

Article

A Proinflammatory Psoriatic Microenvironment Has Early Effects on Keratinocyte Proliferation/Differentiation and Induces Ferroptosis in HaCaT Cells

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Simple Summary

Psoriasis is a chronic immune-mediated skin disease with a highly negative impact on quality of life. It is important to fill the gap between the well-known specific skin plaques and the scenario which takes place before we can visualize them. This can be achieved by using suitable experimental models. This paper shows an experimental model of psoriatic cells' microenvironment and some pathogenetic mechanism of the lesion evaluation, such as, for example, ferroptosis. Ultimately, but not less importantly, the key goal of this effort is to plan an appropriate and targeted therapeutic treatment.

Abstract

Background: The interaction between keratinocytes and proinflammatory cytokines is essential in the development of psoriatic lesions. The synergism among these cytokines and their involvement in ferroptosis are not yet elucidated. This study aimed at evaluating the early impact of a complete proinflammatory microenvironment on keratinocyte differentiation, intercellular adhesion, proliferation, and induction of ferroptosis. **Methods:** HaCaT cells were differentiated with 1.8 mM CaCl₂ and treated with a cytokine combination (MIX) containing IL-17A, IL-22, IL-23, and TNF-alpha for 24 and 48 h. Claudin 1 (CLDN-1), Zonula Occludens 1 (ZO-1), and keratins (K)10/K14 expression was analyzed by immunofluorescence and immunoblot analysis, paralleled by proliferation and ultrastructural analysis. Ferroptosis was induced with erastin and RSL3 and evaluated by testing glutathione (GSH)/glutathione peroxidase 4 (GPX4) protein expression, GSH levels, cell availability/toxicity, intracellular iron and ATP levels. **Results:** After MIX incubation at T48, CLDN-1 and ZO-1 immunofluorescences were reduced in HaCaT cells, while K10 and K14 were unaffected. The proliferative activity was reduced. Psoriatic-like MIX triggered the ferroptotic pathway, as shown by the increase in intracellular iron levels as well as by the reduction in GPX4 protein expression, the decrease in GSH levels, cell availability, and ATP levels. **Conclusions:** This experimental model mimics the early pathogenetic processes underlying psoriatic plaque formation/progression paving the way for new therapeutic strategies.



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1. Introduction

The histoarchitecture of the human epidermis provides our body with a powerful and efficient physiological physicochemical barrier, able to respond promptly to a wide range of stimuli preserving and restoring epidermal homeostasis [1]. The fine-tuning of cell proliferation and terminal differentiation (TD) of the most represented epidermal cytotype, i.e., keratinocytes (KCs), is crucial for the renewal of the epithelial compartment and maintenance of its homeostasis. While the proliferative activity is confined to the lowermost basal layer, the differentiative events involve all the suprabasal epidermal layers, from the spinous layer upward to the stratum corneum, encompassing in parallel the cytoskeletal apparatus and the composition of the intercellular junctions, mainly desmosomes and tight junctions (TJs).

The delicate balance between proliferation and TD can be disturbed by a plethora of exogenous stimuli [2–4], including pro-inflammatory cytokines, as observed in psoriasis [5]. In fact, the formation and progression of the typical psoriatic plaques are a direct consequence of a complex interplay between the imbalance of proliferation/differentiation of resident keratinocytes and the activation of the immune system [6].

The original hypothesis that psoriasis was solely a consequence of KC hyperproliferation rapidly evolved and a key role for the immune system in pathogenesis of the disease has been widely documented. Moreover, proinflammatory cytokines produced by infiltrated immune cells can activate epidermal keratinocytes and other resident cutaneous cells [7]. Tumor Necrosis Factor (TNF)-alpha, interleukin (IL)-17A, and IL-23 have emerged as major drivers finally establishing the dominant pathogenetic role of IL-17/23 axis [8]. The contribution of IL-22 to psoriatic processes other than proliferation has been recently reviewed [9].

The histopathological features of full-blown psoriatic lesions and the contribution of psoriatic inflammatory milieu have been well characterized in the last three decades [10]. A hallmark of psoriatic lesions is epidermal hyperproliferation localized into the suprabasal layers, accompanied by profound alterations in keratinocyte differentiation [10,11]. These changes are reflected by an abnormal keratin expression pattern, mainly consisting of a reduced keratin 10 (K10) distribution and an increased expression of keratin 17 (K17), a keratin of hyperproliferative disease [12]. Moreover, keratin 14 (K14), normally confined to the basal layer, is aberrantly upregulated in psoriatic epidermis, consistent with an accelerated and dysregulated differentiation programme [13]. Keratins are key structural components of KCs, biomarkers of TD and of proliferating and immuno-mediated skin diseases [12,14]. In physiological conditions, K5/K14 are the characteristic isoforms of the proliferative basal layer, whilst the switch to K1/K10 isoforms occurs when KCs enter the suprabasal layer starting TD. This tightly regulated differentiation process is paralleled by a dynamic rearrangement of cell junctions with space-time modifications. In particular, tight junctions (TJs), which are mainly localized in the granular layer, play a crucial barrier role and are composed of transmembrane proteins such as claudin-1 (CLDN-1), and cytoplasmic scaffolds between the cytoskeleton and cell membrane proteins, like zonula occludens-1 (ZO-1) [15,16]. Notably, TJ remodelling has been identified as an early event in psoriatic lesion development and consists of a reduction in CLDN-1 and an altered localization of ZO-1 in the epidermal layers, as pioneeringly reported by Kirschner et al. [17].

While the histopathological features of established psoriatic plaques have been extensively described, less is known about the early cellular events occurring in keratinocytes upon exposure to a psoriatic inflammatory milieu. Previous studies have largely focused on the effects of single cytokines or limited cytokine combinations, demonstrating alterations in keratinocyte proliferation, differentiation markers, and junctional proteins [18–21]. More complex cytokine mixtures, such as the M5 cocktail, which comprehends IL-17A, IL-22, oncostatin M, IL-1 α , and TNF- α , have been shown to reproduce selected aspects of psoriasis in vitro [22–25]. However, these models do not include IL-23, which is considered the master cytokine of the psoriatic cascade [26,27]. Consequently, the early synergistic effects of a complete IL-17/23-centred inflammatory environment on keratinocyte biology remain incompletely understood.

