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ORIGINAL RESEARCH

FLI1 Induces Plaque Psoriasis and Its Inhibition Attenuates Disease Progression

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Plaque Psoriasis: Plaque psoriasis is an inflammatory skin disorder affecting nearly 2% of the world population. Despite recent advances in psoriasis treatment, there is still a need for more effective therapies. The ETS transcription factor FLI1 plays critical roles in hematopoiesis, angiogenesis, immunity, and cancer. Emerging evidence suggests that FLI1 is intricately involved in inflammatory processes underlying psoriasis pathogenesis.

Methods: RNAseq and bioinformatic analysis were used to identify the correlation between FLI1 levels and the expression of inflammatory genes associated with psoriasis. Over-expression of FLI1 in skin cells determined FLI1's role in inducing transcription of psoriasis-related inflammatory genes, including IL6, IL1A, IL1B, IL23, and TNFα. Inhibitors such as chelerythrine (CLT) were tested for their suppressive effects on these genes. Mouse models of plaque psoriasis were employed to assess the therapeutic potential of CLT and tacrolimus (TAC).

Results: Over-expression of FLI1 in skin cells upregulated 24 psoriasis-associated genes, which were identified through RNAseq. Inhibitors of FLI1, such as CLT, suppressed these inflammatory genes in skin cells. In mouse models of plaque psoriasis induced by imiquimod (IMQ) or phorbol ester (TPA), treatment with the anti-FLI1 inhibitor CLT, administered either peritoneally or topically, significantly downregulated inflammatory genes and alleviated psoriasis symptoms. Similarly, TAC, a common immunosuppressive agent, effectively attenuated IMQ-induced psoriasis by acting as a potent anti-FLI1 compound.

Conclusion: These findings demonstrate that FLI1 plays a central role in psoriasis development and highlight it as a potential therapeutic target for this skin disorder.

Keywords: psoriasis, FLI1, protein kinase C, inflammation markers, inhibitors, chelerythrine

Introduction

Psoriasis is a chronic inflammatory skin disease comprising multiple forms including plaque, flexural, guttate, pustular or erythrodermic.[1] The worldwide prevalence of psoriasis is about 2%, with a lower incidence in Asians and some Africans, and a higher percentage (11%) in Caucasian and Scandinavian populations[2,3] Despite the availability of several treatment modalities, more effective drugs are needed for effective treatment of all forms of the disease. New insights into the mechanism of psoriasis may then uncover novel therapies for this disease.

Most psoriasis cases are linked to chronic plaque-type psoriasis with classical appearances of erythematous, pruritic plaques covered in silvery scales.[4] Psoriasis typically affects the skin, but may also involve the joints, and has been associated with several diseases.[5] Inflammation is not limited to the psoriatic skin and has been shown to affect different organ systems.[1,2] Psoriasis is thus a systemic disease rather than a solely dermatological. Compared to control subjects,

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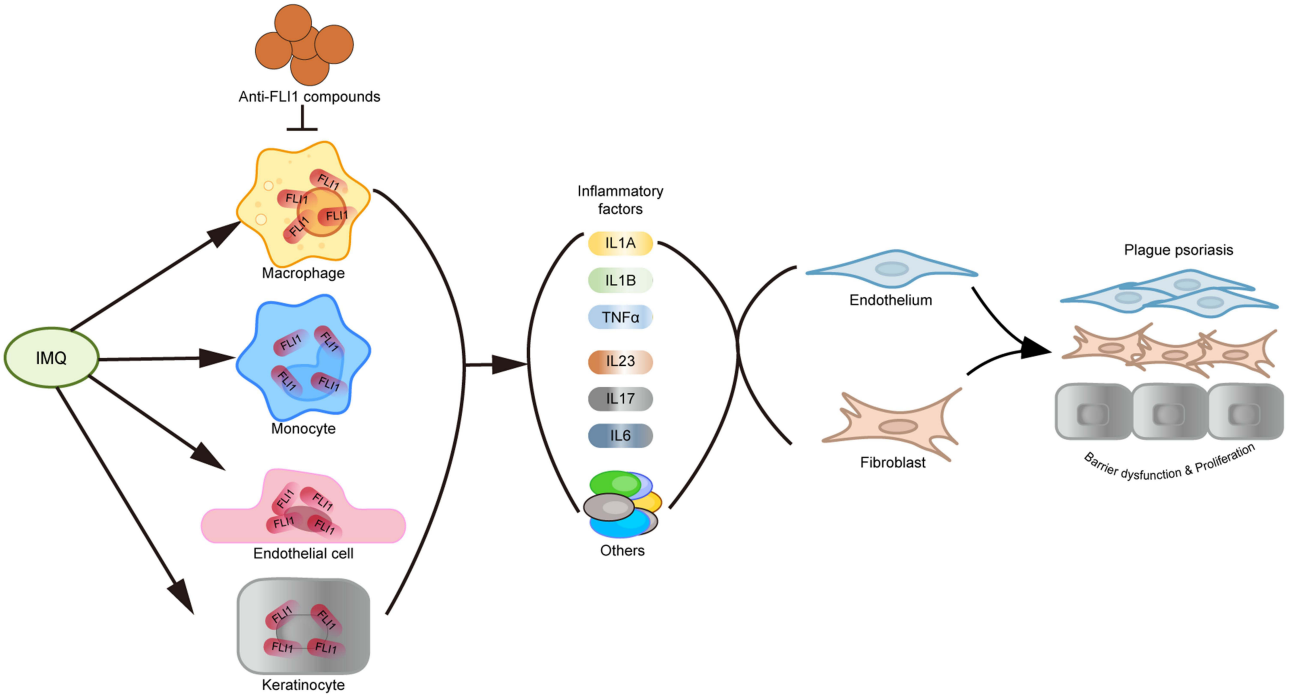
Graphical Abstract



psoriasis patients often exhibited increased hyperlipidemia, hypertension, coronary artery disease, type 2 diabetes, and larger body mass index.[6,7]

Although both innate and adaptative immune responses are associated with psoriatic, there is a strong association with innate immune genes in genetic studies.[8] Several cytokines, including IL1B, IL17, IL23, TNFα, and IL6, secreted from dendritic cells, macrophages, and monocytes, are responsible for the pathogenesis of psoriasis.[9] Induction of these cytokines is facilitated through abnormal expression of LL-37 and different antimicrobial peptides from keratinocytes during stress conditions, such as physical injury.[10]

Interaction between inherited susceptibility alleles and environmental triggers play a critical role in psoriasis's pathogenesis.[11] However, no single genetic variant seems sufficient for full-blown psoriasis. Hence, a multifactorial setting including multiple genetic mutations and environmental factors, which have been attributed up to 30% of disease risk, ought to be considered.[11] In a recent study, a whole-exome SNP array identified 15 new susceptibility loci for psoriasis. These genes include C1ORF141, ZNF683, TMC6, AIM2, IL1RL1, CASR, SON, ZFYVE16, MTHFR, CCDC129, ZNF143, AP5B1, SYNE2, IFNGR2 and 3q26.2-q27.[12] This study also identified four susceptibility loci: TNIP1, NFKBIA, IL12B and LCE3D-LCE3E.

Friend virus-induced leukemia-1 (Fli-1) is an ETS-related oncogenic transcription factor initially identified by our group as a target of retroviral insertional mutagenesis in erythroleukemia induced by Friend virus.[13–15] This oncogene is also activated through translocation (EWS-FLI1) or overexpression in various human solid and liquid tumors.[16,17] In addition to cancer, FLI1 is involved in the development of various illnesses, including inflammatory diseases.[16,18] While overexpression of FLI1 contributes to the development of autoimmune systemic lupus erythematosus (SLE),[19,20] loss of this transcription factor in fibroblasts and epithelial cells underlines systemic sclerosis.[21,22] Finally, Fli-1 is also a major regulator of immunity, hematopoiesis and stem cell maintenance and development.[16] High expression of FLI1 is characteristic to almost all hematopoietic cells.[16]

In this study, we used RNAseq data of FLI1-depleted human leukemic cells and identified a correlation between FLI1 deficiency and reduced expression of markers of psoriasis. Accordingly, overexpression of FLI1 in the epithelial cell line HaCaT increased expression of these markers of inflammation and psoriasis. Moreover, inhibition of FLI1 reversed expression of these psoriasis genes. In a mouse model of psoriasis induced in the skin by imiquimod (IMQ),[23–25] FLI1 inhibitors strongly blocked the induction of inflammation markers and psoriasis. These results, for the first time, implicate FLI1 in the development of psoriasis and point to FLI1 as a potential target for the treatment of this skin disorder.

Materials and Methods

Cells Culture and Drug Therapy

The erythroleukemia HEL and Acute Monocytic THP1 cell lines (human origin) were originally obtained from ATCC (HEL 92.1.7, THP1) and maintained in Dulbecco's Modified Eagle Medium supplemented with HyClone 5% fetal bovine serum (GE Healthcare). HUVEC cell was originally from Thermo Fisher Scientific CN. The spontaneously immortalized human epidermal keratinocytes HaCaT and A431 skin cancer cell lines were also originally obtained from ATCC and were maintained in Dulbecco's modified Eagle's medium (DMEM) containing 10% Fetal Bovine Serum (FBS). Cells were trypsinized (0.25% trypsin-EDTA solution; Invitrogen, US) and sub-cultured when attained 70–80% confluence. Cell lines were thawed from nitrogen and after 6–8 passages discarded. All these cells were cultured in an incubator with 5% CO_2 at 37 °C and maintained mycoplasma free used in all experiments.

Cell treatment procedure with drugs was previously described.[26] Chelerythrine, IMQ, TPA and Camptothecin (CPT) compounds were obtained from MCE (MCE, CN).

Overexpression of FLI1 in HaCaT and A431

DNA was diluted in TE buffer to a final concentration of 4 μg (MigR1; MigR1-Fli1). Lipofectamine transduction of cells was performed according to the manufacturer's protocol (Invitrogen). Transduction of samples at a final concentration of 4 μg was made in 250 μL of Dulbecco Modified Eagle Medium (serum free) and incubated at room temperature for 5 minutes. Lipofectamine 2000 reagent was diluted to 10 μL of 250 μL of media (serum free). Diluted DNA was then added to the lipofectamine 2000 reagent, mixed gently, and incubated for 15 minutes at room temperature to allow for the DNA-lipofectamine complexes to develop. After incubation, DNA-lipofectamine mixture was added to the 70%–80% confluent HaCaT and A431 cell cultures in dishes. Transduced cells were left to incubate at 37°C. After 6 hours, the medium was changed. Two days after transfection, cells were checked for GFP expression, and RNA and protein extracted for further analysis.

RNA Preparation and RT-qPCR

Total RNA was extracted from attached and leukemic cells using TRIzol reagent kit (Life Technologies; Thermo Fisher Scientific, USA). cDNAs were synthesized from extracted mRNAs using the reverse transcription reaction by the PrimeScript RT Reagent kit (Takara Bio, CN). RT-qPCR was carried out using the FastStart Universal SYBR-Green Master Mix (Roche, Shanghai, CN) and the Step One Plus Real-time PCR system (Applied Biosystems/Thermo Fisher Scientific, US). For comparison, the expression of the tested genes was calculated as relative values to the level of GAPDH. Three biological replicates were used for all the RT-qPCRs, each in triplicate (n = 3). The primer sequences are listed in Table 1.

Western Blot Analysis

Western blotting was performed, as previously described.[26] The antibodies used are as follows: Polyclonal rabbit FLI1 (ab133485) was purchased from Abcam (UK), and the GAPDH (G9545) antibody was obtained from Sigma Aldrich (US). The antibody dilutions were performed according to the manufacturer's instructions. The Odyssey system (LI COR Biosciences, US) was used to image and analyze the proteins on the Western blot membranes.

