Mechanical Stretch Exacerbates Psoriasis by Stimulating Keratinocyte Proliferation and Cytokine Production

Pei Qiao1,2, Wei Guo1,2, Yao Ke1,2, Hui Fang1, Yuchen Zhuang1, Man Jiang1, Jieyu Zhang1, Shengxian Shen1, Hongjiang Qiao1, Erle Dang1 and Gang Wang1

Psoriasis is a chronic inflammatory autoimmune skin disease that often occurs in rubbed areas undergoing a strong mechanical stretch, such as the elbows and knees. However, the pathologic role of mechanical tension in psoriasis remains unclear. In this study, we investigated the contribution of keratinocyte mechanical stretch to the clinical features of psoriasis. We found that keratinocyte proliferation and skin barrier-associated gene expression increased significantly after 24 hours of continuous stretching. Additionally, continuous stretching induced the production of psoriasis-associated proinflammatory cytokines, antibacterial peptides, and chemokines in primary human keratinocytes. Furthermore, we established a murine model of skin expansion by implanting a dilator into the dorsum of BALB/c mice to assess the effect of mechanical stretch on the epidermis in vivo. The dilator-implanted mice displayed prominent epidermal hyperproliferation, impaired skin barrier function, and up-regulation of psoriasis-associated cytokines in epidermal keratinocytes. Most importantly, the dilator-implanted psoriatic mice treated with imiquimod or IL-23 displayed an aggravated psoriatic phenotype compared with mice without dilator implantation. Collectively, our results suggest that mechanical stretch can exacerbate psoriatic lesions by promoting cell proliferation and amplifying the production of proinflammatory cytokines by keratinocytes. Thus, our findings provide new insights into the pathogenesis of psoriasis.

INTRODUCTION
Psoriasis is a common chronic inflammatory autoimmune skin disease, characterized by aberrant keratinocyte hyper-proliferation and differentiation and high levels of intradermal infiltration of inflammatory cells (Boehncke and Schön, 2015). Clinically, psoriatic lesions are common and often serious and occur on the scalp and on areas of the trunk and limbs such as the elbows, back, calf, and parts of the leg extension area. A common feature of these sites compared with other parts of the body is that the surface tension of the skin is higher or the skin is more likely to be mechanically stretched, suggesting that mechanical stretch might contribute to the pathogenesis of psoriasis (Albanesi, 2012; Kuchekar et al., 2011); however, the pathogenic mechanisms are unclear. Therefore, we hypothesized that mechanical stretch may affect keratinocyte proliferation, differentiation, and other activities, thus affecting the occurrence and development of psoriatic lesions.

Mechanical stretch is a critical characteristic of the skin that is implicated in many physiological and pathological processes. In response to mechanical stimulation, the skin can stretch and extend to adapt to physical requirements during pregnancy or wound healing (Cho et al., 2010; Mammoto and Ingber, 2010; Takei et al., 1997). In addition, the Koebner phenomenon, which is characterized by the development of new lesions on previously uninvolved skin after mechanical injury, can be observed in many cutaneous diseases (Sagi and Trau, 2011; Weiss et al., 2002). Moreover, mechanical stretching can promote the proliferation of human keratinocytes by inducing calcium influx and the EGFR and ERK1/2 cascades (Yano et al., 2004). Recently, biomechanical stretch was identified as an inducer of epithelial-mesenchymal transition, which can promote epidermal regeneration by regulating keratinocyte proliferation and differentiation (Zhou et al., 2015). Because psoriasis is a skin disorder characterized by abnormal keratinocyte proliferation and is associated with keratinocyte activation (Bata-Csörgő and Szell, 2012), we hypothesized that mechanical stretching of the skin contributes to the pathogenesis of psoriasis by modulating keratinocyte function.

