American Society of Hematology
2021 L Street NW, Suite 900,
Washington, DC 20036
Phone: 202-776-0544 | Fax 202-776-0545
editorial@hematology.org

Malignant T cells induce skin barrier defects through cytokine-mediated JAK/STAT signalling in cutaneous T-cell lymphoma

Tracking no: BLD-2022-016690R1

Maria Gluud (University of Copenhagen, Denmark) Emil Pallesen (University of Copenhagen, Denmark) Terkild Buus (University of Copenhagen, Denmark) Lise Gjerdrum (, Denmark) Lise M. Lindahl (Department of Dermatology, Aarhus University Hospital, Aarhus, Denmark, Denmark) Maria Kamstrup (Bispebjerg Hospital, Denmark) Michael Bzorek (Department of Pathology, Zealand University Hospital, Denmark) Maria Danielsen (Aarhus University Hospital, Denmark) Rikke Bech (Aarhus University Hospital, Denmark) Madalena Monteiro (University of Copenhagen, Denmark) Edda Blümel (University of Copenhagen, Denmark) Andreas Willerslev-Olsen (University of Copenhagen, Denmark) Anders Lykkebo-Velløe (University of Copenhagen, Denmark) Chella Krishna Vadivel (University of Copenhagen, Denmark) Thorbjørn Krejsgaard (University of Copenhagen, Denmark) Charlotte Bonefeld (University of Copenhagen, Denmark) Carsten Geisler (University of Copenhagen, Denmark) Jürgen Becker (German Cancer Consortium (DKTK), Site University Medicine Essen, Germany) Sergei Koralov (New York University School of Medicine, United States) Lars Iversen (Aarhus University Hospital, Denmark) Thomas Litman (University of Copenhagen, Denmark) Anders Woetmann (University of Copenhagen, Denmark) Niels Odum (University of Copenhagen, Denmark)

Abstract:
Cutaneous T-cell lymphoma (CTCL) is a devastating lymphoid malignancy characterised by accumulation of malignant T cells in the dermis and epidermis. Skin lesions cause serious symptoms hampering the quality of life and are entry sites for bacterial infection - a major cause of morbidity and mortality in advanced disease. What drives the pathological processes that compromise the skin barrier remains unknown. Here, we report on increased transepidermal water loss and compromised expression of skin barrier proteins filaggrin and filaggrin-2 in areas adjacent to TOX positive T cells in CTCL skin lesions. Malignant T cells secrete mediators (including cytokines such as IL-13, IL-22 and Oncostatin M) that activate STAT3 signalling and downregulate filaggrin and filaggrin-2 expression in human keratinocytes and reconstructed human epithelium. Consequently, repression of filaggrins could be counteracted by a cocktail of antibodies targeting these cytokines/receptors, by siRNA-mediated knockdown of JAK1/STAT3, and by JAK1 inhibitors. Notably, we show that treatment with a clinically approved JAK inhibitor, Tofacitinib, increases filaggrin expression in lesional skin from mycosis fungoides patients. Taken together, these findings indicate that malignant T cells secrete cytokines, which induce skin barrier defects through a JAK1/STAT3 dependent mechanism. As clinical grade JAK inhibitors largely abrogate the negative effect of malignant T cells on skin barrier proteins, our findings suggest that such inhibitors provide novel treatment options for CTCL patients with advanced disease and a compromised skin barrier.

Conflict of interest: COI declared - see note

COI notes: Niels Ødum has received consulting honoraria from Mindera corp, Micreos Human Health, PS Consulting, and Almirall. Jürgen C. Becker is receiving speaker's bureau honoraria from Amgen, Pfizer, MerckSerono, Recordati and Sanofi, is a paid consultant/advisory board member for Boehringer Ingelheim, eTheRNA, InProTher, MerckSerono, Pfizer, 4SC, Regeneron and Sanofi. His group receives research grants from Bristol-Myers Squibb, Merck Serono, HTG, IQVIA, and Alcedis. Lise Mette Rahbek Gjerdrum receives funding from NanoString Technologies. Thomas Litman is funded by LEO Pharma. Lars Iversen Served as a consultant and/or paid speaker for and/or participated in clinical trials sponsored by: AbbVie, Almirall, Amgen, Astra Zeneca, BMS, Boehringer Ingelheim, Celgene, Centocor, Eli Lilly, Janssen Cilag, Kyowa, Leo Pharma, Micreos Human Health, MSD, Novartis, Pfizer, Regranion, Samsung, Union Therapeutics, UCB. All other authors declare no potential conflicts of interest.

Preprint server: No;

Author contributions and disclosures: Concept and design: MG and NØ. Acquisition, analysis, or interpretation of data: All authors. Drafting of the manuscript: MG and NØ. Critical revision of the manuscript for important intellectual content: All authors. Obtained funding: NØ. Administrative, technical, or material support: EMP, TBB, LMRG, LML, MB, MD, RB, CKV, AL-V, MRK, SBK, LI, TL, AW. Supervision: NØ.

Non-author contributions and disclosures: No;

Agreement to Share Publication-Related Data and Data Sharing Statement: The datasets generated and/or analysed during the current study are public available or from the corresponding author upon request. GSE165623, GSE173205, GSE128531

Clinical trial registration information (if any):

