières syndrome mutations in RNase H2 (cleaves RNA-DNA hybrids) or in adenosine deaminase acting on RNA 1 (ADAR1) invoke innate immune sensing of self-nucleic acids (DNA or RNA), leading to type I IFN signaling and systemic inflammation (13). Biochemical activities of the cytoplasmic RNA exosome are well-studied in the yeast, but its physiological functions in mammals are not known. Here, using a panel of germline, inducible, whole-body, and tissue-specific gene knockout mouse models, we demonstrate that SKIV2L RNA exosome loss-of-function causes skin-specific autoinflammation by disrupting both epidermal and T cell homeostasis without provoking innate immune response to self-RNA. Tissue-specific deletion of SKIV2L also did not trigger type I IFN response in keratinocytes or myeloid cells. Although MX1 expression is moderately increased in the epidermis of the patient with THES2, it is possible that overactive T cells that infiltrate skin tissue induce ISGs expression in keratinocytes in a paracrine manner. Moreover, we did not observe systemic or multi-organ inflammation when the Skiv2l gene was deleted globally in adult mice.

Our in vivo data indicates that the cytoplasmic SKIV2L RNA exosome, under physiological conditions, is unlikely to act as a gatekeeper of self-RNA-mediated innate immune activation. This notion is in line with a recent study investigating substrates of mammalian RNA decay pathways, which reveals that SKIV2L participates widely in surveillance of mRNA with aberrant translation (14). A more comprehensive model could be that the mammalian SKIV2L RNA exosome engages in different functions depending on context or cellular cues. Under physiological conditions, SKIV2L extracts and removes mRNA from stalled ribosomes to avoid activation of mTORC1. Under stress conditions such as viral infection, SKIV2L may expand its role to remove immunogenic host or viral RNA to avoid activation of innate immune sensors.

Our study demonstrates that cytoplasmic RNA quality control failure could activate mTORC1 and cause tissue pathology. The SKI complex directly associates with 80S ribosome and extracts mRNA from stalled ribosomes to avoid activation of mTORC1.
through the mTORC1 pathway and causes epidermal hyperproliferation (15), similar to the Skiv2l-deficient skin phenotypes observed in our study. How stalled ribosomes activate the mTORC1 pathway requires further investigation. Nonetheless, these findings and ours collectively demonstrate how seemingly broad cellular defects from stalled ribosome degradation (7, 8). This mechanism has been proposed to facilitate subsequent dissociation and recycling of stalled ribosomes by the Pelota/Hbs1/ABCE1 complex, though its physiological importance has not been experimentally elucidated. Deletion of Pelota leads to global upregulation of protein translation through the mTORC1 pathway and causes epidermal hyperproliferation (15), similar to the Skiv2l-deficient skin phenotypes observed in our study. How stalled ribosomes activate the mTORC1 pathway requires further investigation. Nonetheless, these findings and ours collectively demonstrate how seemingly broad cellular defects
Table 1. Serology of the patient with THES2

| Serological tests | Reference range | Age, months |
|-------------------|-----------------|-------------|
| ANA titer         | Negative        | 3           | 1:60         |
|                   |                 | 18          | Negative     |
| ANA pattern       | Homogenous      | 20          | 1:80         |
|                   |                 | 25          |              |
|                   |                 | 40          |              |
|                   |                 | 55          |              |
|                   |                 | 77          |              |

(ANA, antinuclear antibody; ANCA, antineutrophil cytoplasmic antibodies; GBM, glomerular basement membrane; LKM1, liver kidney microsome type 1; MPO, myeloperoxidase; RF, rheumatoid factor.)

(RNA quality control, ribosome stalling, and the mTORC1 pathway) can cause remarkably restricted tissue pathology (e.g., skin).