Table 1 Primer Sequences Used for RT-qPCR

Genes	Forward Primer	Reverse Primer
Human		
CMPK2	GTACCTCCTTTATTCCTGAAGCC	ATGGCAACAACCTGGAACTTT
HBEGF	ATCGTGGGGCTTCTCATGTTT	TTAGTCATGCCCAACTTCACTTT
EGR1	GGTCAGTGGCCTAGTGAGC	GTGCCGCTGAGTAAATGGGA
HMOX1	AAGACTGCGTTCCTGCTCAAC	AAAGCCCTACAGCAACTGTCG
IFIT2	AAGCACCTCAAAGGGCAAAAC	TCGGCCCATGTGATAGTAGAC
IFIT1	TTGATGACGATGAAATGCCTGA	CAGGTCACCAGACTCCTCAC
IL1A	TGGTAGTAGCAACCAACGGGA	ACTTTGATTGAGGGCGTCATTC
IL1B	ATGATGGCTTATTACAGTGGCAA	GTCGGAGATTCGTAGCTGGA
ISG20	CTCGTTGCAGCCTCGTGAA	CGGGTTCTGTAATCGGTGATCTC
IGFL1	CACAAGAGATGTGGGGACAAG	CACTGCGACAAAGCCTGTCA
CCL3L3	AGGTCCTCTCTGCACCACTT	ACTCGGTTGTCACCAGACACA
KLK14	TGGGCAAGCACAACCTGAG	ATGGGGCTGGATATAGTTCCC
NCF2	CCCACTCCCGGATTTGCTTC	GTCTCGGTTAATGCTTCTGGTAA
OAS2	CTCAGAAGCTGGGTTGGTTTAT	ACCATCTCGTCGATCAGTGTC
CCL3L1	CACCTCCCGACAGATTCCAC	GGTCACTGACGTATTTCTGGAC
CCL5	CCAGCAGTCGTCTTTGTCAC	CTCTGGGTTGGCACACACTT
IFIH1	TCGAATGGGTATTCCACAGACG	GTGGCGACTGTCCTCTGAA
TNFAIP3	TCCTCAGGCTTTGTATTTGAGC	TGTGTATCGGTGCATGGTTTTA
OASL	CTGATGCAGGAACTGTATAGCAC	CACAGCGTCTAGCACCTCTT
RSAD2	TGGGTGCTTACACCTGCTG	GAAGTGATAGTTGACGCTGGTT
KLF4	CCCACATGAAGCGACTTCCC	CAGGTCCAGGAGATCGTTGAA
ISG15	CGCAGATCACCCAGAAGATCG	TTCGTCGCATTTGTCCACCA
GAPDH	GGAGCGAGATCCCTCCAAAAT	GGCTGTTGTCATACTTCTCATGG
PI3	CACGGGAGTTCCTGTTAAAGG	TCTTTCAAGCAGCGGTTAGGG
CCL3	AGTTCTCTGCATCACTTGCTG	CGGCTTCGCTTGGTTAGGAA
TNFα	CCTCTCTCTAATCAGCCCTCT	GAGGACCTGGGAGTAGATGAG
IL17A	TCCCACGAAATCCAGGATGC	GGATGTTCAGGTTGACCATCAC
IL23	CTCAGGGACAACAGTCAGTTC	ACAGGGCTATCAGGGAGCA
IL6	ACTCACCTCTTCAGAACGAATTG	CCATCTTTGGAAGGTTCAGGTTG
FLI1	CAGCCCCACAAGATCAACCC	CACCGGAGACTCCCTGGAT
GAPDH	GTCTCCTCTGACTTCAACAGCG	ACCACCCTGTTGCTGTAGCCAA
Mouse		
Keratin 17	ACCATCCGCCAGTTTACCTC	CTACCCAGGCCACTAGCTGA
TNFα	CCCTCACACTCAGATCATCTTCT	GCTACGACGTGGGCTACAG
IL17A	TTTAACTCCCTTGGCGCAAAA	CTTTCCCTCCGCATTGACAC
IL23	ATGCTGGATTGCAGAGCAGTA	ACGGGGCACATTATTTTTAGTCT
IL6	TAGTCCTTCCTACCCCAATTTCC	TTGGTCCTTAGCCACTCCTTC
GAPDH	AGGTCGGTGTGAACGGATTTG	GGGGTCGTTGATGGCAACA
CMPK2	ACTTGACCTAGTTGACCAGTGC	GCATCCAGTCCTTCAATGGC
HBEGF	CGGGGAGTGCAGATACCTG	TTCTCCACTGGTAGAGTCAGC
EGR1	TCGGCTCCTTTCCTCACTCA	CTCATAGGGTTGTTCGCTCGG
HMOX1	AGGTACACATCCAAGCCGAGA	CATCACCAGCTTAAAGCCTTCT
IFIT2	GGAGAGCAATCTGCGACAG	GCTGCCTCATTTAGACCTCTG
IFIT1	GCCTATCGCCAAGATTTAGATGA	TTCTGGATTTAACCGGACAGC
IL1A	TCTATGATGCAAGCTATGGCTCA	CGGCTCTCCTTGAAGGTGA
IL1B	GAAATGCCACCTTTTGACAGTG	TGGATGCTCTCATCAGGACAG
ISG20	TGGGCCTCAAAGGGTGAGT	CGGGTCGGATGTACTTGTCATA
IGFL1	ACTGGAACTCTGACAAGAGGT	CGCAGTTTTCCGTGAACTCG
KLK14	CCTGGGCAAGCACAACATAAG	CTTCAGCAGCATGAGGTCATT
NCF2	GGAGAAGTACGACCTTGCTATCA	ACAGGCAAACAGCTTGAACTG

(*Continued*)

Table 1 (Continued).

Genes	Forward Primer	Reverse Primer
OAS2	TTGAAGAGGAATACATGCGGAAG	GGGTCTGCATTACTGGCACTT
PI3	ACTCGAGCCCACATTCCATC	GGTTGTCACCAGACACACTG
CCL3	TGTACCATGACACTCTGCAAC	CAACGATGAATTGGCGTGGAA
CCL5	TTTGCCTACCTCTCCCTCG	CGACTGCAAGATTGGAGCACT
IFIH1	ATGGACGCAGATGTTCGTGG	TCCCTTCTCGAAGCAAGTGTC
TNAIP3	ACCATGCACCGATACACGC	AGCCACGAGCTTCCTGACT
RSAD2	AGCATTAGGGTGGCTAGATCC	CTGAGTGCTGTTCCCATCTTO
KLF4	GGCGAGTCTGACATGGCTG	GCTGGACGCAGTGTCTTCTC
ISG15	GGTGTCCGTGACTAACTCCAT	CTGTACCACTAGCATCACTGTG

RNAseq and Heatmap Analysis

RNAseq was performed using HaCaT-FLI1 and HaCaT-control cells, as previously described.[26] The data for public access was submitted to GEO; accession: GEO Accession viewer (nih.gov). Differentially expressed genes (DEG) were identified from this data using HISAT2 and used for a KEGG pathway analysis. The Supplemental Table 1 listed differentially expressed genes (DEGs) enriched in the KEGG pathway, where $|\log_2(\text{MIGRFLI1A/MIGR1A})| > 1$ and $p < 0.05$. Heatmaps were used to display 24 genes displayed at least two-fold difference between FLI1 expressing and scrambled control cells.

Computer Docking

The three-dimensional structures of Chelerythrine and Tacrolimus were analyzed and drawn in PubChem. The protein crystallographic structure of FLI1 was retrieved from www.rcsb.org(1FLI). Auto Dock tools 1.5.7 used to compute the molecular docking simulations following the standard protocol, as described in the software documentation. Furthermore, the interacting sites were analyzed using PyMol analysis.

Cellular Thermal Shift Assay (CESTA)

The CESTA analysis was performed as previously described by others.[27] Cells (HaCaT-FLI1) were lysed in lysis buffer containing 0.1% TritonX-100 in PBS, and the cell suspension was incubated with CLT/TAC (final concertation of 100 μM) or DMSO for 30 min. The 20 μL aliquots of samples were incubated at temperature 37 °C, 41 °C, 43 °C, 45 °C, 47 °C, 49 °C, 51 °C, 53 °C, 55°C for 8 min, respectively. The lysates were then separated from the precipitated and aggregated proteins by centrifugation at 12,000 rpm for 15 min at 4°C. Equal volume of the supernatant was used in Western blot for protein expression. The control and drug treated groups were used to determine the temperature curve by using the gray value of the images captured from image J. Each experiment was performed in triplicate.

Mice

Balb/c mice (male, 6–7 weeks old, 20 ± 2 g) were kept on a 12 h light/dark cycles under SPF conditions. They were acclimatized for at least 7 days before use.

IMQ-Induced Psoriasis-Like Mouse Model

Mice were randomly divided into three experimental groups (n = 6). (1) Control, (2) IMQ, (3) IMQ + Chelerythrine. An area of 3 cm × 5 cm^2 was shaved from the back of all the mice. A topical dose of 62.5 mg of 5%IMQ cream (Med Shine Pharmaceuticals, Sichuan, CN) was applied to the skin of mice daily for six consecutive days. Chelerythrine Chloride (4 mg/kg) or a vehicle (DMSO) in 100 μL saline was intraperitoneally injected into Balb/c mice every other day. Other details were described in Figure 5a. The administration dose of Chelerythrine chloride was mainly referred to the previous work.[28,29] On day 7, the mice were humanly sacrificed by dislocation of the neck by an experienced technician. Samples of skins, spleens were collected on the seventh day for future experiments, and the psoriasis area and severity

index (PASI) score[30] for mice was recorded. PASI is based on daily assessment of three criteria, epidermal erythema, scaling and ticking of the dorsal skin. PASI is then calculated by adding the scores of the three separate criteria (range from 0 to 12). The spleen from each mouse was isolated, and a photograph was taken before weighing. After experiment, mice were terminated using cervical dislocation, under experienced technical supervision.

Chelerythrine Gel Preparation and Animal Treatment

The composition of CLT gel containing 0.2% CLT is as follows: 20 mg CLT, 0.2 g azone,1.2 g glycerin, TEA, 0.2 g carbopol 940, 2 g 96% ethanol, distilled water q.s. to final weight of 10 g.[31,32] To generate the gel, Carbopol 940 (Mackline, CN) was slowly added to an appropriate quantity of distilled water while stirring. Then, Triethanolamine (TEA) [Aladdin, CN] was added to adjust pH to 6–8, ensuring that the carbopol dissolves completely. The mixture was incubated at room temperature overnight to allow the carbopol to fully swell, then added the glycerin and mixed well. CLT (20 mg) was dissolved in 96% ethanol and supplemented with azone (Macklin, CN). Finally, the CLT solution was transferred to the aqueous solution of carbopol 940 while being stirred continuously until the gel formed. The 0.03% Tacrolimus (TAC) cream [LEO Laboratories Ltd., shanghai, CN] was used as standard.

Mice were randomly divided into four groups (n = 6). Vaseline group (CONTROL), IMQ group (IMQ), IMQ+CLT group (IMQ+CLT) and IMQ+TAC(IMQ+TAC). A daily topical dose of 62.5 mg of 5%IMQ cream or control Vaseline was used on the back skin of the mice for 6 consecutive days. A dose of 25 mg/cm^2 CLT gel or 20 mg TAC cream was applied 2 h later after applying IMQ for 6 consecutive days. Erythema, scaling, and thickness were recorded based on the Psoriasis Area Severity Index (PASI): 0, none; 1, 2, moderate; 3, severe; 4, very severe. On day 7, the mice were humanly sacrificed by the dislocation of the neck by an experienced technician.

TPA-Induced Psoriasis-Like Mouse Model

Psoriasis induction with TPA started at day 0 with the topical application of 20 μL of TPA (2.5 μg/ear) suspended in Methanol/DMSO (99%:1%) solution. Methanol/DMSO solution is applied as control on right ears of each mouse (inner and outer ear surfaces). Thirty minutes before the application of TPA, the inner and outer surface of each ear was treated with 20 μg of CLT (dissolved in Methanol/DMSO) for consecutive 6 days. As a marker of inflammation, changes in right ear thickness during TPA therapy were assessed using a digital Vernier caliper (SMCT shanghai, CN), as described.[33] After the final treatment, the mice were sacrificed, ear biopsies were obtained and used for further analysis.

Histological Analysis

Skin tissues of mice were fixed in 4% paraformaldehyde (XILONG SIENTIFIC, CN) solution and embedded in paraffin. The paraffin-embedded tissues were sectioned and stained with hematoxylin and eosin (H&E) for histological evaluation.

Immunohistochemistry (IHC)

Immunohistochemistry (IHC) was conducted utilizing reagents from Abcam following a standardized protocol. Tissue sections containing the target protein underwent fixation in 4% paraformaldehyde (XILONG SIENTIFIC, CN) solution for 24 hours and were subsequently embedded. Deparaffinization was achieved by immersing the sections in xylene twice for 5 minutes each, followed by rehydration through a series of graded ethanol solutions (100%, 95%, and 70%) and a final rinse in distilled water. Heat-induced antigen retrieval (HIER) was performed using Tris-EDTA buffer (pH 9.0) in a pressure cooker for 20 minutes. Non-specific binding was minimized by incubating the sections with Abcam's Antibody Diluent for 1 hour at room temperature. Primary antibody incubation was carried out overnight at 4°C using Anti-FLI1 Antibody from Abcam in Antibody Diluent. Subsequently, sections were washed with PBS and incubated with HRP-conjugated anti-rabbit IgG secondary antibody in Antibody Diluent for 1 hour at room temperature. Detection was achieved using Abcam's DAB Substrate Kit according to the manufacturer's instructions. Counterstaining was performed with hematoxylin for 1 minute, followed by dehydration and mounting.

