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KLK6 expression in skin induces PAR1-mediated psoriasiform dermatitis and inflammatory joint disease

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KLK6 is a serine peptidase that is highly expressed in skin and can cause keratinocyte apoptosis and keratinocyte hyperproliferation. KLK6 levels are increased in multiple inflammatory and autoimmune diseases, including psoriasis, atopic dermatitis, and rheumatoid arthritis. KLK6 expression in skin induces PAR1-mediated inflammation in the skin alone is sufficient to drive inflammatory joint disease. Further, we identify PAR1 as a promising cytokine-independent target in therapy of psoriasis and psoriatic arthritis.

Introduction

Psoriasis is a chronic, incurable systemic inflammatory disease with complex and incompletely understood pathogenesis. Two percent to 3% of the population worldwide suffers from psoriasis, and affected individuals show higher rates of diverse comorbid conditions. The most classic among these is psoriatic arthritis (PsA), which is found in approximately one-third of patients and can cause severe, disabling joint disease in the peripheral and axial skeletons (1).

Kallikrein-related peptidase 6 (KLK6) belongs to a family of serine proteases that are emerging as prevalent biomarkers of inflammatory and malignant diseases. Cell culture and preclinical animal model studies suggest that KLK6 may promote inflammation and autoimmunity via cleavage of the G protein–coupled protease-activated receptor 1 (PAR1) and PAR2 (2, 3). KLK6 levels are elevated in multiple inflammatory and autoimmune conditions, but no definitive role in pathogenesis has been established. Here, we show that skin-targeted overexpression of KLK6 causes generalized, severe psoriasiform dermatitis with spontaneous development of debilitating psoriatic arthritis-like joint disease. The psoriatic skin and joint phenotypes are reversed by normalization of skin KLK6 levels and attenuated following genetic elimination of PAR1 but not PAR2. Conservation of this regulatory pathway was confirmed in human cell culture and animal models. We further explored a critical role for KLK6/PAR1 signaling in promoting psoriasis, our results demonstrate that KLK6/PAR1-mediated inflammation in the skin alone is sufficient to drive inflammatory joint disease. Further, we identify PAR1 as a promising cytokine-independent target in therapy of psoriasis and psoriatic arthritis.

Results and Discussion

To investigate the role of KLK6 in inflammatory skin disease, we first compared expression of KLK transcripts in skin from healthy individuals to normal-appearing (nonlesional) and lesional skin from patients with psoriasis. KLK6 was significantly upregulated in lesional psoriatic skin, as were multiple other KLKs (Figure 1, A and B). Immunohistochemistry confirmed prominent KLK6 protein upregulation in infiltrating immune cells and keratinocytes of the superficial epidermis (Figure 1C). We further explored KLK6 dynamics during psoriasis treatment with etanercept, a soluble tumor necrosis factor (TNF) receptor that binds TNF to block its proinflammatory effects. KLK6 levels in the skin decreased rapidly in patients who were responsive to etanercept therapy, approximating levels in nonlesional skin by as early as 2 weeks (Figure 1D), sooner than observed for the traditional psoriasis biomarker psoriasin (S100A7). Thus, KLK6 shows disease-specific regulation, correlating closely with local disease activity.
IL-6 protein were highly upregulated in transgenic skin (Figure 2D), and phosphorylated STAT3 was diffusely increased in keratinocytes and inflammatory infiltrate of transgenic skin (Figure 2E). For a broader examination of the transcriptional effects of Klk6 overexpression, we performed RNA sequencing (RNA-seq) of Klk6+ transgenic and control mouse skin and identified differentially expressed genes (DEGs). Upregulated DEGs showed enrichment for Gene Ontology and KEGG terms related to keratinization, epidermal development, cellular proliferation, and steroid biosynthesis (Supplemental Figure 1). Transgenic DEGs showed prominent overlap with genes differentially enriched in lesional versus nonlesional skin of patients with psoriasis (Figure 2F), whereas similar comparison to skin of patients with atopic dermatitis showed only a modest overlap (Supplemental Figure 2). Of note, Klk6 itself has been defined as a gene that differentiates between psoriasis and eczema (10). Strongly upregulated transcripts supporting a psoriasis-like phenotype in Klk6+ transgenic mouse skin include Il17a (580-fold, false discovery rate [FDR] = 3.66 × 10^-42), Il17f (380-fold, FDR = 7.27 × 10^-61), Il22 (1123-fold, FDR = 1.04 × 10^-37), Il24 (293-fold, FDR = 9.92 × 10^-32), Il36a (203-fold, FDR = 3.16 × 10^-31), Klk13 (216-fold, FDR = 1.24 × 10^-19), and Il19 (374-fold, FDR = 1.96 × 10^-14), in comparison with relatively modest changes in key atopic dermatitis transcripts Il4 (5-fold, FDR = 0.00209), Il13 (5-fold, FDR