Figure 1

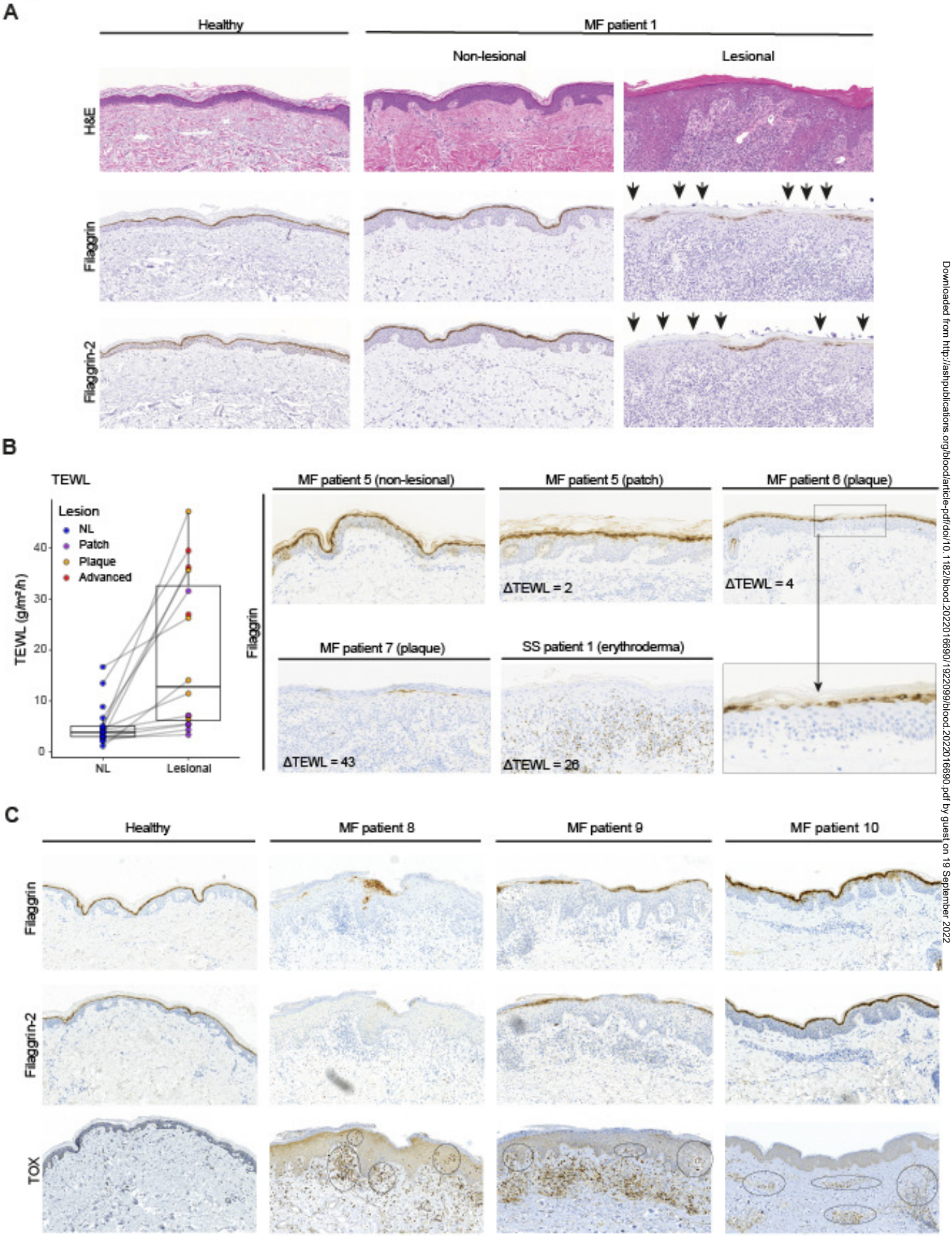


Figure 2

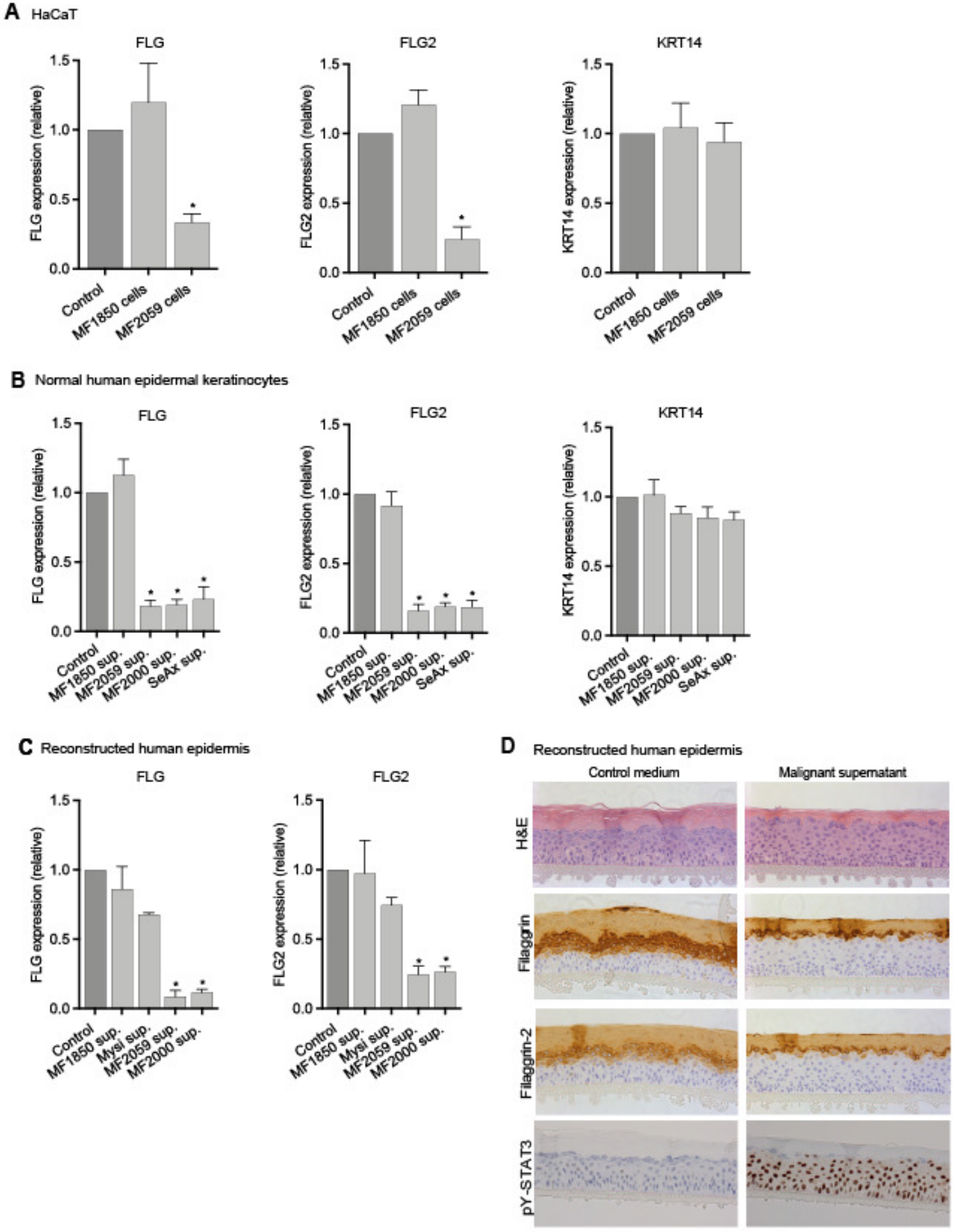


Figure 3

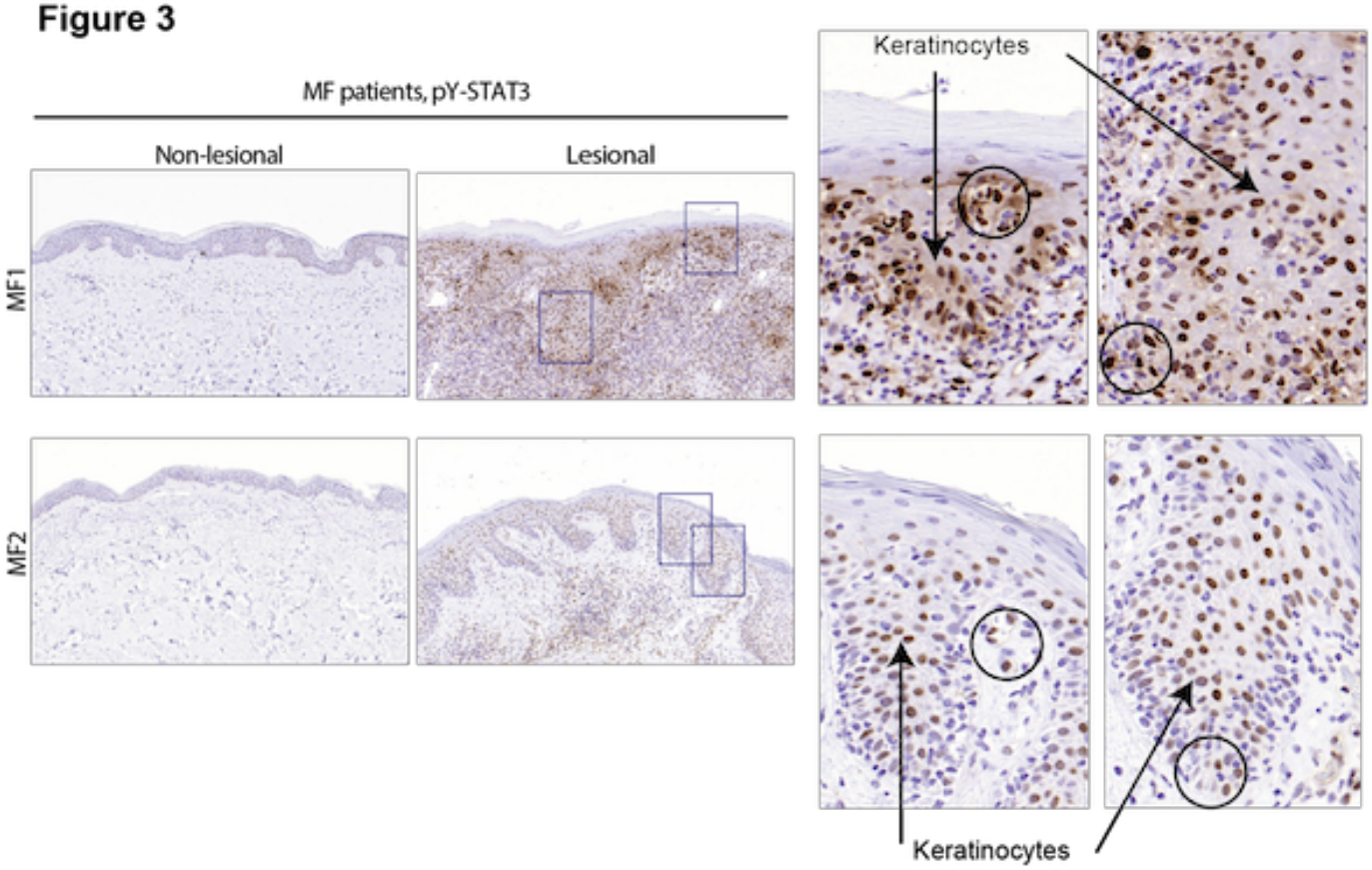


Figure 4

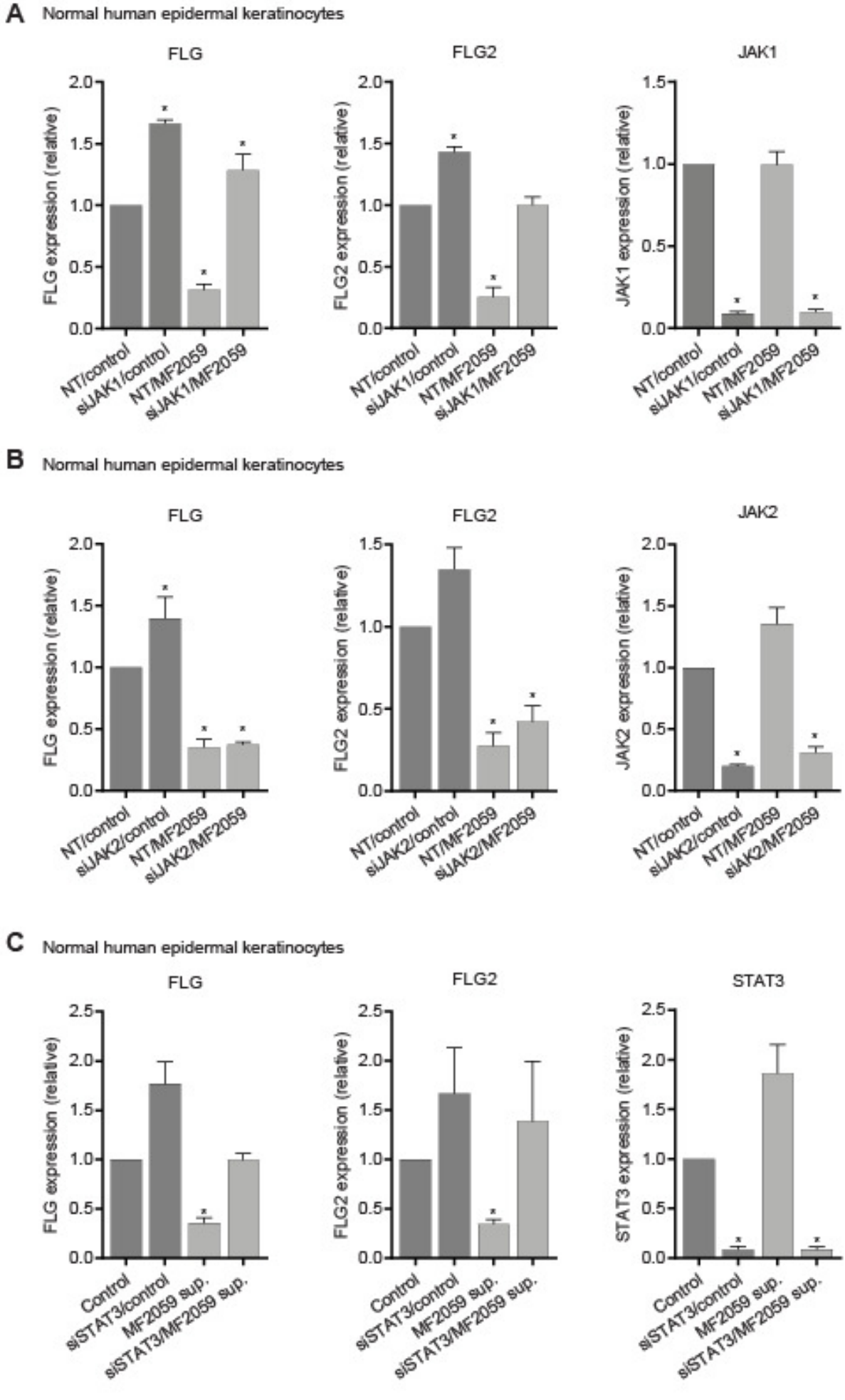


Figure 5

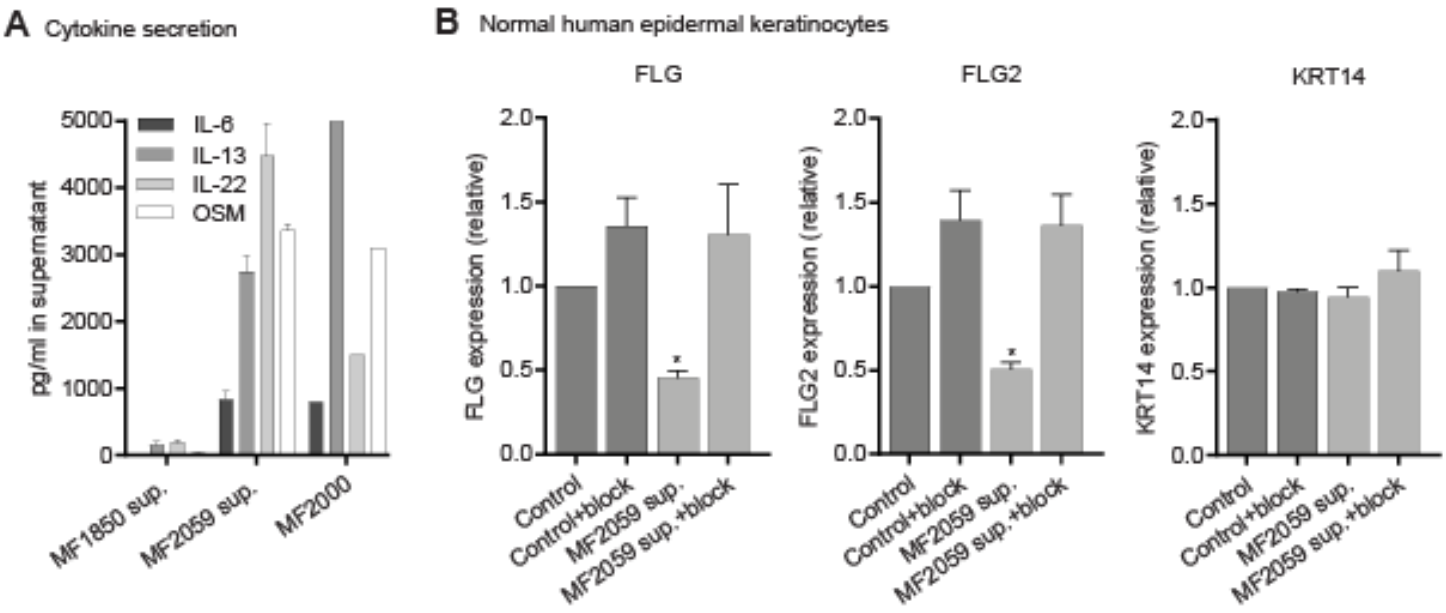


Figure 6

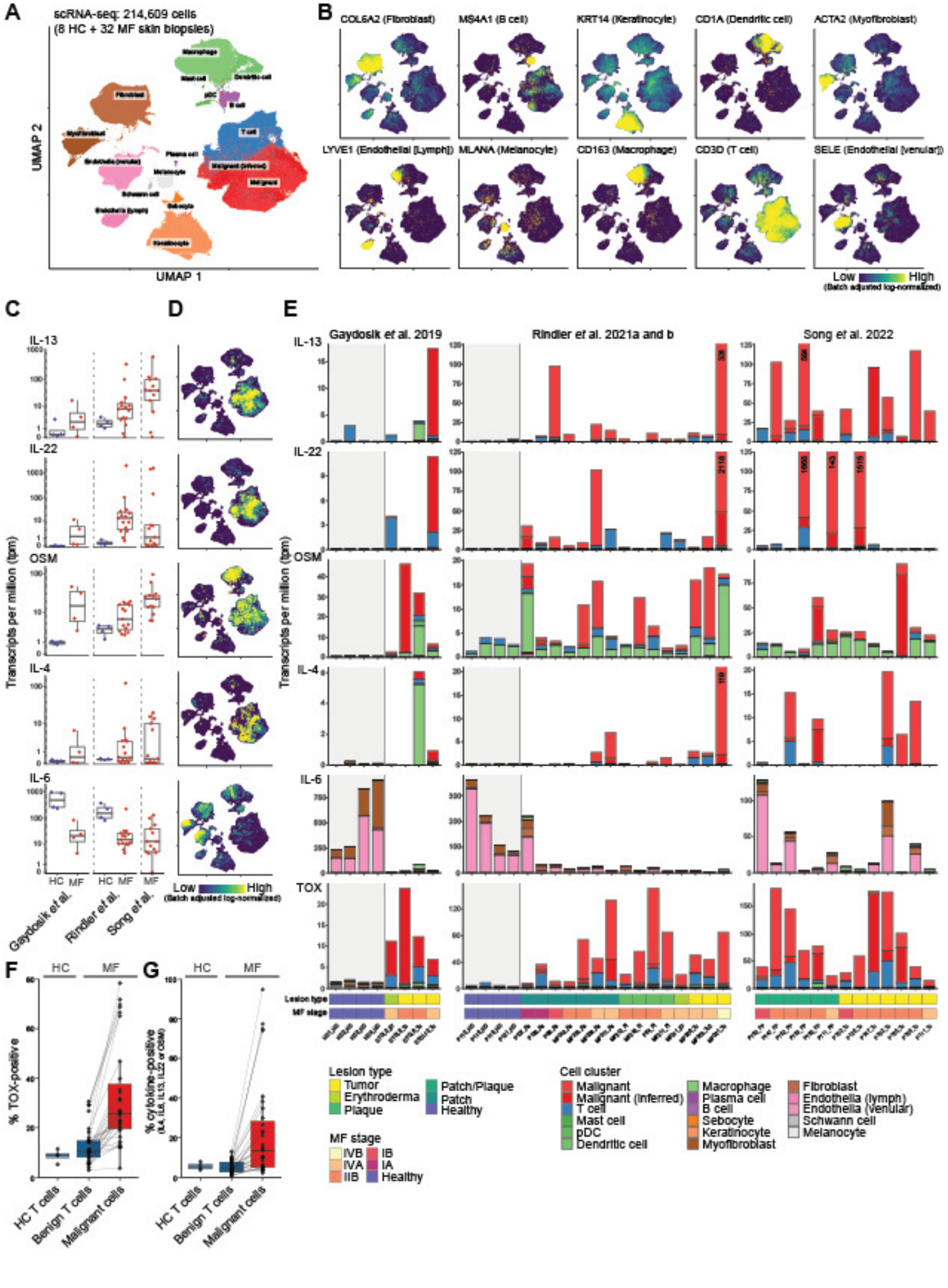
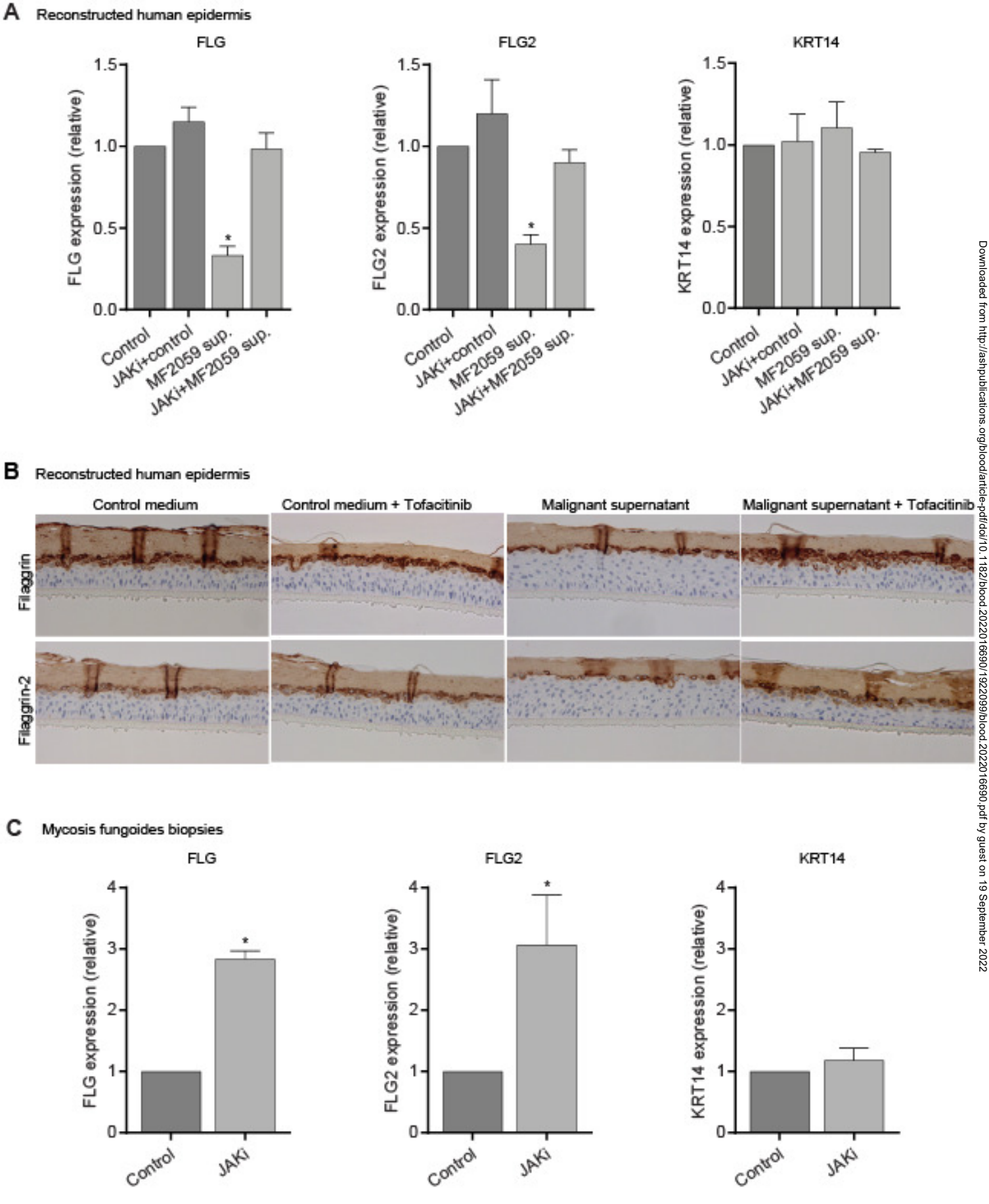


Figure 7



Malignant T cells induce skin barrier defects through cytokine-mediated JAK/STAT signalling in cutaneous T-cell lymphoma

Maria Gluud[1], Emil M. H. Pallesen[1], Terkild B. Buus[1], Lise Mette Rahbek Gjerdrum[2,3], Lise M. Lindahl[4], Maria R. Kamstrup[5], Michael Bzorek[2], Maria Danielsen[4], Rikke Bech[4], Madelena N. Monteiro[1], Edda Blümel[1], Andreas Willerslev-Olsen[1], Anders Lykkebo-Velløe[1], Chella Krishna Vadivel[1], Thorbjørn Krejsgaard[1], Charlotte Menne Bonefeld[1], Carsten Geisler[1], Jürgen C. Becker[6], Sergei B. Koralov[7], Lars Iversen[4], Thomas Litman[1], Anders Woetmann[1], and Niels Ødum[1*]

[1] LEO Foundation Skin Immunology Research Center, Department of Immunology and Microbiology, University of Copenhagen, Copenhagen, Denmark
[2] Department of Pathology, Zealand University Hospital, Roskilde, Denmark
[3] Department of Clinical Medicine, University of Copenhagen, Copenhagen, Denmark
[4] Department of Dermatology, Aarhus University Hospital, Aarhus, Denmark
[5] Department of Dermatology, Bispebjerg and Frederiksberg Hospital, Copenhagen, Denmark
[6] Translational Skin Cancer Research, German Cancer Consortium (DKTK), University Medicine Essen, Essen and Deutsches Krebsforschungszentrum (DKFZ), Heidelberg, Germany
[7] Department of Pathology, New York University School of Medicine, New York, USA

*Correspondence to: Niels Ødum, email: ndum@sund.ku.dk

LEO Foundation Skin Immunology Research Center, Department of Immunology and Microbiology;
The Maersk Tower 07-12, University of Copenhagen,
Blegdamsvej 3, DK2200 N, Copenhagen, Denmark.

Short title: Malignant T cells induce skin barrier defects