Furthermore, the mechanisms underlying cell death observed in psoriatic plaques are still not fully elucidated. Emerging evidence from murine experimental models [28,29] and human psoriatic lesions [30] suggests that ferroptosis-related pathways may be altered in psoriasis. Ferroptosis is a non-apoptotic, regulated, and iron-dependent form of cell death characterized by lipid peroxidation and membrane damage [31]. This process is driven by oxidative stress linked to accumulation of reactive oxygen species (ROS) in the presence of iron and depletion of antioxidants like glutathione (GSH). The ferroptotic cascade is modulated by glutathione peroxidase 4 (GPX4), a GSH-dependent antioxidant enzyme that typically prevents lipid peroxide accumulation [32].

Notably, in psoriatic patients ferroptotic ultrastructural modifications in mitochondria were also reported and the hypothesis that ferrous iron overload in the epidermis might be the cause of ferroptosis was advanced [28]. Moreover, in HaCaT cells the co-exposure of the ferroptosis inducer RSL3 and the M5 cytokine mixture promoted keratinocyte differentiation and inhibited proliferation [33], suggesting that ferroptosis in psoriatic KCs can represent a protective compensatory response inversely correlated with disease severity.

However, whether ferroptosis represents a primary pathogenic mechanism or represents a secondary response to inflammation remains unclear. In particular, little is known about the possible involvement of ferroptosis during the early phases of keratinocyte exposure to a psoriatic proinflammatory microenvironment.

In this context, the present study aimed to investigate the early effects of a complete psoriatic cytokine milieu, composed of IL-17A, IL-22, IL-23, and TNF- α (here defined as MIX) [34,35], on differentiated human keratinocytes. Using HaCaT cells, we analyzed the impact of this cytokine combination on keratinocyte differentiation, intercellular adhesion, proliferation, and ferroptosis-related pathways, with the goal of clarifying whether exposure to a psoriatic inflammatory milieu is sufficient to prime keratinocytes toward ferroptosis-like changes at early stages. Specifically, to investigate the ferroptotic cascade we used two different ferroptosis inducers, erastin and RSL3, which act through diverse mechanisms. In fact, erastin induces ferroptosis by targeting and inhibiting the transporter involved in importing cystine into cells, then used to produce GSH, while RSL3 seems to induce ferroptosis by directly inhibiting GPX4 enzymatic activity [36,37] (Figure 1).

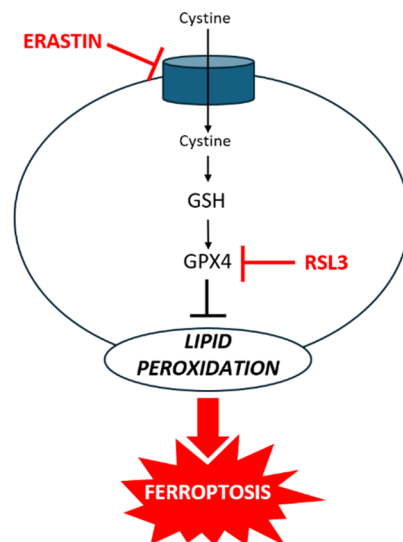


Figure 1. Mechanisms of action of erastin and RSL3. Erastin induces ferroptosis by targeting and inhibiting the transporter involved in importing cystine into cells, used to produce glutathione (GSH). RSL3 induces ferroptosis by inhibiting glutathione peroxidase 4 (GPX4), a GSH-dependent antioxidant enzyme that neutralize toxic lipid peroxides.

2. Materials and Methods

2.1. HaCaT Cell Culture and Treatment with Cytokine Mixture (MIX)

The human transformed keratinocyte cell line (HaCaT), purchased from CLS (Cell Line Service GmbH, Eppelheim, Germany), was seeded into T25 flasks and maintained in complete Dulbecco's modified Eagle's medium (DMEM; Corning, AZ, USA), supplemented with 10% foetal bovine serum (FBS) (Dominique Dutscher, Bernolsheim, France), 1% L-Glutamine 2 mM (Eurobio, Les Ulis, France), and 1% penicillin/streptomycin antibiotics (Eurobio, Les Ulis, France) at 37 °C with 5% CO₂. To perform experiments, cells were harvested using trypsin-EDTA (Biowest, Nuaille, France), centrifuged at 1200 rpm for 3 min, washed with PBS without calcium (Sigma-Aldrich, St. Louis, MO, USA), and resuspended in complete DMEM. Approximately 5×10^3 cells/cm² were seeded of onto glass round coverslips (diameter 12 mm, BioSigma, Cona, Italy) placed into 24-well plates and cultured for 3 days in complete DMEM, until reaching 70% confluence. HaCaT cells were then induced to differentiate by adding 1.8 mM CaCl₂ to the medium (Figure 2). The extracellular CaCl₂ concentration of 1.8 mM was selected to promote early keratinocyte differentiation while preserving proliferative capacity over the experimental time frame [38].

