# Keratin 16 regulates innate immunity in response to epidermal barrier breach

Juliane C. Lessard[a], Sylvia Piña-Paz[a], Jeremy D. Rotty[a], Robyn P. Hickerson[b], Roger L. Kaspar[b,c], Allan Balmain[d], and Pierre A. Coulombe[a,e,f,1]

[a]Department of Biochemistry and Molecular Biology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD 21205; [b]TransDerm, Inc., Santa Cruz, CA 95060; [c]Department of Pediatrics, Stanford University, Stanford, CA 94305; [d]Hellen Diller Comprehensive Cancer Center, University of California, San Francisco, CA 94158; and [e]Department of Biological Chemistry and [f]Department of Dermatology, School of Medicine The Johns Hopkins University, Baltimore, MD 21205

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**Mutations in the type I keratin 16 (*Krt16*) and its partner type II keratin 6 (*Krt6a*, *Krt6b*) cause pachyonychia congenita (PC), a disorder typified by dystrophic nails, painful hyperkeratotic calluses in glabrous skin, and lesions involving other epithelial appendages. The pathophysiology of these symptoms and its relationship to settings in which *Krt16* and *Krt6* are induced in response to epidermal barrier stress are poorly understood. We report that hyperkeratotic calluses arising in the glabrous skin of individuals with PC and *Krt16* null mice share a gene expression signature enriched in genes involved in inflammation and innate immunity, in particular damage-associated molecular patterns. Transcriptional hyper-activation of damage-associated molecular pattern genes occurs following de novo chemical or mechanical irritation to ear skin and in spontaneously arising skin lesions in *Krt16* null mice. Genome-wide expression analysis of normal mouse tail skin and benign proliferative lesions reveals a tight, context-dependent coregulation of *Krt16* and *Krt6* with genes involved in skin barrier maintenance and innate immunity. Our results uncover a role for *Krt16* in regulating epithelial inflammation that is relevant to genodermatoses, psoriasis, and cancer and suggest a avenue for the therapeutic management of PC and related disorders.**

intermediate filament | epidermis

The skin is a highly specialized organ designed to actively prevent and react to a variety of environmental insults such as mechanical trauma, chemical irritation, and exposure to pathogens. Maintaining this first and vital line of defense requires intact structural and immunological barriers, specifically in the stratum corneum, to avoid dehydration and quickly address exterior threats locally. An inadequate or excessive response to an epidermal barrier challenge not only affects the process of acute wound healing, but also can eventually lead to both chronic inflammation and/or tumor development (1). The nature and extent of damage perceived by the epidermis and any downstream actions must therefore be tightly regulated.

Keratinocytes play a special role in sensing epidermal barrier challenges and produce the first signals, known as damage-associated molecular patterns (DAMPs) or "alarmins," to initiate the inflammatory response in the event of a barrier breach (1, 2). Alarmins are a diverse group that includes members of the S100 family of proteins, antimicrobial peptides, and select cytokines and chemokines (3, 4). Most DAMPs, in particular the group of alarmins, are secreted from keratinocytes and act by directly attacking invading pathogens, attracting and activating a wide range of immune cells (e.g., dendritic cells, neutrophils, macrophages, T-cells) and modulating cytokine production (1, 5, 6).

In addition to DAMPs, stressed keratinocytes rapidly induce de novo transcription of keratins (Krt) 6, 16, and 17, whose normal expression pattern in stratified epithelia is restricted to the epidermis of glabrous skin, the oral mucosa, and several appendages (7). Aside from their mechanical properties, these keratins have specialized functions in the progression of inflammation and wound healing. Krt6 impacts cell migration by interacting with Src kinase (8), whereas Krt17 promotes keratinocyte survival (9), growth (10), and a Th1/Th17-dominated immune environment contributing to the development of basaloid skin tumors (11). By contrast, the significance of *Krt16* induction in response to environmental stressors in epithelial cancers and in chronic inflammatory disorders (12) is largely unknown.

Inherited dominant mutations in *KRT6*, *KRT16*, and *KRT17* are causative for pachyonychia congenita (PC), a clinically heterogeneous disorder characterized by dystrophic nails and hyperkeratotic lesions in glabrous skin and oral epithelia (13). In mice, loss of *Krt16*, but not *Krt6a/b* or *Krt17*, results in prominent, chronic lesions on front and hind paws that closely resemble palmoplantar keratoderma (PPK) in PC patients (14). Keratinocyte fragility in these calluses appears sporadic and modest relative to the amount of hyperkeratosis, suggesting an additional, nontraditional role for *Krt16* in glabrous skin. Here, we report that *Krt16* participates in the regulation of early inflammation and innate immunity in a broad range of settings involving skin, revealing a newly defined role relevant for several diseases including PC, psoriasis, and cancer.