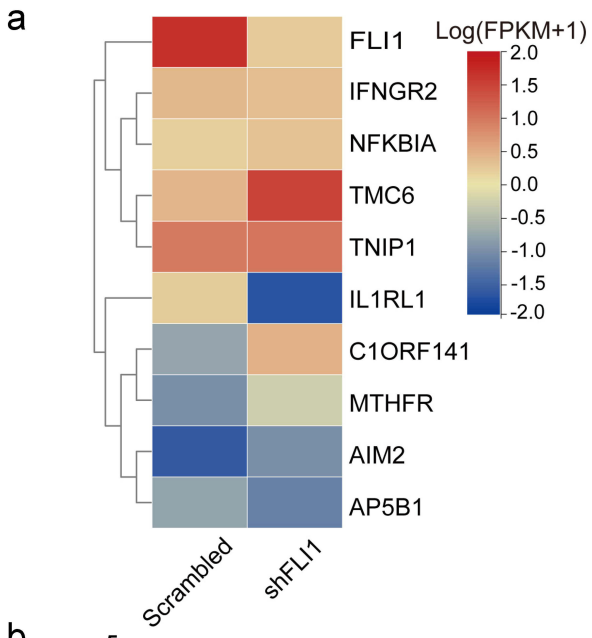
Statistical Analysis

Statistical analysis was performed using a two tailed Student *t* test or a one-way ANOVA with Tukey's post hoc test, using Prism9 GraphPad software. The P values were indicated within the figures using a standard scheme, $P \leq 0.05$ (*), $P \leq 0.01$ (**), $P \leq 0.001$ (***), $P \leq 0.0001$ (****) and $P > 0.05$ (ns). Where appropriate, the data were displayed using the mean ± the SD from at least 3 independent experiments.

Results

FLI1 Expression Correlates with Expression of Psoriasis-Associated Inflammatory Genes

Using RNAseq analysis, we have previously identified over 1000 differential expressed genes (DEGs) whose expression is upregulated or downregulated after knocking down FLI1 in the human erythroleukemia cell line HEL.[26] Within these DEGs, 9 out of 19 genes have been previously linked to psoriasis[12] (Figure 1a), and their expression is significantly altered in FLI1 knockdown cells. To confirm these results, the expression of these nine genes was determined in lentivirus-mediated FLI1 knocked-downed HEL (shFLI1) and scrambled control cells, using RT-qPCR. This analysis



FPKM scores

Genes	Scrambled	ShFLI1
FLI1	122.98	14.99
IFNGR2	27.32	16.9
NFKBIA	21.18	16.37
TMC6	28.13	52.27
TNIP1	53.07	32.11
IL1RL1	22.03	1.57
C1ORF141	6.33	19.21
MTHFR	4.61	8.95
AIM2	1.72	3.98
AP5B1	6.08	3.30

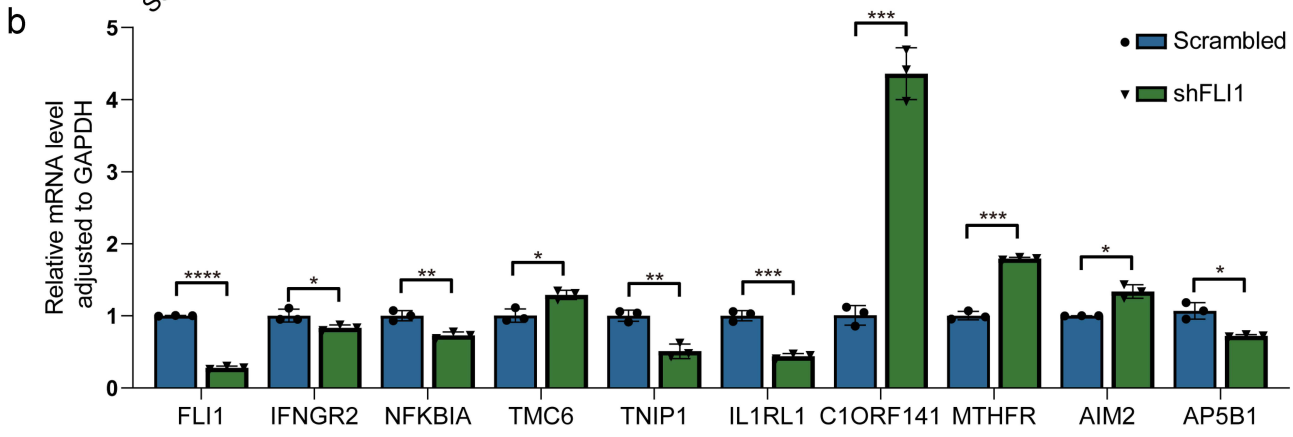


Figure 1 FLI1 regulates genes linked to psoriasis development. (**a**) Heatmap of the indicated psoriasis genes within the differentially expressed genes (DEGs) of the FLI1 knockdown (shFLI1) in HEL cells. (**b**) The relative expression of the indicated genes in shFLI1 versus scrambled control cells, as determined by RT-qPCR. $P \leq 0.05$ (*), $P \leq 0.01$ (**), $P \leq 0.001$ (***) and $P \leq 0.0001$ (****).

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has revealed reduced expression of IFNGR2, NFKBIA, AP5B1, ILIRL1, and TNIP1 and increased expression of TMC6, C1ORF141, MTHFR, and AIM2, in shFLI1 cells versus control (Figure 1b). The expression results from human erythroleukemic cells point to FLI1 as a potential transcriptional regulator of psoriasis-associated genes.

Overexpression of FLI1 in HaCaT Cells Stimulates the Expression of Classical Inflammatory Psoriasis Genes

Previously, the immortalized human keratinocyte cell line HaCaT was used to generate an in vitro model of psoriasis following treatment with IMQ.[25] Interestingly, HaCaT cells exhibit negligible levels of FLI1, and treatment with IMQ did not induce the expression of this transcription factor when compared to erythroleukemia HEL cells, which express high levels of FLI1 (Figure 2a). However, treatment of HEL cells with IMQ significantly induced FLI1 expression in a dose response manner (Figure 2b). As epithelial cells are known to express FLI1,[34] gene silencing due to epigenetic modification is likely responsible for the lack of FLI1 induction in HaCaT cells following IMQ treatment.

To test whether FLI1 alone can induce psoriasis genes, we transfected HaCaT cells with MigR1-FLI1 expression plasmid or vector MigR1, carrying the green fluorescence *GFP*. We obtained a high efficiency of transfection (over 40%) as evident from GFP expression (Figure 2c). Western blot confirmed significant expression of FLI1 in HaCaT (HaCaT-FLI1) versus HaCaT control cells (Figure 2d). Overexpression of FLI1 in HaCaT cells did not increase proliferation (Figure 2e) or changes in cell morphology but induced high expression of pro-inflammatory genes associated with psoriasis including IL23 (Figure 2f), IL6 (Figure 2g), TNFα (Figure 2h),[35] and Keratin 16 (Figure 2i), which is one of the proliferation markers in psoriasis.[36] In contrast, the expression of Keratin 1, a keratinocytes differentiation marker,[37] was downregulated in HaCaT-FLI1 cells (Figure 2j), and the level of IL17A remained unchanged (Figure 2k). Among these,

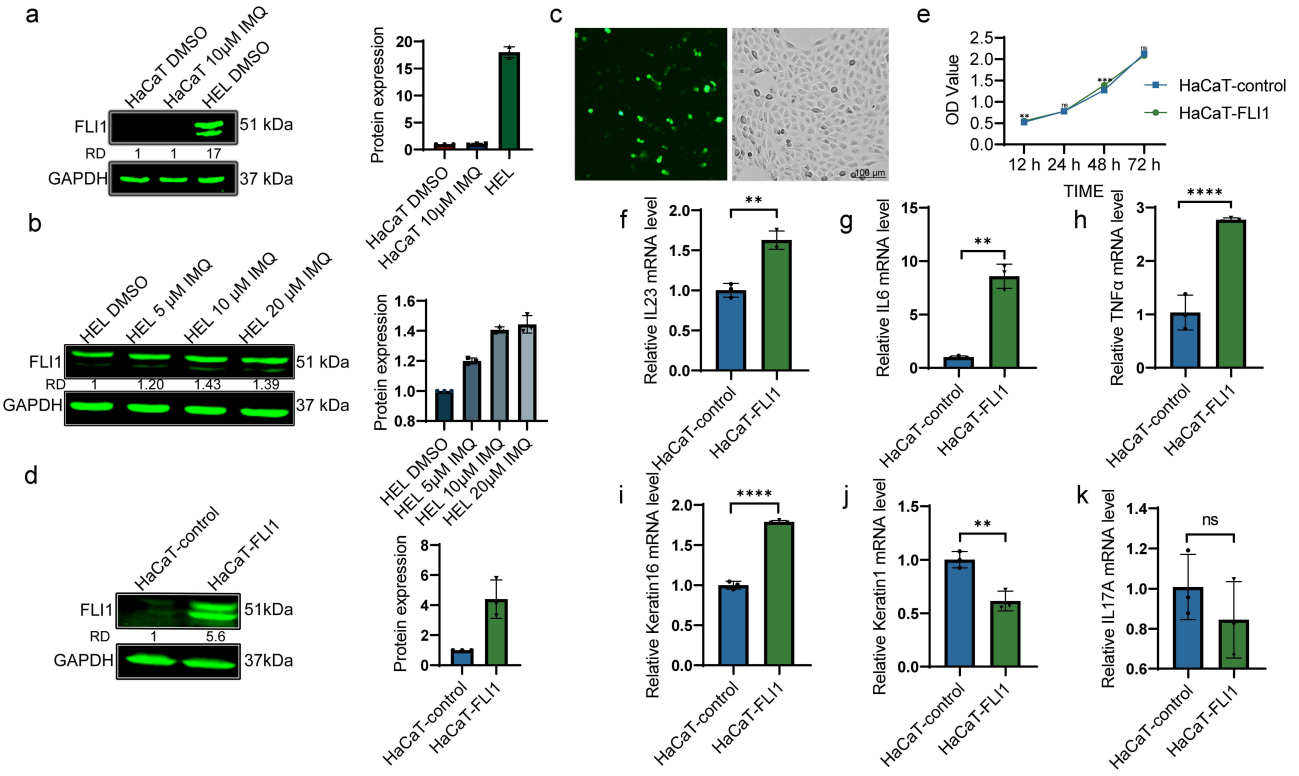

Figure 2 Expression of FLI1 in HaCaT cells induces the transcription of inflammatory genes associated with psoriasis. (**a**) Expression of FLI1 in HaCaT cells treated with 10 μM of IMQ for 24 hours, as determined by Western blotting. The erythroleukemia HEL cells were used as a positive control. (**b**) FLI1 protein expression in HEL cells treated with the indicated concentration of IMQ. RD shows relative density determined through densitometry. Average of protein expression for three experiments shown in right panel. (**c**) Green Fluorescence protein (GFP) expression in HaCaT cells transfected with MigR1-FLI1 (Left panel). Right panel shows the cell microscopic image. (**d**) The expression of FLI1 in HaCaT cells transfected with MigR1-FLI1 and control MigR1 plasmids, as detected by Western blotting. (**e**) The proliferation rate of HaCaT-FLI1 versus HaCaT-control cells, as determined by MTT. (f-k) Relative expression of IL23 (**f**), IL6 (g), TNFα (**h**), Keratin16 (**i**), Keratin1 (**j**) and IL17 (**k**) in HaCaT-FLI1 versus HaCaT-control cells, as determined via RT-qPCR. $P \leq 0.01$ (**), $P \leq 0.0001$ (****) and $P > 0.05$ (ns).

the expression of IL16 and TNFα was previously reported to be regulated by FLI1.[18,38] IL17A was also shown to be a direct target of FLI1 in leukocytes of the MRL/Lpr Mouse Model of Lupus Nephritis.[39] Due to hematopoietic specific expression, IL17A chromatin unacceptability could underlie the lack of induction of this cytokine in HaCaT cells by IMQ.

As FLI1 is also expressed in hematopoietic and endothelial cells,[40] we examined its level in response to IMQ in macrophage THP1 and endothelial HUVAC cells by Western blot analysis. The results show higher expression of FLI1 in these cell lines in response to IMQ treatment (Supplemental Figure 1a and b). Higher FLI1 expression in these cell lines also increased the expression of IL6, IL17, IL1B, IL23, TNFα in THP1 (Supplemental Figure 1c) and IL1B, TNFα in HUVEC cells by RT-qPCR (supplemental Figure 1d). These results demonstrate induction of markers of psoriasis by IMQ in keratinocyte, endothelial and hematopoietic cell line HEL/THP1 associated with higher FLI1 expression.