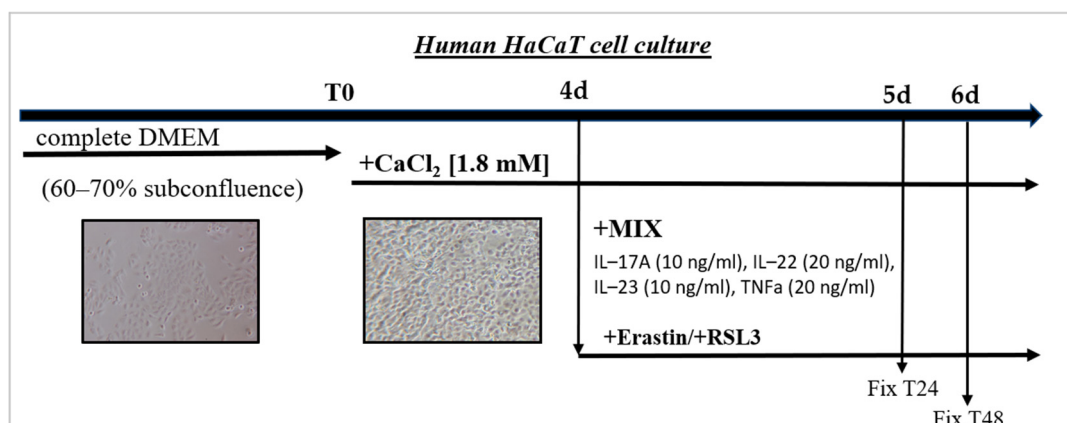


Figure 2. Experimental design. MIX: psoriatic cytokines mixture. IL-17A: interleukin 17A; IL-22: interleukin 22; IL-23: interleukin 23; TNF-alpha: Tumor Necrosis Factor alpha. FIX: fixation.

On the 4th day, the cells were treated for a further 24 h (T24) or 48 h (T48) with a combination (defined as MIX) of IL-17A (10 ng/mL), IL-22 (20 ng/mL), IL-23 (10 ng/mL) and TNF- α (20 ng/mL) (PeproTech, London, UK) as reported in prior models [22,39]. MIX was added to DMEM medium always with 1.8 mM CaCl_2 [40]. Untreated cells were used as controls, and all experiments were repeated three times.

2.2. Transmission Electron Microscopy Analysis on HaCaT Cells

Cells were fixed with 3% glutaraldehyde in sodium cacodylate 0.1 M pH 7.3 for 1 h at 4 °C, post-fixed in 1% osmium tetroxide at room temperature and dehydrated through an ascending series of ethanols.

Cells were embedded in Durcupan (Sigma Aldrich, St. Louis, MO, USA), cut to obtain semithin sections (2 μm thickness), and then stained with toluidine blue (Sigma Aldrich, St. Louis, St. Louis, MO, USA). Ultrathin sections (60 nm thickness) were obtained using an Ultracut ultramicrotome (Reichert Ultracut R, Leica, Wien, Austria), stained with uranyl acetate/lead citrate, and examined by Talos 120 electron microscope (ThermoFisher Scientific Inc., Waltham, MA, USA).

2.3. Immunofluorescence Analysis for Intercellular Adhesion, Cell Differentiation, and Cell Proliferation in HaCaT Cells

HaCaT cells were fixed with paraformaldehyde 4% diluted in PBS 0.1 M were first washed with PBS buffer 0.1 M pH 7.4 at room temperature for 5 min. To block unspecific binding sites, PBS with 0.2% Tween-20 and 1% albumin (PTA) blocking solution was used for 20 min at room temperature with agitation. HaCaT cells were incubated with the primary antibodies as reported in Table 1.

After repeated washes with PTA in agitation, the incubation with the secondary antibodies was performed for 30 min at room temperature. Samples were then washed three times for 10 min in PBS, and nuclei were counterstained for DNA with 0.5 $\mu\text{g}/\text{mL}$ Hoechst 33258 (Sigma–Aldrich, St. Louis, MO, USA) for 5 min. Finally, after two washes with PBS, the slides were mounted with Mowiol 4-88 (Sigma Aldrich, St. Louis, MO, USA) and samples were analyzed after at least 1 h at RT.

To determine the proliferative activity before and after treatment with MIX, DNA synthesis was analyzed by measuring the incorporation of 5-bromo-2'-deoxyuridine (BrdU) as previously described [41]. Briefly, 30 μM BrdU (Sigma-Aldrich, St. Louis, MO, USA) was added to the culture medium for 1 h before cell fixation with 70% ethanol. To hydrolyze the DNA molecule, the dishes were incubated with HCl 2 N for 30 min at room temperature and then sodium tetraborate 0.1 M pH 8.5 was used for 15 min to neutralize the acid solution. Cells were washed twice for 5 min in PBS, incubated for 20 min in the PTA blocking solution, and with a mouse monoclonal anti-BrdU primary antibody (Table 1). Finally, after three washes for 10 min in PTA, cells were incubated for 30 min with a secondary antibody anti-mouse FITC-conjugated (Sigma-Aldrich, St. Louis, MO, USA), diluted 1:100. Samples were washed thoroughly, and the nuclear DNA was counterstained with 0.5 $\mu\text{m}/\text{mL}$ Hoechst 33258 (Sigma-Aldrich, USA). Finally, the coverslips were mounted in Mowiol 4-88 (Sigma Aldrich, St. Louis, MO, USA).

Cells were analyzed for immunofluorescence nuclear positivity with a Zeiss Axiophot Fluorescence microscope (Karl Zeiss, Oberkochen,, Germany) and all images acquired by a Coolpix 950 Digital Camera (Nikon, Tokyo, Japan). For cell proliferation analysis, each counting was repeated by three different operators, and at least 500 cells were counted for each experimental condition.

Images were analyzed using Fiji software (ImageJ 1.54p). Briefly, nuclei were counted using the find maxima tool and, after a background subtraction, the integrated intensity of the green signal in the area covered by cells was evaluated. Data presented represents the

ratio between integrated intensity and number of nuclei, thus providing an average signal expression (evaluation) per cell.

Table 1. Primary and secondary antibodies for indirect immunofluorescence (IF) analysis and Western blot (WB) on HaCaT cells. CLDN-1: Claudin 1; ZO-1: Zonula Occludens 1; K: Keratin; PTA: PBS with 0.2% Tween-20 and 1% albumin; RT: Room temperature; O.N.: Over night; PBT: PBS with 0.2% Tween-20; BSA: Bovine Serum Albumin.