RESULTS
Psoriatic lesions preferably develop on the body sites vulnerable to mechanical stretch
We collected clinical data from 52 random cases of psoriasis vulgaris. The sites of psoriatic lesions were assessed and are documented in Table 1. The incidence of psoriatic lesions...
was significantly higher in regions with greater skin surface tension, such as the exterior of the elbow and the anterior regions of knee and leg extension, than in the corresponding opposite positions, such as the interior of the elbow and the posterior regions of knee and leg flexion ($P < 0.001$). Supplementary Figure S1 online shows a representative case of a psoriasis patient with typical clinical manifestations of silver plaques and scales predominantly distributed on the arm and leg extensors and the scalp. These regions are generally considered to undergo an extensive mechanical stretch, suggesting that mechanical stretch might contribute to the pathogenesis of psoriasis.

Mechanical stretch promoted keratinocyte proliferation in vitro
To explore the contribution of mechanical stretch to the pathogenesis of psoriasis, the effects of mechanical stretch on cell proliferation and differentiation were assessed in vitro. Primary human keratinocytes were cultured on flexible silicone dishes mimicking stretching-induced mechanical force.

<table>
<thead>
<tr>
<th>Table 1. The distribution of skin lesions in patients with psoriasis vulgaris</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>52 Cases of Psoriasis Patients with Lesions of Primary Site</strong></td>
</tr>
<tr>
<td><strong>Site</strong></td>
</tr>
<tr>
<td>Scalp</td>
</tr>
<tr>
<td>Outside the elbow</td>
</tr>
<tr>
<td>Leg extension</td>
</tr>
<tr>
<td>Front of the knee</td>
</tr>
<tr>
<td>Forearm extensor</td>
</tr>
<tr>
<td>Leg flexion side</td>
</tr>
<tr>
<td>Forearm flexion side</td>
</tr>
<tr>
<td>Face</td>
</tr>
<tr>
<td>Inside the elbow</td>
</tr>
<tr>
<td>Back of the knee</td>
</tr>
</tbody>
</table>

Figure 1. Human primary keratinocytes treated with or without mechanical stretch for 24 hours. (a) Cells were harvested and analyzed for cell cycle distribution by flow cytometry. Mechanical stretch significantly increased the proportion of cells in the G2/M phase and decreased the proportion of cells in the G1 phase compared with the control group ($* P < 0.05$). (b) Cell proliferation activity was detected by 5-ethyl-2'-deoxyuridine (EdU) staining, where the red dots represent the population of daughter cells. Hoechst staining (blue dots) was used to label nuclei. Data are presented as mean ± standard error of the mean from five microscopic fields. Scale bar = 25 μm; K6, K16, and K17 expression levels were evaluated using (c) real-time PCR and (d) Western blot analysis in mechanical stretch-treated and nontreated groups. Each experiment was repeated three times, and the results shown represent mean ± standard error of the mean. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ were considered significantly different for the nonstretched versus stretched groups. EdU, 5-ethyl-2'-deoxyuridine; K, keratinocyte.
Keratinocytes with or without stretching for 24 hours were then assessed by flow cytometry, 5-ethynyl-2'-deoxyuridine staining, real-time PCR, and Western blotting. Cell cycle analysis showed that the percentage of stretch-conditioned keratinocytes decreased significantly during the G0/G1 phase, whereas that of cells in the G2/M phase increased (Figure 1a). Furthermore, 5-ethynyl-2'-deoxyuridine (EdU) staining showed that the number of EdU labeled keratinocytes significantly increased in the stretched groups compared with the nonstretched group (Figure 1b). Both the mRNA and protein expression levels of the proliferation markers keratin (K) 6, K16, and K17 were up-regulated in stretched keratinocytes compared with those in nonstretched keratinocytes (Figure 1c and d). Moreover, we also found that the mRNA and protein levels of the differentiation marker K1 were down-regulated in stretched keratinocytes (see Supplementary Figure S2a and b online). Thus, our data indicate that mechanical stretch promoted keratinocyte proliferation and inhibited cell differentiation.