Word count for text: 3996

Word count for abstract: 228

Count for figures: 7 figures

Count for references: 78

Key points

Malignant T cells orchestrate changes in the microenvironment, which lead to skin barrier defects in cutaneous T-cell lymphoma

We provide a novel rationale for Janus Kinase-1 inhibition as an important adjuvant therapy in patients with cutaneous T-cell lymphoma

Abstract

Cutaneous T-cell lymphoma (CTCL) is a devastating lymphoid malignancy characterised by accumulation of malignant T cells in the dermis and epidermis. Skin lesions cause serious symptoms hampering the quality of life and are entry sites for bacterial infection - a major cause of morbidity and mortality in advanced disease. What drives the pathological processes that compromise the skin barrier remains unknown. Here, we report on increased transepidermal water loss and compromised expression of skin barrier proteins filaggrin and filaggrin-2 in areas adjacent to TOX positive T cells in CTCL skin lesions. Malignant T cells secrete mediators (including cytokines such as IL-13, IL-22 and Oncostatin M) that activate STAT3 signalling and downregulate filaggrin and filaggrin-2 expression in human keratinocytes and reconstructed human epithelium. Consequently, repression of filaggrins could be counteracted by a cocktail of antibodies targeting these cytokines/receptors, by siRNA-mediated knockdown of JAK1/STAT3, and by JAK1 inhibitors. Notably, we show that treatment with a clinically approved JAK inhibitor, Tofacitinib, increases filaggrin expression in lesional skin from mycosis fungoides patients. Taken together, these findings indicate that malignant T cells secrete cytokines, which induce skin barrier defects through a JAK1/STAT3 dependent mechanism. As clinical grade JAK inhibitors largely abrogate the negative effect of malignant T cells on skin barrier proteins, our findings suggest that such inhibitors provide novel treatment options for CTCL patients with advanced disease and a compromised skin barrier.