## Results

**Molecular Convergence Between *Krt16*$^{-/-}$ Front Paw Calluses and Human Palmoplantar Keratoderma Lesions.** Skin lesions in adult *Krt16*$^{-/-}$ front paws have an impaired outside–inside epidermal barrier, correlating with loss of the stratum corneum protein filaggrin, induction of the wound healing-associated Krt17, and hyperproliferation (14). We now confirm the presence of inflammation by showing that CD4+ T-cells, monocytes, macrophages, and neutrophils accumulate in lesional *Krt16*$^{-/-}$ front-paw

### Significance

Here we report that keratin 16 (Krt16), a type I intermediate filament cytoskeletal protein, is an integral and functionally important component of a genetic network regulating danger signals, innate immunity, and barrier function in skin epidermis. Our findings help explain the pathogenesis of the conspicuous skin lesions arising in genetic skin disorders caused by mutations in Krt16, such as pachyonychia congenita and focal palmoplantar keratoderma, and in diseases in which Krt16 is induced and misregulated, such as psoriasis and cancer.

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The authors declare no conflict of interest.

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[1]To whom correspondence should be addressed. E-mail: coulombe@jhsph.edu.

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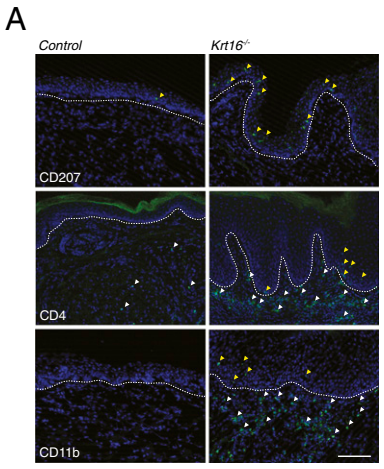
skin (Fig. 1*A*). CD207+ Langerhans cells (LCs), normally rare in murine glabrous skin, are also markedly increased in *Krt16*$^{-/-}$ calluses, especially in areas immediately adjacent to tissue showing hyperproliferation (Fig. 1*A*) (14).

Genome-wide association and gene expression profiling studies have linked *Krt16* to key players in cutaneous inflammation and cancer susceptibility (15–18). We used quantitative RT-PCR (qPCR) to look at a panel of proinflammatory signature mRNAs (Table S1) relevant to these settings. At 8 wk after birth, *Krt16*$^{-/-}$ front paws feature prominent lesions whereas hind-paw pads still appear normal (Fig. S1). Using hind-paw–derived data as an internal control for each mouse, we observe prominent expression of several DAMPs and proinflammatory cytokines in *Krt16*$^{-/-}$ front-paw skin (Fig. 1*B* and Fig. S1). Particularly notable are the high levels of *Sprr2d*, *Sfn*, and *Krt6*a mRNAs as they are selectively induced in keratinocytes at the wound edge (10, 15, 19), are connected to each other in cancer susceptibility networks (20), and, in the case of *Sprr2d*, possess antioxidant properties (21, 22). *Krt16*$^{-/-}$ front-paw lesions also feature high transcript levels for *Stfa1* and *SerpinB3a*, which are keratinocyte-specific protease inhibitors associated with proliferation, differentiation, and increased susceptibility to skin cancer (23, 24). Up-regulation of *DefB3* and *DefB4* further suggests an impairment of both the permeability and the antimicrobial barrier in *Krt16*$^{-/-}$ glabrous skin. By comparison, genes involved in LC trafficking (*Ccr6*, *Ccl20*), apoptosis (*Casp8*), the inflammasome (*Nlrp3*), and the amplification and coordination of the adaptive immune response (*IL-22*) are only moderately induced in *Krt16*$^{-/-}$ front-paw lesions (Fig. 1*B*). Of note, the rupture or lysis of keratinocytes is not a predominant feature in *Krt16*$^{-/-}$ glabrous epidermis as confirmed by transmission electron microscopy (Fig. S1).

We next analyzed global gene expression in plantar keratoderma biopsies from human PC patients carrying mutations in *KRT16*, *KRT6,* or *KRT17* and normalized the data to nonlesional glabrous skin from the same individuals. Interestingly, although expression of proinflammatory cytokines was generally low, several genes encoding for S100s, Sprr proteins, and β-defensins were among the most abundant transcripts in lesional tissue (Fig. 1*C*). Expression of *KRT6* paralogs (a and b) was also markedly elevated (Fig. 1*C*), consistent with their wound-inducible nature (25) and their newly proposed status as DAMPs (26). Such a DAMP-centric profile occurs independently of the disease-causing mutation and is strikingly similar to our findings in *Krt16*$^{-/-}$ front-paw calluses, suggesting that the misregulation of barrier-related genes is a general feature of PC-related palmoplantar keratoderma. These data validate the *Krt16*$^{-/-}$ mouse as a relevant model in which to study the pathogenesis of PPK.