Identification of Differentially Expressed Genes Regulated by FLI1 That are Associated with Psoriasis

A recent single-cell RNA sequencing database reporting gene expression in psoriasis patients untreated or receiving IL23 blockade.[41] In this study, while fibroblasts and myeloid populations displayed the most extensive changes during psoriasis evolution, with WNT5A+/IL24+ fibroblasts shown to upregulate multiple inflammatory genes in keratinocytes that could be blocked by IL23 treatment. Analysis of this scRNA database also revealed changes in the level of FLI1 expression in both hematopoietic and non-hematopoietic cells within normal and disease skin, while the highest level was found in endothelial cells (Supplemental Figure 2). Treatment with IL23 at days 3 and 14 resulted in significant reduction in FLI1 level in endothelial, Melanoma, T and luminal epithelial cells when compared to skin lesions obtained from day zero and non-lesion biopsies from the same patients. Despite positive effect of IL23 treatment on FLI1 expression, this cytokine is shown here to be downstream of FLI1 and likely regulated by this transcription factor. While this analysis clearly revealed FLI1 induction in specific psoriasis skin cells, it may not reflect the events associated with expression of this transcription factor during the development of the disease. To independently test for the role of FLI1 in psoriasis, we performed RNAseq analysis on HaCaT cells over-expression FLI1 versus control cells, as previously described.[26] This analysis identified a total of 102 DEGs significantly induced by overexpression of FLI1 (Supplemental Table 1). A GO analysis of the HaCaT-FLI1 versus HaCaT-control cells revealed pathways involved in virus infection, TNF and cytokine signaling pathways and other diseases (Figure 3a and supplemental Figure 3). According to protein–protein interaction (PPI) network, several psoriasis factors including IL1B, IL1A and TNFα were among DEGs involved in the TNF and cytokine pathway (Supplemental Figure 3). Among 102 DEGs, 24 genes displayed at least two-fold difference between FLI1 expressing and scrambled control cells (Figure 3b), and induction of these DEGs by FLI1 was confirmed by RT-qPCR analysis (Figures 2g, h, 3c and Supplemental Figure 4). Expression of IL6 and TNFα was induced, but slightly less than 2-fold in HaCaT-FLI1 cells and excluded from the list. Interestingly, 20 of these 24 genes induced in HaCaT-FLI1 cells were previously linked to psoriasis including KLF4,[42] EGR1,[43] IL1A,[44] CCL3,[45] KLK14,[46] FLI1[47], CCL3L3,[48] HBGEF,[49] HMOX1[50] IGFL1,[51] NCF2,[52] RSAD2,[53] CCL5,[54] TNFAIP3,[55] IL1B,[56] IFIT1,[57] OAS2[58], OASL[58] PI3,[59] ISG15.[60]

To further corroborate these results, we overexpressed FLI1 in another keratinocyte cell line A431 (Supplemental Figure 5a), which induced similar psoriasis genes (Supplemental Figure 5b). To validate whether FLI1 indeed regulates these psoriasis-related genes, we inhibited the activity of this transcription factor using the previously established anti-FLI1 compound camptothecin (CPT)[61–63] (Figure 3d). Camptothecin was previously shown to exert strong anti-FLI1 activity, independent of its topoisomerase inhibitory function, when applied at very low concentrations.[61,62] Treatment of HaCaT-FLI1 cells with a low dose of CPT (20 nM) reverted transcription of eight tested psoriasis genes (IL-6, IL1B, TNFAIP3, IFIT1, IL23, EGR1, CCL3, KLK14; Supplemental Figure 6), further demonstrating the effect of FLI1 on expression of these psoriasis-related genes.

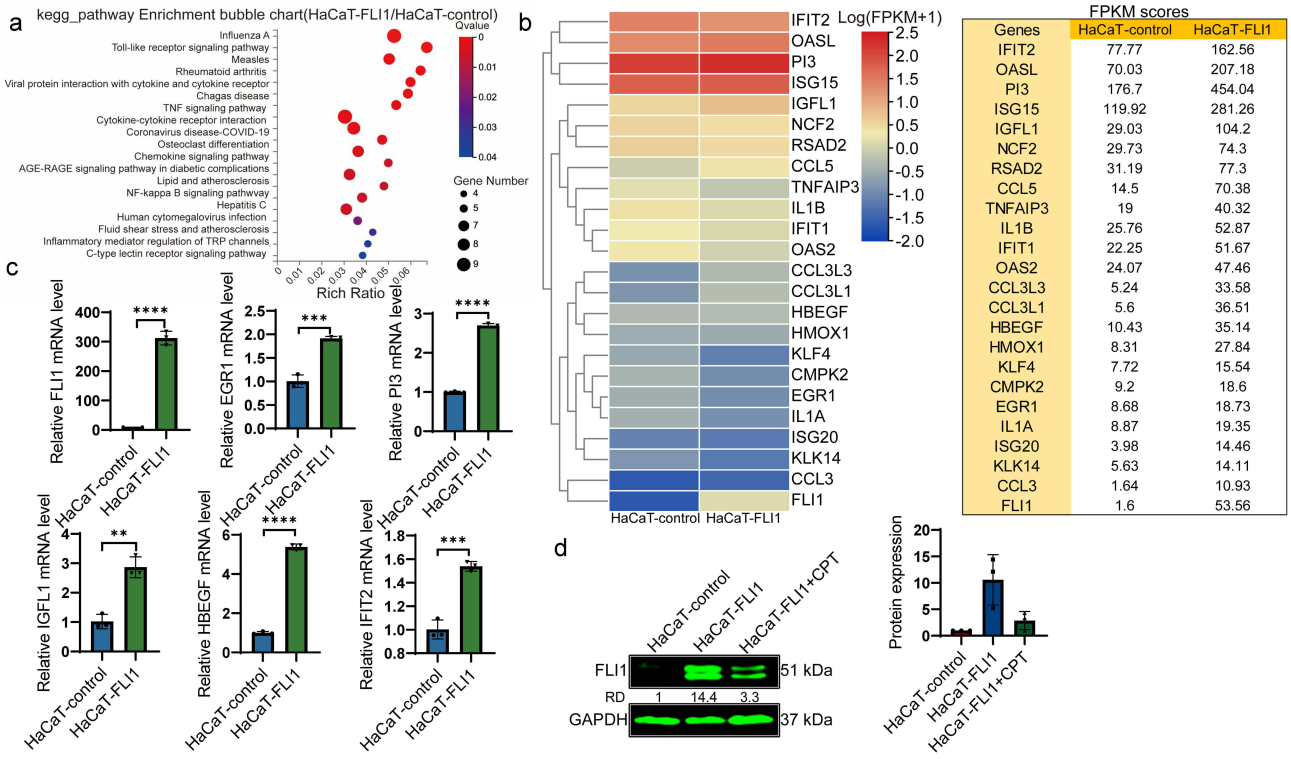

Figure 3 FLI1 expression in HaCaT cells induces a spectrum of genes associated with psoriasis. (**a**) The Kegg pathway enrichment analysis of DEGs identified in HaCaT-FLI1 versus HaCaT-control cells. (**b**) The left panel shows the heatmap of DEGs with significant differences derived from HaCaT-FLI1 versus HaCaT-control cells. The right panel presents the FPKM scores of the indicated genes. (**c**) Relative expression of the indicated genes in the HaCaT-FLI1 and HaCaT-control cells, as determined by RT-qPCR. (**d**) The expression analysis of FLI1 in HaCaT-FLI1 cells treated with either DMSO or 20 nM of Camptothecin (CPT) for 24h, as determined by Western blotting. $P \leq 0.01$ (**), $P \leq 0.001$ (***) and $P \leq 0.0001$ (****).

Chelerythrine Inhibits FLI1 and Blocks the Induction of Psoriasis Markers

While CPT inhibits FLI1 at a very low dose (20nM), this compound is also known to exhibit type 1 topoisomerase activity.[61,63] We therefore sought out specific inhibitors of FLI1 that may reduce the risk of drug-induced side effects. In search of new FLI1 inhibitors, our drug screening identified the benzophenanthridine alkaloid chelerythrine (Figure 4a), a PKCα/β inhibitor[64] that reduced the expression of FLI1 protein in leukemia HEL cells expressing high levels of this transcription factor (Figure 4b). Indeed, the effect of chelerythrine (CLT) on FLI1 downregulation was significantly diminished in the scrambled control cells versus FLI1 knockdown HEL cells (Figure 4c).[26] This result further indicates the specificity of CLT to FLI1. Inhibition was previously reported to induce FLI1 downstream target MIR145, further suppressing FLI1 protein expression in leukemic cells.[65,66] Accordingly, significant upregulation of MIR145 was detected in CLT-treated HaCaT-FLI1 cells associated with lower FLI1 protein (Figure 4d).

Docking analysis revealed strong binding of CLT to the DNA-binding ETS domain of FLI1, as presented by the two- (Figure 4e) and three-dimensional views (Figure 4f). This interaction revealed a high binding energy of −7.36 kJ/mol (Figure 4f, bottom). Cellular thermal shift assay (CESTA) was then used to further confirm this affinity of CLT to FLI1 in HaCaT-FLI1 cells. CESTA technique is designed to detect changes in the thermal stability of the target protein upon CLT binding.[27] When a target protein binds to a compound, it becomes stable and less susceptible to thermal denaturation. Accordingly, when the temperature was gradually increased, the stability of the target protein FLI1 increased after CLT treatment compared with the control (Supplemental Figure 7). Thus, CLT, in addition to inactivating FLI1, also down-regulates FLI1 protein through induction of its downstream target MIR145. To determine whether CLT inhibits FLI1 in HaCaT cells, HaCaT-FLI1 cells were treated with CLT, and the effect on psoriasis genes was determined using RT-qPCR. This analysis revealed the slight upregulation of FLI1, but downregulation of some of its target genes (IL1A, TNFα, IL1B, IL23, and IL6) by CLT (Figure 4g-I).

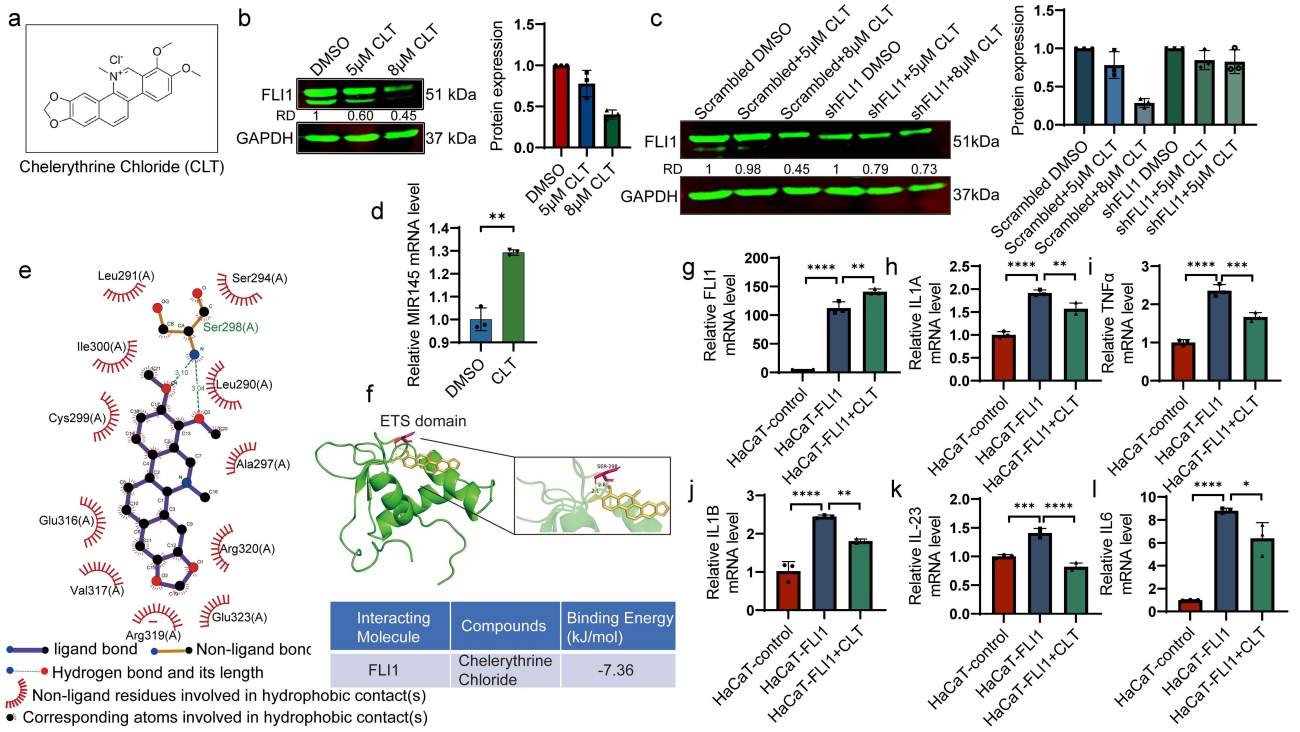

Figure 4 Chelerythrine inhibits FLI1 and blocks the expression of the FLI1 target genes in HaCaT-FLI1 cells. (**a**) The chemical structure of chelerythrine chloride (CLT). (**b**) CLT downregulates the expression of FLI1 in HEL cells, as determined via Western blotting. GAPDH was used as a loading control. (**c**) The expression of FLI1 in shFLI1-HEL versus scrambled-HEL cells treated with the indicated concentrations of CLT for 24h, by Western blotting. (**d**) The expression of MIR145 in HaCaT-FLI1 vs HaCaT-control cells, as determined via RT-qPCR. (**e**) Docking analysis of the interaction of CLT to the ETS-DNA binding domain of FLI1 through 2-dimensional analysis. (**f**) Properties of CLT binding affinity to the FLI1 DNA binding domain through 3-dimensional analysis. (**g-l**) The expression of FLI1 (**g**), IL1A (**h**), TNFα (**i**), IL1B (**j**), IL23 (**k**), and IL6 (**i**) in HaCaT-FLI1 cells treated with DMSO or CLT for 24h, as determined by RT-qPCR. HaCaT-control cells were used as control. $P \leq 0.05$ (*), $P \leq 0.01$ (**), $P \leq 0.001$ (***), $P \leq 0.0001$ (****).