Introduction

Cutaneous T-cell lymphoma (CTCL) represents a heterogeneous group of malignant extranodal non-Hodgkin lymphoproliferative disorders arising from neoplastic T cells[1–4]. The most prevalent variant, mycosis fungoides (MF), is characterised by epidermotropism of malignant T cells associated with inflammatory skin lesions[5]. MF lesions are chronically inflamed and the tumour microenvironment (TME) is dominated by the presence of TH2-associated cytokines including IL-13 secreted by malignant T cells[6–8]. Early-stage MF presents with scaly patches and plaques, and some patients progress to advanced stages suffering from tumours and extensive erythroderma[2]. Sézary Syndrome (SS) is a rare and aggressive, leukemic CTCL variant characterised by malignant T cells in the blood and the skin, causing extensive erythroderma[9].

Attempts to identify key mutational drivers suggest that the disease is not caused by a single genetic event[10,11]. Instead, dysregulation of JAK/STAT signalling and several other signalling pathways has been linked to malignant T cell proliferation, apoptosis resistance, and inflammation in CTCL[12,13]. Mutations and fusion-proteins involving the JAK/STAT pathway have been described in some patients, while deficiencies and abnormal activity of regulators of the pathway have been described in other patients[14–17]. Of note, STAT3 activation in malignant T cells has for long been suspected to play a key role in the pathogenesis, and constitutive STAT3 activation in $CD4^+$ T cells drives disease development in a mouse model of CTCL[18]. Moreover, environmental factors, such as enterotoxin-producing *Staphylococcus aureus* (*S. aureus*), drive STAT3/5 activation and proliferation of malignant T cells[19–21]. Thus, it is likely that genetic, epigenetic, environmental factors, as well as immune-deregulation, are all implicated in the pathogenesis of CTCL[2,11,22–28].

A key function of the epidermis is to serve as a barrier to the external world. The uppermost layer of the epidermal compartment, stratum corneum, is essential for the barrier function and integrity of the skin[29]. Two important components of the stratum corneum are filaggrin (FLG) and the filaggrin family member-2 (filaggrin-2; FLG2)[30,31]. Filaggrin is synthesised in granular keratinocytes and filaggrin monomers aggregate keratin filaments into bundles[30–32]. Filaggrin deficiencies have for long been associated with impaired stratum corneum properties; decreased levels of natural moisturizing factors, decreased barrier function, and increased susceptibility to bacterial colonisation, microbial dysbiosis and infection - highlighting the importance of filaggrins in maintaining a functional skin barrier[33,34].

It has recently been reported that skin barrier proteins such as filaggrins are decreased in CTCL lesions, suggesting that the skin barrier is compromised in CTCL[35,36]. This may at least partly explain why CTCL patients are susceptible to cutaneous colonisation and infection by bacteria such as enterotoxin-producing *S. aureus* and *Bacillus safensis*[37–42]. Clinical data indicate that colonisation of skin lesions by *S. aureus* and other bacteria fuels the disease in CTCL. Consequently, a broken skin barrier is likely to play an important pathogenetic role in disease activity and progression[19,37,42–45]. What drives the development of skin lesions in CTCL remains largely unknown. Here, we provide first evidence that malignant T cells deteriorate the skin barrier through cytokine-driven, JAK1/STAT3- mediated repression of filaggrins in keratinocytes. Thus, our findings indicate that malignant T cells orchestrate profound changes in the skin TME, which in turn may pave the way for further disease escalation and bacterial complications.

Materials and methods

Cell line cultures

The malignant MF T-cell lines MF2059, MF2000, and the non-malignant T cells lines MyLa1850 and MySi were cultured as described in details elsewhere[46–48]. Supernatants were obtained from the T cell lines cultured for 24h at a cell density of $1x10^6$ cells/ml.

Epidermal models

Normal human epidermal keratinocytes (NHEK) were cultured in medium containing supplements (Promocell, C20111). Keratinocyte differentiation was induced by adding 1.25 mM $CaCl_2$ for 3 days prior to experimental setups. Reconstructed human epidermis models (Episkin, SkinEthic RHE, S-17, 0.5 cm^2) were applied according to the manufacturer's instructions. Paired MF biopsies obtained from the same plaque were washed in PBS supplemented with P/S and gentamycin and then cultured in DMEM/HAM's F12 (1:1, Thermo Fisher, #3966047/31966021) media supplemented with 10% FBS, 1% P/S, 10 µg/ml insulin and 10 ng/ml EGF for 72h with 1µM Tofacitinib.

Patient material

Experiments with samples from CTCL patients were performed in accordance with the Declaration of Helsinki. Biopsies from the patients were obtained after approval by the Regional Ethical Committee, Denmark (1-10-72-151-16 and M-20090102 and H-16025331). A table of the patients is presented in Suppl. Table 1.

Transepidermal water loss (TEWL)

Skin barrier function was measured by assessing transepeidermal water loss using Dermalab Evaporimeter (Cortex technology) according to the guidelines. Skin sites from 12 patients (15 non-lesional and 16 leisonal) were measured (in triplicates) on anatomically paired lesional and non-lesional skin sites. Results were shown as mean ($g/m^2/h$). ΔTEWL was calculated by subtracting site matched non-lesional TEWL from lesional TEWL.

Inhibitors and blocking antibodies

Cells were treated with 0.001-1µM of the pan-JAK inhibitor Tofacitinib (InvivoGene, CP6905590) and 0.5-1 µM of the JAK1 inhibitor Abrocitinib or DMSO. R&D blocking antibodies were applied;

1 µg/ml IL-22Rα1 (#AF2770), 10 µg/ml gp130 (#MAB228), 30 µg/ml IL-13Rα1 (#AF152) and 250 µg/ml Dupilumab.

Lipofectamine transfection

NHEK were transfected with 10 pmol (20 nmol/L) siRNA targeting JAK1, JAK2, STAT3 or control non-targeting siRNA (NT siRNA, Dharmacon, ON-TARGETplus) using Lipofectamine® RNAiMAX Transfection Reagent (ThermoFisher). 48h following transfection, NHEK were cultured in the presence of supernatants.

Quantitative reverse transcription PCR (RT-qPCR)

RNA from skin samples and from NHEK/HaCaT cells and biopsies was purified (Qiagen 217004/74134) and transcribed to cDNA and subjected to RT-qPCR (Lightcycler480) using β-actin as reference gene for normalization as described in details[49]. Data was calculated according to the $2^{\Delta\Delta CT}$ method.

Immunohistochemistry (IHC)

IHC studies were performed on skin sections using anti-human pY-STAT3, filaggrin, filaggrin-2 and TOX. The method is described in detail in supplementary and elsewhere[19].

ELISA

Duoset® ELISA's detecting IL-6, IL-13, IL-22 and oncostatin M (OSM) (R&D system) was performed according to the manufacturer's instructions.

Cytokine expression in skin biopsies using RNA single cell sequencing (scRNA-seq)

Count matrices from four studies were obtained or generated from Gene Expression Omnibus GSE165623[50], GSE173205[50], GSE128531[51] or from Sequence Read Archive SRP332550[52]. Only skin biopsies from healthy controls or lesional CTCL skin containing an identifiable malignant T cell population were included in the analysis. Detailed description is included in supplementary.

Statistics

All graphs and statistical analysis were performed using the software GraphPad Prism v8.0. Student's t-test and one-way analysis of variance (ANOVA) were applied, followed by post hoc analysis. For

qPCR results, the relative expression of each intervention was compared to the control. Error bars are shown as SEM, and the level of statistical significance was set to p-value <0.05 (*).

Results

As malignant T cells reside in a unique tumor microenvironment (TME) in the epidermis, with keratinocytes as the major cell type, we hypothesized that malignant T cells may interact with adjacent keratinocytes to orchestrate the characteristic pathological changes seen in CTCL skin. Comparing lesional MF skin (plaques) to non-lesional MF and healthy skin, we found a reduction in filaggrin and filaggrin-2 protein expression in stratum granulosum/stratum corneum (Fig. 1A and Suppl. Fig. S1A). Notably, the expression was decreased to varying degree within patient biopsies and among patients (Fig. 1A-C and Suppl. Fig. S1A). To assess barrier function in CTCL patients, paired non-lesional and lesional skin sites were evaluated for transepidermal water loss (TEWL) in 12 patients. In all patients, TEWL measurements were higher in lesional compared to matched non-lesional skin sites (Fig. 1B). Importantly, TEWL correlated with severity of the lesions. Most patch lesions displayed minor increases in TEWL compared to non-lesion skin. In contrast, advanced lesions (tumor/erythroderma) exhibited much higher increase in TEWL, indicating a severely compromised barrier (Fig. 1B). Increased ΔTEWL values appeared to correlate with decreased expression of filaggrin (Fig. 1B) and filaggrin-2 (Suppl. Fig. S1B-C).

TOX is highly expressed in malignant T cells from MF patients and may be weakly expressed in some reactive non-malignant T cells[53–56]. We found TOX positive cells in lesional biopsies from all investigated patients whereas TOX expression was absent in healthy skin (Fig. 1C and Suppl. Fig S1B-D). Interestingly, strong epidermal staining for TOX (Fig. 1C and Suppl. Fig. S1B-D) was observed in patients with weak or absent filaggrin and filaggrin-2 staining. In contrast, in lesional skin displaying normal or near normal expression of filaggrins we did not detect TOX staining in the epidermis (patient MF5 and MF10). In these patients, positive TOX staining was observed in deeper layers of the dermis (Fig. 1C and Suppl. Fig. S1B). This suggests that the proximity between malignant T cells and epidermal keratinocytes might be associated with a deficient filaggrin expression. In support, increased numbers of TOX-positive cells in the epidermis were associated with both increased TEWL values and downregulated filaggrin (Fig. 1B and Suppl. Fig. S1B-C).