**Keratinocytes Lacking Krt16 Hyper-Activate Alarmin Expression in Response to Chemical and Mechanical Challenges to the Epidermis.** To test the hypothesis that the absence of *Krt16* alters the course of acute cutaneous inflammation, we treated ear skin of 8-wk-old *WT* or *Krt16*$^{+/-}$ and *Krt16*$^{-/-}$ mice twice with 12-*O*-tetradecanoylphorbol-13-acetate (TPA). TPA is a well-known activator




C

| Patient Registry # | Mutation | OASL | S100A1 | S100A2 | S100A7 | S100A8 | S100A9 | S100A12 | S100A16 |
|---|---|---|---|---|---|---|---|---|---|
| PC-1009 | KRT16 R127C | 8.35 | -1.08 | 2.42 | 19.32 | 19.44 | 10.25 | 1.94 | 3.84 |
| PC-1015 | KRT16 R127C | 5.33 | -1.44 | 4.25 | 35.03 | 96.37 | 41.11 | 2.88 | 3.20 |
| PC-10 | KRT6A N171 | 23.09 | -2.10 | 7.42 | 1.95 | 5.81 | 8.50 | 21.69 | 2.12 |
| PC-661 | KRT6B E472K | 64.27 | -1.20 | N/A | 53.41 | 79.14 | 72.98 | 8.92 | 2.49 |
| PC-394 | KRT17 N92S | 2.66 | -1.34 | 3.70 | 24.32 | 100.00 | 71.21 | 1.54 | 2.88 |

| Patient Registry # | Mutation | DEFB103A | DEFB4 | DEFB129 | SPRR1A | SPRR1B | SPRR2A | SPRR2C | SPRR3 |
|---|---|---|---|---|---|---|---|---|---|
| PC-1009 | KRT16 R127C | 5.53 | 15.11 | 2.39 | 20.41 | 18.05 | 3.40 | 2.70 | 5.78 |
| PC-1015 | KRT16 R127C | 4.12 | 18.06 | 1.42 | 9.29 | 9.28 | 3.02 | 4.14 | 4.95 |
| PC-10 | KRT6A N171 | 6.76 | 15.58 | 3.15 | 5.83 | 6.07 | 2.75 | 6.20 | 20.68 |
| PC-661 | KRT6B E472K | 37.17 | 27.00 | 1.39 | 20.30 | 21.24 | 12.52 | 32.24 | N/A |
| PC-394 | KRT17 N92S | 3.61 | 11.04 | 1.82 | 7.22 | N/A | 2.37 | 4.16 | 9.10 |

| Patient Registry # | Mutation | SERPINB4 | SERPINB13 | CSTA | SFN | KRT6A | KRT6B | KRT16 | KRT17 | HO-1 |
|---|---|---|---|---|---|---|---|---|---|---|
| PC-1009 | KRT16 R127C | 8.25 | 9.09 | 3.42 | 6.92 | 66.13 | 94.15 | 41.67 | 12.91 | 1.50 |
| PC-1015 | KRT16 R127C | 23.26 | 7.26 | 6.36 | 3.07 | 41.92 | 92.22 | 25.21 | 25.21 | 2.42 |
| PC-10 | KRT6A N171 | 6.95 | 6.36 | 3.50 | 2.03 | 4.56 | 6.52 | 2.34 | 4.10 | 3.80 |
| PC-661 | KRT6B E472K | 11.98 | 3.15 | 7.62 | 2.77 | 21.09 | N/A | 7.82 | 6.33 | 9.20 |
| PC-394 | KRT17 N92S | 9.55 | 4.63 | 5.08 | 2.60 | 13.31 | 44.10 | N/A | 2.42 | 1.09 |

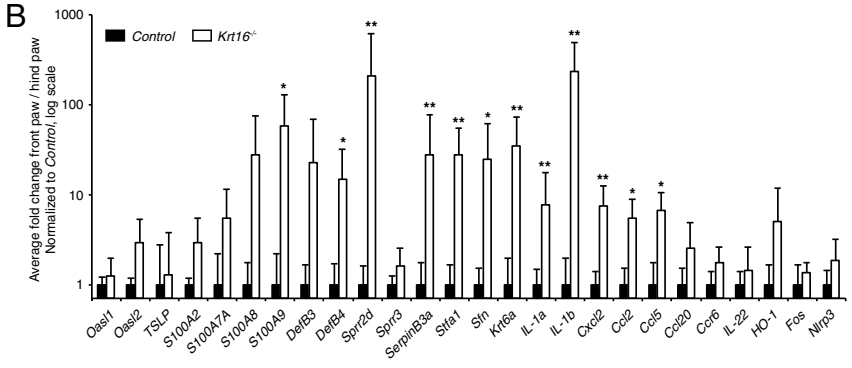



**Fig. 1.** *Krt16*$^{-/-}$ front-paw lesions and PPK biopsies from pachyonychia congenita patients share a DAMP-enriched gene expression signature. (*A*) Langerhans cells (CD207+), CD4+ T-cells, and CD11b+ neutrophils, macrophages, and monocytes are highly abundant in *Krt16*$^{-/-}$ lesions. White arrowheads indicate presence in the dermis, yellow arrowheads highlight immune cells present in the epidermis (scale bar, 100μm). (*B*) qPCR profile of front-paw gene expression relative to hind paws, normalized to control mice. DAMPs, protease inhibitors, and select cytokines are significantly up-regulated in *Krt16*$^{-/-}$ lesions. Each bar represents the mean + SD of five to eight biological replicates. **P* < 0.05, ***P* < 0.01, Mann-Whitney test, two-tailed. (*C*) Excerpt of qPCR microarray data obtained from plantar biopsies of pachyonychia congenita patients. Data are shown as fold changes of lesioned skin relative to unlesioned skin from the same individual.