Mechanical stretch induced the production of psoriasis-related cytokines in keratinocytes in vitro

To investigate the effects of mechanical stretch on keratinocytes, we investigated the expression and secretion of proinflammatory cytokines and chemokines in keratinocytes. Mechanical stretching of keratinocytes significantly up-regulated IL-1α, IL-6, and IL-23 mRNA (Figure 2a); however, mechanical stretch did not significantly affect the mRNA level of tumor necrosis factor (TNF)-α (P = 0.1143). The production of neutrophil chemoattractants (CXCL1 and CCL20) and antimicrobial peptides (LCN2 and β-defensin) also increased significantly in stretched keratinocytes (Figure 2c, and see Supplementary Figure S2c and d). To confirm these findings, ELISA was performed to determine the
Mechanical Stretch Exacerbates Psoriasis Progression

Mechanical stretch inhibited skin barrier-associated molecules and affected skin barrier function

Because changes in skin barrier function represent a main characteristic of psoriasis, we next examined the expression levels of the predominant epidermal components filaggrin (FLG), loricrin (LOR), and involucrin (IVL), which play crucial roles in skin permeability. Both IVL and FLG were significantly down-regulated after a 24-hour continuous stretch in vitro (Figure 3a), suggesting that mechanical stretch affected the skin function. To further evaluate the functional impact of mechanical stretch on the skin, 19 patients (including those with pigmented nevus, sebaceous glands, scars, and keloids) who needed dilator implant surgery for skin transplantation were enrolled to compare changes in skin barrier function before and after surgery. Dilator implantation significantly increased transepidermal water loss (TEWL) and decreased skin moisture (Figure 3b). Taken together, these results indicate that mechanical stretch might be an important factor that affects skin barrier function.

Skin function was impaired in a dilator-implanted mouse model in vivo

To confirm our in vitro findings and further explore the effects of mechanical stretch on the skin, we established a cutaneous expansion model by implanting a mini silicone dilator into the dorsal of 8- to 10-week-old BALB/c mice with daily injection of H2O into the dilator. This mouse model can emulate the skin under mechanical stretch in vivo. Hematoxylin and eosin staining showed that the thickness of the dorsal epidermis was slightly greater in dilator-implanted mice than in untreated mice, whereas the thickness of the dorsal epidermis significantly increased in dilator-implanted mice with H2O-expansion compared with that in mice without H2O expansion (Figure 4a and b). Additionally, expression of the proliferation markers K6, K16, and K17 in the dorsal epidermis increased in dilator-implanted mice with or without H2O expansion (Figure 4c and d). Dilator-implanted mice with H2O expansion showed much higher Ki67 staining than mice without H2O expansion (Figure 4d and e). The mRNA and protein levels of the differentiation markers K1 and K10 were lower in dilator-treated mice with H2O expansion than in those in mice without H2O expansion or wild-type (WT) mice (see Supplementary Figure S3a and b online). These results suggest that mechanical stretch induced by the dilator implants enhanced keratinocyte proliferation and inhibited differentiation in vivo.

Furthermore, we assessed the expression of cytokines, chemokines, and antimicrobial peptides in the epidermis of dilator-implanted mice. Cytokines (IL-6, IL-23, and TNF-z), chemokines (CXCL1 and CCL20), and antimicrobial peptides (S100A8, S100A9, LCN2, and b-defensin) mRNAs were significantly up-regulated in the epidermis of dilator-implanted mice (Figure 4f and g, and see Supplementary Figure S4a online). IL-1z mRNA showed no significant difference (see Supplementary Figure S3b). Dilator-implanted mice with H2O expansion displayed significantly up-regulated CCL20 mRNA compared with both untreated and dilator-implanted mice. The dilator-implanted mice with H2O expansion showed significantly decreased skin moisture and increased TEWL, suggesting that dilator-induced mechanical stretch impaired skin barrier functions in vivo (Figure 4h). In addition, mRNA expression of the skin barrier function-related gene IVL was significantly down-regulated in dilator-implanted mice in response to stretch (see Supplementary Figure S3c). These results indicate that dilator-induced mechanical stretch caused activation of keratinocytes, thereby inducing cytokine secretion associated with skin inflammation.