To test the hypothesis, we performed co-culture experiments mimicking the close interplay between keratinocytes and malignant T cells. A keratinocyte cell line (HaCaT) was cultured for 24h in the presence or absence of the malignant T-cell line MF2059 prior to quantification of filaggrin (FLG) and filaggrin-2 (FLG2) gene expression. As shown in Fig. 2A, FLG and FLG2 expression was

significantly decreased in the presence of malignant MF2059 T cells. In contrast, FLG and FLG2 expression was not repressed in co-cultures of HaCaT cells in the presence of the non-malignant MF1850 cell line. The gene encoding keratin protein 14 (KRT14), which is expressed in basal keratinocytes, was not affected by co-culture with either of the cell lines (Fig. 2A). To determine if cell-cell contact was required for the reduction of FLG and FLG2 expression, culture supernatants were collected from malignant T cell lines and added for 24h to cultures of HaCaT cells. Supernatants from malignant T cells significantly reduced the expression of FLG and FLG2 (Suppl. Fig. S2A). To validate these observations, normal human epidermal keratinocytes (NHEK) and reconstructed human epidermis (RHE) were incubated with supernatants from malignant and non-malignant T cell lines as above. mRNA expression of FLG and FLG2 was significantly reduced in NHEK cultured for 24h with supernatants from the malignant T cell lines MF2059, MF2000 and SeAx, but not from non-malignant MF1850 cells (Fig. 2B). Similar changes in mRNA expression were observed in RHE samples (Fig. 2C). Additionally, supernatants from malignant T cells also reduced protein expression of filaggrin and filaggrin-2 in stratum granulosum (Fig. 2D). The staining intensity and the number of keratinocytes positive for filaggrin and filaggrin-2 decreased, supporting the hypothesis that malignant T cells secrete soluble factors, which inhibit expression of filaggrins.

The JAK/STAT3 pathway plays a key role in the repression of filaggrin in chronic inflammatory skin conditions such as atopic dermatitis (AD)[57,58]. Thus, we examined whether repression of filaggrins is also mediated through STAT3 signalling in CTCL. Staining of RHE cultures for the activated form of STAT3 (pY-STAT3) showed that supernatants from malignant T cells induced profound pY-STAT3 expression in keratinocytes in RHE samples (Fig. 2D). Comparable results were observed using western blotting for pY-STAT3 in HaCaT cells and NHEK (Suppl. Fig. S2C-D).

Although constitutive activation of STAT3 has been implicated in malignant T cell proliferation, apoptosis resistance, and inflammation in CTCL, little is known about STAT3 activation in keratinocytes in lesional skin of CTCL patients[12]. As shown in Fig. 3, staining for pY-STAT3 in MF patients with deficient filaggrin expression (patients MF1 and MF2) demonstrated strong positive pY-STAT3 staining in epidermal layers of lesional skin, whereas non-lesional skin did not exhibit staining. Importantly, STAT3 activation was not only seen in lymphocytes with a neoplastic morphology (circles) but also in a large fraction of keratinocytes (arrows) as shown in close-up

micrographs of lesional skin. These findings demonstrate that STAT3 activation is indeed observed in keratinocytes in lesional skin with concomitant filaggrin deficiencies.

To address the role of JAK/STAT3 signalling in the repression of filaggrins in CTCL, we performed siRNA-mediated JAK1 knockdown in NHEK following incubation with supernatant from malignant T cells MF2059. Knockdown of JAK1 trigged an enhanced base-line expression of FLG and FLG2, when compared to non-targeting control siRNA (Fig. 4A), suggesting that JAK1 regulates endogenous level of filaggrins. As expected, malignant supernatant induced a profound inhibition of FLG and FLG2 expression, but had no effect on JAK1 expression, whereas JAK1 siRNA induced an almost complete knockdown of JAK1 (Fig. 4A). Importantly, the inhibition of FLG and FLG2 expression by the malignant T cell supernatant was largely blocked by siRNA mediated knockdown of JAK1, indicating that supernatant-induced repression of filaggrins was mediated via JAK1 (Fig. 4A). The effect was highly selective as siRNA-mediated knockdown of JAK2 had little effect (Fig. 4B). STAT3 knockdown almost completely blocked STAT3 expression and enhanced the basal level of FLG and FLG2 expression (Fig. 4C), which is in line with other findings showing that STAT-signalling regulates endogenous filaggrin expression in cultured keratinocytes[57]. Of note, knockdown of STAT3 partly blocked the effect of malignant supernatant on FLG and FLG2 expression (Fig. 4C), supporting the hypothesis that malignant T cells repress expression of filaggrins through a JAK1/STAT3 dependent pathway.

Several cytokines including IL-4, IL-6, IL-13, IL-22, IL-31, IFN-γ and oncostatin M (OSM) have been reported to induce filaggrin repression via JAK1/STAT3 signalling in keratinocytes[59]. High concentrations (> 2500 ng/mL) of IL-13, IL-22 and OSM and medium concentrations of IL-6 (~1000 ng/mL) were detected in supernatants from malignant T cells (Fig. 5A), confirming and extending that these cytokines are upregulated in MF[6,60,61]. Very low concentrations of the cytokines were measured in supernatants from non-malignant T cells. IL-4, IL-17A, IL-17F, IL-31 and IFN-γ were not detected in the supernatant from malignant T cells (Suppl. Fig. S3A). To evaluate the effect of IL-6, IL-13, IL-22 and OSM on FLG and FLG2 expression, keratinocytes were pre-treated with cytokine receptor blocking antibodies targeting: IL-6 and OSM (gp130: also blocking IL-11, LIF), IL-13 (IL-13Rα1 and Dupilumab) and IL-22 (IL-22Rα1). In combination, these receptor antibodies blocked supernatant-induced repression of FLG and FLG2 (Fig. 5B). In contrast, addition of the individual receptor blocking antibodies was not sufficient to fully block the FLG and FLG2 repression

induced by malignant supernatant (Suppl. Fig. S3B). This supports that in combination IL-6, IL-13, IL-22, and OSM may play a key role in the repression of filaggrins by malignant T cells.

To evaluate the physiological and clinical relevance of the cytokines investigated, we analysed four publicly available scRNA-seq datasets containing a total of 32 lesional skin samples (patch/plaque/tumour) from 21 MF patients and 8 healthy controls[50–52,62]. Cluster analysis showed a clear separation of various cell types, including T cells, dendritic cells, macrophages and keratinocytes (Fig. 6A-B). TCR clonality and expression of malignant-associated genes were used to distinguish between malignant and non-malignant T-cells. IL-13, IL-22, OSM and IL-4 were highly expressed in lesional skin biopsies from MF patients compared to healthy control skin (Fig. 6C-E). While we found large heterogeneity between patients as well as between different lesions from the same patient, IL-13, IL-22, OSM and IL-4 were highly expressed by malignant T cells in multiple patients (Fig. 6E). IFN-γ was produced in lesions of some patients, IL-17A/F was expressed by two of the 21 included patients whereas IL-31 was largely absent (Suppl. Fig. S4A-C). IL-6 was not higly expressed and primarily expressed by macrophages, fibroblasts and endothelial cells (Fig. 6C-E). TOX expression was higher and more frequent within malignant cells compared to non-malignant cells (Fig. 6F). Furthermore, malignant cells were more likely to produce IL-13, IL-22, OSM, IL-4 or IL-6 than their non-malignant counterparts from the same biopsies (Fig. 6G). Taken together, these data confirm that the investigated cytokines, especially IL-13, IL-22, OSM and IL-4 are highly expressed in lesional skin from MF patients and expressed by malignant T cells and to some extent by other cells in the TME.

Given our findings that JAK1 knockdown blocked cytokine-mediated repression of filaggrins in keratinocytes, we hypothesized that JAK inhibitors may also block the effect of malignant T cell supernatants. Accordingly, RHE samples were treated with 1μM of the pan-JAK inhibitor, Tofacitinib, and cultured with supernatant from malignant MF2059 T cells. Importantly, supernatant from malignant cells induced a profound downregulation in FLG and FLG2 mRNA expression in RHE that was reversed in the presence of Tofacitinib (Fig. 7A). The effect of JAK inhibition was also observed for NHEK treated with supernatants from both malignant MF2059 and MF2000 cells and was shown to be dependent on the JAK inhibitor concentration (Suppl. Fig. S5A-B). Furthermore, the effects of JAK inhibition were confirmed using another JAK inhibitor (Abrocitinib, 0.5-1 μM) which is more JAK1-selective as compared to Tofacitinib (Suppl. Fig. S5C-D). IHC analysis of RHE samples cultured with malignant supernatants showed reduced protein expression of filaggrin and

filaggrin-2 - an effect, which was strongly inhibited by Tofacitinib when compared to control cultures (Fig. 7B). The skin barrier of RHE became functionally compromised after incubation with malignant supernatants as judged by an increased permeability of Lucifer yellow (Suppl. Fig. S5E). Importantly, Tofacitinib, which had little effect on its own, seemed to block the effect of malignant supernatant on barrier permeability in RHE (Suppl. Fig. S5E). To evaluate the effect of Tofacitinib in MF skin, we cultured biopsies from lesional skin of MF patients *ex vivo* in culture medium in the presence or absence of 1μM Tofacitinib. As illustrated in Fig 7C, FLG and FLG2 expression increased in the lesional skin biopsies cultured with Tofacitinib, highlighting the potential of JAK inhibitors to reverse deficient FLG and FLG2 expression in MF skin of patients.

Discussion

Skin lesions are characteristic manifestations of T cell lymphomas and play an important role in the symptomatology and secondary bacterial colonisation, infections, and comorbidity in CTCL[2]. Yet, relatively little is known about the pathological processes that drive the disease-associated skin changes. Here, we provide first evidence that malignant T cells, through secretion of cytokines (IL-13, IL-22, OSM and potentially IL-6) and JAK1/STAT3 signalling, induce transcriptional repression of FLG and FLG2 in keratinocytes. Thus, supernatants from malignant T cells triggered an enhanced STAT3 activation and a decreased expression of filaggrins in human keratinocytes and in a human skin equivalent 3D model. Importantly, repression of filaggrins was strongly inhibited by a cocktail of relevant cytokine-receptor blocking antibodies and by knockdown of JAK1 and STAT3. Data from scRNA-seq showed that malignant T cells in lesional skin expressed IL-13, IL-22, OSM confirming the pathological relevance of the cytokines identified in supernatants of malignant T cell lines. Interestingly, a fraction of non-malignant T cells derived from lesional skin also expressed IL-13 and IL-22, and IFN-γ and OSM suggesting that other cell types may also contribute to filaggrin repression in skin lesions.

It is well-established that CTCL is a highly heterogeneous disease displaying large differences in gene expression between malignant cells from different individuals and even between different subpopulations of the malignant T cell clone in each individual patient[63,64]. Therefore, it was not surprising that the relative abundance of malignant T cells producing the cytokines in question differed between patients. IL-13 is the dominating cytokine driving repression of filaggrins in AD and our scRNA-seq analysis displayed high expression of IL-13 in lesional skin in several patients. This suggests that IL-13 may also play an important role in skin barrier defects in CTCL. In support, blocking IL-13 partially inhibited the effect of malignant supernatants. However, blockage of other cytokines was also needed to fully negate the effect of malignant supernatants. This supports the notion that multiple cytokines - in concert - repress the skin barrier proteins. We cannot exclude that additional cytokines (such as IL-17[59]) may also contribute to the repression of filaggrins but only two of the analysed 21 MF patients displayed significant expression of IL-17A/F suggesting that IL-17 may not play a general role in repression of filaggrins in CTCL. Although IL-4 was not detected in the supernatants from malignant T cells it was produced by a fraction of malignant T cells from lesional skin samples consistent with previous reports[65]. This suggests that IL-4 - like IL-13 - may contribute to filaggrin repression in CTCL. Interestingly, targeting IL-4 and IL-13 signalling by

Dupilumab rapidly inhibited itch and the TH2 bias in a SS patient[66], which may be due to an improvement of the skin barrier following IL-4/IL-13 blockage as hypothesized in the present study. As Dupilumab may unmask or promote CTCL in AD[67,68], it is uncertain whether IL-4/IL-13 blockage and, in turn, JAK1 inhibition comes with a risk in CTCL patients. On the other hand, disease aggravation was not reported in a clinical phase 2 study on Ruxolitinib, a JAK1/JAK2 inhibitor[69,70].