of protein kinase C that initiates epidermal inflammation and promotes tumor formation (27). Topical TPA application to mouse ear skin causes epidermal thickening, hyperproliferation, up-regulation of Krt16 and Krt17 proteins, and recruitment of wound-associated immune cells (10) (Fig. 2*A* and Fig. S2). Before TPA treatment, histology and epidermal thickness are normal in *Krt16*$^{-/-}$ ear skin (Fig. 2*A*). At 48 h following the last of two TPA treatments, expansion of the postmitotic suprabasal layers is modestly but significantly greater in *Krt16*$^{-/-}$ relative to control (Fig. 2*A*). The origin of this expansion is unknown, as the mitotic index remains the same in *Krt16*$^{-/-}$ and control TPA-treated ears (Fig. 2*A*). Onset of Krt16 expression precedes epidermal thickening and thus can be uncoupled from hyperproliferation in such settings (19, 28).

TPA treatment of ear skin tissue also results in the increased expression of a group of proinflammatory and barrier-related gene targets similar to *Krt16*$^{-/-}$ front-paw lesions (Fig. S2). Normalization of gene expression fold changes to the control genotype highlights a significant over-induction of several genes in *Krt16*$^{-/-}$ TPA-treated skin, in particular several DAMPs (*TSLP*, *S100A7A*, *DefB4*), select cytokines (*Ccl5*, *IL-1a*), *Sprr2d*, *HO-1*, and *Sfn* (Fig. 2*B*). We do not observe a significant increase in *Nlrp3* mRNA indicative of inflammasome activation (18, 29), suggesting that this aspect of the inflammatory response may not be a major contributor to the molecular phenotype of TPA-treated *Krt16*$^{-/-}$ skin, despite enhanced levels of IL-18 mRNA. We note that IL-18 secretion from keratinocytes was recently shown to depend on Krt1 (30), whereas IL-1b production is enhanced in the skin of newborn *Krt5*$^{-/-}$ mice (31).

At 48 h after the topical application of acetone as the vehicle control, which in itself elicits a mild epidermal barrier disruption in mouse skin (32), we find that neither Krt16 nor Krt17 are detectable in ear interfollicular epidermis (Fig. 2*A* and Fig. S2) and that most of the transcripts analyzed show similar levels between genotypes (Fig. S2). This is significant because it suggests a similar basal state of adult *Krt16*$^{-/-}$ and control epidermis. This said, we observed a modest decrease in mRNA levels for *IL-1b*, *Ccl2*, *Ccl5*, and *HO-1* in acetone-treated *Krt16*$^{-/-}$ ear epidermis (Fig. S2). However, these changes do not overlap with