We present a new paradigm of tissue-specific autoinflammation, where metabolically dysregulated T cells (without innate immune activation or priming) are attracted by skin-intrinsic pathology to confer tissue specificity and fuel inflammation. This mechanism is distinct from other skin-specific autoimmunity diseases, such as psoriasis and cutaneous lupus erythematosus, which are generally believed to be initiated by aberrant innate immune activation as the primary trigger of pathogenesis (16, 17). Notably, the metabolic perturbation in T cells by *Skiv2l* deficiency seems insufficient to breach global immune tolerance, and we did not observe systemic T cell-mediated autoimmune pathologies that are common in patients with FOXP3 or AIRE mutations (18, 19). While hyper-responsiveness of *iSkin2l*–/– naïve T cells to ex vivo TCR stimulation highly suggest a cell-intrinsic role for SKIV2 in regulating T cell response, it’s worth noting that soluble or secreted factors that naïve T cells have engaged with in vivo could also contribute to enhanced T cell response. The patient with THES2 in this study did not develop hemophagocytic syndrome, which is characterized by hyperactivation of lymphocytes and histiocytes, and which is noted in about 60% of patients with THES (20). However, the patient developed ANCA-associated vasculitis, including pauci-immune necrotizing and crescentic glomerulonephritis, which, to our knowledge, is first reported in patients with THES in the present study. Besides autoreactive B cells, expanded effector T cells that are in a persistent activation state are also involved in the pathogenesis (21). The T cell hyperactivation phenotype in our mouse model merits further clinical investigation in patients with THES. The increased mTORC1 activity and decreased mTORC2 activity in *Skiv2l*-deficient T cells are similar to that in systemic lupus erythematosus (SLE) (22–25). Treatment with sirolimus, an mTORC1 inhibitor, corrects the proinflammatory T cell phenotype and improves clinical outcomes in patients with SLE (23, 26). Hyperactivation of the mTORC1 pathway has also been associated with other autoimmune hematological diseases, including autoimmune lymphoproliferative syndrome (ALPS) (27) and idiopathic multicentric Castleman disease (iMCD) (28–30). Aberrant mTOR signaling also contributes to pathogenic lymphoproliferation in those diseases and administration of sirolimus has been shown to induce cellular or clinical benefits in patients (27–32).

Patients with THES develop other clinical symptoms besides skin inflammation, including intractable diarrhea, primary immunodeficiency, and liver disease (11). Some patients with THES2 do not develop characteristic diarrhea, suggesting phenotypic heterogeneity of the disease (33). The whole-body inducible *iSkin2l*–/– mice do not fully replicate all THES phenotypes; this is likely due to incomplete or delayed gene deletion in certain tissues or differences in mouse versus human. Nonetheless, numerous studies have demonstrated a critical role for mTORC1 in intestinal homeostasis (34–36). We demonstrated remarkable therapeutic efficiency of mTOR inhibitor rapamycin in ameliorating skin disease of *Skiv2l*-deficient mice. Therefore, our findings provide a scientifically rationalized and immediately actionable treatment for the THES disease.

Methods

*Mice.* The C57BL/6N-A^tm1a(EUCOMM)Rkhsd/BayMmucd strain (catalog 037780-UCD) carrying the “knockout-first” *Skiv2l* allele was recovered from the cryo-archive at the Mutant Mouse Resource & Research Centers (https://www.mmrrc.org). The derived heterozygous mice with black appearance were further crossed with B6N.129S4-Gt(ROSA)26Sor^+^/+^/B6^+/+^/J mice (B6N.ROSA26:FLPe, Jackson Laboratory, catalog 016226) to delete neomycin selection cassette and generate *Skiv2l*-floxed mice. *Skiv2l^fl/fl* mice were further crossed with B6.Cg-Ndor1^+^/J (UBC-Cre-ERT2, Jackson Laboratory, catalog 007001), B6.129P2-Lyz2^tm1(cre)^/J (Ly5-M-Cre, Jackson Laboratory, catalog 004781) or B6N.Cg-Tg(KRT14-cre)1Amc/J (K14-Cre, Jackson Laboratory, catalog 018964) to generate tamoxifen-inducible (*Skiv2l^fl/fl^UBC-Cre-ERT2*), myeloid-specific (*Skiv2l^fl/fl^LysM-Cre*), and epidermis-specific (*Skiv2l^fl/fl^K14-Cre*) knockout mice, respectively. As for the crossing of *Skiv2l^fl/fl^* and *Skiv2l^fl/+^K14-Cre* mice, we named the progenies *Skiv2l^fl/+^*, *Skiv2l^fl/fl^*, and *Skiv2l^fl/fl^K14-Cre* as *Skiv2l^fl/+^* since all 3 genotypes showed no phenotypic difference. The schematic graph of different *Skiv2l* alleles and mouse breeding scheme are illustrated in Supplemental Figure 1A. PCR genotyping of knock-out-first and floxed alleles was carried out using the following primers: knockout first allele, forward 5′-CGAATTCTCAGTTGAG -3′, knockout-first and floxed alleles was carried out using the following primers: knockout-first allele, forward 5′-TACACATGCCTCCATTAGCCTG-3′, reverse 5′-CCACAACGGGTTCTTCTGTTAG-3′; forward 5′-TACACATGCCTCCATTAGCCTG-3′, reverse 5′-CCACAACGGGTTCTTCTGTTAG-3′. PCR genotyping of LysM-Cre, UBC-Cre/ERT2, and K14-Cre mice was carried out following protocols of the Jackson Laboratory. All mice were housed in pathogen-free barrier facilities at UT Southwestern Medical Center.
To generate postnatal whole-body Skiv2l knockout mice, tamoxifen (Sigma-Aldrich, catalog T5648) was dissolved in corn oil (20 mg/mL) and injected intraperitoneally into 4-week-old Skiv2l<sup>fl/fl</sup>/UBC-Cre/ERT2 and Skiv2l<sup>fl/fl</sup> littermate control mice (75 mg/kg body weight) once daily for 5 consecutive days. To delete Skiv2l locally in a small area of skin, Skiv2l<sup>fl/fl</sup>/UBC-Cre/ERT2and Skiv2l<sup>fl/fl</sup> littermate control mice were depilated on both lower flanks. Five microliters of (Z)-4-OHT, Sigma-Aldrich, catalog H7904, dissolved in DMSO to a concentration of 5 μM or vehicle DMSO was topically applied on right or left lower flank within a circle area (diameter 1 cm) respectively, once daily for 5 consecutive days.