TOX is an important, but not an exclusive marker for malignant T cells, as it can also be expressed at lower levels in reactive T cells[53,55]. However, there is an overall correlation between high TOX expression and accumulation of malignant T cells in CTCL[53,54,56]. Indeed, scRNA-seq analysis showed that malignant cells accounted for the vast majority of TOX transcripts. Moreover, malignant cells exhibited a consistently higher percentage of TOX-positive cells than their benign T cell counterparts within the same biopsies. Coupled with the overall higher abundance of malignant cells in most biopsies, the TOX-positive cells in the histological stainings are much more likely to represent malignant than benign T cells. Similarly, malignant cells were much more likely to be cytokine-positive (IL-4, IL-6, IL-13, IL-22, OSM) than their benign counterparts. Thus, our observations suggest that a high frequency of TOX positive malignant cells in the epidermis correlates with a deficient filaggrin barrier. Interestingly, we only observed deficient filaggrin expression in lesions where TOX-positive cells were located in the epidermis, whereas the skin barrier was unaffected in lesions where epidermal TOX-positive cells were absent - despite large clusters of TOX positive cells in the dermis. This may indicate that the basal membrane could function as a diffusion-barrier for cytokines from TOX-positive cells.

Our data suggest that local skin defects were driven by cytokine-producing malignant T cells and other cells in the TME. In addition to cytokines, malignant T cells release galectins, which may enhance proliferation of keratinocytes[47]. Of note, malignant T cells may also regulate other cells in the TME, which in turn stimulate T cells, supporting the concept of malignant inflammation[8,71–73].

Bacteria pose an important clinical problem in CTCL[38,74]. In advanced disease, the majority of patients harbour enterotoxin-producing *S. aureus* and more than half of the patients die from infection rather than the cancer *per se*[74]. A compromised skin barrier is an important point for bacterial entry and recent evidence indicates that SE-producing *S. aureus* drive changes in the TME that fuel proliferation of malignant T cells and disease activity[19,21,43,46,75]. Thus, novel treatments are warranted

that could heal the skin lesions. JAK inhibitors decrease severity of symptoms and improve epidermal barrier markers in AD patients and are also used for treating vitiligo, alopecia areata and rheumatoid arthritis[57,58,76,77]. Accordingly, we examined the effect of JAK inhibitors and demonstrated that Tofacitinib reversed the repression of filaggrins and decreased skin permeability in an epidermal model and enhanced filaggrin expression in freshly isolated specimens from MF skin lesions. These findings suggest that JAK inhibitors have the potential to improve skin lesions in CTCL patients. As Tofacitinib inhibits ectopic expression of onco-microRNA-155 and IL-2Rg-driven proliferation in primary malignant T cells[20], it may have the potential to simultaneously target multiple disease mechanisms in CTCL (in addition to an effect on skin barrier defects as shown here).

As anti-tumor immune responses are important in early-stage MF[78], JAK inhibition may pose a risk related to a weakened anti-tumor defence. Our findings indicate that structural and functional skin barrier defects were predominately seen in plaque, tumor or erythrodermic lesions whereas the skin barrier was relatively structurally and functionally intact in patch lesions. We therefore propose that therapeutic intervention with JAK inhibition is most relevant in more advanced disease, where skin defects are most pronounced and anti-tumor immunity already seems to have failed.

In conclusion, we provide evidence for the existence of cytokine-mediated crosstalk between malignant T cells and keratinocytes leading to a marked reduction in skin barrier proteins in CTCL. Moreover, our data demonstrate that JAK1 inhibitors largely abrogate the negative effect of malignant T cells on skin barrier proteins, suggesting that they provide novel treatment options for CTCL patients with compromised skin.

Acknowledgment

This research was funded by LEO Foundation through the LEO Foundation Skin Immunology Research Center and LEO Foundation Grant (LF-OC-20-000351) to SBK, The Danish Cancer Society (Kræftens Bekæmpelse), the Fight Cancer Program (Knæk Cancer), Novo Nordisk Research Foundation, Novo Nordisk Foundation Tandem Program (grant number NNF21OC0066950), Lundbeck Foundation (AWO), and The Danish Council for Independent Research (Danmarks Frie Forskningsfond, 2 project grants for N.Ø., a Sapera Aude Talent Grant [DFF-4092-00122] for T.K.). LINAK A/S, Nordborg, Aage Bangs Foundation. Role of the funding source: The funding source had no influence on the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

K. Rindler, P. M. Brunner, X. Song and P-L. Chen graciously assisted in the annotation and elaboration of their respective scRNA-seq datasets.

Author contributions

Concept and design: MG and NØ. *Acquisition, analysis, or interpretation of data*: All authors. *Drafting of the manuscript*: MG and NØ. *Critical revision of the manuscript for important intellectual content*: All authors. *Obtained funding*: NØ. *Administrative, technical, or material support*: EMP, TBB, LMRG, LML, MB, MD, RB, CKV, AL-V, MRK, SBK, LI, TL, AW. *Supervision*: NØ.

Conflicts of interest

Niels Ødum has received consulting honoraria from Mindera corp, Micreos Human Health, PS Consulting, and Almirall. Jürgen C. Becker is receiving speaker's bureau honoraria from Amgen, Pfizer, MerckSerono, Recordati and Sanofi, is a paid consultant/advisory board member for Boehringer Ingelheim, eTheRNA, InProTher, MerckSerono, Pfizer, 4SC, Regeneron and Sanofi. His group receives research grants from Bristol-Myers Squibb, Merck Serono, HTG, IQVIA, and Alcedis. Lise Mette Rahbek Gjerdrum receives funding from NanoString Technologies. Thomas Litman is funded by LEO Pharma. Lars Iversen Served as a consultant and/or paid speaker for and/or participated in clinical trials sponsored by: AbbVie, Almirall, Amgen, Astra Zeneca, BMS, Boehringer Ingelheim, Celgene, Centocor, Eli Lilly, Janssen Cilag, Kyowa, Leo Pharma, Micreos Human Health, MSD, Novartis, Pfizer, Regranion, Samsung, Union Therapeutics, UCB. All other authors declare no potential conflicts of interest.

References

1. Wilcox RA. Cutaneous T-cell lymphoma: 2017 update on diagnosis, risk-stratification, and management. *American Journal of Hematology*. 2017;92(10):1085-1102. doi:10.1002/ajh.24876
2. Hristov AC, Tejasvi T, Wilcox RA. Mycosis fungoides and Sézary syndrome: 2019 update on diagnosis, risk-stratification, and management. *Am J Hematol*. 2019;94(9):1027-1041. doi:10.1002/ajh.25577
3. The 2018 update of the WHO-EORTC classification for primary cutaneous lymphomas. Accessed January 16, 2021. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6473500/
4. Mehta-Shah N, Horwitz SM, Ansell S, et al. NCCN Guidelines Insights: Primary Cutaneous Lymphomas, Version 2.2020. *J Natl Compr Canc Netw*. 2020;18(5):522-536. doi:10.6004/jnccn.2020.0022
5. Raghavan SS, Kim J. Histopathologic approach to epidermotropic lymphocytic infiltrates. *Seminars in Cutaneous Medicine and Surgery*. 2018;37(1):56-60. doi:10.12788/j.sder.2018.003
6. Geskin LJ, Viragova S, Stolz DB, Fuschiotti P. Interleukin-13 is overexpressed in cutaneous T-cell lymphoma cells and regulates their proliferation. *Blood*. 2015;125(18):2798-2805. doi:10.1182/blood-2014-07-590398
7. Kim EJ, Hess S, Richardson SK, et al. Immunopathogenesis and therapy of cutaneous T cell lymphoma. *J Clin Invest*. 2005;115(4):798-812. doi:10.1172/JCI200524826
8. Krejsgaard T, Lindahl LM, Mongan NP, et al. Malignant inflammation in cutaneous T-cell lymphoma—a hostile takeover. *Semin Immunopathol*. 2017;39(3):269-282. doi:10.1007/s00281-016-0594-9
9. Querfeld C, Zain J, Rosen ST. Primary Cutaneous T-Cell Lymphomas: Mycosis Fungoides and Sezary Syndrome. *Cancer Treat Res*. 2019;176:225-248. doi:10.1007/978-3-319-99716-2_11
10. Odum N, Lindahl LM, Wod M, et al. Investigating heredity in cutaneous T-cell lymphoma in a unique cohort of Danish twins. *Blood Cancer Journal*. 2017;7(1):e517. doi:10.1038/bcj.2016.128
11. Yumeen S, Girardi M. Insights Into the Molecular and Cellular Underpinnings of Cutaneous T Cell Lymphoma. *Yale J Biol Med*. 2020;93(1):111-121.
12. Netchiporouk E, Litvinov IV, Moreau L, Gilbert M, Sasseville D, Duvic M. Deregulation in STAT signaling is important for cutaneous T-cell lymphoma (CTCL) pathogenesis and cancer progression. *Cell Cycle*. 2014;13(21):3331-3335. doi:10.4161/15384101.2014.965061
13. Seffens A, Herrera A, Tegla C, et al. STAT3 Dysregulation in Mature T and NK Cell Lymphomas. *Cancers (Basel)*. 2019;11(11). doi:10.3390/cancers11111711
14. Kiel MJ, Sahasrabuddhe AA, Rolland DCM, et al. Genomic analyses reveal recurrent mutations in epigenetic modifiers and the JAK–STAT pathway in Sézary syndrome. *Nature Communications*. 2015;6:8470. doi:10.1038/ncomms9470
15. Pérez C, Mondéjar R, García-Díaz N, et al. Advanced-stage mycosis fungoides: role of the signal transducer and activator of transcription 3, nuclear factor-κB and nuclear factor of activated T cells pathways. *British Journal of Dermatology*. 2020;182(1):147-155. doi:10.1111/bjd.18098
16. Tensen CP, Quint KD, Vermeer MH. Genetic and epigenetic insights into cutaneous T-cell lymphoma. *Blood*. 2022;139(1):15-33. doi:10.1182/blood.2019004256
17. Bastidas Torres AN, Cats D, Mei H, et al. Genomic analysis reveals recurrent deletion of JAK-STAT signaling inhibitors HNRNPK and SOCS1 in mycosis fungoides. *Genes Chromosomes Cancer*. 2018;57(12):653-664. doi:10.1002/gcc.22679
18. Fanok MH, Sun A, Fogli LK, et al. Role of Dysregulated Cytokine Signaling and Bacterial Triggers in the Pathogenesis of Cutaneous T-Cell Lymphoma. *J Invest Dermatol*. Published