Mechanical stretch induced by dilator implantation aggravated the psoriatic phenotype in the imiquimod-induced mouse model in vivo

To further investigate the contribution of mechanical stretch to the pathogenesis of psoriasis in vivo, a dilator-implanted...
Figure 4. The established dilator-implanted mouse model in vivo. (a, b) Epidermal thickness was determined by immunochemistry. (c) Real-time PCR and (d) immunofluorescence staining for proliferation markers K6, K16, K17, and Ki67 in dilator-implanted mice treated with up to 4 ml of H2O. (e) Results for

P Qiao et al.
Mechanical Stretch Exacerbates Psoriasis Progression

Journal of Investigative Dermatology (2019), Volume 139
cutaneous expansion mouse model was established, followed by topical application of imiquimod (IMQ)-containing cream to induce psoriasis-like lesions for further evaluation (see Supplementary Figure S5 online).

As shown in Figure 5a and b, dilator implantation followed by H2O-treatment significantly increased the epidermal thickness induced by IMQ compared with that in WT mice or mice treated with dilator implantation alone. Concurrently, after IMQ treatment, the mRNA and protein levels of K6 were up-regulated, and those of K10 were down-regulated, in the epidermis of mice implanted with the dilator followed by H2O expansion compared with WT mice or mice implanted with the dilator alone (Figure 5c and d, and see Supplementary Figure S6a and b online). Moreover, dilator implantation followed by H2O expansion significantly increased Ki67 staining induced by IMQ compared with WT mice or mice treated with dilator implantation alone (Figure 5d and e). Immunohistochemistry analysis showed elevated neutrophil infiltration into both the epidermis and dermis of the dorsal skin of dilator-implanted mice with H2O expansion (Figure 5f). After IMQ application, CXCL1, S100A8, S100A9, LCN2, and β-defensin mRNA expression levels significantly increased in the epidermis of dilator-implanted mice with H2O expansion compared with those in the epidermis of WT mice or mice subjected to dilator implantation alone (Figure 5g and i, and see Supplementary Figure S4b), similar to the characteristic up-regulation of chemokines and antimicrobial peptides found in lesions of psoriasis patients. Additionally, IL-6 mRNA expression was up-regulated in the dorsal skin of dilator-implanted mice with H2O expansion after IMQ treatment compared with that in WT mice or mice treated with dilator implantation alone (Figure 5h). No significant difference was observed in IL-1α mRNA expression among the four groups (see Supplementary Figure S6b). Dilator-implanted mice with H2O expansion showed significantly decreased skin moisture and increased TEWL after IMQ treatment (Figure 5j). IMQ treatment significantly down-regulated LOR and ILV mRNA expression levels in dilator-implanted mice with H2O expansion compared with that in WT mice or mice subjected to dilator implantation alone (see Supplementary Figure S6c). These findings indicate that mechanical stretch in dilator-implanted mice can enhance psoriasis by promoting keratinocyte proliferation, skin barrier function, neutrophil recruitment, and the production of proinflammatory cytokines.

To confirm these results, we also performed validation in an IL-23–induced psoriasis mouse model (see Supplementary Figure S7 online). Dilator implantation followed by H2O expansion significantly increased epidermal thickness induced by IL-23 compared with untreated or dilator-implanted mice (see Supplementary Figure S7a and b). Concurrently, dilator-implantation followed by H2O expansion significantly increased K16 and K17 mRNA expression and protein induced by IL-23 compared with WT mice or mice subjected to dilator implantation alone (see Supplementary Fig S7c and d). Dilator implantation followed by H2O expansion significantly increased IL-23–induced Ki67 staining compared with WT mice or mice subjected to dilator implantation alone (see Supplementary Figure S7d and e). Additionally, dilator implantation with H2O expansion significantly increased the mRNA levels of a cytokine (TNF-α), chemokines (CCL20 and CXCL1), and antimicrobial peptides (S100A8 and S100A9) after IL-23 treatment, compared with those in mice without H2O expansion (see Supplementary Figure S7f). All of these results were consistent with the data obtained with dilator- and IMQ-treated psoriasis mouse models.