Chelerythrine Attenuates Skin Psoriasis in Mice Treated With IMQ

To generate a mouse model of plaque psoriasis, 2-month-old Balb/c mice (n = 6) were shaved and treated with daily doses of topical IMQ cream,[30] as depicted in Figure 5a. Another group of IMQ-treated mice (n = 6) was treated with CLT dissolved in saline and injected intravenously (4 mg/kg) a day before IMQ administration. Images in Figure 5b-top show significant attenuation in IMQ-induced psoriasis following CLT treatment. H&E staining analysis of the dorsal skin of the IMQ group on day 7 revealed significant epidermal hyperplasia, increased acanthosis, parakeratosis, telangiectasia, and inflammatory cell infiltration. CLT injection significantly reversed these histological changes caused by IMQ, showing less parakeratosis and less epidermal thickening than the IMQ group (Figure 5b-bottom). The psoriasis area severity index (PASI) score[30] for these mice shows an intermediate improvement in IMQ+CLT versus IMQ alone treated groups (Figure 5c). Psoriasis in mice is characterized by spleen enlargement.[24] Indeed, IMQ-treated mice had a larger spleen size. In contrast, IMQ plus CLT-treated mice exhibited a significantly smaller spleen size (Figure 5d and e). As controls, CLT injection of normal mice had no effect on the skin feature or the size of spleen (Supplemental Figure 8a and b).

Although Fli1 expression was not prominently observed in the epidermis in our study, based on our immunohistochemistry (IHC) analysis, the Fli1 expression appeared to be in different skin cells. FLI1 expression was more evident in the dermis and hair follicle regions, which is why we focused on these areas for illustration. These findings are consistent with the observed increase in FLI1 levels following IMQ treatment and its suppression upon co-treatment with CLT (Figure 5f). As CLT blocks Fli1 protein, RT-qPCR expression analysis showed that IMQ-induced Fli1 expression was slightly but not significantly affected by CLT (Figure 5g). IMQ treatment induced whereas CLT co-treatment suppressed RNA levels of the psoriasis-related inflammatory gene Krt17 (Supplemental Figure 9a), Il1a (Figure 5h), Il17 (Figure 5i), Tnfα (Figure 5j), and Il6 (Figure 5k). Moreover, CLT inhibited expression of the selected psoriasis genes (Supplemental

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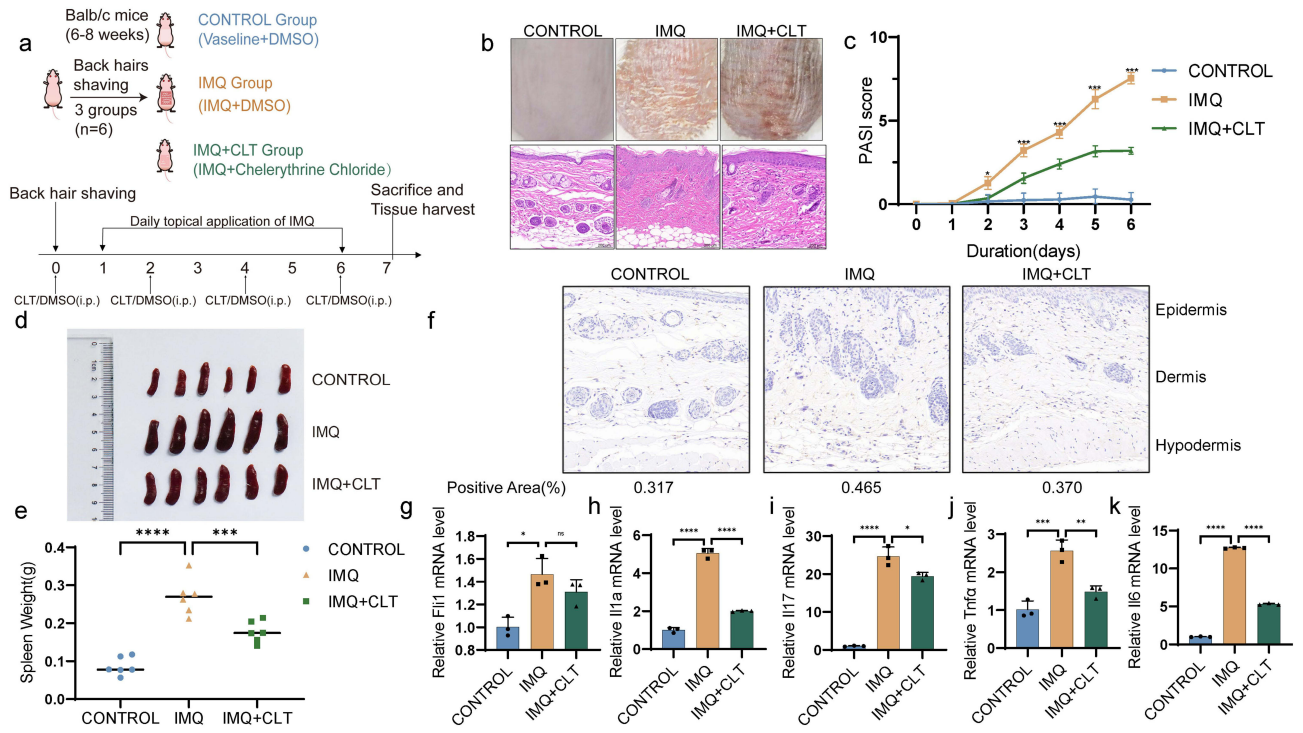

Figure 5 CLT inhibits IMQ-induced psoriasis-like symptoms in mice. (**a**) Depicted diagram shows the methodology used to induce psoriasis by IMQ and the strategy applied to treat these mice with CLT. (**b**) Images of IMQ-induced psoriasis-like symptoms within the shaved region of mice skins (n=6) treated with or without CLT (top panel). Bottom panel shows histological views and H&E staining of skins at day seven of the experiment for the indicated groups. (**c**) PASI score for IMQ and IMQ+CLT treated mice when compared to the control group. (**d**) Spleen images of the indicated experimental groups removed from the sacrificed mice. (**e**) Spleen weight of the indicated mouse groups. (**f**) Immunohistochemistry analysis of Fli1 expression in skin section of control, IMQ and IMQ+CLT treated mice. The overall positive area of Fli1 protein expression indicated below. (**g-k**) The expression of Fli1 (**g**), Il1a (**h**), Il17 (**i**), Tnfα (**j**) and Il6 (**k**) in skin samples of the indicated mouse groups, as determined by RT-qPCR. *P*≤0.05 (*), *P*≤0.01 (**), *P*≤0.001 (***), *P*≤0.0001 (****) and *P*>0.05 (ns).

Figure 9b-i) in skin that induced in HaCaT-FLI1 cells (Supplemental Figure 4). These results demonstrate the critical role Fli1 plays in the induction of plaque psoriasis and markers associated with the disease.

Chelerythrine Also Attenuates Skin Psoriasis Induced by TPA

The phorbol ester 12-*O*-tetradecanoylphorbol-13-acetate (TPA) is a protein kinase C activator known to induce inflammation and epidermal hyperplasia and recapitulated some of the hallmarks of psoriasis.[33,67] The experimental strategy depicted in Figure 6a was used to determine the effect of CLT on the severity of TPA-induced ear inflammation in mice. Ear edema was observed 24 h after treatment in all TPA-treated animals and was retained on day 6 until the last application. In animals treated with vehicle alone, initial ear thickness equaled 0.25 ± 0.011 mm (mean ± SD). Ear thickness increased to 0.53 ± 0.008 mm (mean ± SD) by day 6 after TPA treatment. When TPA-treated mice were also administrated topically with CLT solution, significant improvement in right ear thickness (0.43 ±0.019 mm) was observed compared to TPA alone mice treated with control solution (Figure 6b-left). H&E staining confirmed the attenuation of TPA-induced inflammation in mice treated with TPA plus CLT versus TPA alone (Figure 6b-right). Topical CLT treatment significantly improved ear thickness (Figure 6c) and PASI score (Figure 6d). As control, treatment of ears with CLT alone had no effect on skin thickness (Supplemental Figure 8c). While CLT treatment does not affect the expression of Fli1 (Figure 6e), it reduced the psoriasis gene Krt17 (Figure 6f), Il1a (Figure 6g), Il17 (Figure 6h), Tnfα (Figure 6i), Il6 (Figure 6j) induced by TPA in ear samples. In addition, CLT blocked the expression of some of the tested genes induced by FLI1 in HaCaT cells (Figure 3c and supplemental Figure 4) in RNA samples isolated from the mice ears (Supplemental Figure 10). Thus, Fli1 inhibition attenuated psoriasis induced by two different agents (IMQ and TPA), associated with downregulation of various Fli1 target genes.

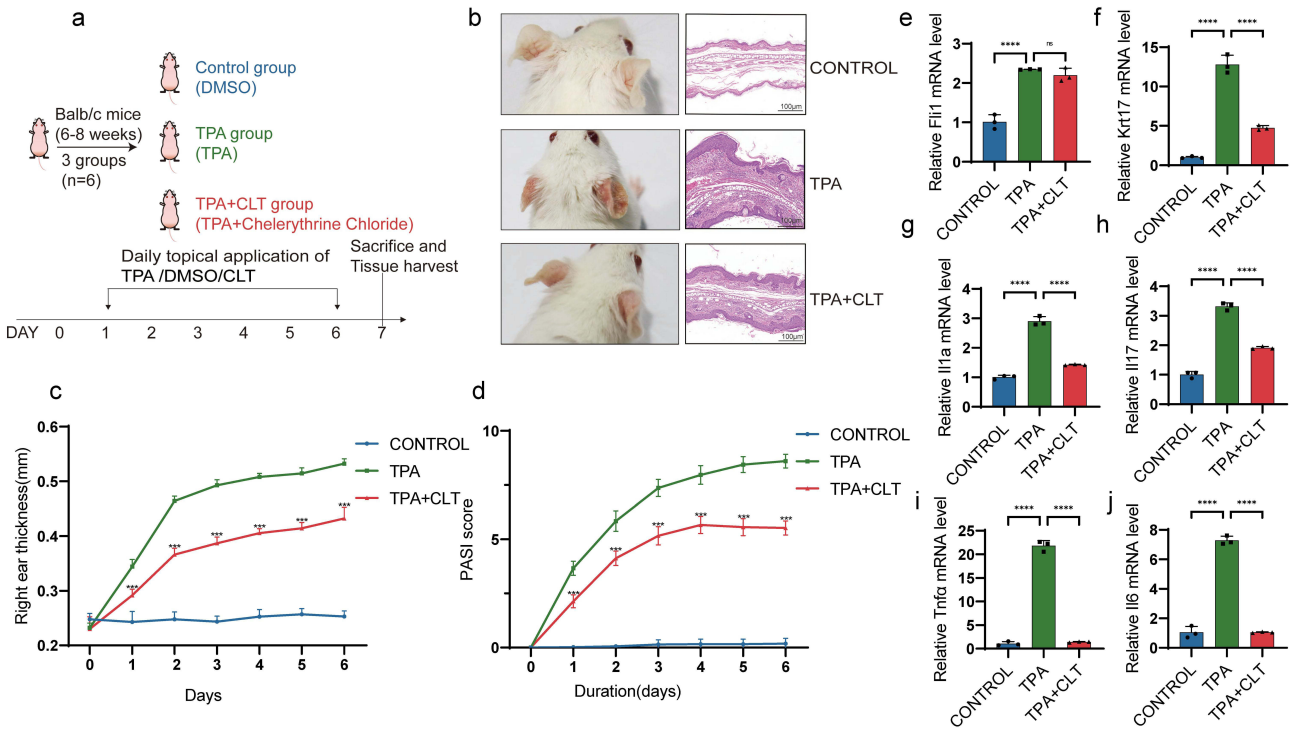

Figure 6 CLT suppresses TPA-induced psoriasis-like symptoms in the ears of the treated mice. (**a**) The depicted strategy used to induce psoriasis-like symptoms in mouse ears with TPA and following treatment with CLT. (**b**) Images of psoriasis-like symptoms induced by TPA on right ear of mice (n=6) treated with or without CLT (left panel). Right panel shows histological views and H&E staining of ear samples from the indicated mouse groups. (**c**) The right ear thickness score for the indicated mouse groups measured at the termination of the experiment. (**d**) The PASI score of the indicated groups obtained from the recorded daily condition of the treated mice. (**f-j**) The transcriptional level of Fli1 (**e**), Krt17 (**f**), Il1a (**g**), Il17 (**h**), Tnfα (**i**) and Il6 (**j**) in ear samples of the indicated treated mice groups, as determined by RT-qPCR. $P \leq 0.001$ (***), $P \leq 0.0001$ (****) and $P > 0.05$ (ns).