Primary and Secondary Antibodies	IF (Dilution in PTA)	WB (Dilution in PBT)
rabbit polyclonal anti-human CLDN-1 (ThermoFisher Scientific, Invitrogen, Carlsbad, CA, USA)	1:100 2 h at 37 °C	1:1000 1 h at 37 °C
rabbit polyclonal anti-human ZO-1 (ThermoFisher Scientific, Invitrogen, Carlsbad, CA, USA)	1:50 2 h at 37 °C	1:1000 1 h at 37 °C
mouse monoclonal anti-human K10 (Santa Cruz Biotechnology, Dallas, TX, USA)	1:100 2 h at RT	1:1000 1 h at 37 °C, then O.N. at 4 °C
rabbit monoclonal anti-human K10 (Bioss, Woburn, MA, USA)		1:500 1 h at 37 °C, then O.N. at 4 °C
mouse monoclonal anti-human K14 (Santa Cruz Biotechnology, Dallas, TX, USA)	1:100 2 h at RT	1:1000 1 h at 37 °C, then O.N. at 4 °C
rabbit polyclonal anti-K14 (Cell Signalling Technology, Danvers, MA, USA)		1:1000 1 h at 37 °C, then O.N. at 4 °C
mouse monoclonal anti-BrdU (clone BU-1, Cytiva uk Ltd. Amersham, UK)	1:100 1 h at RT	
goat anti-mouse Alexa Fluor 488 FITC-conjugated (ThermoFisher Scientific, Invitrogen, Carlsbad, CA, USA)	1:100 30 min at RT	-
goat anti-rabbit Alexa Fluor 488 FITC-conjugated (ThermoFisher Scientific, Invitrogen, Carlsbad, CA, USA)	1:100 30 min at RT	-
mouse monoclonal anti-GPX4 (Cell Signalling Technology, Danvers, MA, USA)		1:500 O.N. at 4 °C
mouse monoclonal anti-HSP60 (ThermoFisher Scientific, Invitrogen, Carlsbad, CA, USA)		1:1000 O.N. at 4 °C
goat anti-mouse HRP-conjugated (Cell Signaling Technology, Danvers, MA, USA)		1:2000 1 h at 37 °C
goat anti-rabbit HRP-conjugated (Cell Signaling Technology, Danvers, MA, USA)		1:2000 1 h at 37 °C

2.4. Western blot Analysis on HaCat Cells

HaCaT cells grown at the same cell density described above into T75 flasks were differentiated with CaCl_2 and treated on the 4th day with psoriatic MIX, or erastin 20 μM or RSL3 5 μM , or with the combination of MIX/erastin and MIX/RSL3 and harvested at T24 or T48 after treatment. Cells were dissolved directly in ice-cold cytoplasm isolation RIPA lysis buffer-PAGE (150 mM NaCl, 50 mM HEPES, 1% NP-40, 0.1% SDS in distilled water) with protease inhibitor cocktail (Cell Signalling Technology, Danvers, MA, USA) for 5 min on ice. After centrifugation for 5 min at $500 \times g$, the supernatants with protein cytoplasmic fraction were collected. Total protein concentration was determined by using BCA assay (Pierce™ BCA Protein Assay Kit, 23225; Thermo Fisher Scientific, Carlsbad, CA, USA).

Cell lysate proteins (10–20 μg for each sample) were loaded onto an SDS-PAGE gel with a specific percentage of polyacrylamide (7.5–15%) related to the detectable antigen.

Proteins were then transferred by electroblot (Amersham, Little Chalfont, UK) onto a nitrocellulose membrane (Amersham, Little Chalfont, UK).

The membrane was saturated for 30 min with blocking solution (PBS with 0.2% Tween20 and 5% non-fat dried milk) to block unspecific binding of antibodies and then incubated with the different primary antibodies (Table 1). Mouse monoclonal anti-HSP60 antibody (Invitrogen, Carlsbad, CA, USA, #MA3-012) was used as internal control (1:1000). Three consecutive washes with TBS-0.1% Tween20 solution were followed by incubation at room temperature with the secondary horseradish (HRP)-conjugated antibody. After three TBS washes, the detection of the protein bands on the membrane was revealed by chemiluminescence using the ECL detection kit (Euroclone, Milan, Italy). Images were acquired using the Biorad Chemidoc. Images were cropped and bands quantified using Image J (1.53e Java 1.8.0_172 version) with the values being calculated after normalization to the amount of HSP60. All protein expression levels are presented as fold changes normalized to 1 (mean expression of CTR cells). Each experiment was performed at least in triplicate.

2.5. Determination of GSH Content

Approximately 5×10^3 HaCaT cells/cm² were seeded into a 96-well plate and, on the 4th day of treatment with CaCl₂, cells were treated with erastin 20 µM, RSL3 5 µM, psoriatic MIX, combination of MIX/erastin and combination MIX/RSL3 and harvested at T24 or T48 after treatment. Once the treatment was completed, GSH levels were evaluated by following the instruction of the GSHG/GSSG-Glo Assay (Promega Italia, Milan, Italy). All data are presented as fold induction normalized to 1 (mean values of CTR cells). Each experiment was performed at least in triplicate.

2.6. Cytotoxicity Test

Approximately 5×10^3 HaCaT cells/cm² were seeded into a 96-well plate and, on the 4th day of differentiation with CaCl₂, cells were treated with erastin 20 µM, RSL3 5 µM, psoriatic MIX, combination of MIX/erastin and combination MIX/RSL3 and harvested at T24 or T48 after treatment. Once the treatment was completed, the MTT assay was performed and the cell toxicity was evaluated by following the manufacture instructions of the CellTox Green Cytotoxicity assay (Promega Italia, Milan, Italy). All data are presented as fold induction normalized to 1 (mean values of CTR cells). Each experiment was performed at least in triplicate.

2.7. ATP Determination

Approximately 5×10^3 HaCaT cells/cm² were seeded into a 96-well plate and, on the 4th day of treatment with CaCl₂ for cell differentiation, cells were treated with erastin 20 µM, RSL3 5 µM, psoriatic MIX, combination of MIX/erastin and combination MIX/RSL3 and harvested at T24 or T48 after treatment. Once treatment was completed, ATP levels were evaluated by following the manufacturer's instructions of the Mitochondrial ToxGlo assay (Promega Italia, Milan, Italy). All data are presented as fold induction normalized to 1 (mean values of CTR cells). Each experiment was performed at least in triplicate.