**DISCUSSION**

As a typical chronic inflammatory disease of the skin, psoriasis is mainly characterized by epidermal hyperproliferation and impaired skin barrier function (Liang et al., 2017). Multiple reports have shown an interesting clinical pattern: psoriasis occurs in skin areas with stronger mechanical tension, such as the elbows, knees, and scalp (Boehncke and Schôn, 2015). In this study, we systematically evaluated the effect of mechanical stretch on keratinocytes to explore the mechanism underlying its contribution to psoriasis pathogenesis both in vitro and in vivo. Our results indicated that mechanical stretch affected keratinocyte function with regard to cell proliferation, antibacterial peptide production, skin barrier function, and cytokine production. Concurrently, mice exposed to mechanical stretch of the skin at a higher intensity displayed prominent epidermal hyperproliferation and up-regulation of psoriasis-associated cytokines, which probably contributed to the impaired skin barrier function that led to the aggravated phenotype in IMQ-induced psoriatic mice. To our knowledge, these data provide the first direct evidence of the impact of mechanical stretch on clinical features of psoriasis.

The pathogenesis of psoriasis involves a complex network of cells and inflammatory molecules, which are primarily involved in hyperproliferation of epidermal keratinocytes and cytokine-mediated chronic inflammation (Albanesi, 2012; Kim and Krueger, 2015). Data from multiple studies have indicated the contribution of keratinocyte malfunction to the pathogenesis of psoriasis (Kim and Krueger, 2015; Perera et al., 2012). Keratinocytes can respond to and integrate different signals under stretched conditions. Specifically, during the pathogenesis of psoriasis, activated keratinocytes can direct the migration of neutrophils or new T-cell subsets into the skin by producing chemokines such as CXCL1 and CCL20 (Homey et al., 2000; Lowes et al., 2007). Furthermore, they can express and secrete antimicrobial peptides to trigger the innate immune response (Morizane and Gallo, 2012; Schröder and Harder, 2006). Moreover, activated keratinocytes can synthesize cytokines and cause a cytokine storm, which is considered a potential initiator of T helper

---

K67-positive cell ratio represent the means ± standard error of the mean from five microscopic fields. mRNA expression levels of (f) CXCL1, CCL20, S100A8, and S100A9 and (g) IL-6, IL-23, and TNF-α in the epidermis of dilator-implanted mice treated with up to 4 ml of H2O. (h) Skin moisture and TEWL of the dilator-implanted mice. Five mice were included in each group, and the results show represent the mean ± standard error of the mean. *P < 0.05, **P < 0.01, and ***P < 0.001 were considered significantly different for each group versus WT mice. 4P < 0.05 and 44P < 0.01 were considered significantly different for dilator-implanted mice with or without H2O expansion. Scale bars = 100 μm. K, keratin; TEWL, transepidermal water loss; TNF, tumor necrosis factor; WT, wild type.
type 17-mediated immune responses in psoriatic lesions (Perera et al., 2012). Mechanical stretch has been intensively analyzed for decades because of its crucial contribution to cellular biologic phenomena such as proliferation, differentiation, and signal transduction (Takei et al., 1997; Yano et al., 2004, 2006). Data from several studies have shown that mechanical stretch changes the nuclear shape and influences the chromatin structure, thus influencing gene expression and mobility (Heo et al., 2015; Martins et al., 2012). Mechanical stretch-induced increases in H3K27me3 occupancy at promoters and decreased RNAPII-S2p along gene bodies resulted in silenced expression of genes associated with keratinocyte differentiation (LOR, TGM1, PPL, CRC1, and LCE1A) and cell cycle regulation (CDKN2A), which in turn affected the differentiation and proliferation of keratinocytes (Le et al., 2016). In agreement with previous studies, we observed that stable mechanical stretch promoted cell cycle progression and keratinocyte proliferation and inhibited keratinocyte differentiation. In addition, we found that epidermally expressed antimicrobial peptides including S100A8 and S100A9 were up-regulated in keratinocytes during mechanical stretch. More importantly, up-regulation of psoriasis-related cytokines including TNF-α, IFN-γ, and IL-6 in keratinocytes under mechanical stretch was observed both in vivo and in vitro. These findings indicate that mechanical stretch activated keratinocytes and increased the severity of inflammation in sites undergoing more intense mechanical stretching, thereby accelerating psoriasis progression in situ.