Dermal Chelerythrine Treatment Moderates Psoriasis Symptoms Induced by IMQ in Mice

As systemic treatment of mice with CLT via IP injection strongly blocked skin psoriasis symptoms induced by IMQ (Figure 5), we examined whether such an effect can also be recapitulated using topical treatment. We prepared CLT gel for topical treatment in this experiment, as described in materials and methods. We also used the immunosuppressive compound Tacrolimus (TAC) as a control, which can effectively block psoriasis when used as a topical gel.[68–70] Indeed, treatment with IMQ for two hours followed by CLT or TAC moderated IMQ-induced psoriasis effect (Figure 7a, top) with a better improvement in skin disfiguration than the control group (Figure 7a, bottom). Treatment with both IMQ and with CLT or TAC also significantly reduced the spleen size relative to IMQ (Figure 7b and c). The attenuation of psoriasis was accompanied by a lower score than IMQ alone treated mice (Figure 7d). While IMQ treatment significantly increased Fli1 staining in spleen sections, treatment with IMQ+CLT or IMQ+TAC significantly moderated this expression (Figure 7e). As control, both intraperitoneal and topical treatment of CLT gel or TAC cream had no effect on the skin of control mice (Supplemental Figure 8a and b). The double treatment condition (IMQ+CLT and IMQ+TAC) reduced expression of Il1b (Figure 7f) Tnfα (Figure 7g), Il6 (Figure 7h), Il17 (Figure 7i) and Il23 (Figure 7j), but not Fli1 (Figure 7k) induced by IMQ, as determined by RT-qPCR using skin samples isolated from treated mice.

While CLT significantly reduced the expression of Fli1 targets, a similar reduction was observed after TAC administration (Figure 7f-k). These results raised the possibility that TAC, like CLT, may have anti-FLI1 activity. Indeed, in docking analysis, TAC binds to the ETS DNA-binding domain of FLI1 (Supplemental Figure 11a), with binding energy of −8.64 kJ/mol (Supplemental Figure 11b), which is higher than CLT (binding energy −7.36 kJ/mol) [Figure 4f]. In CESTA analysis, TAC increased the stability of FLI1 protein after gradual temperature increase (Supplemental Figure 11c). Treatment of HaCaT-FLI1 cells with CLT or TAC significantly reduced FLI1 protein expression as determined by Western blotting (Supplemental Figure 12a). TAC treatment was also reduced FLI1

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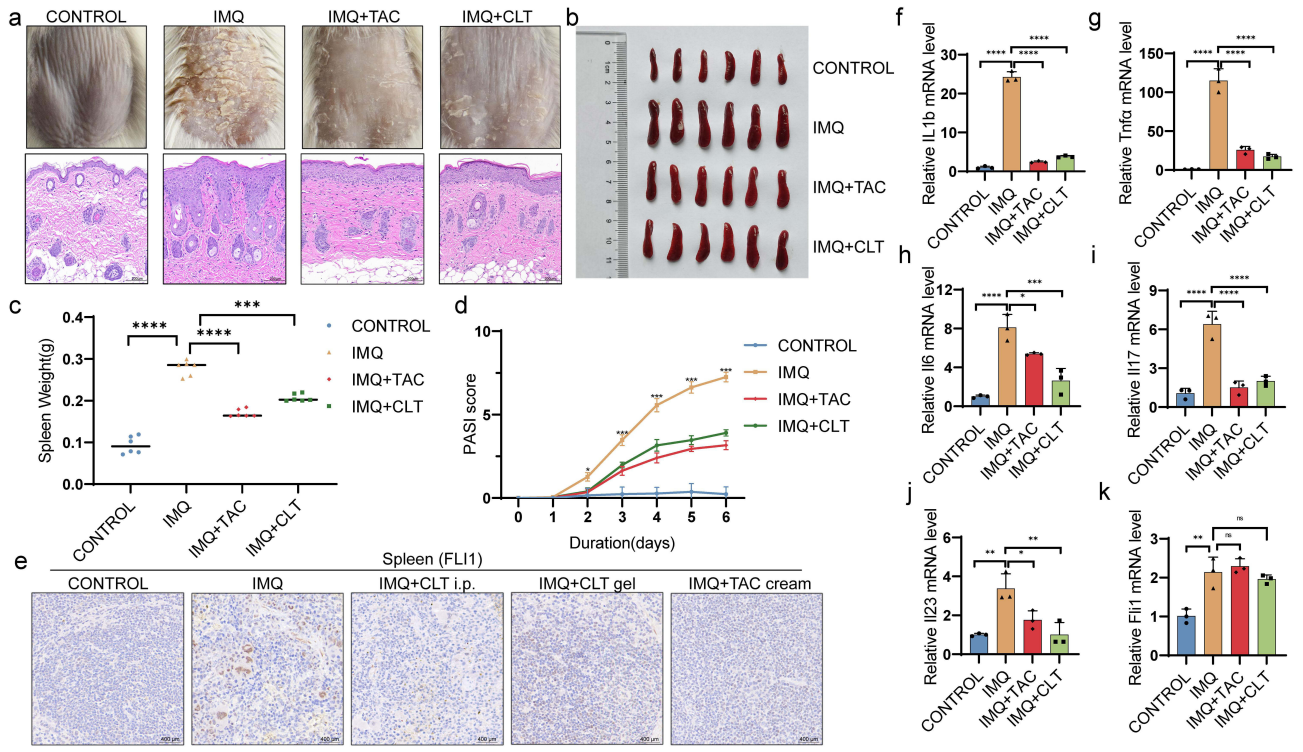

Figure 7 CLT administered as a topical treatment suppresses IMQ-induced psoriasis symptoms in mice. (**a**) Images of psoriasis-like symptoms induced in mice skin by IMQ (n=6) treated with CLT or TAC (top panel). Bottom panel represents histological views and H&E staining of skin samples from the indicated mouse groups. (**b, c**) Spleen weight (**b**) and spleen images (**c**) of mice from the indicated groups treated with topical CLT gel or TAC cream. (**d**) The PASI score of the indicated treated mice obtained from the recorded daily condition of the treated mice. (**e**) Immunohistochemistry analysis of spleens isolated from the indicated groups. (**f-k**) The expression of Il1b (**f**), Tnfα (**g**), Il6 (**h**), Il17 (**i**), Il23 (**j**), Fli1 (**k**) in skin samples of the indicated groups, as determined by RT-qPCR. $P \leq 0.05$ (*), $P \leq 0.01$ (**), $P \leq 0.001$ (***), $P \leq 0.0001$ (****) and $P > 0.05$ (ns).

expression in the leukemic HEL cell line (Supplemental Figure 12b). These results point to FLI1 inhibitors as a potential new target for psoriasis.

Discussion

Previous studies indicated the critical role FLI1 plays in hematopoiesis, angiogenesis, and development of various cancers and diseases.[16,18–22] Herein, we report a novel role for FLI1 in the induction of skin psoriasis as a potential therapeutic target for this disease. We show that FLI1 expression controls the transcription of several classical markers of psoriasis and that can be blocked by treatment with anti-FLI1 inhibitors both in tissue culture and in animal models of psoriasis.

Knockdown of FLI1 in leukemic cells identified many regulated genes, among which we identified nine that were previously linked to loci associated with the development of psoriasis.[12] This association raised the possibility of involvement of FLI1 in psoriasis, which we confirmed through overexpression of this transcription factor in epithelial cells. Interestingly, a recent study has identified FLI1 among DEGs differentially expressed in psoriasis patients, although these authors failed to show a role for this gene in initiating this disease.[47] Here, we demonstrated that FLI1 induces several pre-inflammatory genes (IL1A, IL1B, IL6, TNFα, and IL23) associated with psoriasis in human epithelial cells that can be blocked by anti-FLI1 inhibitors CPT, and the new discovered TAC and CLT compounds. In RNAseq analysis, the overexpression of FLI1 causes significant changes in the expression of 24 genes, most of which was previously linked to psoriasis.[42–60] Among these genes, the peptidase inhibitor 3 (PI3) gene expression, previously identified as a serum biomarker for psoriasis, was strongly induced in HaCaT-FLI1 epithelial cells.[59,71,72] Moreover, expression of the psoriasis markers IL1B, IL16, IL17 and TNFα was previously shown to be directly controlled by FLI1 resulting in inflammation and leukemia progression.[18,38,39] Expression of the remaining genes was also correlated with FLI1 as drug-

mediated inhibition of this ETS transcription factor moderately restored normal expression. As FLI1 regulates many of these genes, it is possible that a combination of these factors controls psoriasis. Further analysis is then required for each of these genes to determine their contribution alone or in combination in development of psoriasis and whether FLI1 directly or indirectly regulates them.

IL23 and IL17 production by immune cells was identified as the critical driver of psoriasis pathogenesis.[73] Excellent response of patients to anti-IL23/IL17A therapies indicates a crucial role for these cytokines in psoriasis.[73,74] While FLI1 expression in HaCaT cells only slightly, but significantly increases IL23, we detected a significant increase in the expression of both cytokines in skin samples of IMQ-treated mice partially suppressed by the anti-FLI1 inhibitors TAC and CLT. The induction of IL17/IL23 into skin by IMQ is likely partly driven by hematopoietic and other cells infiltrating the skin. Indeed, expression of these cytokines as well as other markers of psoriasis was also detected in HaCaT-FLI1 and in macrophage cell line THP1 treated with IMQ. As FLI1 is a major regulator of hematopoiesis[16] and its expression is induced in the skin by IMQ, this transcription factor may be responsible for higher IL17 and IL23 expression mostly in hematopoietic and may also in non-hematopoietic compartment of psoriasis, a notion that remains to be investigated in future studies.

Recently, scRNA-seq analysis of psoriasis in patients received IL23 blockade revealed a subset of WNT5A+/IL24+ fibroblasts capable of upregulating multiple inflammatory genes in keratinocytes that were blocked by IL23 therapy.[41] Analysis of this scRNA-seq database identified a reduction in expression of FLI1 in hematopoietic, endothelial, and epithelial cells, in lesion treated with systemic IL23 blockade. This result is consistent with our observation using HaCaT-FLI1 cells and mice treated with IMQ in which activation of FLI1 upregulates IL23. FLI1 downregulation is associated with skin Systemic Sclerosis (SSC)[21,22] However, high FLI1 expression is associated with lupus erythematosus (LE)[19,20] and higher psoriasis cases reported in LE patients.[75] Interestingly, the Th17 pathway is shared by these two disease entities 76 and targeting IL17/IL23 is used to treat both diseases.[76] These results further strengthen the role of FLI1 in psoriasis.

Previously, camptothecin (CPT) was classified as a potent inhibitor of FLI1 in leukemic cells while also possessing a potent topoisomerase I activity.[63] Herein, we identified CLT as a new inhibitor of FLI1 transcriptional activity by binding to its DNA binding domain. In mice treated with CLT, plaque psoriasis induced by IMQ was significantly attenuated with downregulation of many downstream genes associated with psoriasis. Similar results were also observed in the TPA-induced psoriasis-like model, further confirming the role of FLI1 in the induction of this disease. Notably, as a protein kinase C-α/-β inhibitor, the CLT compound is shown to have major health benefits against bacteria, inflammation, cancers, and others.[64] Interestingly, FLI1 is activated by PKC agonists through phosphorylation at serine site[77]. Thus, in addition to directly inhibiting FLI1, CLT also blocks its activity through suppression of the PKC pathway.

