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REVIEW ARTICLE

Mouse models of psoriasis and their relevance

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ABSTRACT

Psoriasis is an inflammatory skin disorder that includes dynamic interactions between the immune system and skin and is clinically characterized by keratinocyte proliferation and distinct inflammatory cell infiltrates. Cross-talk between keratinocytes and immunocytes is essential for the development of psoriasis given that it mediates the production of cytokines, chemokines and growth factors. To resolve the pathogenesis of psoriasis, numerous experimental animal models have been generated. In this review, we discuss recent findings from mouse models, their relevancy to psoriasis and use, including the discovery of new therapies.

Key words: immune system, keratinocytes, mouse model, psoriasis, signal transducer and activator of transcription 3.

INTRODUCTION

Psoriasis is one of the most common inflammatory disorders of the skin that affects greater than 2% of the population in Western countries. Disease prevalence varies depending on ethnicity and geographical regions. The pathogenesis of psoriasis is multifactorial with genetic, environmental and immunological factors contributing to the phenotypes. Psoriasis involves excessive proliferation and altered differentiation of epidermal keratinocytes, likely mediated by the immune systems.[1,2] Genetic alterations that affect the key signaling pathways involved in inflammatory immune responses in keratinocytes and inflammatory cells can alter skin homeostasis and induce psoriasis. Although the specific cells that initiate the development of psoriasis are unknown, mouse models have provided important insights into this aspect and mainly focused on keratinocytes and immunocytes, especially T cells, as disease initiators.[3–6] The pathogenesis of psoriasis is complex and dynamic, involving epidermal cells and immune cells. Cross-talk between keratinocytes and immune cells, which results in the production of cytokines, chemokines and growth factors, is thought to mediate this disease.

Psoriatic lesions are characterized by histopathological changes, including: (i) a thickened epidermis (acanthosis) arising from rapid keratinocyte proliferation; (ii) a reduced or absent granular layer (hypogranulosis) due to the aberrant differentiation of keratinocytes; (iii) a marked dilation of blood vessels in papillary dermis, which causes visible erythema; (iv) accumulation of neutrophils in parakeratosis stratum corneum (Munro's microabscesses); and (v) a dense inflammatory infiltrate composed of clusters of $CD4^+$ T-helper (Th) cells and antigen-presenting dendritic cells (DC) in the dermis and $CD8^+$ cells in the epidermis.

Previous studies have demonstrated that the interleukin (IL)-23/IL-17 pathway is involved in the induction of psoriasis.[1,2,7] Inflammatory myeloid DC release IL-23 and IL-12 to activate Th17 cells to produce abundant psoriatic cytokines, including IL-17, tumor necrosis factor (TNF) and IL-22. These cytokines mediate effects on keratinocytes to amplify psoriatic inflammation. The most distinct evidence for the role of IL-23/Th17 in psoriasis is derived from clinical studies. Therapeutic studies with anti-cytokine antibodies have demonstrated the importance of the key cytokines IL-23, TNF and IL-17 in this process.[8–10] IL-22, a cytokine produced from Th17, is elevated in the blood of psoriatic patients,[11] and triggering the IL-22R mediates the proliferation and migration of keratinocytes via signal transducer and activator of transcription (Stat)3 activation.[12] In addition to IL-22, other IL-20 subfamily cytokines, such as IL-19, IL-20, IL-24 and IL-26, have been implicated in psoriasis. In addition to adaptive immunity, innate immunity also plays an important role in the pathogenesis of psoriasis.[13] Stimulation of Toll-like receptor (TLR)9 by complexes of the antimicrobial peptide LL37 and self-DNA results in the activation of plasmacytoid DC (pDC), which initiate the onset of psoriasis.[14] In addition, a previous study demonstrated that self-RNA–LL37 complexes trigger TLR8 of myeloid DC and TLR7 of pDC in human and drive autoinflammatory responses in psoriasis.[15] Furthermore, psoriasis is susceptible to an increase in copy number variation in the *β-defensin* gene locus.[16] These findings suggest that an altered innate defense mechanism and the resulting enhanced IL-23 signaling may initiate psoriasis.

Given that psoriasis exclusively affects humans among all animals, the lack of a virtual animal model has hindered experimental *in vivo* research of the pathogenesis of psoriasis. Over the past decades, murine models of psoriasis have been

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developed as tools for understanding the pathogenesis of this disease and as preclinical models. These models include transgenic mice, knockout mice and reconstituted models of psoriasis.[3–6] Despite their limitations, these models reproduce some but not all features of psoriasis, such as epidermal hyperplasia, vascular proliferation and skewed T immunity. Before these mouse models are discussed in detail, it is important to recognize the differences between mouse and human skin.

In 2007, Gudjonsson *et al.* described various mouse models of the immunological pathways that contributed to the psoriatic phenotype and compared them in detail.[3] They classified the models as xenografts, allografts, transgenic, targeted mutations and spontaneous models, and ranked features of human psoriasis in each model.[3] In 2011, Swindell *et al.* explored that transcriptional profiling of the five most representative models (K5.Stat3C, K14-amphiregulin, K5.Tie2, K5.TGF-β1 and imiquimod treatment) and assessed them via comparison with human psoriasis.[6] Strong and statistically significant similarities in the gene expression profiling were noted between human psoriasis and each of these mouse models, in particular gene expression patterns in the epidermis.[6] However, marked differences existed in immune-associated gene expression across the models.

Signal transducers and activators of transcription constitute a family of latent cytoplasmic proteins involved in transmitting extracellular signals to the nucleus.[17] One member, Stat3, has a critical role in various biological activities, including cell proliferation, survival and cell migration.[17,18] Persistent activation of Stat3 has been implicated in carcinogenesis of squamous cell carcinoma.[19]

In the skin, Stat3 has an essential role in wound healing[20,21] and is activated in psoriatic lesions.[22] Stat3 is a key regulator of keratinocyte proliferation and survival following UV irradiation.[23] The transgenic mice, K5.Stat3C in which a constitutively active Stat3 mutation was expressed under the keratin 5 promoter, were generated as a psoriasis model.[22] These mice developed skin lesions resembling psoriasis either spontaneously or in response to tape stripping or topical treatment with tumor promoter 12-*O*-tetradecanoylphorbol-13-adetate (TPA) (Fig. 1). The skin lesions exhibited epidermal hyperplasia with parakeratosis but loss of the granular layer, dilated blood vessels and leukocytic infiltration. *In vivo* reconstitution experiments in nude mice using combinational skin and lymphocytes revealed that the development of skin lesions required Stat3 activation in the skin together with activated CD4 T lymphocytes. In addition, K5.Stat3C mice exhibited cytokine profiles in their lesions with close similarities to plaque psoriasis (Fig. 2).[24,25] Therefore, anti-IL-12/23p40, anti-IL-17A and anti-IL-23p19 antibodies inhibited the development of lesions, demonstrating similar therapeutic efficacies in psoriasis.[24] In this review, we describe our studies on model mice, including K5.Stat3C and K5-SPTcKO. We investigated