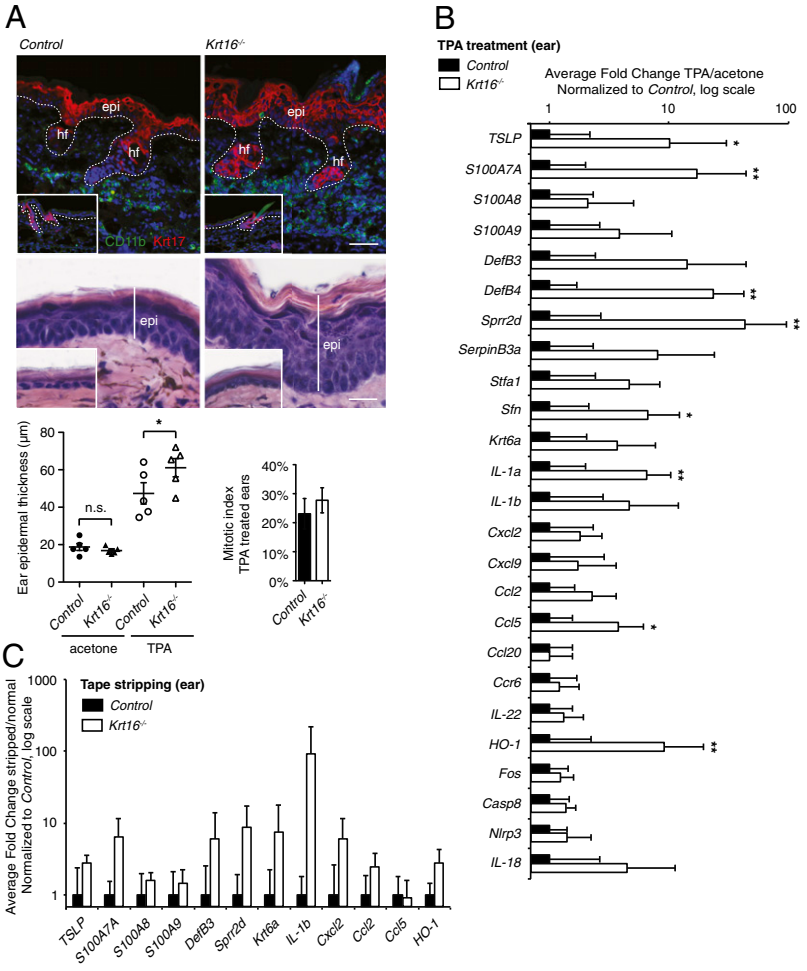



**Fig. 2.** Chemical and mechanical irritation leads to hyper-activation of DAMPs and cytokines in *Krt16*$^{-/-}$ skin. (*A*) Phorbol ester (TPA) treatment of ear skin in 8-wk-old mice induces Krt17 expression in suprabasal keratinocytes and infiltration by nonresident immune cells (CD11b+). epi, epidermis; hf, hair follicle. (Scale bar, 50 μm.; the first scale bar refers to the IF stainings, the second to the H&E stainings.) H&E stainings illustrate the epidermal expansion in response to TPA. (Scale bar, 25 μm.) *Insets* show ears treated with acetone vehicle. Note the equal epidermal thickness as well as the absence of Krt17 or immune cell staining in the interfollicular epidermis of vehicle-treated ears. *Krt16*$^{-/-}$ mice develop significantly more epidermal thickening in response to TPA than controls without a change in the mitotic index. *$P < 0.05$, n.s., not significant, one-way ANOVA with Bonferroni correction. (*B*) qPCR data from TPA-treated ear skin represented as fold changes (TPA/acetone) and normalized to control mice. *Krt16*$^{-/-}$ mice show a significant over-induction of alarmins and cytokines. Each bar represents the mean + SD of 5–10 biological replicates. *$P < 0.05$, **$P < 0.01$, Mann–Whitney test, two-tailed. (*C*) Mechanical disruption of the epidermal barrier via tape stripping also leads to an over-induction of DAMP and cytokine RNA in *Krt16*$^{-/-}$ mice. Data represent the mean fold changes (stripped ear/normal ear) + SD of three biological replicates relative to control mice.

findings in hind-paw skin epidermis (Fig. S1), normal ear epidermis (Fig. S3), or newborn keratinocytes in primary culture (Fig. S3) and could be related to the very low steady-state levels of these mRNAs in normal skin. Consistent with Krt17's proposed role as a proinflammatory immunomodulator (11) and in contrast to our findings in *Krt16*$^{-/-}$ skin, DAMP mRNA levels are essentially unchanged in *Krt17*$^{-/-}$ ear skin after TPA treatment (Fig. S2). An over-reaction to external stimuli is a wide-ranging characteristic of *Krt16*$^{-/-}$ epidermis because tape stripping of ear skin—a superficial mechanical insult that removes the stratum corneum and induces *Krt16* mRNA and protein expression (33)—also consistently leads to exaggerated expression of several alarmins, *IL-1b*, *Sprr2d*, *HO-1*, and *Krt6a* in *Krt16*$^{-/-}$ ear skin (Fig. 2*C*).

Unbalanced DAMP expression in response to trauma has long-term implications for an organism. Patients with atopic dermatitis (AD) overreact to injury with increased production and secretion of DAMPs (34). Likewise, chronically elevated levels of thymic stromal lymphopoietin (TSLP) in mouse epidermis trigger the formation of AD-like lesions (35), and overexpression of *S100A7A* leads to an immunological overreaction to mechanical stress (36). In addition to their PPK-like paw lesions, older *Krt16*$^{-/-}$ mice also develop spontaneous chronic dermatitis, which is fully penetrant yet variable in onset, severity, and location (Fig. S4). Such lesions appear in areas where *Krt16* is not normally expressed and show markedly elevated levels of *Krt6*, *Krt17*, *TSLP*, and *S100A7A* mRNAs and proteins (Fig. S4).