To investigate a pathogenic role for Klk6 in driving inflammatory disease in the skin, we developed Tet-off transgenic mice that overexpressed C-terminal Myc/His-tagged murine Klk6 in keratinocytes in the absence of doxycycline, hereafter referred to as Klk6+ transgenic mice. In these mice, transgene overexpression is under control of the bovine keratin 5 (K5) promoter, which drives expression in epidermal keratinocytes and some other stratified squamous epithelia (see Supplemental Methods and refs. 8, 9; supplemental material available online with this article; https://doi.org/10.1172/JCI133159DS1). By 2 months of age, all Klk6+ mice developed diffuse scaling and redness of the skin (Figure 2A). Histology revealed features of psoriasis including hyperplasia and focal parakeratosis, an indicator of abnormal keratinocyte maturation (Figure 2B). Ki67 staining demonstrated epidermal hyperproliferation (Figure 2C). Immunohistochemistry for inflammatory cell markers showed a brisk mixed infiltrate in the superficial dermis as well as collections of intraepidermal neutrophils (Figure 2C, GR1) comparable to the hallmark Munro’s microabscesses of psoriasis. Increased dermal vascularity was observed (Figure 2C, MECA), consistent with the hypervascularity of psoriatic plaques. Thus, epidermal overexpression of Klk6 causes a severe, generalized rash with histological features of psoriasis.

To further characterize the inflammation in Klk6+ transgenic mouse skin, we assessed activation of key psoriasis inflammatory pathways. Il-17A, the p40 and p19 subunits of IL-23, and IL-6 were highly upregulated in transgenic skin (Figure 2D), and phosphorylated STAT3 was diffusely increased in keratinocytes and inflammatory infiltrate of transgenic skin (Figure 2E). For a broader examination of the transcriptional effects of Klk6 overexpression, we performed RNA sequencing (RNA-seq) of Klk6+ transgenic and control mouse skin and identified differentially expressed genes (DEGs). Upregulated DEGs showed enrichment for Gene Ontology and KEGG terms related to keratinization, epidermal development, cellular proliferation, and steroid biosynthesis (Supplemental Figure 1). Transgenic DEGs showed prominent overlap with genes differentially enriched in lesional versus nonlesional skin of patients with psoriasis (Figure 2F), whereas similar comparison to skin of patients with atopic dermatitis showed only a modest overlap (Supplemental Figure 2). Of note, Klk6 itself has been defined as a gene that differentiates between psoriasis and eczema (10). Strongly upregulated transcripts supporting a psoriasis-like phenotype in Klk6+ transgenic mouse skin include Il17a (580-fold, false discovery rate [FDR] = 3.66 × 10^-42), Il17f (380-fold, FDR = 7.27 × 10^-61), Il22 (1123-fold, FDR = 1.04 × 10^-37), Il24 (293-fold, FDR = 9.92 × 10^-32), Il36a (203-fold, FDR = 3.16 × 10^-31), Klk13 (216-fold, FDR = 1.24 × 10^-19), and Il19 (374-fold, FDR = 1.96 × 10^-14), in comparison with relatively modest changes in key atopic dermatitis transcripts Il4 (5-fold, FDR = 0.00209), Il13 (5-fold, FDR