**Human patient study.** The patient included in this study is a female (< 10 years old) born to nonconsanguineous non-Hispanic White parents. Genetic and histopathological analyses of the affected patient were performed with informed consent. Skin fibroblasts were obtained from the patient and expanded in the laboratory. Formalin-fixed paraffin-embedded lesional skin section was sectioned for fluorescence immunohistochemistry analysis. Deidentified nondiseased skin samples were obtained from the Tissue Management Shared Resource (TMSR) at UT Southwestern Medical Center. Skin biopsy slides from an unrelated case of AD and an unrelated case of seborrheic keratosis (SK) were kindly provided by Richard C. Wang (UTSW) and used for comparison to the THES2 patient’s skin biopsy.

**Histopathology, immunohistochemistry, and fluorescence immunohistochemistry.** Mouse tissues were fixed in 4% paraformaldehyde and paraffin embedded. H&E staining of mouse tissue was performed at the UT Southwestern Medical Center HistoPathology Core. Immunohistochemistry of mouse skin samples was performed by HistoWiz. H&E staining and immunohistochemistry of the patient’s biopsies were performed and interpreted by a pathologist in Children’s Medical Center Dallas. Fluorescence immunohistochemistry was performed as previously described (37). Briefly, 5 μm paraffin-embedded tissue sections mounted on a slide were deparaffinized and rehydrated. Antigen retrieval was performed in citric acid solution at 121°C for 20 minutes. Slides were blocked with 10% normal goat serum in 1% BSA/PBS with indicated antibodies for 30 minutes at 4°C. For intracellular staining following surface marker staining, cells were fixed and permeabilized using Cytofix/Cytoperm Fixation/Permeabilization Solution Kit (BD Biosciences, catalog 554714), then stained with antibodies for intracellular markers. Dead cells were excluded by using Zombie Aqua Fixable Viability Kit (BioLegend, catalog 423102) during surface marker staining.

For BrdU incorporation assay, BrdU was injected intraperitoneally (2 mg/mouse) and splenocytes were collected 20 hours later for analysis. BrdU staining was performed following BD Pharmingen BrdU Flow Kits (BD Biosciences, catalog 559619) instruction. Phospho-flow cytometry of p-S6 was performed following BD Phosflow Protocols for Mouse Spleenocytes or Thymocytes. Data were collected on LSRII flow cytometer (BD Biosciences) or CytoFLEX (Beckman Coulter) and was analyzed using FlowJo software.

**Magnetic-activated cell sorting and T cell in vitro activation.** Magnetic-activated cell sorting (MACS) was performed using autoMACS Pro Separator. The cell isolation kits used were as follows: Pan T Cell Isolation Kit II (Miltenyi Biotec, catalog 130-095-130), Pan B Cell Isolation Kit II, mouse (Miltenyi Biotec, catalog 130-104-443), CD4<sup>+</sup> T Cell Isolation Kit, mouse (Miltenyi Biotec, catalog 130-104-454), CD8<sup>+</sup> T Cell Isolation Kit, mouse (Miltenyi Biotec, catalog 130-104-075), Naive CD4<sup>+</sup> T Cell Isolation Kit, mouse (Miltenyi Biotec, catalog 130-104-453). Cell labeling and sorting was performed per manufacturer’s instructions. Sorted cells were cultured in plates precoated with anti-CD3 (145-2C11, eBioscience, catalog 16-0031-85) and anti-CD28 (93, eBioscience, catalog 16-0281-85) antibodies at the indicated concentration. For CFSE dilution assay, T cells were loaded with CFSE (2.0 μM, Thermo Fisher Scientific, catalog C34570) for 10 minutes at 37°C, then washed with twice complete medium. After culture for indicated time, cells were collected and stained for cell surface or intracellular markers for flow cytometry analysis.