The analyses of our TPA and tape-stripping experiments were conducted at 48 h posttreatment, which allows for the arrival of systemic immune cells at the site of inflammation (Fig. 2*A* and Fig. S2). These immune cells likely make a contribution to the elevated mRNA levels for several genes, including *S100A8*, *S100A9*, and *IL-1b* (6, 37). Induction of targets such as the epithelial-specific *Krt6a* and *Sprr2d*, however, suggests a keratinocyte-autonomous component in this phenomenon. To test this hypothesis, newborn skin keratinocytes were seeded in primary culture, treated once with TPA, and processed for qPCR analysis. In this setting, DAMP gene expression peaks at 3–6 h posttreatment and returns to baseline within 24 h (Fig. 3*A*). Relative to controls, *Krt16*$^{-/-}$ but not *Krt17*$^{-/-}$ keratinocytes overexpress several DAMPs after TPA exposure (Fig. 3*B*). Before TPA treatment, *Krt16*$^{-/-}$ and control keratinocytes express similar levels of all mRNAs tested (Fig. S3). Other keratins do not appear to compensate for the loss of *Krt16* in the primary culture setting (Fig. S3). At this time, we cannot comment on whether the absence of Krt16 alters the secretion of DAMPs from keratinocytes. The induction of alarmin transcription 3 h after TPA addition is largely mediated by the Erk1/2 arm of MAPK signaling and does not appear to require IL-1–dependent amplification (Fig. 3*C* and Fig. S3). The ex vivo findings strongly suggest that the specific induction of *Krt16* in skin keratinocytes subject to cellular stress is critical for the proper transcriptional regulation of innate danger signals.

**Systems Genetics Analysis Independently Links *Krt16* to Alarmin and Skin Barrier Genes.** In 2009, Quigley et al. reported an unbiased systems genetics analysis yielding genome-wide expression and association networks for adult mouse skin during normal homeostasis and different stages of carcinogenesis (20). Here, we reanalyzed this data set for genes correlated with *Krt16*. In normal tail epidermis, *Krt16* expression is constitutive (similar to glabrous epidermis) and strongly positively correlated with several DAMPs and other regulators of skin barrier function (Fig. 4*A*, top 30 hits shown). Many of the top-scoring genes in this *Krt16*-anchored network—e.g., *Krt6a*, *S100A8*, *S100A9*, *DefB3*, *SerpinB3a*, and *Stfa1* (Fig. 4*A*)—are markedly misregulated in *Krt16*$^{-/-}$ mouse skin subject to barrier challenges and in PC-related PPK (Figs. 1–3). *Krt16* expression in benign papillomas sampled from back skin in the same set of mice is notably higher and more uniform relative to normal tail skin (Fig. 4*B*). A striking proportion of the top-scoring genes lose their correlation with *Krt16* in papillomas (Fig. 4*A*), suggesting that the interrelationships between *Krt16* and barrier-related genes may differ in settings of chronic inflammation. These findings significantly extend the notion that *Krt16* is an integral part of a genetic network that includes DAMP-encoding and skin barrier-associated genes.

## Discussion

We show here that when the epidermal barrier is experimentally challenged by acute proinflammatory and mechanical stimuli, keratinocytes lacking *Krt16* fail to properly regulate the production of innate danger signals and overactivate the expression of DAMPs, cytokines, and other regulators of skin barrier function. Our results imply a role for *Krt16* in this form of innate immunity, provide an innovative framework to understand the complex pathogenesis of several chronic inflammatory skin diseases, and, finally, may have direct implications for the treatment of the painful and debilitating palmoplantar keratoderma associated with PC and related genodermatoses. Early activation of *Krt16* expression after various types of insults to the skin is therefore functionally relevant to the progression of cutaneous inflammation. We infer that loss of *Krt16* eliminates an important inflammatory checkpoint, leaving the organism vulnerable to inappropriate immune responses, and we further speculate that loss of Krt16 function, whether complete or partial, impairs the resolution of PPK-like calluses in glabrous skin.