online November 8, 2017. doi:10.1016/j.jid.2017.10.028
19. Lindahl LM, Willerslev-Olsen A, Gjerdrum LMR, et al. Antibiotics inhibit tumor and disease activity in cutaneous T-cell lymphoma. *Blood*. 2019;134(13):1072-1083. doi:10.1182/blood.2018888107
20. Kopp KL, Ralfkiaer U, Gjerdrum LMR, et al. STAT5-mediated expression of oncogenic miR-155 in cutaneous T-cell lymphoma. *Cell Cycle*. 2013;12(12):1939-1947. doi:10.4161/cc.24987
21. Willerslev-Olsen A, Krejsgaard T, Lindahl LM, et al. Staphylococcus aureus enterotoxin A (SEA) stimulates STAT3 activation and IL-17 expression in cutaneous T-cell lymphoma. *Blood*. Published online January 1, 2016:blood-2015-08-662353. doi:10.1182/blood-2015-08-662353
22. Litvinov IV, Shtreis A, Kobayashi K, et al. Investigating potential exogenous tumor initiating and promoting factors for Cutaneous T-Cell Lymphomas (CTCL), a rare skin malignancy. *Oncoimmunology*. 2016;5(7):e1175799. doi:10.1080/2162402X.2016.1175799
23. Ghazawi FM, Alghazawi N, Le M, et al. Environmental and Other Extrinsic Risk Factors Contributing to the Pathogenesis of Cutaneous T Cell Lymphoma (CTCL). *Front Oncol*. 2019;9. doi:10.3389/fonc.2019.00300
24. Querfeld C, Leung S, Myskowski PL, et al. Primary T Cells from Cutaneous T-cell Lymphoma Skin Explants Display an Exhausted Immune Checkpoint Profile. *Cancer Immunol Res*. 2018;6(8):900-909. doi:10.1158/2326-6066.CIR-17-0270
25. Durgin JS, Weiner DM, Wysocka M, Rook AH. The Immunopathogenesis and Immunotherapy of Cutaneous T Cell Lymphoma: Part I, Pathways and Targets for Immune Restoration and Tumor Eradication. *J Am Acad Dermatol*. Published online December 19, 2020. doi:10.1016/j.jaad.2020.12.027
26. Shalabi D, Bistline A, Alpdogan O, et al. Immune evasion and current immunotherapy strategies in mycosis fungoides (MF) and Sézary syndrome (SS). *Chin Clin Oncol*. 2019;8(1):11. doi:10.21037/cco.2019.01.01
27. Hodak E, Amitay-Laish I. Mycosis fungoides: A great imitator. *Clin Dermatol*. 2019;37(3):255-267. doi:10.1016/j.clindermatol.2019.01.004
28. Scarisbrick JJ, Bagot M, Ortiz-Romero PL. The changing therapeutic landscape, burden of disease, and unmet needs in patients with cutaneous T-cell lymphoma. *Br J Haematol*. Published online October 23, 2020. doi:10.1111/bjh.17117
29. Nestle FO, Di Meglio P, Qin JZ, Nickoloff BJ. Skin immune sentinels in health and disease. *Nature Reviews Immunology*. 2009;9(10):679-691. doi:10.1038/nri2622
30. Sandilands A, Sutherland C, Irvine AD, McLean WHI. Filaggrin in the frontline: role in skin barrier function and disease. *J Cell Sci*. 2009;122(9):1285-1294. doi:10.1242/jcs.033969
31. Pendaries V, Lamer ML, Cau L, et al. In a three-dimensional reconstructed human epidermis filaggrin-2 is essential for proper cornification. *Cell Death & Disease*. 2015;6(2):e1656. doi:10.1038/cddis.2015.29
32. Mohamad J, Sarig O, Godsel LM, et al. Filaggrin 2 Deficiency Results in Abnormal Cell-Cell Adhesion in the Cornified Cell Layers and Causes Peeling Skin Syndrome Type A. *Journal of Investigative Dermatology*. doi:10.1016/j.jid.2018.04.032
33. Pendaries V, Malaisse J, Pellerin L, et al. Knockdown of Filaggrin in a Three-Dimensional Reconstructed Human Epidermis Impairs Keratinocyte Differentiation. *J Invest Dermatol*. 2014;134(12):2938-2946. doi:10.1038/jid.2014.259
34. Kezic S, Kemperman PMJH, Koster ES, et al. Loss-of-Function Mutations in the Filaggrin Gene Lead to Reduced Level of Natural Moisturizing Factor in the Stratum Corneum. *J Invest Dermatol*. 2008;128(8):2117-2119. doi:10.1038/jid.2008.29

35. Trzeciak M, Olszewska B, Sakowicz-Burkiewicz M, et al. Expression Profiles of Genes Encoding Cornified Envelope Proteins in Atopic Dermatitis and Cutaneous T-Cell Lymphomas. *Nutrients*. 2020;12(3). doi:10.3390/nu12030862
36. Suga H, Sugaya M, Miyagaki T, et al. Skin barrier dysfunction and low antimicrobial peptide expression in cutaneous T-cell lymphoma. *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research*. 2014;20(16):4339-4348. doi:10.1158/1078-0432.CCR-14-0077
37. Willerslev-Olsen A, Krejsgaard T, Lindahl LM, et al. Bacterial Toxins Fuel Disease Progression in Cutaneous T-Cell Lymphoma. *Toxins (Basel)*. 2013;5(8):1402-1421. doi:10.3390/toxins5081402
38. Axelrod PI, Lorber B, Vonderheid EC. Infections complicating mycosis fungoides and Sézary syndrome. *JAMA*. 1992;267(10):1354-1358.
39. Talpur R, Bassett R, Duvic M. Prevalence and treatment of Staphylococcus aureus colonization in patients with mycosis fungoides and Sézary syndrome. *Br J Dermatol*. 2008;159(1):105-112. doi:10.1111/j.1365-2133.2008.08612.x
40. Jackow CM, Cather JC, Hearne V, Asano AT, Musser JM, Duvic M. Association of erythrodermic cutaneous T-cell lymphoma, superantigen-positive Staphylococcus aureus, and oligoclonal T-cell receptor V beta gene expansion. *Blood*. 1997;89(1):32-40.
41. Mirvish ED, Pomerantz RG, Geskin LJ. Infectious agents in cutaneous T-cell lymphoma. *J Am Acad Dermatol*. 2011;64(2):423-431. doi:10.1016/j.jaad.2009.11.692
42. Dehner CA, Ruff WE, Greiling T, et al. MALIGNANT T CELL ACTIVATION BY A BACILLUS SPECIES ISOLATED FROM CUTANEOUS T CELL LYMPHOMA LESIONS. *JID Innovations*. Published online December 16, 2021:100084. doi:10.1016/j.xjidi.2021.100084
43. Emge DA, Bassett RL, Duvic M, Huen AO. Methicillin-resistant Staphylococcus aureus (MRSA) is an important pathogen in erythrodermic cutaneous T-cell lymphoma (CTCL) patients. *Archives of Dermatological Research*. 2020;312(4):283-288. doi:10.1007/s00403-019-02015-7
44. Kadin ME, Hamilton RG, Vonderheid EC. Evidence linking atopy and staphylococcal superantigens to the pathogenesis of lymphomatoid papulosis, a recurrent CD30+ cutaneous lymphoproliferative disorder. *PLoS One*. 2020;15(2):e0228751. doi:10.1371/journal.pone.0228751
45. Tokura Y, Yagi H, Ohshima A, et al. Cutaneous colonization with staphylococci influences the disease activity of Sézary syndrome: a potential role for bacterial superantigens. *Br J Dermatol*. 1995;133(1):6-12. doi:10.1111/j.1365-2133.1995.tb02485.x
46. Woetmann A, Lovato P, Eriksen KW, et al. Nonmalignant T cells stimulate growth of T-cell lymphoma cells in the presence of bacterial toxins. *Blood*. 2007;109(8):3325-3332. doi:10.1182/blood-2006-04-017863
47. Thode C, Woetmann A, Wandall HH, et al. Malignant T Cells Secrete Galectins and Induce Epidermal Hyperproliferation and Disorganized Stratification in a Skin Model of Cutaneous T-Cell Lymphoma. *J Invest Dermatol*. 2015;135(1):238-246. doi:10.1038/jid.2014.284
48. Gluud M. MicroRNA-93 targets p21 and promotes proliferation in Mycosis Fungoides T cells. *Dermatology*. Published online in press.
49. Fredholm S, Willerslev-Olsen A, Met Ö, et al. Special AT rich-binding1 protein (SATB1) in malignant T cells. *J Invest Dermatol*. Published online May 8, 2018. doi:10.1016/j.jid.2018.03.1526
50. Rindler K, Bauer WM, Jonak C, et al. Single-Cell RNA Sequencing Reveals Tissue Compartment-Specific Plasticity of Mycosis Fungoides Tumor Cells. *Front Immunol*. 2021;12:666935. doi:10.3389/fimmu.2021.666935
51. Gaydosik AM, Tabib T, Geskin LJ, et al. Single-Cell Lymphocyte Heterogeneity in

Advanced Cutaneous T-cell Lymphoma Skin Tumors. *Clin Cancer Res*. 2019;25(14):4443-4454. doi:10.1158/1078-0432.CCR-19-0148
52. Song X, Chang S, Seminario-Vidal L, et al. Genomic and single-cell landscape reveals novel drivers and therapeutic vulnerabilities of transformed cutaneous T-cell lymphoma. *Cancer Discov*. Published online February 28, 2022:candisc.1207.2021. doi:10.1158/2159-8290.CD-21-1207
53. McGirt LY, Degesys CA, Johnson VE, Zic JA, Zwerner JP, Eischen CM. TOX expression and role in CTCL. *J Eur Acad Dermatol Venereol*. 2016;30(9):1497-1502. doi:10.1111/jdv.13651
54. Huang Y, Litvinov IV, Wang Y, et al. Thymocyte selection-associated high mobility group box gene (TOX) is aberrantly over-expressed in mycosis fungoides and correlates with poor prognosis. *Oncotarget*. 2014;5(12):4418-4425. doi:10.18632/oncotarget.2031
55. Schrader AMR, Jansen PM, Willemze R. TOX expression in cutaneous T-cell lymphomas: an adjunctive diagnostic marker that is not tumour specific and not restricted to the CD4(+) CD8(-) phenotype. *Br J Dermatol*. 2016;175(2):382-386. doi:10.1111/bjd.14508
56. Nielsen PR, Eriksen JO, Lindahl LM, et al. Diagnostic Two-Gene Classifier in Early-Stage Mycosis Fungoides: A Retrospective Multicenter Study. *Journal of Investigative Dermatology*. 2021;141(1):213-217.e5. doi:10.1016/j.jid.2020.04.026
57. Amano A, Amano W. The Janus kinase inhibitor JTE-052 improves skin barrier function through suppressing signal transducer and activator of transcription 3 signaling. *Journal of Allergy and Clinical Immunology*. 20150901;136(3):667-677.
58. Nakagawa H, Nemoto O, Igarashi A, Nagata T. Efficacy and safety of topical JTE-052, a Janus kinase inhibitor, in Japanese adult patients with moderate-to-severe atopic dermatitis: a phase II, multicentre, randomized, vehicle-controlled clinical study. *Br J Dermatol*. 2018;178(2):424-432. doi:10.1111/bjd.16014
59. Hänel KH, Cornelissen C, Lüscher B, Baron JM. Cytokines and the Skin Barrier. *International Journal of Molecular Sciences*. 2013;14(4):6720-6745. doi:10.3390/ijms14046720
60. Miyagaki T, Sugaya M, Suga H, et al. IL-22, but Not IL-17, Dominant Environment in Cutaneous T-cell Lymphoma. *Clin Cancer Res*. 2011;17(24):7529-7538. doi:10.1158/1078-0432.CCR-11-1192
61. Lawlor F, Smith NP, Camp RD, et al. Skin exudate levels of interleukin 6, interleukin 1 and other cytokines in mycosis fungoides. *Br J Dermatol*. 1990;123(3):297-304. doi:10.1111/j.1365-2133.1990.tb06288.x
62. Rindler K, Jonak C, Alkon N, et al. Single-cell RNA sequencing reveals markers of disease progression in primary cutaneous T-cell lymphoma. *Mol Cancer*. 2021;20(1):124. doi:10.1186/s12943-021-01419-2
63. Buus TB, Willerslev-Olsen A, Fredholm S, et al. Single-cell heterogeneity in Sézary syndrome. *Blood Adv*. 2018;2(16):2115-2126. doi:10.1182/bloodadvances.2018022608
64. Litvinov IV, Tetzlaff MT, Thibault P, et al. Gene expression analysis in Cutaneous T-Cell Lymphomas (CTCL) highlights disease heterogeneity and potential diagnostic and prognostic indicators. *Oncoimmunology*. 2017;6(5):e1306618. doi:10.1080/2162402X.2017.1306618
65. Vowels BR, Lessin SR, Cassin M, et al. Th2 cytokine mRNA expression in skin in cutaneous T-cell lymphoma. *J Invest Dermatol*. 1994;103(5):669-673.
66. Steck O, Bertschi NL, Luther F, et al. Rapid and sustained control of itch and reduction in Th2 bias by dupilumab in a patient with Sézary syndrome. *J Eur Acad Dermatol Venereol*. 2021;35(6):1331-1337. doi:10.1111/jdv.17001
67. Espinosa ML, Nguyen MT, Aguirre AS, et al. Progression of cutaneous T-cell lymphoma after dupilumab: Case review of 7 patients. *J Am Acad Dermatol*. 2020;83(1):197-199.