Figure 1. KLK6 expression is significantly elevated in psoriatic lesions and parallels disease activity. (A) Detection of KLK transcripts by RNA-seq in healthy control skin (NN, gray; N = 90), psoriatic patient nonlesional skin (PN, yellow; N = 26), and psoriatic patient lesional skin (PP, blue; N = 99). P values were computed using Wilcoxon’s rank-sum test; false discovery rate (FDR) was used to control the multiple testing. *P < 0.05 in PP vs. PN. (B) Relative expression of select KLK transcripts by qRT-PCR in a unique cohort of healthy controls and psoriatic patients (N = 6). Mean is indicated by the horizontal line. Box, 25th–75th percentile. Whiskers, minimum and maximum. *P < 0.005 in PP vs. both NN and PN by ordinary 1-way ANOVA with post hoc Tukey’s multiple-comparisons test. (C) Immunostaining of KLK6 in human skin. Results are representative of 4 biological replicates. Scale bar: 100 μm. (D) Detection of KLK5 and S100A7 transcripts by RNA-seq in PN and PP at the indicated weeks’ duration of etanercept therapy in responsive patients (N = 14). Mean is indicated by the horizontal line. Box, 25th–75th percentile. For definition of whiskers and additional details, see Supplemental Methods. *P < 0.05 vs. PN at w0; +P < 0.05 vs. PP at w0 by negative binomial test.
Intriguingly, Klk6+ transgenic mouse skin is more transcriptionally similar to lesions of patients with psoriasis who have PsA than those who do not (PsC) (Figure 2F and Supplemental Figure 3). We therefore examined Klk6+ transgenic mice for manifestations of inflammatory arthritis. Transgenic forelimbs showed severe erythe-
ma and scaling (Figure 3A), with variable nail dystrophy and digital swelling akin to the characteristic dactylitis, or sausage digit, of PsA. Additionally, transgenic mice developed excessive cervico-thoracic spinal curvature, termed kyphosis, by 10 weeks of age that led to a hunched posture and impaired mobility (Supplemental Video 1). To better characterize the bony abnormalities, we performed micro-computed tomography of transgenic mice. This revealed kyphosis (Figure 3B), decreased joint space in the sacroiliac joints and pubic symphysis, and erosions in the pubic symphysis in some, but not all, Klk6+ transgenic mice (Figure 3C and Figure 4B). Transgenic mice also showed significantly ($P = 1.4 \times 10^{-6}$) decreased vertebral bone mineral density (Figure 3D), which is commonly seen in patients with inflammatory arthritis, including those with PsA (11). H&E-stained spine sections revealed thinning of the trabecular bone and subchondral plate, impaired calcification of the vertebral cartilage, and abnormalities of the vertebral disc and surrounding fibrous tissue in Klk6+ transgenic mice (Figure 3E), supporting our gross and radiographic observations of axial skeleton disease. As peripheral joints, in particular the digits, are the most common site of inflammation in PsA, we examined transgenic paws for evidence of inflammatory arthritis. Klk6+ paws showed features of spondyloarthritis including enthesal inflammation and synovitis in approximately 80% of the mice examined, with severely affected mice showing signs of bone erosion (Figure 3F). Together, these gross, radiographic, and histologic findings demonstrate that overexpression of Klk6 in the skin alone is sufficient to cause progressive axial skeletal bony changes and peripheral joint inflammatory arthritis consistent with PsA.

We next investigated whether the Klk6+ transgenic phenotype is dependent on continued Klk6 overexpression. We allowed transgenic mice to develop robust skin and joint manifestations and then repressed transgene expression with doxycycline for 4 weeks to restore Klk6 to wild-type levels (Supplemental Figure 4A). This fully ameliorated the cutaneous phenotype (Supplemental Figure 4, B and C), indicating that KLK6-induced skin inflammation is not self-perpetuating. Unexpectedly, this also led to partial and in some cases complete reversal of bony abnormalities (Supplemental Figure 4, D and E), suggesting that persistence of inflammatory arthritis requires ongoing KLK6-driven skin inflammation.