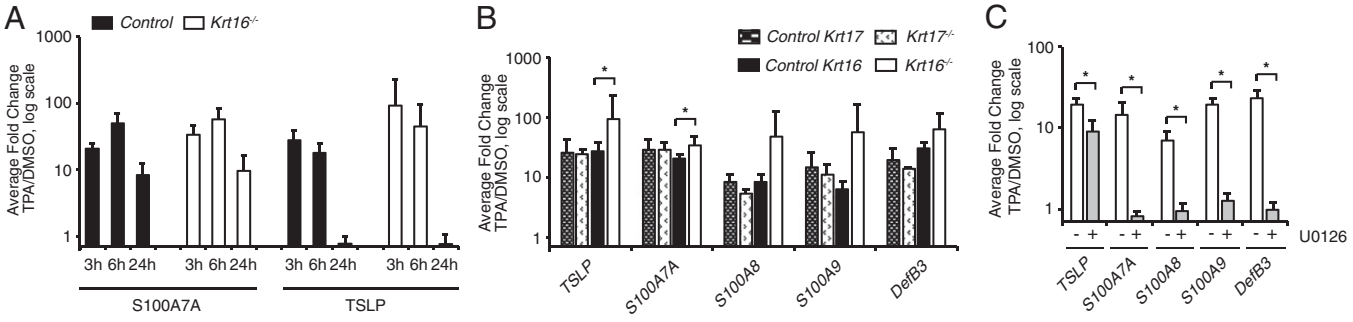



**Fig. 3.** Misregulation of innate danger signals is specific to *Krt16*$^{-/-}$ keratinocytes. (*A*) DAMP expression peaks at 3–6 h post-TPA treatments in newborn keratinocytes in primary culture and returns to baseline by 24 h. (*B*) Cultured *Krt16*$^{-/-}$ primary keratinocytes retain the ability to hyper-activate alarmins 3 h after TPA treatment. *Krt17*$^{-/-}$ cells do not show a difference compared with controls. Data represent the mean + SD of three to eight biological replicates. *$P < 0.05$, Mann–Whitney test, two-tailed. (*C*) DAMP transcription in *Krt16*$^{-/-}$ keratinocytes in response to TPA is mediated by the Erk arm of MAPK signaling. Data represent the mean + SD of four biological replicates. *$P < 0.05$, Student *t* test, two-tailed.

A

| Gene | Chromosome | Normal tail | Papilloma |
|---|---|---|---|
| *Krt6b* | 15 | 0.90871 | 0.81112 |
| *S100a8* | 3 | 0.85108 | -0.42041 |
| *S100a9* | 3 | 0.84008 | -0.46594 |
| *Krt6a* | 15 | 0.81958 | 0.88599 |
| *Defb3* | 8 | 0.79299 | 0.21072 |
| *Sprr2d* | 3 | 0.77642 | N/A |
| *Stfa1* | 16 | 0.76976 | -0.16943 |
| *Cd44* | 2 | 0.76633 | 0.13698 |
| *Serpinb3c* | 1 | 0.74718 | 0.15256 |
| *Pkm2* | 9 | 0.73883 | 0.20405 |
| *Fetub* | 16 | 0.7357 | 0.47238 |
| *Serpinb3a* | 1 | 0.7349 | 0.16322 |
| *Galnt6* | 15 | 0.73452 | 0.14721 |
| *Wfdc12* | 2 | 0.73027 | 0.5922 |
| *Tgm3* | 2 | 0.7231 | 0.82736 |
| *Rptn* | 3 | 0.72293 | 0.65618 |
| *Rps6ka4* | 19 | 0.72126 | 0.56679 |
| *Ehf* | 2 | 0.72002 | -0.05234 |
| *Sprr2i* | 3 | 0.71945 | 0.50755 |
| *2610528A11Rik* | 14 | 0.71649 | N/A |
| *Eno1* | 4 | 0.71145 | 0.59627 |
| *Mlze* | 15 | 0.70513 | 0.53037 |
| *Pdzk1ip1* | 4 | 0.70488 | 0.45805 |
| *Tmprss11a* | 5 | 0.70429 | 0.05486 |
| *Egln1* | 8 | 0.70106 | 0.12945 |
| *Pcdh21* | 14 | 0.69647 | 0.54218 |
| *Klk9* | 7 | 0.69344 | 0.51229 |
| *Pfkl* | 10 | 0.6934 | -0.0549 |
| *Ptges* | 2 | 0.69313 | N/A |
| *C130090K23Rik* | 5 | 0.69268 | -0.15678 |

Positive correlation with *Krt16*

Decrease in or loss of correlation with *Krt16*

B

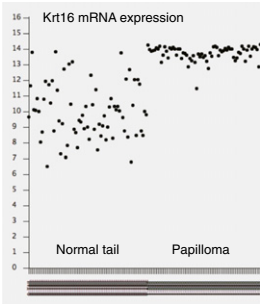



**Fig. 4.** *Krt16* is a member of a barrier- and DAMP-centric gene network in skin. (*A*) Genome-wide expression data set (20) analyzed for correlations with *Krt16*. Shown are the top 30 hits that strongly correlate with *Krt16* in normal tail skin and their relative correlation with *Krt16* in papilloma of back skin. (*B*) *Krt16* expression is constitutive yet variable in normal tail skin. By contrast, papilloma in back skin consistently express high, uniform levels of *Krt16*. Each dot represents an individual mouse.

Recent advances in the field support the modulation of innate immunity by select keratins and their necessity for epidermal barrier maintenance. Krt1, a type II keratin constitutively expressed in the differentiating layers of the epidermis, is essential for controlling inflammasome activity, specifically the amount of IL-18, S100A8, and S100A9 secretion from keratinocytes (30).