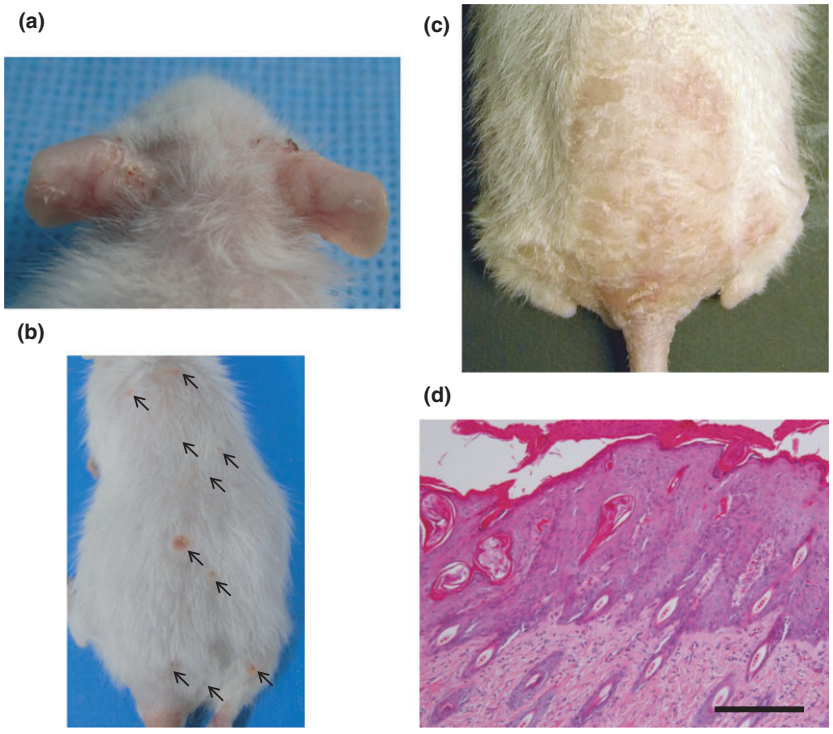

Figure 1. (a,b) Spontaneously developed psoriasis-like lesions in K5.Stat3C mice. (K5.Stat3C mice which are 6 months old). (c) 12-*O*-Tetradecanoylphorbol-13-acetate (TPA) (6.8 nmol in acetone) was topically treated three times weekly for 8 weeks on the dorsal skin of K5.Stat3C mice. (d) The histopathological findings including parakeratosis, hyperkeratosis, loss of granular layer, acanthosis, dilated vessels and inflammatory cells infiltrate in psoriasis-like lesions in K5.Stat3C mice. Bar = 200 μm (hematoxylin–eosin).

new strategies for psoriasis therapies and novel insights into its pathophysiology.

STUDIES OF K5.STAT3C MOUSE FOR CLINICAL RELEVANCY TO PSORIASIS

Stat3 as a novel target for the treatment of psoriasis

We demonstrated that STA-21, a small Stat3 inhibitor, could be useful in ameliorating psoriatic lesions not only in the K5.Stat3C mice but also in human psoriasis.[26] Treatment with STA-21 markedly inhibited the cytokine-dependent nuclear translocation of Stat3 in normal human keratinocytes *in vitro* (Fig. 3a). Keratinocyte proliferation was inhibited by STA-21 in a dose-dependent manner through downregulation of c-Myc and cyclin D1, whereas involucrin, transglutaminase 1 and keratin 10 levels were upregulated (Fig. 3b,c). Moreover, topical application of STA-21 abolished the generation of skin lesions in K5.Stat3C mice and human psoriatic lesions (Fig. 3d). This study clearly indicates the clinical relevance of Stat3 targeting as a novel therapy for psoriasis.

Targeting IL-22 alone is not sufficient for anti-psoriasis therapy

We demonstrated that psoriasis-like lesions are dependent on IL-23 but develop in the absence of IL-22 in K5.Stat3C mice (Fig. 4).[27] Topical treatment with TPA on the back skin for

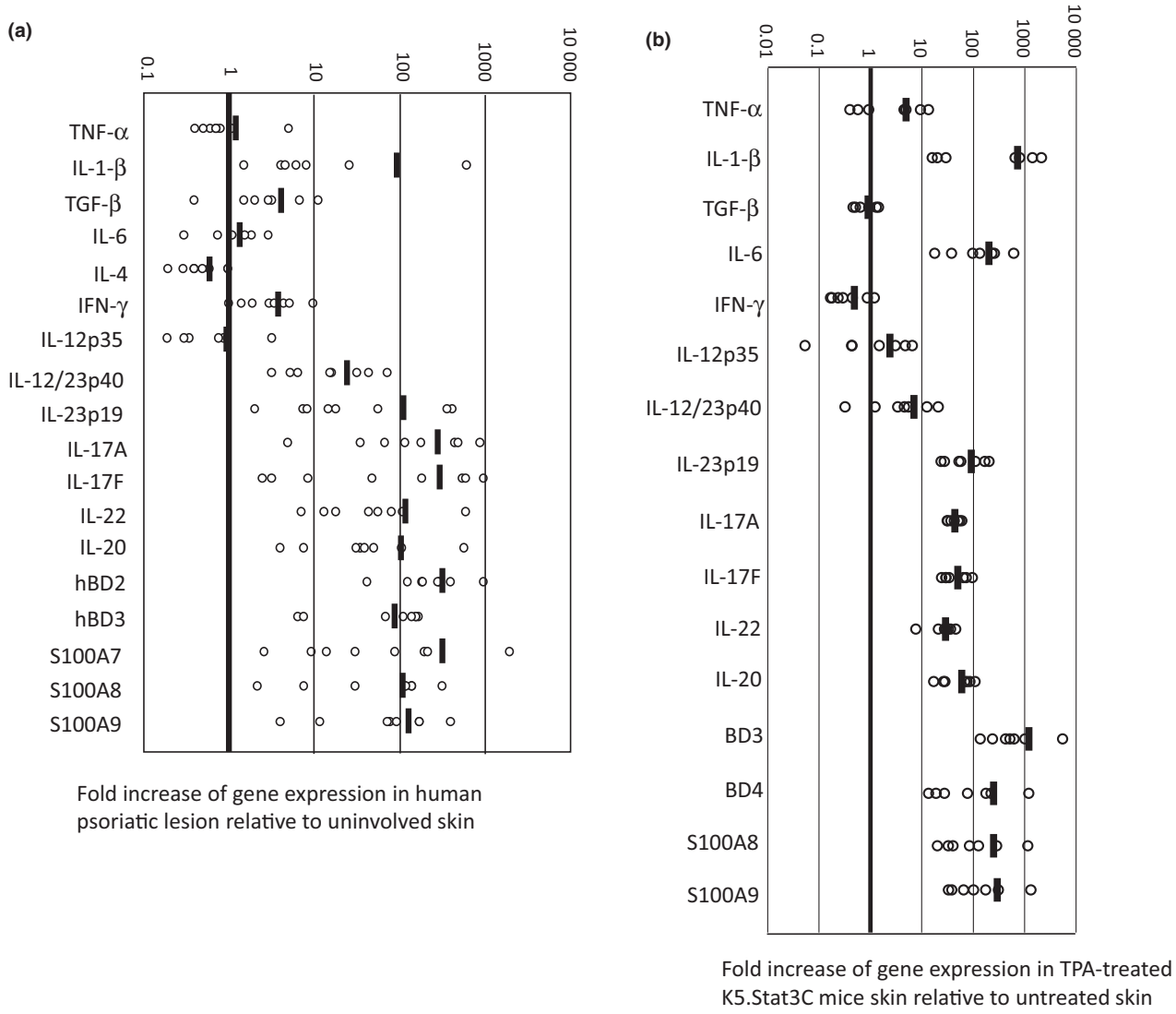

Figure 2. (a) Profiles of cytokines, β-defensins and S100A family proteins in human psoriatic skin relative to contiguous non-lesional skin. (b) Similar profiles in 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced psoriasis-like lesions in K5.Stat3C mice. IFN, interferon; IL, interleukin; TGF, transforming growth factor; TNF, tumor necrosis factor. (Reproduced from Nakajima *et al.*,[24] with permission.).