Finally, we sought to define the mechanism through which KLK6 induces psoriatic skin and joint disease. We crossed the Klk6+ Tet-off transgenic mice with Par1- and Par2-knockout lines and assessed
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3155

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concept, we then applied vorapaxar, an FDA-approved PAR1 antagonist, to cultured explanted lesional skin from psoriatic patients. Vorapaxar therapy decreased psoriasis-associated proinflammatory markers CXCL1, CXCL2, and IL1B (Figure 4F), confirming that KLK6/PAR1 signaling drives inflammation in psoriasis in humans.

Despite intensive investigation, the mechanistic connection between PsA and psoriasis remains unclear. Although many mouse psoriasis models have been developed through epidermal genetic alterations, very few show spontaneous evolution of joint disease (12, 13), challenging the hypothesis that PsA reflects spillover of cutaneous inflammation. Here, we have shown that KLK6 overexpression limited to the epidermis is sufficient to drive not only psoriasis-like cutaneous disease but also inflammatory arthritis via signaling through PAR1. Whether joint disease requires intermediary inflammation in other compartments such as the gut is of considerable interest and remains to be investigated.

Figure 4. KLK6 induces psoriatic skin and joint disease through PAR1, but not PAR2. (A) Gross images and H&E dorsal skin staining of 10-week-old mice of the indicated genotypes. (B) Micro-CT images of the sacroiliac (SI) joint, public symphysis, and cervico-thoracic kyphosis (spine) in mice of the indicated genotypes. (C) Quantitative analysis of micro-CT images of the SI joint, public symphysis (Symph), and cervico-thoracic kyphosis in mice of the indicated genotypes. Mean and SEM are indicated. N ≥ 5 per group. *P < 0.05 vs. control; †P < 0.05 for Klk6+ vs. Klk6+ Par1−/− by ordinary 1-way ANOVA with post hoc Tukey’s multiple-comparisons test (SI joint and Symph) or a post hoc uncorrected Fisher’s LSD test (Spine). Control and Klk6+ data are reproduced in Supplemental Figure 4E. (D) PAR1 immunostaining in human healthy control and lesional psoriatic skin. (E) PAR1 (orange) and CD3 (green) immunofluorescence in human healthy control and lesional psoriatic skin. (F) Detection of indicated proinflammatory marker transcripts by qRT-PCR of lesional psoriatic skin explants (N = 4) treated with 0 or 100 nM of the PAR1 antagonist vorapaxar. P < 0.05 by paired t test for all. Scale bars: 100 μm (A, D, and E).
The mechanisms by which KLK6/PAR1 signaling promotes psoriasis and joint inflammation remain to be determined. STAT3 was diffusely increased in Klk6+ mouse skin (Figure 2E) and has previously been linked with KLK6/PAR1 signaling in injured spinal cord (14). STAT3 activation is a hallmark of psoriatic epidermis, and STAT3 strongly correlates with KLK6 in human psoriatic lesional skin (15) (Spearman’s correlation coefficient $\rho$ of 0.71; $P = 3.3 \times 10^{-6}$), providing a mechanistic link between KLK6/PAR1 and STAT3 activation. STAT3 plays a critical role in the feedback loop between keratinocytes and immune cells, driving production of TNF, IL-23, and IL-17, which in turn feed back onto keratinocytes to drive further generation of KLK6 and downstream activation of STAT3. Recently, expression of hyperactive STAT3 in mouse CD4+ T cells was shown to promote psoriasis-like skin disease, enthesis/tendonitis, and periarticular bone erosion accompanied by osteopenia (16). Klk6+ transgenic mice have high levels of IL22 (>1000-fold relative to WT), IL17a (569-fold), IL17f (378-fold), IL19 (372-fold), and IL24 (292-fold) (Supplemental Figure 1), cytokines that contribute to STAT3-mediated inflammation between keratinocytes and immune cells in psoriatic lesional skin and joints, suggesting KLK6-mediated PAR1 cleavage occurs upstream of these pathways.