The preferential development of PPK and *Krt16*$^{-/-}$ paw lesions in areas of high mechanical pressure suggests that Krt16's function could be related to mechanically activated signal transduction in keratinocytes. For example, intermediate filaments are required for hemidesmosome function in mice (38). Hemidesmosomal integrins can transduce mechanical signals (39) and, like Krt16, are inducibly expressed in suprabasal keratinocytes during wound healing, chronic inflammation, and in response to phorbol esters (40). Keratinocyte-specific loss of murine Rac1, which interacts with hemidesmosomal integrins, causes epidermal hypersensitivity to proinflammatory stimuli (41), similar to TPA-treated *Krt16*$^{-/-}$ ear skin. Phorbol esters heighten keratinocyte sensitivity to outside stressors and potentiate EGF receptor (EGFR) activation via Erk1/2 signaling, resulting in the increased production of IL-1a (42). IL-1a is known to interact functionally with MAPK signaling to activate and amplify keratinocyte proliferation and epidermal inflammation, creating an autoimmune feedback loop (37, 43). Integrins, the IL-1 receptor, and the EGF receptor are all located in focal adhesion complexes at the plasma membrane (44), which are altered in epidermal keratinocytes null for Krt6, the type II keratin partner for Krt16 (8). In addition, focal adhesion kinase links mechanical stress to Erk1/2 signaling and cytokine production in dermal fibroblasts (45). Possibly, Krt16 may be involved in regulating a pathway that is activated when the skin experiences increased or altered mechanical forces, e.g., in normal glabrous skin or at the wound edge.

DAMP and cytokine transcription in cultured keratinocytes depends on the MAPK signaling cascade, a major switchboard for relaying and amplifying stress signals (46 and this study). *Krt16* is a direct target for EGFR and Erk1/2-mediated signaling (47–50), and its overexpression in mice dose-dependently enhances EGFR activity (51). Following stress, Krt16 could conceivably impact MAPK and/or EGF signaling to modulate the total level of DAMPs in a keratinocyte-autonomous fashion. *Krt16* is also a direct target for signaling mediated by the Nrf2 transcription factor, a master regulator of ROS levels and the oxidative stress response in skin (50, 52, 53). In adult mice, misregulation of Nrf2 levels raises the risk for tumorigenesis (54, 55) and promotes cutaneous inflammation secondary to stratum corneum abnormalities (22). In utero, Nrf2 stimulates epidermal barrier repair via the up-regulation of Sprr2d and Sprr2h (56). We observe high levels of *Sprr2d* as well as *HO-1* in *Krt16*$^{-/-}$ TPA-treated ears, suggesting the activation of the Nrf2-mediated oxidative stress response and raising the possibility that Krt16 may play a role in this cellular defense mechanism.

Autoantibodies to KRT16 have been tied to an exaggerated activation of innate immunity signaling pathways in psoriatic lesions (57, 58). Furthermore, IL-1a treatment of human primary keratinocytes elicits a transcriptional profile enriched in antimicrobial peptides and genes from the epidermal differentiation complex (59) that is strikingly similar to challenged *Krt16*$^{-/-}$ skin and to Krt16's association with skin barrier-related factors as revealed by computational analysis. In the absence of *Krt16*, improper control of the IL-1a–signaling pathway and/or its proinflammatory feedback loop could explain the phenotypes that we observe in both *Krt16*$^{-/-}$ skin and human PPK lesions. In our hands, inhibiting IRAK1/4 did not alter DAMP transcription in response to TPA in *Krt16*$^{-/-}$ keratinocytes in culture. However, in vivo cellular architecture and feedback from other cell types, e.g., fibroblasts as well as resident and infiltrating immune cells, play a major role in IL-1a–mediated autoimmune feedback (37). The lack of an intact tissue microenvironment could thus account for the modest induction of DAMP expression occurring in newborn skin keratinocyte cultures compared with adult whole ear tissue.

Various strategies have been applied toward the therapeutic management of PC (60–62) or for palmoplantar keratoderma of various etiologies (63, 64) with mixed results and, in the end, limited relief for the patient. This includes topical treatments (e.g., with corticosteroids or retinoids) designed to antagonize inflammation in a broad and rather nonspecific fashion (64–66). A recent trial involving the use of a mutant *Krt6a* allele-specific siRNA in plantar skin led to the recession of calluses and associated pain in one patient, but the extreme pain associated with the direct injection is problematic considering the large areas covered by PPK (61). The development of therapies designed to attenuate the alarmin response in skin, especially when combined with keratin mutant allele-specific interventions, could prove beneficial for PC patients.

## Materials and Methods

Procedures for the collection, processing, and analysis of patient plantar biopsies, gene expression correlation analysis, epidermal barrier challenges in Krt16 and Krt17 null mice, qPCR, cell culture, histology, reagents, and the system genetics analysis are described in the *SI Materials and Methods*. Animal experiments involving mice were approved by The Johns Hopkins University Institutional Animal Care and Use Committee. *Krt16*$^{-/-}$ and *Krt17*$^{-/-}$ mouse lines (C57BL/6 background) (10, 14) were maintained under specific pathogen-free conditions and fed chow and water ad libitum. De-identified plantar human skin samples were obtained, with informed consent, from one affected and one unaffected site of five nonrelated, adult PC patients harboring a KRT16 R127C, KRT6A N171K, KRT6B E472K, or KRT17 N92S single nucleotide mutation (patients #1009, #1015, #10, #661, and #394 from the International Pachyonychia Congenita Research Registry).

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