Our data clearly showed that CLT directly binds to FLI1, inhibiting its function and its downstream pathway. In addition to FLI1 functional inactivation, CLT is also shown to reduce the level of this transcription factor at protein level, through upregulation of its target gene MIR145.[65,66] Interestingly, the transcription of Fli1 was increased in FLI1-HaCaT cells by CLT, but same treatment resulted in lower mRNA expression in skin cells isolated from IMQ-treated mice. This difference in expression is likely generated due to cell context specify and/or expression of FLI1 in HaCaT cells under an exogenous promoter.

Tacrolimus (TAC) was previously reported to suppress psoriasis[69,70] and is used in the clinic as a potent immunosuppressive agent along with other drugs to prevent organ rejection.[69] Interestingly, similar to CLT, we showed here that TAC suppresses the psoriasis gene expression, leading to attenuation of skin psoriasis induced by IMQ. Furthermore, we found that TAC has a higher affinity to the FLI1 binding domain than CLT, leading to strong transcriptional inactivation. These results further confirm the role of FLI1 in the development of psoriasis and implicate inhibitors of this transcription factor as potent agents for the treatment of this disease.

Previous studies revealed that CLT has low selectivity and can promote apoptosis of normal cells, thus displaying cytotoxicity behavior in humans.[78,79] However, we showed herein that topical administration of CLT on TPA- and IMQ-induced lesions in mice successfully inhibited psoriasis, which was accompanied by downregulation of markers of this disease. It would be then interesting to investigate whether topical application of both CLT and TAC will better inhibit psoriasis than either of these agents alone.

In conclusion, we discovered that FLI1 regulates several genes associated with psoriasis in epithelial cells. Accordingly, FLI1 overexpression in HaCaT cells alters the spectrum of known and new psoriasis genes that can be blocked by anti-FLI1 inhibitors. Increased FLI1 expression and its regulation of the psoriasis genes was also detected in psoriasis skin cells including hematopoietic, epithelial and endothelial cells. In an animal model of IMQ-induced psoriasis, the anti-FLI1 agents CLT and TAC attenuated disease symptoms, and this was associated with the downregulation of the psoriasis-associated genes. These results implicate FLI1, for the first time, in the development of psoriasis and point to the potential usefulness of anti-FLI1 inhibitors such as CLT and TAC and possibly others[16] for the treatment of this skin disorder.

Data Sharing Statement

All data generated or analyzed during this study are included in this manuscript.

Ethics Approval

In accordance with Article 32, Items 1 and 2 of the "Measures for Ethical Review of Life Science and Medical Research Involving Human Beings", issued by the National Health Commission of China in 2023, this study is exempt from ethical review. The research involves the use of legally obtained public data, non-interfering observation of public behavior, and anonymized data. As no direct interaction with human subjects is involved, and only publicly available or anonymized data are utilized, the study meets the criteria outlined in the aforementioned measures for exemption. This exemption is intended to reduce unnecessary burdens on researchers and facilitate the advancement of life science and medical research.

The research adheres to ethical principles, ensuring the confidentiality of all data, and is conducted in a transparent and responsible manner, in line with the ethical standards of scientific inquiry. Animal care and procedures are followed by the criteria for the use of laboratory animals. The animal protocol was reviewed and approved by the Guizhou Medical University Animal Care Committee under the guidelines of the China Council of Animal Care (Approval ID #1900373, approval date: 5 March 2023).

Author Contributions

All authors made significant contributions to the work reported, including but not limited to the conception, study design, execution, acquisition of data, analysis, and interpretation. They were involved in drafting, writing, substantially revising, or critically reviewing the article. All authors agreed on the journal to which the article was submitted and reviewed and approved all versions of the manuscript, including during revision, the final version accepted for publication, and any significant changes introduced at the proofing stage. Furthermore, all authors agree to take full responsibility for and be accountable for the content of the article.

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Disclosure

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to Bimini Biotech, a patent PCT/B2024/058089 pending, a patent EP4241766 A1 issued. The authors report no other conflicts of interest in this work.

References

1. Christophers E. Psoriasis–epidemiology and clinical spectrum. *Clinical and Experimental Dermatology*. 2001;26(4):314–320. doi:10.1046/j.1365-2230.2001.00832.x
2. Parisi R, Symmons DP, Griffiths CE, Ashcroft DM. Global epidemiology of psoriasis: a systematic review of incidence and prevalence. *J Investigative Dermatol*. 2013;133(2):377–385. doi:10.1038/jid.2012.339
3. Rendon A, Schäkel K. Psoriasis pathogenesis and treatment. *Int J mol Sci*. 2019;20(6):1475. doi:10.3390/ijms20061475
4. Ortonne J, Chimenti S, Luger T, Puig L, Reid F, Trüeb RM. Scalp psoriasis: European consensus on grading and treatment algorithm. *Journal of the European Academy of Dermatology and Venereology: JEADV*. 2009;23(12):1435–1444. doi:10.1111/j.1468-3083.2009.03372.x
5. Dhir V, Aggarwal A. Psoriatic arthritis: a critical review. *Clinical Reviews in Allergy & Immunology*. 2013;44(2):141–148. doi:10.1007/s12016-012-8302-6
6. Garshick MS, Ward NL, Krueger JG, Berger JS. Cardiovascular risk in patients with psoriasis: JACC review topic of the week. *Journal of the American College of Cardiology*. 2021;77(13):1670–1680. doi:10.1016/j.jacc.2021.02.009
7. Sommer DM, Jenisch S, Suchan M, Christophers E, Weichenthal M. Increased prevalence of the metabolic syndrome in patients with moderate to severe psoriasis. *Archives of Dermatological Res*. 2006;298(7):321–328. doi:10.1007/s00403-006-0703-z
8. Harden JL, Krueger JG, Bowcock AM. The immunogenetics of Psoriasis: a comprehensive review. *Journal of Autoimmunity*. 2015;64:66–73. doi:10.1016/j.jaut.2015.07.008
9. Petit RG, Cano A, Ortiz A, et al. Psoriasis: From pathogenesis to pharmacological and nano-technological-based therapeutics. *Int J mol Sci*. 2021;22(9):4983. doi:10.3390/ijms22094983
10. Takahashi T, Yamasaki K. Psoriasis and antimicrobial peptides. *Int J mol Sci*. 2020;21(18):6791. doi:10.3390/ijms21186791
11. Grjibovski AM, Olsen AO, Magnus P, Harris JR. Psoriasis in Norwegian twins: contribution of genetic and environmental effects. *Journal of the European Academy of Dermatology and Venereology: JEADV*. 2007;21(10):1337–1343. doi:10.1111/j.1468-3083.2007.02268.x
12. Zuo X, Sun L, Yin X, et al. Whole-exome SNP array identifies 15 new susceptibility loci for psoriasis. *Nat Commun*. 2015;6(1):6793. doi:10.1038/ncomms7793
13. Ben-David Y, Bernstein A. Friend virus-induced erythroleukemia and the multistage nature of cancer. *Cell*. 1991;66(5):831–834. doi:10.1016/0092-8674(91)90428-2
14. Ben-David Y, Giddens EB, Bernstein A. Identification and mapping of a common proviral integration site Fli-1 in erythroleukemia cells induced by Friend murine leukemia virus. *Proceedings of the National Academy of Sciences of the United States of America*. 1990;87(4):1332–1336. doi:10.1073/pnas.87.4.1332
15. Ben-David Y, Giddens EB, Letwin K, Bernstein A. Erythroleukemia induction by Friend murine leukemia virus: insertional activation of a new member of the ets gene family, Fli-1, closely linked to c-ets-1. *Genes & Development*. 1991;5(6):908–918. doi:10.1101/gad.5.6.908
16. Ben-David Y, Gajendran B, Sample KM, Zacksenhaus E. Current insights into the role of Fli-1 in hematopoiesis and malignant transformation. *Cellular and Molecular Life Sciences: CMLS*. 2022;79(3):163. doi:10.1007/s00018-022-04160-1
17. Delattre O, Zucman J, Plougastel B, et al. Gene fusion with an ETS DNA-binding domain caused by chromosome translocation in human tumours. *Nature*. 1992;359:6391):162–5. doi:10.1038/359162a0
18. Chen B, Sheng D, Wang C, et al. FLI1 regulates inflammation-associated genes to accelerate leukemogenesis. *Cell. Signalling*. 2022;92:110269. doi:10.1016/j.cellsig.2022.110269
19. Zhang L, Eddy A, Teng YT, et al. An immunological renal disease in transgenic mice that overexpress Fli-1, a member of the ets family of transcription factor genes. *Molecular and Cellular Biology*. 1995;15(12):6961–6970. doi:10.1128/mcb.15.12.6961
20. Zhang XK, Gallant S, Molano I, et al. Decreased expression of the Ets family transcription factor Fli-1 markedly prolongs survival and significantly reduces renal disease in MRL/lpr mice. *Journal of Immunology)*. 2004;173(10):6481–6489. doi:10.4049/jimmunol.173.10.6481
21. Asano Y, Bujor AM, Trojanowska M. The impact of Fli1 deficiency on the pathogenesis of systemic sclerosis. *J Dermatological Sci*. 2010;59(3):153–162. doi:10.1016/j.jdermsci.2010.06.008
22. Asano Y, Stawski L, Hant F, et al. Endothelial Fli1 deficiency impairs vascular homeostasis: a role in scleroderma vasculopathy. *The American Journal of Pathology*. 2010;176(4):1983–1998. doi:10.2353/ajpath.2010.090593
23. Jia HY, Qiu HY, Zhang MD, Hou JJ, Zhou ML, Wu Y. Lenalidomide attenuates IMQ-induced inflammation in a mouse model of psoriasis. *Biomedicine & Pharmacotherapy = Biomedecine & Pharmacotherapie*. 2022;156:113883. doi:10.1016/j.biopha.2022.113883
24. Min M, Yan BX, Wang P, et al. Rottlerin as a therapeutic approach in psoriasis: evidence from in vitro and in vivo studies. *PLoS One*. 2017;12(12):e0190051. doi:10.1371/journal.pone.0190051
25. Varma SR, Sivaprakasam TO, Mishra A, Prabhu S, M R, P R, M R, P R. Imiquimod-induced psoriasis-like inflammation in differentiated Human keratinocytes: its evaluation using curcumin. *European Journal of Pharmacology*. 2017;813:33–41. doi:10.1016/j.ejphar.2017.07.040.
26. Wang C, Sample KM, Gajendran B, et al. FLI1 induces megakaryopoiesis gene expression through WAS/WIP-dependent and independent mechanisms; implications for Wiskott-Aldrich Syndrome. *Front Immunol*. 2021;12:607836. doi:10.3389/fimmu.2021.607836
27. Martinez Molina D, Jafari R, Ignatushchenko M, et al. Monitoring drug target engagement in cells and tissues using the cellular thermal shift assay. *Science*. 2013;341:6141):84–7. doi:10.1126/science.1233606
28. Kamal RM, Sabry MM, El-Halawany AM, et al. GC-MS analysis and the effect of topical application of essential oils of Pinus canariensis C.Sm. Cupressus lusitanica Mill. and Cupressus arizonica Greene aerial parts in Imiquimod-Induced Psoriasis in Mice. *Journal of Ethnopharmacology*. 2024;318(Pt B):116947. doi:10.1016/j.jep.2023.116947
29. Peng L, Wen L, Shi Q, Gao F, Huang B, Wang C. Chelerythrine ameliorates pulmonary fibrosis via activating the Nrf2/ARE signaling pathway. *Cell Biochemistry and Biophysics*. 2021;79(2):337–347. doi:10.1007/s12013-021-00967-0
30. Thatikonda S, Pooladanda V, Sigalapalli DK, Godugu C. Piperlongumine regulates epigenetic modulation and alleviates psoriasis-like skin inflammation via inhibition of hyperproliferation and inflammation. *Cell Death Dis*. 2020;11(1):21. doi:10.1038/s41419-019-2212-y