**Flow cytometry analysis of cell cycle and protein synthesis in vivo.** To analyze cell cycle, keratinocytes were isolated from adult mice tail skin and P0 neonate skin using dispase II digestion (Sigma, catalog
RNA sequencing and pathway enrichment analysis. Total RNA was isolated from the epidermis of PO pups using RNeasy Mini Kit (Qiagen, catalog 74104). DNA was removed by on-column DNase digestion. RNA integrity was measured by Bioanalyzer (Agilent) at the UT Southwestern Microarray & Immune Phenotyping Core Facility, and samples with RNA Integrity Number greater than 9.0 were used for sequencing. Total RNA sequencing was performed at BGI Americas. Raw sequencing data were processed with Astrocyte RNASeq Analysis Workflows at the UT Southwestern Bioinformatics Core Facility (https://astrocyte.biohpc.swmed.edu/workflow/12/version/324/docs) to generate fragments per kilobase million (FPKM) for each transcript. Protein coding genes with FPKM value greater than 0.5 in at least 1 sample were selected for DEG analysis using iDEP (http://bioinformatics.sdstate.edu/idep/). For MA plot, average gene expression (log 10-transformed, bioinformatics.sdstate.edu/idep/) were plotted using GraphPad. RNA sequencing data are available at NCBI GEO (GSE184418). For GSEA, protein-coding transcripts of total RNA sequencing data set were run on GSEA 4.1.0 software (Broad Institute) using H: hallmarks gene sets database with 1000 "gene_set" permutations. The gene set size was specified between 15 and 500, and t test was used as the metric for ranking genes. RNA isolation and RT-qPCR. Total RNA was isolated from the epidermis using TRI reagent (Sigma) per the manufacturer’s instructions, and cDNA was synthesized with iScript CDNA Synthesis Kit (Bio-Rad). iTag Universal SYBR Green Supermix (Bio-Rad) was used to quantify mRNA expression with CFX96 Real-Time PCR Detection System. Gene expression was normalized to internal controls. Primer sequences are shown in Supplemental Table 1. Interferon score. Total RNA was isolated from the patient’s and 2 healthy individuals’ PBMCs using the RNeasy Mini Kit (Qiagen, catalog 74104). RNA integrity was measured by Bioanalyzer (Agilent).
Rapamycin treatment. Rapamycin was purchased from LC Laboratory (purity >99%, catalog R-5000). For intraperitoneal treatment, rapamycin was first dissolved in DMSO to 200 mg/mL. Before injection, rapamycin was further diluted in 5% PEG-400/5% Tween-80 to the final concentration of 1.6 mg/mL. After the last dose of tamoxifen injection, mice received intraperitoneal injection of rapamycin (8 mg/kg body weight) or equal volume vehicle daily for 4 weeks. For topical treatment, rapamycin was dissolved in acetone to 1% (wt/vol). Dorsal skin was shaved after the last dose of tamoxifen injection (the day before treatment). Mice received topical applications of 50 μL 1% rapamycin daily on the right dorsal skin and equal volume of acetone on the left dorsal skin for 4 weeks.

Statistics. Graphpad Prism was used for illustration of graphs and statistical analysis. Statistical tests performed are indicated in the figure legends. Numerical data were represented as mean ± SEM (error bars). *P less than 0.05 was considered statistically significant. Schemes and models were created with Biorender.com.

Study approval. Mouse studies were carried out in accordance with the NIH’s Guide for the Care and Use of Laboratory Animals (National Academies Press, 2011) and the animal protocol was approved by the IACUC at UT Southwestern Medical Center (APN 2017-101968). Human patient studies were reviewed and approved by the IRB at UT Southwestern Medical Center.

Author contributions
KY performed most of the experiments. JH assisted with mouse breeding and mouse experiments. MA and LAG performed some skin tissue staining and advised on experimental design. JG, JYP, MNS, JGG, TW, CAV, and MTM performed all clinical immunology and pathology analysis. KY and NY designed the project, analyzed the data, and wrote the manuscript with input from all authors.

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