4 weeks resulted in the development of psoriasis-like lesions in K5.Stat3C:IL-23p19$^{+/-}$ mice, whereas IL-23p19 deficiency significantly attenuated the development of psoriasis-like lesions. This result confirmed that IL-23 is essentially required for the formation of psoriasis-like lesions in K5.Stat3C mice. To examine gene expression in the back skins treated with TPA treatment, we performed quantitative reverse transcription polymerase chain reaction (RT–PCR) analysis. IL-23p19 deficiency reduced IL-17A, IL-17F and IL-22 transcriptional levels. In contrast to IL-23p19, IL-22 deficiency did not affect the development of skin lesions in K5.Stat3C mice. The transcriptional profile demonstrated that psoriasis-related genes were equally upregulated in both K5.Stat3C:IL-22$^{+/-}$ mice and K5.Stat3C:IL-22$^{-/-}$ mice. However, administration of anti-IL-17A antibody resulted in significant attenuation of psoriasis-like lesions in K5.Stat3C:IL-22$^{-/-}$ mice. This observation suggests that IL-17 serves as an alternate pathway to psoriatic changes in the absence of IL-22 and that IL-22 is not essential for TPA-induced psoriatic changes in K5.Stat3C mice. Furthermore, *in vitro* stimulation of mouse keratinocytes with IL-17 resulted in upregulation of IL-19 and IL-24, both of which are additional IL-20 subfamily cytokines and are also involved in psoriasis, suggesting their compensatory role in the absence of IL-22. This finding endorsed not only the role for IL-23 but also previous failure of anti-IL-22 antibody in clinical trials for the treatment of psoriasis.

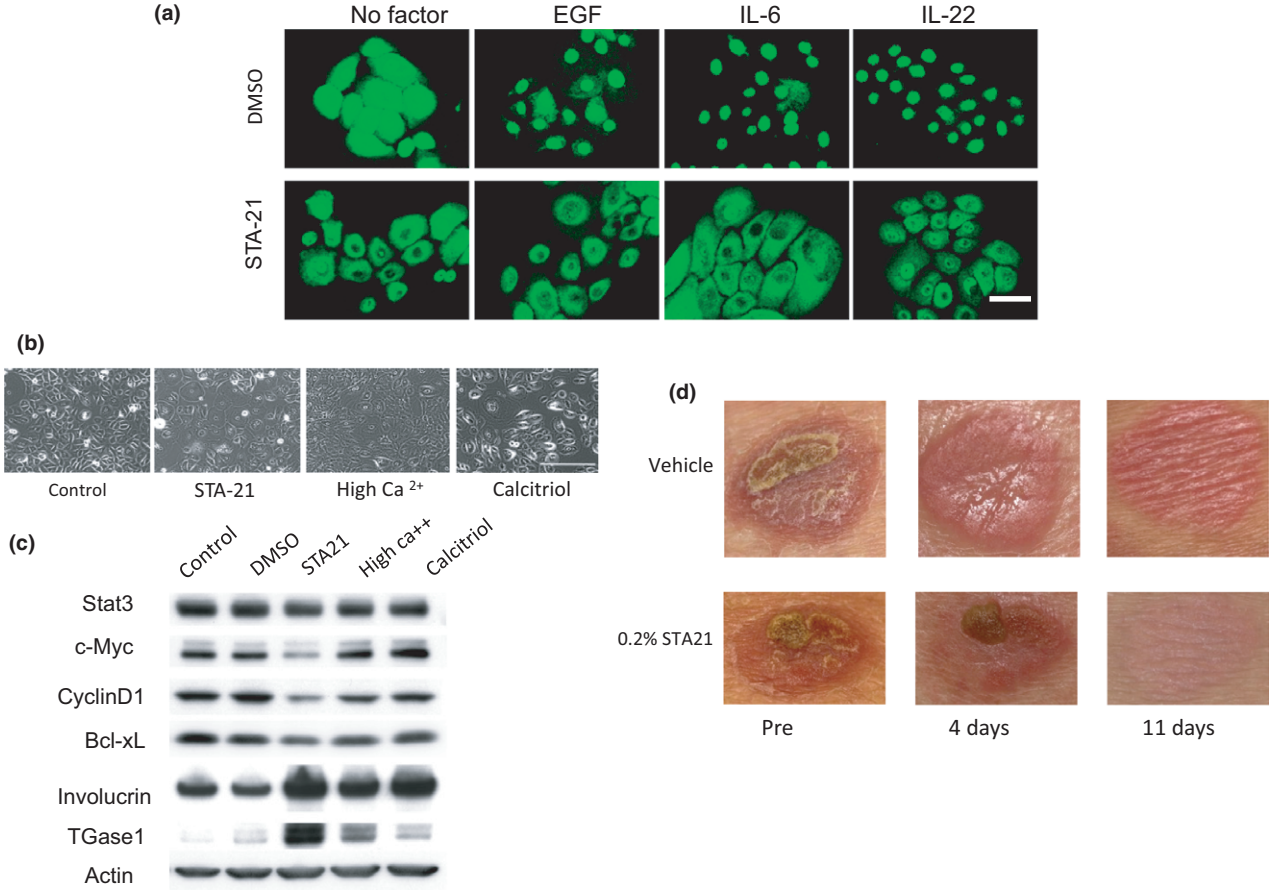

Figure 3. (a) STA-21 inhibits signal transducer and activator of transcription (Stat)3 signals in normal human keratinocytes (NHK). Pretreatment of NHK in culture with 20 μmol/L STA-21 for 1 h before stimulation with epidermal growth factor (EGF), interleukin (IL)-6 or IL-22 (100 ng/mL for 30 min). Immunostaining with an anti-Stat3 antibody showed that EGF, IL-6 or IL-22 induced nuclear translocation of Stat3, which was inhibited by STA-21. Bar = 50 μm. (b) STA-21 induces keratinocyte differentiation markers *in vitro*. Morphology of NHK cultured 24 h in the presence of STA-21 (10 μmol/L), high calcium (1.2 mmol/L) or calcitriol (10^{-7} mol/L). Cells became flattened and large when treated with STA-21, similar to cells treated with calcium and calcitriol. Bar = 250 μm. (c) Western blotting of NHK cells treated with STA-21 (20 μmol/L), high calcium (1.2 mmol/L) or calcitriol (10^{-7} mol/L) for 48 h. STA-21 increased involucrin and transglutaminase proteins up to 1.5- and 7.6-fold, respectively, compared with the dimethylsulfoxide (DMSO)-treated control. In contrast, STA-21 decreased c-Myc and cyclin D1 protein to 0.5- and 0.4-fold, respectively. (d) Efficacy of topical treatment with STA-21 on human psoriatic lesions. Psoriatic lesions of patient during course (patient, a 56-year-old man). Improvement of psoriatic lesions following application of STA-21 on the indicated days compared with vehicle control (Vaseline). (Reproduced from Miyoshi *et al.*,[26] with permission.).