31. Gao J, Chen F, Fang H, Mi J, Qi Q, Yang M. Daphnetin inhibits proliferation and inflammatory response in human HaCaT keratinocytes and ameliorates imiquimod-induced psoriasis-like skin lesion in mice. *Biol. Res.* 2020;53(1):48. doi:10.1186/s40659-020-00316-0
32. Patel NA, Patel NJ, Patel RP. Formulation and evaluation of curcumin gel for topical application. *Pharmaceutical Development and Technology.* 2009;14(1):80–89. doi:10.1080/10837450802409438
33. Hiraganahalli Bhaskarmurthy D, Evan Prince S. Effect of Baricitinib on TPA-induced psoriasis like skin inflammation. *Life Sciences*. 2021;279:119655. doi:10.1016/j.lfs.2021.119655
34. Takahashi T, Asano Y, Sugawara K, et al. Epithelial Fli1 deficiency drives systemic autoimmunity and fibrosis: possible roles in scleroderma. *J Exp Med*. 2017;214(4):1129–1151. doi:10.1084/jem.20160247
35. Ben Abdallah H, Seeler S, Bregnhøj A, et al. Heat shock protein 90 inhibitor RGRN-305 potently attenuates skin inflammation. *Front Immunol*. 2023;14:1128897. doi:10.3389/fimmu.2023.1128897
36. Leigh IM, Navsaria H, Purkis PE, McKay IA, Bowden PE, Riddle PN. Keratins (K16 and K17) as markers of keratinocyte hyperproliferation in psoriasis in vivo and in vitro. *British J Dermatol*. 1995;133(4):501–511. doi:10.1111/j.1365-2133.1995.tb02696.x
37. Zeng J, Xie Y, Zhang H, et al. Protective roles of tRNA-derived small RNA tRF-Ile-AAT-019 in pathological progression of psoriasis. *Experimental Dermatol*. 2023;32(2):135–145. doi:10.1111/exd.14689
38. Sato S, Lennard Richard M, Brandon D, et al. A critical role of the transcription factor fli-1 in murine lupus development by regulation of interleukin-6 expression. *Arthritis Rheumatol*. 2014;66(12):3436–3444. doi:10.1002/art.38818
39. Sato S, Zhang XK, Temmoku J, et al. Ets family transcription factor Fli-1 promotes leukocyte recruitment and production of IL-17A in the MRL/Lpr mouse model of lupus nephritis. *Cells*. 2020;9(3):714. doi:10.3390/cells9030714
40. Bujor AM, El Adili F, Parvez A, Marden G, Trojanowska M. Fli1 downregulation in scleroderma myeloid cells has profibrotic and proinflammatory effects. *Front Immunol*. 2020;11:800. doi:10.3389/fimmu.2020.00800
41. Francis L, McCluskey D, Ganier C, et al. Single-cell analysis of psoriasis resolution demonstrates an inflammatory fibroblast state targeted by IL-23 blockade. *Nat Commun*. 2024;15(1):913. doi:10.1038/s41467-024-44994-w
42. Kim KJ, Park S, Park YH, et al. The expression and role of krüppel-like factor 4 in psoriasis. *Annals of Dermatology.* 2014;26(6):675–680. doi:10.5021/ad.2014.26.6.675
43. Fang M, Wee SA, Ronski K, Fan H, Tao S, Lin Q. Evidence of EGR1 as a differentially expressed gene among proliferative skin diseases. *Genomic Medicine*. 2007;1(1–2):75–85. doi:10.1007/s11568-007-9010-9
44. Doo C, Bao L, Shen K, Yang JF, Shen RR, Chan LS. Diacerein alone and in combination with infliximab suppresses the combined proinflammatory effects of IL-17A, IL-22, Oncostatin M, IL-1A, and TNF-alpha in keratinocytes: a potential therapeutic option in psoriasis. *Journal of Interferon & Cytokine Research: the Official Journal of the International Society for Interferon and Cytokine Research*. 2021;41(8):302–306. doi:10.1089/jir.2021.0036
45. Chen L, Wu J, Pier E, Zhao Y, Shen Z. mTORC2-PKBα/Akt1 Serine 473 phosphorylation axis is essential for regulation of FOXP3 Stability by chemokine CCL3 in psoriasis. *J Investigative Dermatol*. 2013;133(2):418–428. doi:10.1038/jid.2012.333
46. Komatsu N, Saijoh K, Kuk C, Shirasaki F, Takehara K, Diamandis EP. Aberrant human tissue kallikrein levels in the stratum corneum and serum of patients with psoriasis: dependence on phenotype, severity and therapy. *British J Dermatol*. 2007;156(5):875–883. doi:10.1111/j.1365-2133.2006.07743.x
47. Zhang Z, Liu L, Shen Y, et al. Characterization of chromatin accessibility in psoriasis. *Frontiers of Medicine*. 2022;16(3):483–495. doi:10.1007/s11684-021-0872-3
48. Shehwana H, Ijaz S, Fatima A, et al. Transcriptome analysis of host inflammatory responses to the ectoparasitic mite Sarcoptes scabiei var. hominis. *Front Immunol*. 2021;12:778840. doi:10.3389/fimmu.2021.778840
49. Yoshida A, Kanno H, Watabe D, Akasaka T, Sawai T. The role of heparin-binding EGF-like growth factor and amphiregulin in the epidermal proliferation of psoriasis in cooperation with TNFalpha. *Archives of Dermatological Res*. 2008;300(1):37–45. doi:10.1007/s00403-007-0809-y
50. Hanselmann C, Mauch C, Werner S. Haem oxygenase-1: a novel player in cutaneous wound repair and psoriasis? *The Biochemical Journal*. 2001;353(Pt 3):459–466. doi:10.1042/0264-6021:3530459
51. Lobito AA, Ramani SR, Tom I, et al. Murine insulin growth factor-like (IGFL) and human IGFL1 proteins are induced in inflammatory skin conditions and bind to a novel tumor necrosis factor receptor family member, IGFLR1. *J Biol Chem*. 2011;286(21):18969–18981. doi:10.1074/jbc.M111.224626
52. Su W, Zhao Y, Wei Y, Zhang X, Ji J, Yang S. Exploring the pathogenesis of psoriasis complicated with atherosclerosis via microarray data analysis. *Front Immunol*. 2021;12:667690. doi:10.3389/fimmu.2021.667690
53. Zhang YJ, Sun YZ, Gao XH, Qi RQ. Integrated bioinformatic analysis of differentially expressed genes and signaling pathways in plaque psoriasis. *Molecular Medicine Reports*. 2019;20(1):225–235. doi:10.3892/mmr.2019.10241
54. Johansen C, Rittig AH, Mose M, et al. STAT2 is involved in the pathogenesis of psoriasis by promoting CXCL11 and CCL5 production by keratinocytes. *PLoS One*. 2017;12(5):e0176994. doi:10.1371/journal.pone.0176994
55. van den Reek J, Coenen MJH, van de L'Isle Arias M, et al. Polymorphisms in CD84, IL12B and TNFAIP3 are associated with response to biologics in patients with psoriasis. *British J Dermatol*. 2017;176(5):1288–1296. doi:10.1111/bjd.15005
56. Nakamizo S, Dutertre CA, Khalilnezhad A, et al. Single-cell analysis of human skin identifies CD14+ type 3 dendritic cells co-producing IL1B and IL23A in psoriasis. *J Exp Med*. 2021;218(9). doi:10.1084/jem.20202345
57. Sevimoglu T, Turanli B, Bereketoglu C, Arga KY, Karadag AS. Systems biomarkers in psoriasis: integrative evaluation of computational and experimental data at transcript and protein levels. *Gene*. 2018;647:157–163. doi:10.1016/j.gene.2018.01.033
58. Huang YZ, Zheng YX, Zhou Y, et al. OAS1, OAS2, and OAS3 contribute to epidermal keratinocyte proliferation by regulating cell cycle and augmenting IFN-1–induced jak1–signal transducer and activator of transcription 1 phosphorylation in psoriasis. *J Investigative Dermatol*. 2022;142(10):2635–2645.e9. doi:10.1016/j.jid.2022.02.018
59. Elgharib I, Khashaba SA, Elsaid HH, Sharaf MM. Serum elafin as a potential inflammatory marker in psoriasis. *Int J Dermatol*. 2019;58(2):205–209. doi:10.1111/ijd.14217
60. Luo Y, Luo Y, Chang J, Xiao Z, Zhou B. Identification of candidate biomarkers and pathways associated with psoriasis using bioinformatics analysis. *Hereditas*. 2020;157(1):30. doi:10.1186/s41065-020-00141-1

61. Li YJ, Zhao X, Vecchiarelli-Federico LM, et al. Drug-mediated inhibition of Fli-1 for the treatment of leukemia. *Blood Cancer J*. 2012;2(1):e54. doi:10.1038/bcj.2011.52
62. Schutt SD, Wu Y, Kharel A, et al. The druggable transcription factor Fli-1 regulates T cell immunity and tolerance in graft-versus-host disease. *J Clin Invest*. 2022;132(21). doi:10.1172/jci143950
63. Wang X, Oates JC, Helke KL, Gilkeson GS, Zhang XK. Camptothecin and topotecan, inhibitors of transcription factor Fli-1 and Topoisomerase, markedly ameliorate lupus nephritis in (NZB × NZW)F1 mice and reduce the production of inflammatory mediators in human renal cells. *Arthritis Rheumatol*. 2021;73(8):1478–1488. doi:10.1002/art.41685
64. Ghashghaeinia M, Dreischer P, Wieder T, Köberle M. Coronavirus disease 2019 (COVID-19), human erythrocytes and the PKC-alpha/-beta inhibitor chelerythrine -possible therapeutic implication. *Cell Cycle*. 2020;19(24):3399–3405. doi:10.1080/15384101.2020.1859197
65. Liu T, Xia L, Yao Y, et al. Identification of diterpenoid compounds that interfere with Fli-1 DNA binding to suppress leukemogenesis. *Cell Death Dis*. 2019;10(2):117. doi:10.1038/s41419-019-1363-1
66. Zhang J, Guo H, Zhang H, et al. Putative tumor suppressor miR-145 inhibits colon cancer cell growth by targeting oncogene Friend leukemia virus integration 1 gene. *Cancer*. 2011;117(1):86–95. doi:10.1002/cncr.25522
67. Stanley PL, Steiner S, Havens M, Tramposch KM. Mouse skin inflammation induced by multiple topical applications of 12-O-tetradecanoylphorbol-13-acetate. *Skin Pharmacology: the Official Journal of the Skin Pharmacology Society*. 1991;4(4):262–271. doi:10.1159/000210960
68. Gottlieb AB. Psoriasis: emerging therapeutic strategies. *Nat Rev Drug Discov*. 2005;4(1):19–34. doi:10.1038/nrd1607
69. Malecic N, Young H. Tacrolimus for the management of psoriasis: clinical utility and place in therapy. *Psoriasis*. 2016;6:153–163. doi:10.2147/ptt.S101233
70. Vissers WH, van Vlijmen I, van Erp PE, de Jong EM, van de Kerkhof PC. Topical treatment of mild to moderate plaque psoriasis with 0.3% tacrolimus gel and 0.5% tacrolimus cream: the effect on SUM score, epidermal proliferation, keratinization, T-cell subsets and HLA-DR expression. *British J Dermatol*. 2008;158(4):705–712. doi:10.1111/j.1365-2133.2008.08442.x
71. Glickman JW, Dubin C, Renert-Yuval Y, et al. Cross-sectional study of blood biomarkers of patients with moderate to severe alopecia areata reveals systemic immune and cardiovascular biomarker dysregulation. *Journal of the American Academy of Dermatology*. 2021;84(2):370–380. doi:10.1016/j.jaad.2020.04.138
72. Holmannova D, Borsky P, Borska L, et al. Metabolic syndrome, clusterin and elafin in patients with Psoriasis Vulgaris. *Int J mol Sci*. 2020;21(16):5617. doi:10.3390/ijms21165617
73. Griffiths CEM, Armstrong AW, Gudjonsson JE, Barker J. Psoriasis. *Lancet)*. 2021;397(10281):1301–1315. doi:10.1016/s0140-6736(20)32549-6
74. Ghoreschi K, Balato A, Enerbäck C, Sabat R. Therapeutics targeting the IL-23 and IL-17 pathway in psoriasis. *Lancet*. 2021;397(10275):754–766. doi:10.1016/s0140-6736(21)00184-7
75. Hsieh CY, Tsai TF. Aggravation of discoid lupus erythematosus in a patient with psoriasis and psoriatic arthritis during treatment of secukinumab: a case report and review of literature. *Lupus*. 2022;31(7):891–894. doi:10.1177/09612033221095692
76. Santacruz JC, Pulido S, Arzuaga A, Mantilla MJ, Santos AM, Londono J. Current Evidence for IL-17/23 blockade for the treatment of lupus nephritis. *Cureus*. 2021;13(12):e20087. doi:10.7759/cureus.20087
77. Liu T, Yao Y, Zhang G, et al. A screen for Fli-1 transcriptional modulators identifies PKC agonists that induce erythroid to megakaryocytic differentiation and suppress leukemogenesis. *Oncotarget*. 2017;8(10):16728–16743. doi:10.18632/oncotarget.14377
78. Shen Y, Zhu C, Wang Y, et al. Evaluation the binding of chelerythrine, a potentially harmful toxin, with bovine serum albumin. *Food Chemical Toxicology*. 2020;135:110933. doi:10.1016/j.fct.2019.110933
79. Vieira SM, de Oliveira VH, Valente Rdo C, Moreira Oda C, Fontes CF, Mignaco JA. Chelerythrine inhibits the sarco/endoplasmic reticulum Ca(2+)-ATPase and results in cell Ca(2+) imbalance. *Archives of Biochemistry and Biophysics*. 2015;570:58–65. doi:10.1016/j.abb.2015.02.019