2.8. Detection of the Intracellular Fe²⁺ Levels

Approximately 5×10^3 HaCaT cells/cm² were seeded into 6-well plates and, on the 4th day of treatment with CaCl₂ for cell differentiation, cells were treated with erastin 20 µM, RSL3 5 µM, psoriatic MIX, combination of MIX/erastin and combination MIX/RSL3 and harvested at T24 or T48 after treatment. Then, cells were collected and labile iron was assessed by using the Fe²⁺ fluorescent probe FerroOrange (1 µM FerroOrange working solution, Cell Signalling Technology, USA) at 37 °C for 30 min. Then, the iron level was

detected by Cytex Northern Lights™ flow cytometer (Cytex Biosciences, Fremont, CA, USA), that incorporates the Full Spectrum Profiling™ (FSP™) technology in a three-laser system (405, 488, 603 nm). Dead cells were excluded using the Zombie Aqua™ Fixable Viability Kit (Biolegend, San Diego, CA, USA). Samples containing only the Zombie Aqua™ dye were used as negative controls. Unstained samples were used to check the relative cellular autofluorescence and subtract it from the analyzed samples. Data analysis was performed with the FlowJo software (FlowJo, LLC, Ashland, OR, USA).

2.9. Statistical Analysis

The results are presented as mean $\pm$ SEM. Statistically significant differences were obtained after carrying out the Mann–Whitney test for independent two-tailed samples using Prism software (version 10.6.1; 799 SEpt. 8/2025). Differences were considered statistically significant when the p -value was * $p < 0.05$, ** $p < 0.01$ or $p^{***} < 0.001$. Statistical significance was reported when present.

3. Results

3.1. Structure and Ultrastructure Are Not Perturbed by Psoriatic-like MIX Treatment in HaCaT Cells

Light microscopy analysis showed that no evident changes were observed in HaCaT cells (Figure 3A). After 48 h of treatment with the cytokine mixture, cell cultures showed rounded clusters of cells (Figure 3A, inserts). By TEM, the ultrastructural analysis showed that the monolayer was flattened with cytoskeletal filaments (white arrows) and scattered desmosomes (arrow heads) (Figure 3B). These observations are in accordance with the ultrastructural features previously reported in the literature [42–44]. No evident differences were observed among the different experimental groups at both experimental time points (T24 and T48).

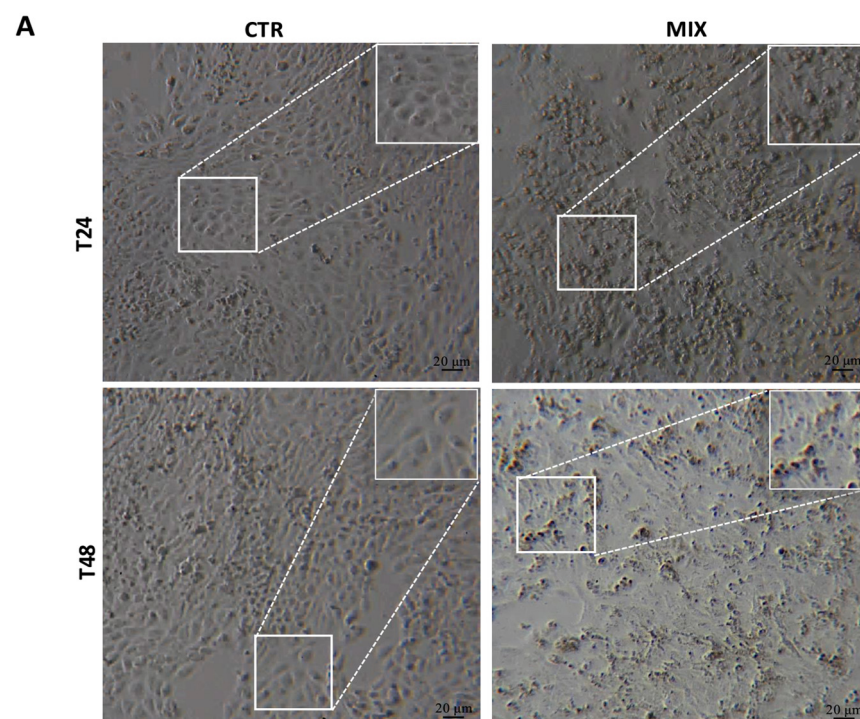


Figure 3. Cont.