TACE as a novel target for the treatment of psoriasis

We suggested TNF-α converting enzyme (TACE) inhibition as a potential therapeutic target for the treatment of psoriasis.[28] TNF-α is involved in the development of psoriasis, as evidenced by the therapeutic efficacy of TNF-α inhibitors on psoriasis.[8] TNF-α is produced as a membrane-bound form and is processed by TACE to become a soluble form that exerts biological activity.[29,30] In addition to TNF-α, membrane-bound epidermal growth factor receptor (EGFR) ligands, including amphiregulin, heparin-binding epidermal growth factor (HB-EGF) and transforming growth factor (TGF)-α, are TACE substrates. These EGFR ligands contribute to the pathogenesis of psoriasis.[31,32] Moreover, TACE is expressed by epidermal keratinocytes and inflammatory cells in the dermis in psoriatic lesions.[33] To evaluate the possible role of TACE in the pathogenesis of psoriasis, we investigated the involvement of TACE in TPA-induced psoriasis-like lesions in K5.Stat3C mice (Fig. 5a–c).[28] EGFR signaling was associated with the development of psoriasis-like lesions in K5.Stat3C mice similar to human psoriasis. We demonstrated that TACE released TNF-α from various cells and induced the release of EGFR ligands from keratinocytes. Treatment of K5.Stat3C mice with TNF-α and EGFR inhibitors improved psoriasis-like skin lesions, suggesting the roles of TACE substrates in psoriasis. We also demonstrated the downregulation of tissue inhibitor of metalloproteinase-3 (Fig. 5d,e), an endogenous TACE inhibitor, and increased TACE enzymatic activity in psoriasis-like skin lesions

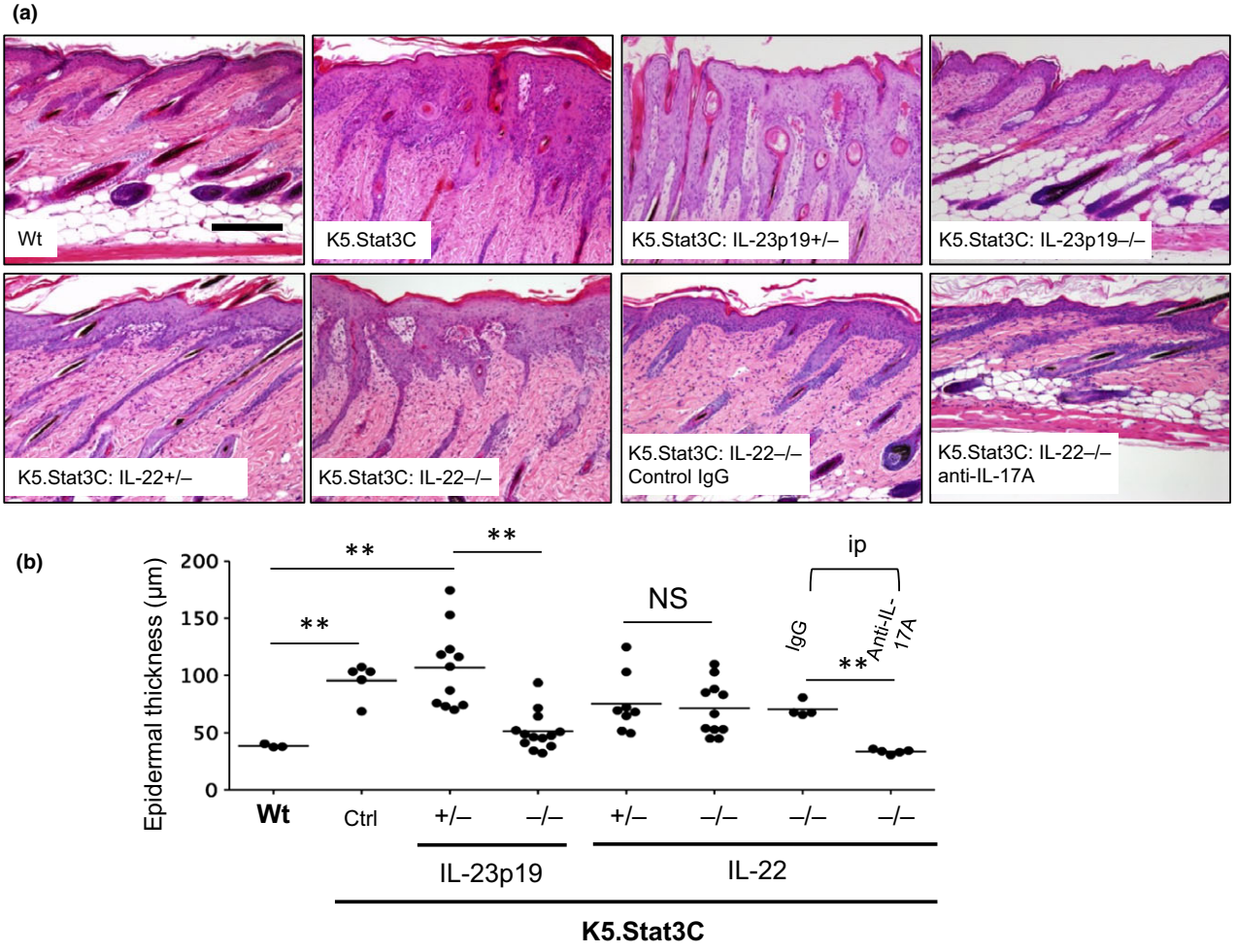

Figure 4. Psoriasis-like lesions are dependent on interleukin (IL)-23 but develop in the absence of IL-22 in a mouse model. 12-*O*-Tetradecanoylphorbol-13-acetate (TPA) (6.8 nmol in acetone) was topically treated three times weekly for 4 weeks on the dorsal skin of mice indicated in the figure. The administration of the anti-mouse IL-17A and rat/mouse chimeric immunoglobulin (Ig)G was performed as previously described.[24] (a) Representative histology with hematoxylin–eosin staining of skin lesions. Thickness of the interfollicular epidermis from 10 areas was measured under a microscope. Bar = 200 μm. (b) The epidermal thickness (μm) of each mouse is shown as a dot and mean thickness is shown as a bar. Statistical significance was calculated by Student's *t*-test. $^{**}P < 0.01$. ip, intraperitoneal injection of antibodies; NS, not significant. (Reproduced from Takaishi *et al.*,[27] with permission.).

of K5.Stat3C mice.[28] To assess whether TACE plays a role in releasing TNF-α from various cells, including keratinocytes, DC, macrophages and lymphocytes, we added the selective inhibitor of TACE TNF-α processing inhibitor-1 (TAPI-1) to an *in vitro* culture of bone marrow-derived DC (BMDC), peritoneal macrophages and primary keratinocytes. TAPI-1 dose-dependently suppressed the lipopolysaccharide- or TPA-induced release of TNF-α from BMDC, peritoneal macrophages and keratinocytes of K5.Stat3C newborn mice (Fig. 5f).[28] Moreover, a TACE inhibitor abrogated EGFR ligand-dependent keratinocyte proliferation and vascular endothelial growth factor production *in vitro* due to inhibition of processing of amphiregulin (Fig. 5g) and HB-EGF, respectively. These findings suggest that TACE was involved in both epidermal hyperplasia and angiogenesis during psoriasis development. Collectively, we concluded that TACE contributed to the development of psoriatic lesions through releasing two types of psoriasis mediators, TNF-α and EGFR ligands.