Both clinical and experimental molecular studies support the emerging premise that psoriasis progression could be driven by a primary defect in skin-barrier function (Coto et al., 2011; Wolf et al., 2012). Epithelial sites, including extenders of the extremities and the scalp, are vulnerable to epidermal trauma and are preferentially affected by psoriasis (Boyd, 1988; Crowley, 2010; Ruano et al., 2016). Our results show reduced moisture and increased TEWL in patients with dilator implantation, indicating that dilator-induced mechanical stretch could impair skin barrier function. Moreover, keratinocytes, as the predominant cellular component of the epidermis, express major proteins, including FLG, LOR, and I VL, which facilitate skin barrier function, primarily contributing to skin permeability (Candi et al., 2005; Kalinín et al., 2002). The present in vitro data indicate that skin barrier function-associated genes encoding FLG and IVL were significantly down-regulated after 24 hours of continuous stretching. Concurrently, Gutman-Yassky et al. (2009) reported down-regulation of both FLG and LOR in psoriatic skin compared with with normal skin, indicating that the epidermal barrier capacity was compromised. Thus, mechanical stretch potentially causes skin barrier function impairment, thereby accelerating psoriasis progression.

Our findings, together with previous findings, indicate that mechanical stretch drives keratinocyte proliferation and activation. To further investigate the effect of mechanical stretch on psoriasis pathogenesis, we established a skin expansion mouse model to mimic the actual microenvironment of keratinocytes under mechanical stretch. As expected, dilator-implanted mice with H2O treatment displayed significant epidermal hyperproliferation, impaired skin barrier function, and release of psoriasis-associated cytokines, in agreement with the findings of our in vitro experiments. IMQ (a TLR7 agonist) and IL-23, which induce psoriasis-like dermatitis, are two agents that are directly used to generate mouse models for studying psoriasis (Bocheriska et al., 2017). The role of mechanical stretch in psoriasis pathogenesis is further supported by our observation that inflammation increased markedly in dilator-implanted mice after IMQ or IL-23 treatment. These data provide experimental evidence that excessive tension might be critical for the development of psoriasis at high-tension sites, including the elbows and knees.

Our study has the following limitations. Mechanical stretch in human joints or in the scalp is three dimensional and rather complex (Yu et al., 2018) and, thus, cannot be completely imitated by the present flex cell-tension system in keratinocytes. Although IMQ and IL-23–induced mouse models exhibited the same reaction after mechanical stretch, there were still slight differences in gene expression (IL-6, TNF-α, and CCL20) between the two models. We speculate that this may be partially explained by the fact that both IMQ and IL-23 induce different inflammation environments in vivo. Moreover, different immune responses induced by IMQ and IL-23 might conversely affect skin sensitivity to mechanical stretch, although this possibility needs to be tested in a future study. Third, our observations that several inflammatory cytokines were up-regulated after a 24-hour stretch appeared to contradict the results of a previous study, which indicated that mechanical strain induces transcriptional repression (Le et al., 2016). We speculate that the different time points (6 or 12 hours after stretch in the literature versus 24 hours in this study) might have resulted in gene expression differences. In addition, whether mechanical stretch induced changes of RNAPII-S2p and H3K27me3 occupancy at genes encoding inflammatory cytokines and regulatory proteins needs further investigation. Overall, we believe that our model will provide more evidence to explain the changes in keratinocytes after mechanical traction with the emergence of more advanced equipment. Therefore,
further studies are required to investigate the role of mechanical stretch during the pathogenesis of psoriasis.

In conclusion, these results indicate that mechanical stretch contributes to psoriasis pathogenesis by modulating keratinocyte proliferation and cytokine/chemokine expression in keratinocytes, thereby providing new insights into the pathogenesis of psoriasis.