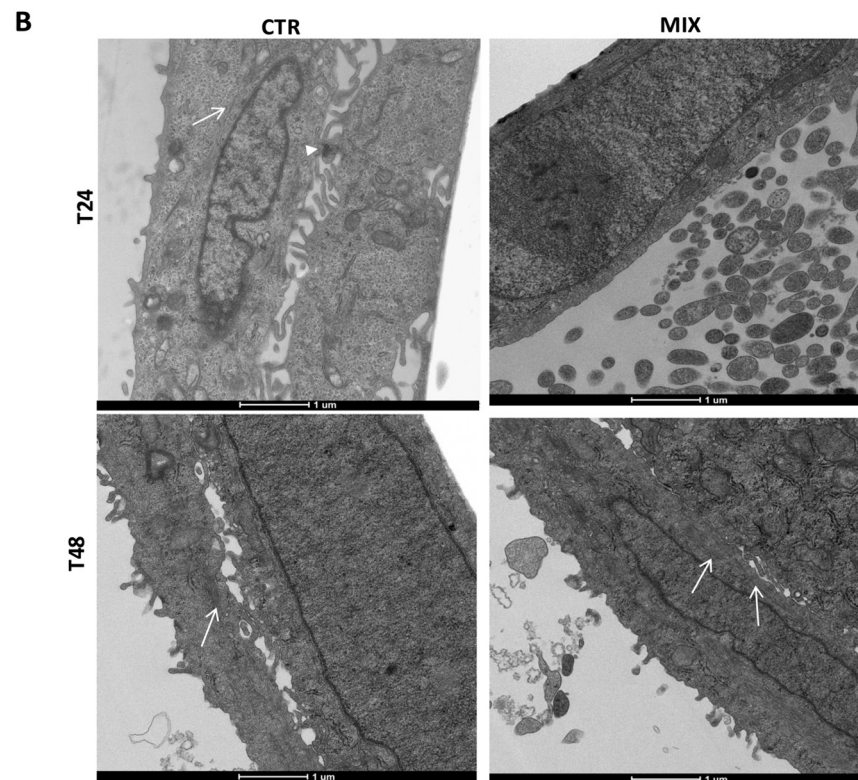


Figure 3. Morphological analysis of HaCaT cells. HaCaT cells after four days of differentiation with CaCl_2 were treated for T24 or T48 with MIX. (A) Phase contrast photomicrographs of keratinocytes. In the upper right corner, a zoomed-in view of a specific area from each image. (B) Transmission electron microscopy analysis of araldite ultrathin sections. CTR: control; MIX: cytokine mixture. Arrows: cytoskeletal filaments. Arrowheads: desmosomes. (A) Scale bar: 20 μm ; (B) Scale bar: 1 μm .

3.2. Cell Adhesion, Differentiation, and Proliferation Are Affected by Psoriatic-like MIX Treatment in HaCaT Cells

In HaCaT cells, in control conditions, CLDN-1 immunofluorescence was homogeneously distributed in the keratinocyte membrane at T24 and T48 (Figure 4A). After MIX incubation, a clear decrease in the membrane immunostaining was detectable at both experimental time points (Figure 4A). The total quantitative protein expression was not changed after MIX incubation. (Figure 4B).

For the scaffold plaque TJ cytoplasmic protein ZO-1, a clustered intracellular distribution was observed at T24 in control HaCaT cells, which is enhanced in T24 MIX group (Figure 4C). Notably, a clear increase in this protein was evident in the control group at T48, whereas in the MIX samples at T48, the immunostaining decreased and was comparable to T24 groups (Figure 4C). Accordingly, ZO-1 total protein expression tendentially increased in a time-dependent way in control samples from T24 to T48 (Figure 4D), whereas in samples treated with MIX a tendency to decrease at T48 was confirmed (Figure 4D).

Regarding the changes in the expression of K14 and K10, it is important to note that immunofluorescence was always localized in keratinocyte cytoplasm (Figure 5A,C). In control HaCaT cells, K14 immunofluorescence intensity was increased at T48 (Figure 5A), although the quantitative Western blot analysis did not show any differences (Figure 5B). Notably, the presence of cytokine MIX induced an increase in both cytoplasmatic and total K14 levels at T24, but not at T48 (Figure 5A,B). In the case of K10, both Western blot and quantitative immunofluorescence analyses showed relatively stable expression levels (Figure 5C,D).