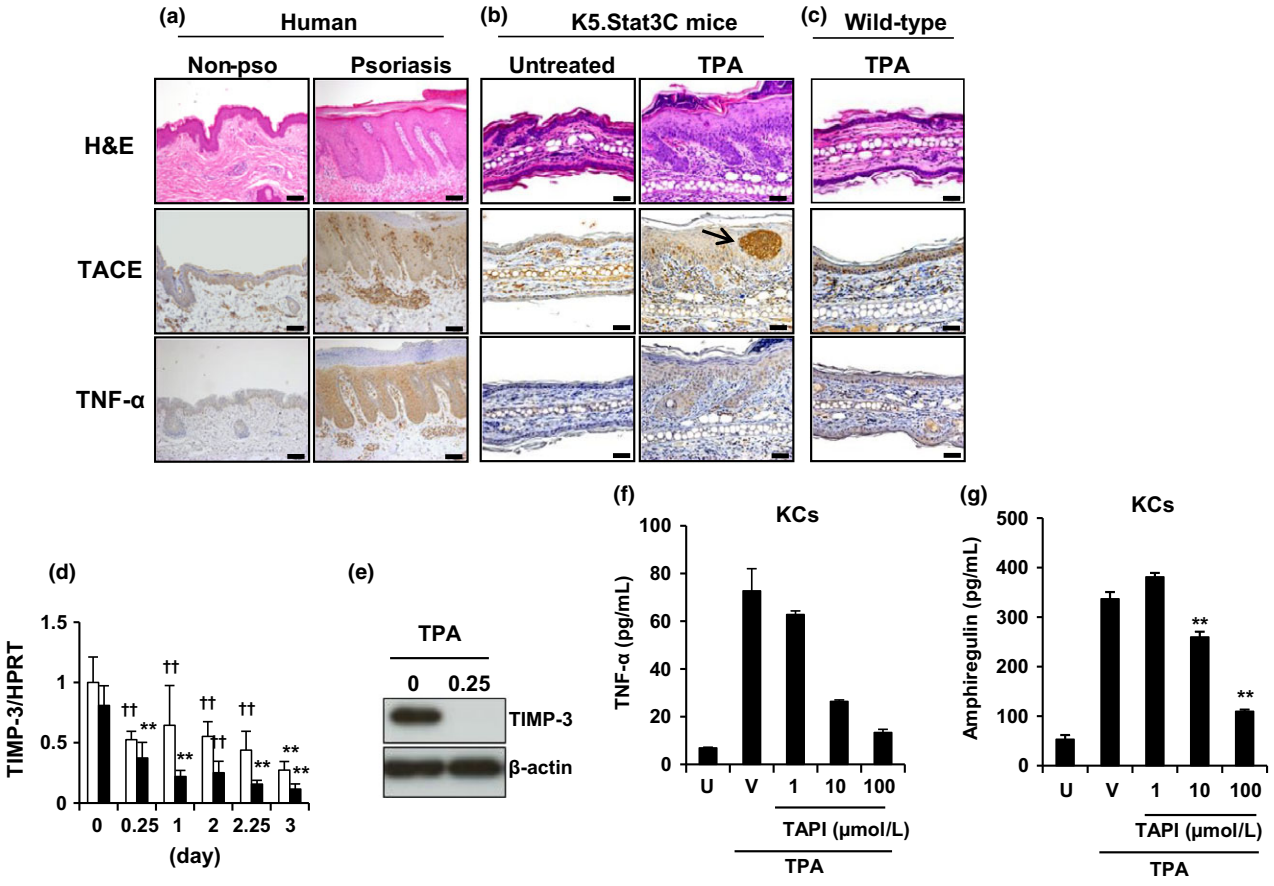

Figure 5. (a–c) Expression of tumor necrosis factor (TNF)-α converting enzyme (TACE) and TNF-α in the development of psoriasis-like skin lesions in K5.Stat3C mice. Representative histology and immunohistochemistry of (a) human skins, (b) ear skins in K5.Stat3C mice and (c) wild-type mice. 12-*O*-Tetradecanoylphorbol-13-acetate (TPA)-treated ear skins sampled at day 3. Immunohistochemical staining for TACE (middle panels) and TNF-α (bottom panels). Arrow, intraepidermal pustule of neutrophils. Bars = 100 μm (human), 50 μm (mouse). (d) Downregulation of tissue inhibitor of matrix metalloproteinase (TIMP)-3, an endogenous TACE inhibitor, and TACE enzymatic activity in the development of psoriasis-like skin lesions. Gene expression of TIMP-3 in the TPA-treated ear skins of K5.Stat3C mice (black bars) and wild-type mice (white bars). Data represent means ± standard deviation (SD) of 3–8 mice. $^{**}P < 0.01$, versus K5.Stat3C mice at day 0, $^{\dagger\dagger}P < 0.01$, versus wild-type mice at day 0, by Dunnett's test. (e) Western blot analysis of TIMP-3 in ear skins of K5.Stat3C mice collected before TPA application (0) or 6 h (0.25) after TPA application. (f,j) Contribution of TACE to the production of soluble TNF-α and epidermal growth factor receptor (EGFR) ligands from murine cells. TNF-α proteins in the 24-h culture supernatants of cells pretreated with or without TNF-α processing inhibitor-1 (TAPI-1), selective inhibitor of TACE, at the indicated concentrations. (h) TPA-stimulated primary keratinocytes from K5.Stat3C newborn mice. (i) Production of amphiregulin from TPA-treated primary keratinocytes of K5.Stat3C newborn mice for 24 h. Cells were pretreated with or without TAPI-1 (TAPI) for 30 min prior to stimulation. Data represent means ± SD of triplicate wells. $^{*}P < 0.05$, $^{**}P < 0.01$, versus vehicle alone by Dunnett's test. non-pso, non-psoriasis control; U, unstimulated control; V, vehicle alone. (Reproduced from Sato *et al.*,[28] with permission.).