MATERIALS AND METHODS

Patients
All psoriatic patients were diagnosed based on clinical symptoms and did not receive any systemic treatment for at least 4 weeks before the procedures, including immunosuppressive agents or phototherapy. Healthy control individuals did not have a positive family history of psoriasis or other autoimmune diseases. This study was approved by the ethics review board of the Fourth Military Medical University. Written informed consent was obtained from all participants before the study.

Cell culture and mechanical stretch
Primary human keratinocytes (American Type Culture Collection, Manassas, VA) were cultured in DMEM containing 0.05 mmol/L calcium chloride (Gibco-Invitrogen, Carlsbad, CA) and supplemented with 10% fetal bovine serum (Gibco-Invitrogen) in a humidified incubator at 37 °C under 5% CO2. Keratinocytes were plated at 2 × 10⁵ cells per well into six-well flexible silicone rubber Mechanical Forcelex plates coated with type I collagen (Flexcell International Corporation, Hillsborough, NC). Before the application of mechanical stretch, keratinocytes were cultured in DMEM (10% fetal bovine serum) containing 1.05 mmol/L calcium chloride for 24 hours, and then the cells were stretched by 20% using an FX-5000T Flexercell Tension Plus unit (Flexcell International Corporation) for elongation, with a 0.2-Hz sinusoidal curve (Yano et al., 2004). Cells were cultured at 37 °C and 5% CO2 during stretch stimulation. Cells were harvested after 24 hours of stretching, and control cells were cultured in the same plates in the same incubator but not subjected to mechanical stretch.

Establishment of a mouse model of psoriasis through minidilator insertion and IMQ induction of psoriasis
Male BALB/c mice aged 8–10 weeks were obtained from the Department of Laboratory Animal Medicine of the Fourth Military Medical University (Xi’an, China) and housed in a specific pathogen-free animal facility. Linear full-thickness, 20-mm–long incisions were made into the dorsal skin near the neck after removing the dorsal hair. Five mice were involved in each group. Group A comprised mice with a silicone dilator (10 ml; China Winner Plastic Surgery Company, Shanghai, China) surgically implanted, whereby the dilator was injected with up to 4 ml H2O (0.5 ml per day, ending on day 8). Group B comprised mice with a dilator implantation, whereby the dilator was injected with up to 4 ml H2O (0.5 ml per day, ending on day 8). Group C comprised mice with a silicone dilator (0.5 ml per day, ending on day 8). IMQ cream was applied daily on the dorsum of group C mice. Petroleum jelly was used as the control.

As reported, the IL-23–induced psoriatic mouse model was established by intradermal injection of recombinant murine IL-23 (1 μg/day for 6 days) into the dorsal skin of mice after dilator implantation (Getschman et al., 2017). Intradermal injection of phosphate buffered saline was used as a control. The biopsy samples were harvested after the last injection of IL-23 for real-time PCR and immunofluorescence staining.

The animal studies were approved by the institutional review board. The mice were killed at the conclusion of all the experiments; thereafter, the entire expanded skin was harvested and divided into three parts for subsequent experiments, including real-time PCR, hematoxylin and eosin staining, and immunofluorescence analysis.

Statistical analysis
All results represent the mean ± standard error of the mean of at least three separate experiments, and each experiment was performed in triplicate. Statistical analyses were performed using Student t test or one-way analysis of variance, followed by Bonferroni corrections for post hoc comparisons. Pearson chi-square test was performed to analyze the proportions at each site in psoriasis patients. All statistical analyses were performed using SPSS software, version19.0 (IBM, Armonk, NY).

CONFLICT OF INTEREST
The authors state no conflict of interest.

ACKNOWLEDGMENTS
This work was supported by the National Natural Science Foundation of China (nos. 81430073, 81673051, and 81703116).

SUPPLEMENTARY MATERIAL
Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2018.12.019.

REFERENCES
Heo SJ, Thorpe SD, Driscoll TP, Duncan RL, Lee DA, Mauck RL. Biophysical regulation of chromatin architecture instills a mechanical memory in mesenchymal stem cells. Sci Rep 2015;5:16895.