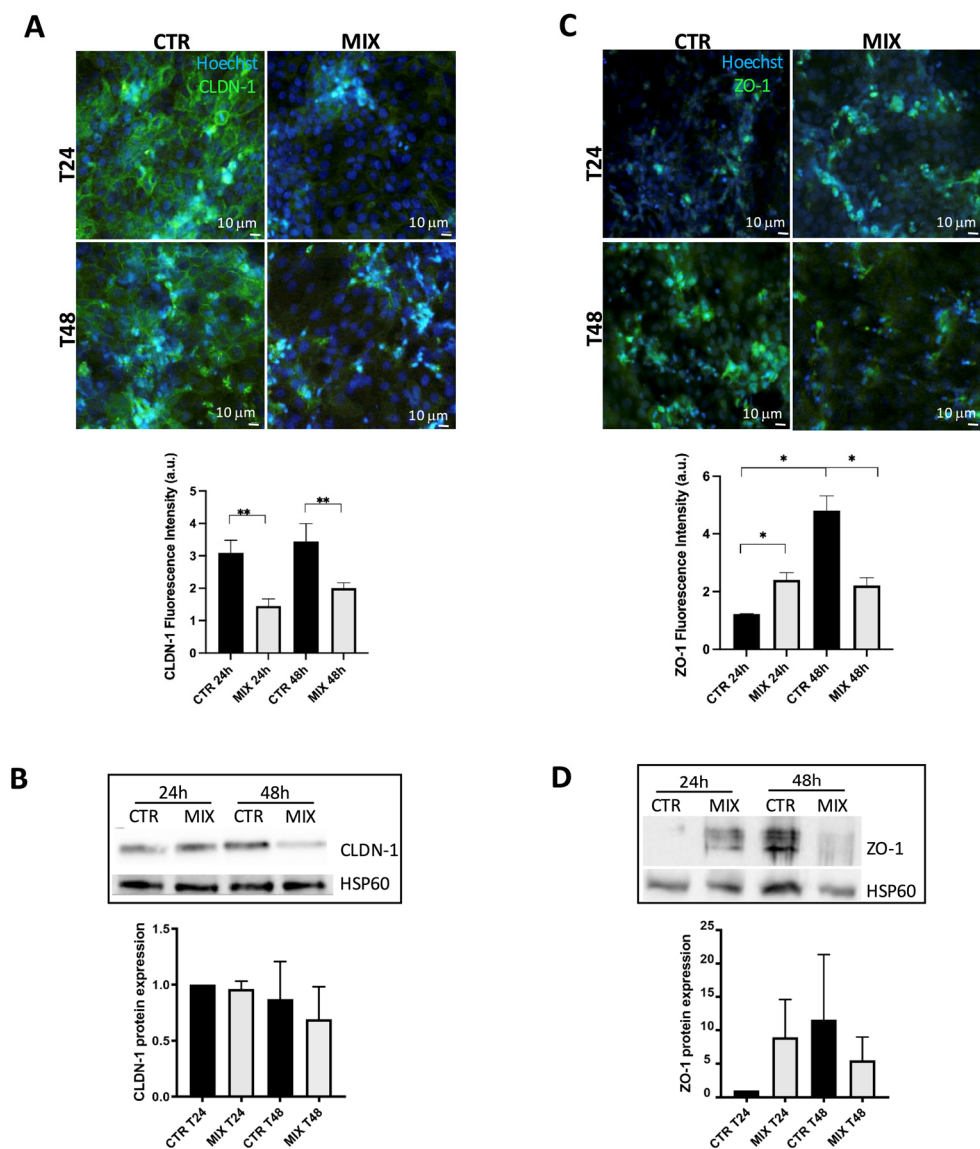


Figure 4. Expression of CLDN-1 and ZO-1 in HaCaT cells. HaCaT cells after four days of differentiation with CaCl_2 were treated for T24 or T48 with MIX. **(A)** Quantification of immunofluorescence of CLDN-1; representative immunofluorescence images are shown. Nuclei are counterstained with Hoechst 33258 (blue fluorescence). Scale bars: 10 μ m. Data are presented as mean values $\pm$ SEM. $n = 3$ independent experiments. $** p < 0.01$. See Materials and Methods for immunofluorescence quantification details. a.u. = arbitrary unit. **(B)** Quantification of immunoblot analysis of CLDN-1; representative immunoblots are shown. The values were normalized to HSP60 and expressed as a fraction of CTR cells normalized to 1. Data are presented as mean values $\pm$ SEM. $n = 6$ independent experiments. **(C)** Quantification of immunofluorescence of ZO-1; representative immunofluorescence images are shown. Nuclei are counterstained with Hoechst 33258 (blue fluorescence). Scale bars: 10 μ m. Data are presented as mean values $\pm$ SEM. $n = 3$ independent experiments. $* p < 0.05$. See Materials and Methods for immunofluorescence quantification details. a.u. = arbitrary unit. **(D)** Quantification of immunoblot analysis of ZO-1; representative immunoblots are shown. The values were normalized to HSP60 and expressed as a fraction of CTR cells normalized to 1. Data are presented as mean values $\pm$ SEM. $n = 3$ independent experiments.