doi:10.1016/j.jaad.2020.03.050
68. Poyner EFM, Bacon CM, Osborne W, Frew JA, Weatherhead SC. Dupilumab unmasking cutaneous T-cell lymphoma: report of a fatal case. *Clin Exp Dermatol*. 2022;47(5):974-976. doi:10.1111/ced.15079
69. Moskowitz AJ, Ghione P, Jacobsen ED, et al. Final Results of a Phase II Biomarker-Driven Study of Ruxolitinib in Relapsed and Refractory T-Cell Lymphoma. *Blood*. 2019;134(Supplement_1):4019-4019. doi:10.1182/blood-2019-125017
70. Moskowitz AJ, Ghione P, Jacobsen E, et al. A phase 2 biomarker-driven study of ruxolitinib demonstrates effectiveness of JAK/STAT targeting in T-cell lymphomas. *Blood*. 2021;138(26):2828-2837. doi:10.1182/blood.2021013379
71. Aronovich A, Moyal L, Gorovitz B, et al. Cancer-Associated Fibroblasts in Mycosis Fungoides Promote Tumor Cell Migration and Drug Resistance through CXCL12/CXCR4. *J Invest Dermatol*. 2021;141(3):619-627.e2. doi:10.1016/j.jid.2020.06.034
72. Takahashi N, Sugaya M, Suga H, et al. Thymic Stromal Chemokine TSLP Acts through Th2 Cytokine Production to Induce Cutaneous T-cell Lymphoma. *Cancer Res*. 2016;76(21):6241-6252. doi:10.1158/0008-5472.CAN-16-0992
73. Mehdi SJ, Moerman-Herzog A, Wong HK. Normal and cancer fibroblasts differentially regulate TWIST1, TOX and cytokine gene expression in cutaneous T-cell lymphoma. *BMC Cancer*. 2021;21(1):492. doi:10.1186/s12885-021-08142-7
74. Posner LE, Fossieck BE, Eddy JL, Bunn PA. Septicemic complications of the cutaneous T-cell lymphomas. *Am J Med*. 1981;71(2):210-216.
75. Krejsgaard T, Willerslev-Olsen A, Lindahl LM, et al. Staphylococcal enterotoxins stimulate lymphoma-associated immune dysregulation. *Blood*. 2014;124(5):761-770. doi:10.1182/blood-2014-01-551184
76. He H, Guttman-Yassky E. JAK Inhibitors for Atopic Dermatitis: An Update. *Am J Clin Dermatol*. 2019;20(2):181-192. doi:10.1007/s40257-018-0413-2
77. Luo Y, Alexander M, Gadina M, O'Shea JJ, Meylan F, Schwartz DM. JAK-STAT signaling in human disease: From genetic syndromes to clinical inhibition. *Journal of Allergy and Clinical Immunology*. 2021;148(4):911-925. doi:10.1016/j.jaci.2021.08.004
78. Vieyra-Garcia P, Crouch JD, O'Malley JT, et al. Benign T cells drive clinical skin inflammation in cutaneous T cell lymphoma. *JCI Insight*. 4(1). doi:10.1172/jci.insight.124233

Figure legends

Figure 1: Filaggrin and filaggrin-2 protein expression is reduced in lesional MF skin, transepidermal water loss is increased and epidermal immune infiltrates correlate with the reduced expression levels. A: Lesional- and non-lesional skin biopsies obtained from a MF patient (MF1) as well as a biopsy from a healthy donor were stained for H&E, filaggrin and filaggrin-2. The arrowheads highlight areas of deficient filaggrin and filaggrin-2 protein expression. B (left): TEWL ($g/m^2/h$) measurements were performed for anatomically matched lesional and non-lesional sites on 12 patients with either patches, plaques or advanced leasions (tumor/erythroderma). Some patients had multiple lesions that were analysed. B (right): Lesional skin biopsies from three MF patients and one SS pateint (MF5, MF6, MF7, SS1) and non-lesional biopsy from patient MF5 were stained for filaggrin. Micrograph close up for MF6 illustrates areas of lack of filaggrin expression. ΔTEWL values (subtracting non-lesional from lesional values) were given for each of the patients. C: Lesional skin site biopsies obtained from three MF patients (MF8, MF9, MF10) as well as a biopsy from a healthy donor were stained for filaggrin, filaggrin-2 and TOX. Areas of positive TOX staining in the epidermis/dermis are highlighted by black circles. Images are scanned by the Zeiss Axio Scan.Z1 in 20x.

Figure 2: Malignant T cells induce alterations in the epidermis. A-C: mRNA expression was analysed by qPCR using β-actin as a reference gene. Gene expression was given as relative expression. A: HaCaT cells were cultured for 24h with the non-malignant MF1850 or the malignant MF2059 T-cell line. B: NHEK were cultured for 24h with supernatants obtained from the T-cell lines MF1850, MF2059, MF2000 and SeAx. C: RHE samples were cultured in the presence of supernatants for 24h. D: Protein levels were analysed by IHC for RHE samples cultured for 48h with supernatants from the malignant T-cell line MF2059. The samples were stained for H&E, filaggrin, filaggrin-2 and pY-STAT3. n=3. (*) indicates statistical significance ($P<0.05$).

Figure 3: pY-STAT3 is induced in lesional MF skin. Lesional- and non-lesional skin site biopsies obtained from two MF patients (MF1 and MF2) donors were stained for pY-STAT3. Micrograph close ups illustrate areas with pY-STAT3 positive keratinocytes (arrows) close to areas with immune-infiltrates, potentially pY-STAT3 positive malignant T cells (circles).

Figure 4: JAK1 and pY-STAT3 knockdown blocks tumour cell induced repression of FLG and FLG2 in the epidermis. A-C: NHEK cells were transfected with siRNA targeting either JAK1, JAK2, STAT3 or a non-targeting siRNA control. Following 48h, samples were cultured for 24h with supernatant from the malignant MF2059 T-cell line. mRNA expression was analysed by qPCR using β-actin as a reference gene. Gene expression was given as relative expressions. A: mRNA expression of FLG, FLG2 and JAK1 was analysed following siRNA mediated knockdown of JAK1 in the presence or absence of supernatant from MF2059. B: mRNA expression of FLG, FLG2 and JAK2 (B) or STAT3 (C) was analysed following siRNA mediated knockdown of JAK2 (B) or STAT3 (C) in the presence or absence of supernatant from MF2059. n=3. (*) indicates statistical significance ($P<0.05$).

Figure 5: Malignant T cells produce cytokines that induce alteration of FLG1 and FLG2 in the epidermis. A: Supernatants obtained from MF1850, MF2059, MF2000 or cells, which had been cultured for 24h, were analysed for the cytokines IL-6, IL-13 IL-22 and OSM by ELISA. Concentrations were given as pg cytokine/mL supernatant. B: NHEK were cultured for 2h in the presence or absence of receptor blocking antibodies; IL-13Rα1 and Dupilumab (IL-13), gp130 (IL-6 and OSM) and IL22Rα1 (IL-22) to block cytokine signalling. NHEK were then cultured for 24h with supernatants obtained from MF2059. mRNA expression levels were analysed by qPCR using β-actin as a reference gene. Gene expression was given as relative expressions. n=3 for MF2059 and MF1850 and 1 for MF2000. (*) indicates statistical significance ($P<0.05$).

Figure 6: IL-13, IL-22, OSM and IL-4 are expressed in lesional skin from MF patients. scRNA-seq samples from 8 healthy controls (HC) and 32 Mycosis Fungoides (MF) biopsies across 4 publicly available studies[50–52,62] were integrated and batch corrected using SCVI. A, B: Cell type annotation visualized on Uniform Manifold Approximation and Projection (UMAP) showing distinct clustering of major cell types annotated by expression of lineage markers. C: Quantification of IL-13, IL-22, OSM, IL-4 and IL-6 transcripts across each sample divided into HC and MF. Values denote number of unique cytokine transcripts per million total transcripts (tpm). D: UMAP visualization of which cell clusters express each cytokine. Due to sparsity of the transcripts, positive cells are plotted on top. E: Quantification of IL-13, IL-22, OSM, IL-4 IL-6 and TOX transcripts across cell clusters within each sample. Bars depicting samples with extreme expression have been truncated to allow visualization together with samples with lower expression levels. Truncation is indicated by dashed lines and numbers in bars denote tpm for the given bar at full height. Grey areas mark

HC samples. Samples were ordered by study and disease status (HC vs. MF), lesion type and MF stage as shown in the bottom. Multiple biopsies across multiple lesion types are included for some patients and can be identified by the sample names below the plot. F, G: Fraction of cells expressing at least one transcript of TOX (F) or IL-13, IL-22, OSM, IL-4 or IL-6 (G) within T cells from healthy controls (HC), benign T cells or malignant cells from MF biopsies. Lines link values from matched benign T cells and malignant cells from the same sample.

Figure 7: JAK inhibition blocks tumour cell induced reduction of FLG and FLG2 in keratinocytes and in *ex vivo* MF skin. A: RHE samples were cultured for 24h with the malignant MF2059 T-cell line in the presence of either 1 μM JAK inhibitor (Tofacitinib) or DMSO (control). B: RHE samples were cultured in the presence of supernatants for 48h with 1 μM Tofacitinib or DMSO. C: Skin biopsies from MF plaques were cultured with 1 μM Tofacitinib or DMSO for 72h. A+C: mRNA expression was analysed by qPCR using β-actin as a reference gene. Gene expression was given as relative expression. B: Protein expression was analysed by IHC for RHE samples cultured for 48h with supernatants from the malignant T-cell line MF2059 and in the presence or absence of Tofacitinib. The RHE samples were stained for H&E, filaggrin and filaggrin-2. n=3. (*) indicates statistical significance ($P<0.05$).