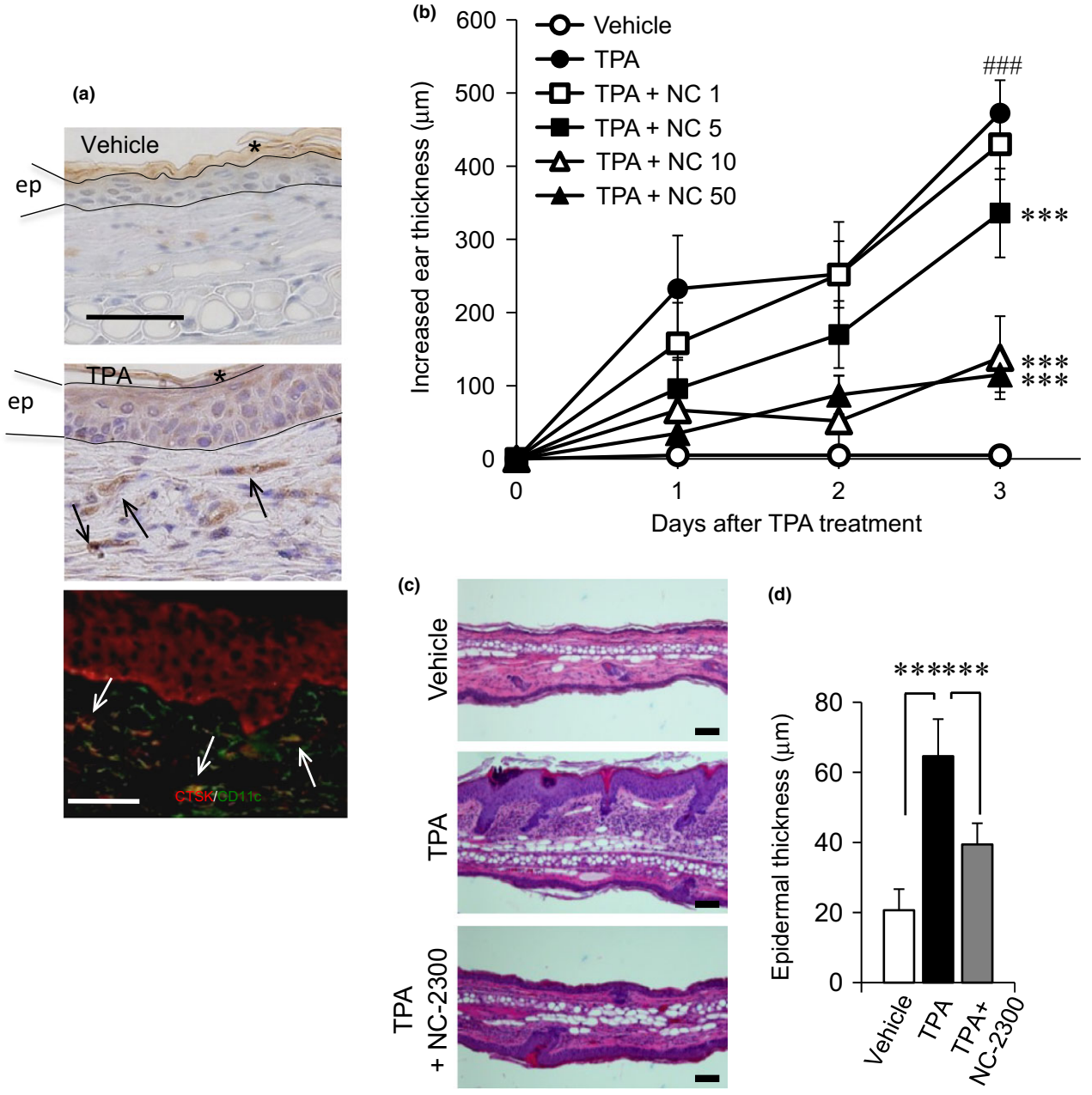

Figure 6. Effect of topical treatment with NC-2300 on the development of the psoriasis-like lesions in K5.Stat3C mice. (a) Immunostaining of K5.Stat3C mouse skin treated for 3 days with acetone (top) and 12-*O*-tetradecanoylphorbol-13-acetate (TPA) (middle and bottom). Note that TPA-treated epidermis (ep) is positive for cathepsin K (CTSK) compared with control epidermis. Arrows indicate CTSK-expressing dermal inflammatory cells. Asterisks, unspecific staining in the cornified layer. Immunofluorescence double staining of psoriasis-like lesion with anti-CD11c and anti-CTSK antibodies (bottom panel). Arrows indicate CTSK-expressing $CD11c^{+}$ cells, which were both positive for CTSK (red) and CD11c (green). Bars = μm. (b) The dose-dependent effect of NC-2300 on the psoriasis-like lesion. The ear skin of K5.Stat3C mice was treated with acetone (open circles) or TPA (filled circle) and then acetone instead of NC-2300, respectively. NC-2300 was topically applied at 1 mg/mL (open squares), 5 mg/mL (filled squares), 10 mg/mL (open triangles) and 50 mg/mL (filled triangles) on TPA-treated K5.Stat3C mice. Results are shown as mean increased ear thickness (μm) ± standard deviation (SD), $^{###}P < 0.001$ versus vehicle-treated mice; $^{***}P < 0.001$ versus TPA-treated mice. Tukey–Kramer test. (c) Representative histological features of TPA-treated ear skin following treatment with 10 mg/mL NC-2300. Bars = 100 μm (hematoxylin–eosin). (d) Assessment of the epidermal thickness. White bar, vehicle control; black bar, TPA control; gray bar, TPA plus 10 mg/mL NC-2300 treatment. The data are reported as mean thickness (μm) ± SD, $^{***}P < 0.001$. Tukey test. (Reproduced from Hirai *et al.*,[38] with permission.).

Cathepsin K as a novel target for the treatment of psoriasis

Lysosomal cystein proteases, generally known as the cathepsins (CTS), exert enzymatic activity under low pH conditions.[34,35] Cathepsin K (CTSK) is highly expressed in osteoclasts and is involved in the degradation of bone matrices, such as type I collagen.[36] CTS also play an important role in the turnover of intracellular proteins and extracellular proteins, such as the degradation of extracellular matrices and the processing of antigen proteins. The CTSK inhibitor NC-2300 not only suppresses bone erosion by inhibition of CTSK but also ameliorates paw swelling at inflamed joints in adjuvant-induced arthritis in rats.[37] The amelioration of joint inflammation by NC-2300 is mediated by the downregulation of cytokine expression in DC, which are essential for Th17 activation. *Ctsk* and *Ctss* mRNA levels were increased in psoriasis-like lesions in K5.Stat3C mice compared with uninvolved skin and wild-type (WT) mice.[38] We also demonstrated that CTSK activities, which were sensitive to NC-2300, were increased in the epidermis and dermis of K5.Stat3C mice compared with WT mice. Furthermore, TPA-induced psoriasis-like lesions are ameliorated by topical treatment with NC-2300 (Fig. 6). *In vitro* experiments revealed that TLR7 activation of bone marrow-derived myeloid DC led to increases in IL-23, which were