Neutrophils and other innate immune cells are important in the pathogenesis of PsA, and IL-23/IL17A signaling and IL-22 expression are increased in PsA patient synovial membranes and entheses contributing to joint inflammation and bone remodeling (17). Klk6+ transgenic mice have high levels of IL-23, IL-17A/F, and IL-22 (Figure 2D and Supplemental Figure 1), and IL-23/IL-17 signaling promotes production of CXCL1, CXC2L, and CXCL5, which promote neutrophil recruitment and migration into joint spaces. CXCL1 strongly correlates with KLK6 ($\rho$ of 0.75; $P = 9.6 \times 10^{-6}$) in psoriatic patient lesional skin, and voropaxar treatment of psoriatic skin explants decreases CXCL1 expression (Figure 4F). Other transcripts strongly associated with KLK6 in psoriatic lesional skin include IL36A ($\rho$ of 0.7; $P = 6 \times 10^{-6}$) and PI3 ($\rho$ of 0.81; $P = 1.7 \times 10^{-4}$), which are also critically involved in keratinocyte–immune cell hyperactivation and inflammation and may link skin inflammation with joint and bone disease.

Using the Klk6+ transgenic mouse, we have identified a critical role for KLK6 in promoting psoriasis-like inflammation via PAR1 signaling, suggesting that targeting PAR1 may offer a cytokine-independent approach for treating psoriasis. The unexpected discovery that normalization of murine KLK6 levels largely reverses KLK6-driven bony disease may reflect the plasticity of the skeletal and articular system of young mice; however, there is precedent for reversal of PsA with therapy in adult patients (18). Ongoing investigation of development and regression of PsA-like joint and bone disease in the Klk6+ mouse and, perhaps, future evaluation of the effects of KLK6 normalization in patients with psoriasis will provide further insight.

Methods

Refer to Supplemental Methods for details.

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Data availability. The RNA-seq data sets have been deposited in the NCBI’s Gene Expression Omnibus (GEO) database under accession number GSE144312.

Statistics. Experimental data are presented as mean ± SEM unless otherwise indicated. $P$ values for experiments comparing 2 groups were calculated using either a 2-tailed Student’s $t$ test or a Mann-Whitney test. For experiments comparing more than 2 groups, an ordinary 1-way ANOVA was used with post hoc Tukey’s multiple-comparisons test, unless otherwise noted. $P$ less than 0.05 was considered statistically significant.

Study approval. Human samples were obtained from volunteer patients with psoriasis and healthy controls with informed written consent before inclusion in the study in accordance with Declaration of Helsinki principles. All protocols were approved by the University of Michigan institutional review board. All animal experiments were approved by the Case Western Reserve University institutional animal care and use committee and conformed to the American Association for Accreditation of Laboratory Animal Care guidelines.

Author contributions

JEG, RJM, and NLW conceived the study and designed experiments. WRS, LCT, RR, RJM, JEG, and NLW analyzed the data. JEL, YY, DD, RR, XX, PAK, DG, RU, MIC, MKS, and NLW conducted the experiments. DG, RR, RJM, MEH, CM, SAR, JUS, JMK, JEG, ACB, and NLW contributed to the phenotyping and understanding of the arthritic phenotype. ACB, JEG, and NLW wrote the manuscript with input from all authors.

Assigning of order of shared first author position was done as follows: ACB interpreted the data, generated the figures, and wrote the manuscript and was assigned first position; JEL conducted the majority of the animal analyses and was placed second; and YY designed the mouse cloning and completed critical experiments and analyses in the initial years of the study and was placed third.

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3. Radulovic M, Yoon H, Wu J, Mustafa K, Fehlings MG, Scarisbrick IA. Genetic targeting of protease activated receptor 2 reduces inflammatory astro-