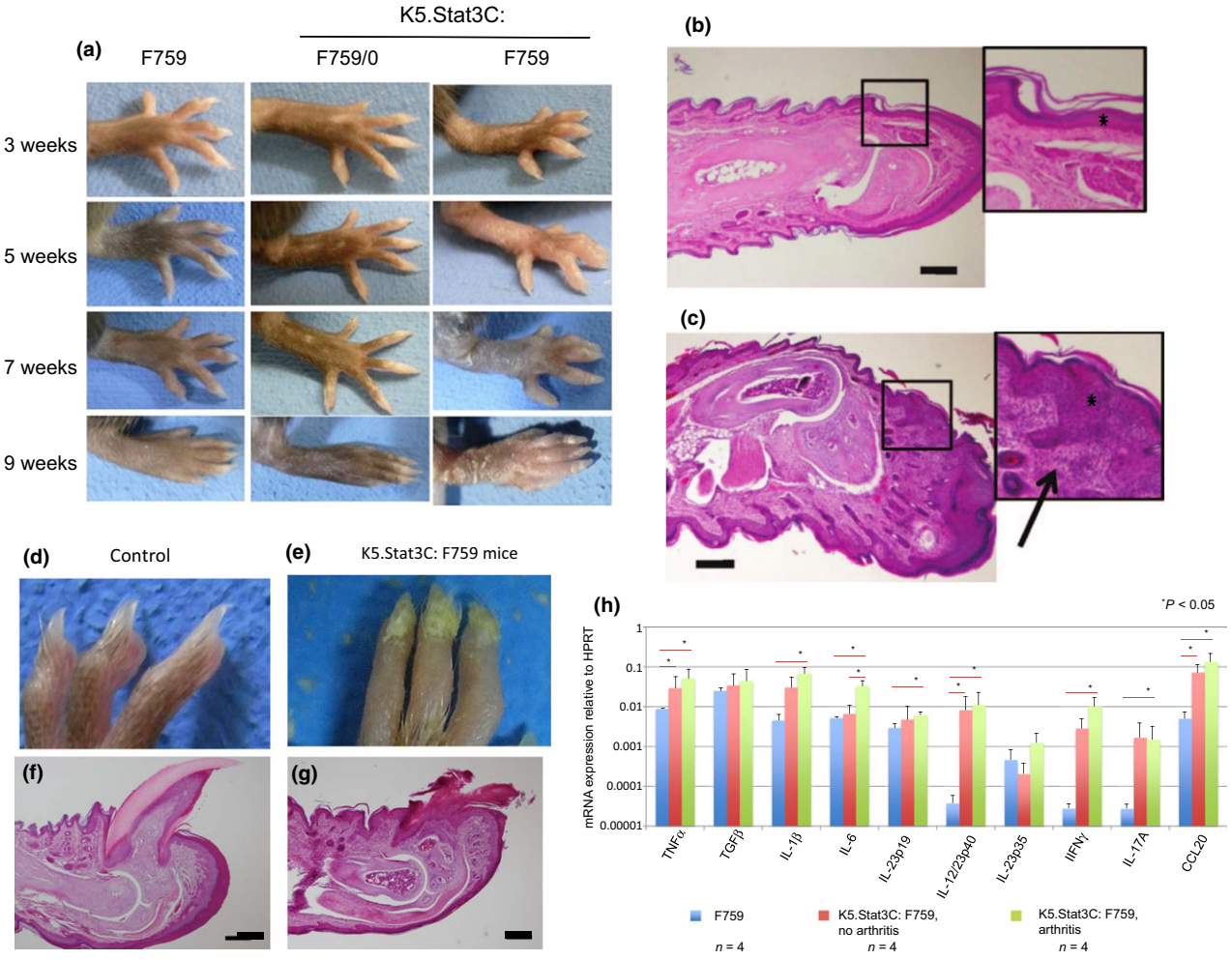

Figure 7. Early development of skin lesions and arthritis in the paws of K5.Stat3C:F759 mice. (a) Representative views of paw digits. Progression of skin lesions and swelling of paw digits are shown from 3 to 9 weeks of age in K5.Stat3C:F759 mice, whereas F759 and K5.Stat3C:F759/0 mice remain unaffected. (b) Histology of paws in the hindlimbs of F759 mice and (c) K5.Stat3C:F759 mice (hematoxylin–eosin [HE]). Square areas are shown at high magnification in insets. Note the hyperplastic epidermis and dermal infiltrates (arrow) in K5.Stat3C:F759 mice. Bars = 200 μm. (d–g) Nail lesions of K5.Stat3C:F759 mice. (e) Representative features of nail symptoms of K5.Stat3C:F759 mice at 6 weeks of age (d) compared with control mice. (g) Histology of nails in K5.Stat3C:F759 mice and (f) control (HE). Note the hyperkeratosis, acanthosis, dermal cell infiltrates and capillary proliferation. Bar = 80 μm. (h) Periarticular cytokine profiles in diseased K5.Stat3C:F759 mice. Relative mRNA levels of cytokines in periarticular tissues from K5.Stat3C:F759 mice with arthritis (green bars), unaffected K5.Stat3C:F759 mice (red bars) and F759 mice (blue bars). Data are reported as mean ± SD; *$P < 0.05$ by Mann–Whitney *U*-test. (Reproduced from Yamamoto *et al.*,[43] with permission.).

downregulated by NC-2300. Our data suggest that CTSK plays a role in the development of psoriatic lesions through the TLR7-mediated IL-23/Th17 pathway. In conclusion, CTSK is involved in development of psoriasis-like skin lesions through TLR-dependent Th17 activation, and inhibition of CTSK may be relevant as a novel therapy for psoriasis.

NEW PSORIATIC ARTHRITIS MODEL MOUSE IN CONJUNCTION OF POTENTIALS FOR PSORIASIS AND AUTOIMMUNE ARTHRITIS

Joint involvement in patients with psoriasis was recognized as a distinct clinical entity.

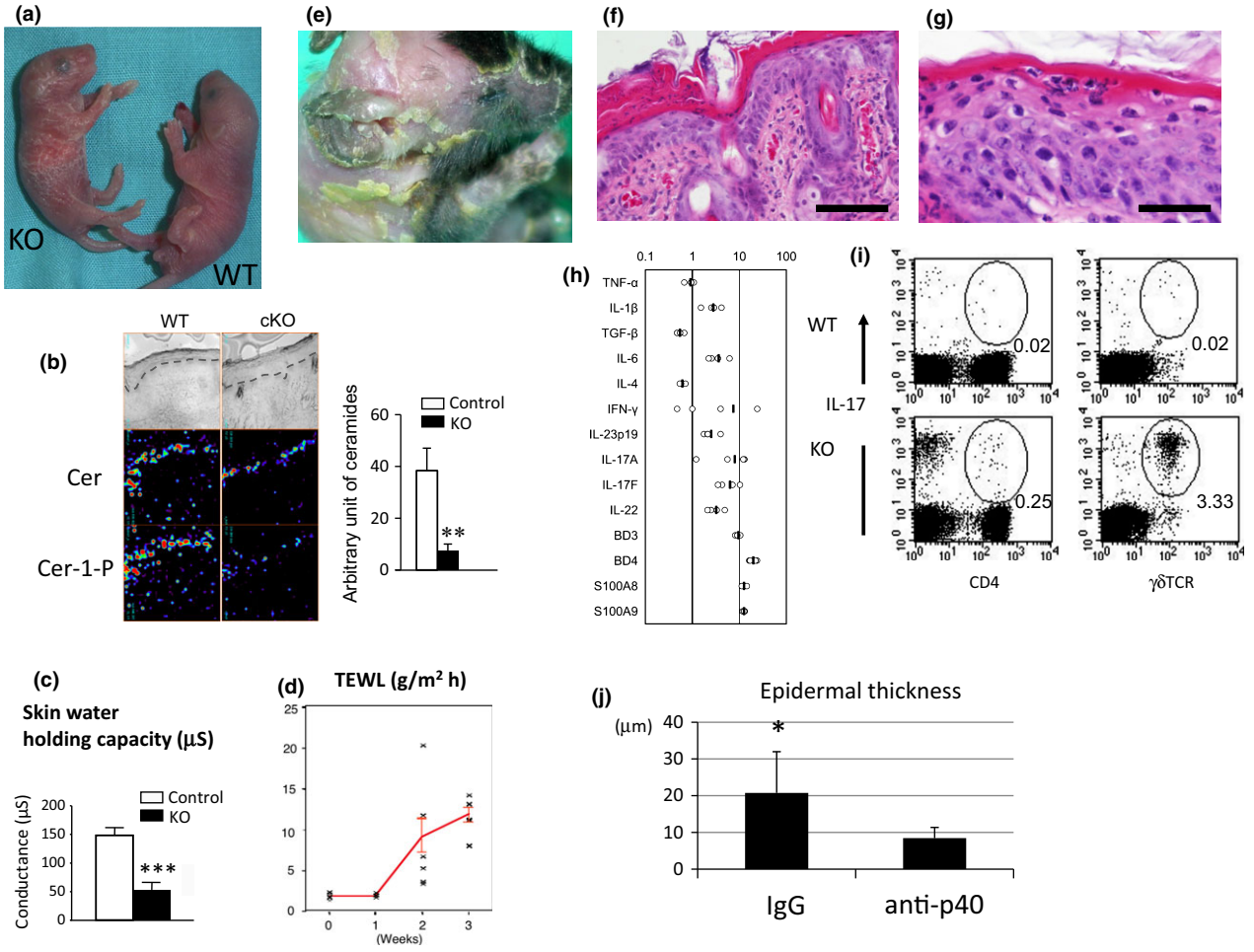

Figure 8. The phenotype of newborn serine palmitoyltransferase (SPT)-cKO mice. (a) The skin of SPT-cKO mice look dry. (b) Imaging mass spectrometry analysis using footpad skin sections of SPT-cKO and wild-type (WT) mice at postnatal day (PD)3. Ion images of the epidermis of SPT-cKO mice reveal that ceramide is markedly decreased compared with WT mice. (c) Decreased water retention in newborn SPT-cKO mice (black bar) and compared with WT mice (white bar) ***$P < 0.001$. Unpaired Student's *t*-test. SPT-cKO mice at 2 weeks of age and older develop psoriasis-like lesions. Barrier disruption develops in SPT-cKO mice from 2 weeks of age and older. (e) Severe desquamation and generalized erythema are evident at PD21. (f,g) Histopathological findings of the skin in SPT-cKO mice. Hyperkeratosis, parakeratosis, loss of granular layers, acanthosis, dermal cell infiltrates, dilated vessels and accumulation of neutrophils. Bar = 200 μm, 250 μm. (h) Gene expression profiles of SPT-cKO mice and increased number of interleukin (IL)-17 producing γδ T cells. Elevated gene expression of psoriasis-associated molecules such as IL-17A, IL-17F, IL-22, β-defensins and S100A family members in the skin lesions of SPT-cKO mice at PD18 compared with WT skin. mRNA transcript levels of each gene are normalized to hypo-xanthine phosphoribosyltransferase mRNA. Symbols show mean mRNA transcript levels in skin-draining lymph nodes (SDLNs) from SPT-cKO mice compared with WT mice. (i) Both IL-17-producing $CD4^+$ cells and γδ T cells are increased in SDLNs from SPT-cKO mice compared with WT mice analyzed by flow cytometry. Involvement of IL-23 in the generation of skin lesions in SPT-cKO mice. (j) Attenuation of epidermal hyperplasia by anti-IL-12/23p40 treatment. Bars show epidermal thickness (mean μm ± standard deviation of skin lesions of SPT-cKO mice treated with control immunoglobulin (Ig)G or with anti-IL-12/23p40. *$P < 0.05$, unpaired Student's *t*-test. IFN, interferon; TEWL, transepidermal water loss; TGF, transforming growth factor; TNF, tumor necrosis factor. (Reproduced from Nakajima *et al.*,[47] with permission.).

Psoriatic arthritis (PsA) has distinct clinical features ranging from arthritis of the distal interphalangeal joints to spondylitis. The prevalence of PsA in psoriasis is approximately 10% according to the PsA classification criteria established in 2016.[39] PsA is well-associated with scalp, nail, or intergluteal/buttock psoriasis.[40]

K5.Stat3C mice do not develop arthritis even if the skin lesions become severe. We took advantage of the gp130$^{F759/F759}$knock-in mouse autoimmune arthritis model (referred to as F759), which involves a mutant variant of gp130 where Y759 is substituted for phenylalanine (F), leading to Stat3 activation due to the absence of suppressor of cytokine signaling 3-mediated suppression.[41] The IL-6/gp130/Stat3 pathway in F759 mice induces Th17 cell activation with age, leading to the development of rheumatoid arthritis-like disease in the limbs after 1 year of age.[42] We crossed F759 mice with K5.Stat3C mice, in which keratinocytes express constitutive active Stat3 (Stat3C).[43] K5.Stat3C:F759 mice spontaneously develop severe psoriasis-like lesions as wells as joint diseases in their paws as early as 3 weeks of age (Fig. 7a–c). The joint lesions included swelling of the peripheral paws and nail deformities contiguous with skin lesions closely resembling PsA (Fig. 7d–g). Severe skin and joint lesions were observed in older K5.Stat3C:F759 mice. Histopathological findings revealed enthesitis and bone erosions with mononuclear cell infiltration. Additionally, quantitative RT–PCR, immunohistochemical analyses and flow cytometry revealed upregulation of the IL-23/Th17 pathway in affected joints of K5.Stat3C:F759 mice (Fig. 6h). We also confirmed Stat3 activation in both the epidermis and periarticular fibroblasts in K5.Stat3C:F759 mice. Similar to the effect of introduction of the Stat3C transgene, enforced development of psoriasis-like lesions in F759 mice by topical application of TPA led to swelling of the underlying joints, suggesting that psoriatic inflammation facilitated arthritis.

SPT-cKO MOUSE AS A PSORIASIS MODEL WITH EPIDERMAL BARRIER DYSFUNCTION

Inflammatory skin diseases are often associated with skin barrier disruption; however, the cause and effect relationship is complex. The discovery of loss-of-function mutations in the *filaggrin* gene in patients with atopic dermatitis (AD) demonstrated that the disruption of the skin barrier is the primary cause of the disease.[44] Both patients with AD and psoriasis exhibit barrier dysfunction with distinct lipid composition profiles in the epidermis.[45,46] Moreover, a reduction in the ceramides levels in the cornified layer has been recognized in both diseases.[45] Then, we investigated the effects of the lack of ceramides in the epidermis in mice. To this end, we generated serine palmitoyltransferase (SPT)-targeted mice under the keratin 5 promoter (K5.SPT-cKO mice); thus, ceramide biosynthesis was defective in keratinocytes.[47] K5.SPT-cKO mice were viable and the skin appeared dry (Fig. 8a). We confirmed severe ceramide deficiency in the epidermis of K5.SPT-cKO mice by immunohistochemistry and mass spectrometry imaging (Fig. 8b). The newborn K5.SPT-cKO mice exhibited impaired water-holding capacity (Fig. 8c). Previous studies demonstrated that SPT in the epidermis is required for barrier recovery.[48,49] To verify this finding, the process of barrier recovery after tape-stripping of the skin was examined in K5.SPT-cKO mice. As expected, SPT-cKO mice at postnatal day 3 demonstrated a significant elevation of transepidermal water loss at 2.5 and 6 h in K5.SPT-cKO mice compared with WT mice. K5.SPT-cKO mice at 2 weeks of age onward, which reflects the timeframe of permeability barrier impairment (Fig. 8d), developed skin lesions with histopathological aberrations, including hyperkeratosis, parakeratosis, acanthosis, loss of the granular layers, neutrophil accumulation in the stratum corneum and inflammatory cell infiltrates (Fig. 8e–g). We confirmed the activation and enhanced migration of Langerhans cells to lymph nodes in SPT-cKO mice. The skin lesion exhibited upregulation of psoriasis-associated genes, such as IL-17A, IL-17F, IL-22, S100A8, S100A9 and β-defensins (Fig. 8h). The skin lesion and skin-draining lymph nodes harbored increased numbers of γδ T cells that produce IL-17 (γδ-17 cells) (Fig. 8i). We also confirmed that γδ-17 cells produced IL-22. Furthermore, IL-23-producing DC were observed in the skin lesions. *In vivo* treatment of K5.SPT-cKO mice with anti-IL-12/23p40 antibody inhibited the development of skin lesions (Fig. 8j). SPT expression was reported to be significantly reduced in psoriatic lesions compared with psoriatic uninvolved skins and inversely correlated with Psoriatic Area Severity Index score.[50] We conclude that ceramide deficiency in the epidermis leads to severe barrier disruption and simultaneous development of psoriasis-like lesions mediated by IL-23-dependent-producing γδ-17 cells. This finding highlighted the involvement of the dysfunction of permeability barrier in the pathophysiology of psoriasis development given that barrier-associated genes, including late cornified envelope 3, have also been classified as psoriasis-susceptibility genes.[51]

CONCLUSION

Advances in genetics and immunology have demonstrated the relevance of abnormal immune responses to the pathogenesis of psoriasis. Despite the accumulation of knowledge in recent years, numerous questions remain. We have resolved some of the questions using the K5.Stat3C mouse and other mice. Mouse models have provided important molecular insights into the pathogenesis of psoriasis and have led to the identification of new therapies. Improvements in the molecular characterization of psoriasis will result in new treatments in the near future.

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