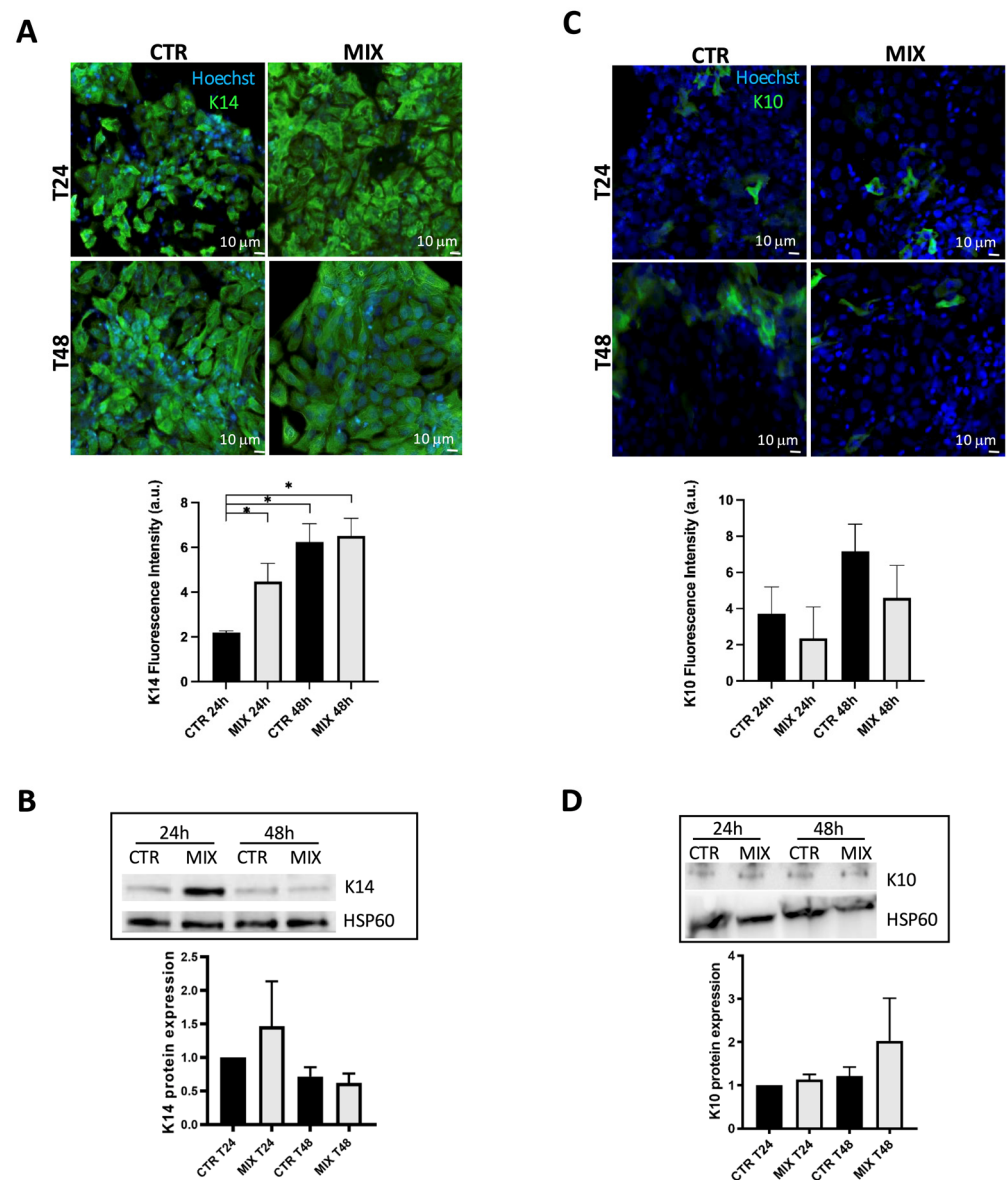


Figure 5. Expression of K14 and K10 in HaCaT cells. HaCaT cells after four days of differentiation with CaCl_2 were treated for T24 or T48 with MIX. (A) Quantification of immunofluorescence of K14; representative immunofluorescence images are shown. Nuclei are counterstained with Hoechst 33258 (blue fluorescence). Scale bars: 10 μ m. Data are presented as mean values $\pm$ SEM. $n = 3$ independent experiments. * $p < 0.05$. See Materials and Methods for immunofluorescence quantification details. a.u. = arbitrary unit. (B) Quantification of immunoblot analysis of K14; representative immunoblots are shown in the inset. The values were normalized to HSP60 and expressed as a fraction of CTR cells normalized to 1. Data are presented as mean values $\pm$ SEM. $n = 4$ independent experiments. (C) Quantification of immunofluorescence of K10; representative immunofluorescence images are shown. Nuclei are counterstained with Hoechst 33258 (blue fluorescence). Scale bars: 10 μ m. Data are presented as mean values $\pm$ SEM. $n = 3$ independent experiments. See Materials and Methods for immunofluorescence quantification details. a.u. = arbitrary unit. (D) Quantification of immunoblot analysis of K10; representative immunoblots are shown in the inset. The values were normalized to HSP60 and expressed as a fraction of CTR cells normalized to 1. Data are presented as mean values $\pm$ SEM. $n = 7$ independent experiments.

In parallel, cell proliferation was analyzed. BrdU incorporation was consistently observed in the nuclei of S-phase proliferating keratinocytes (Figure 6A). A quantitative analysis of cell proliferation revealed no significant differences between the control and MIX groups at T24 (Figure 6B). However, at T48 the number of proliferating cells statistically

significantly increased in the control samples (Figure 6A,B). In contrast, the MIX treatment resulted in a statistically significant decrease in proliferation at T48, bringing levels down to those like T24 samples (Figure 6B).

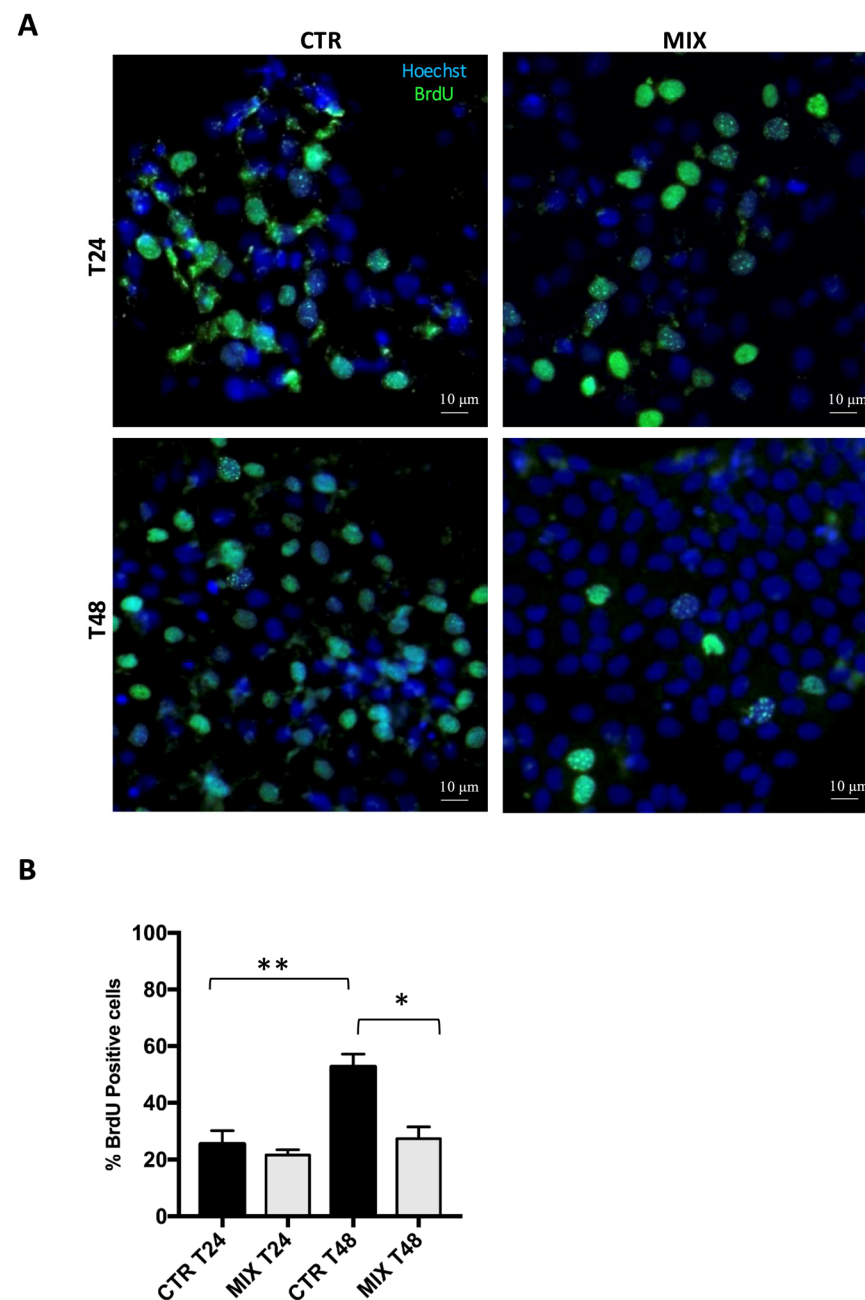


Figure 6. Cell proliferation analysis on HaCaT cells. HaCaT cells after four days of differentiation with CaCl₂ were treated for T24 or T48 with MIX. (A) Qualitative and (B) quantitative analysis of cell proliferation after BrdU incorporation. (A) Scale bars: 10 μm. (B) Percentage of BrdU-positive proliferating cells versus total counted cells. Data are presented as mean values ± SEM. * $p < 0.005$, ** $p < 0.01$. $n = 3$ independent experiments.

3.3. GPX4 Expression in HaCaT Cells

The GPX4 protein expression after treatment with erastin was reduced. Interestingly also the MIX and the combination MIX/erastin induced a decrease in the GPX4 protein expression (Figure 7). This effect was even more evident at T48, with a statistically significant reduction compared to controls. As expected, RSL3, alone or in combination with MIX, had no effect on GPX4 protein levels expression, as it acts on its enzymatic activity [36,37].

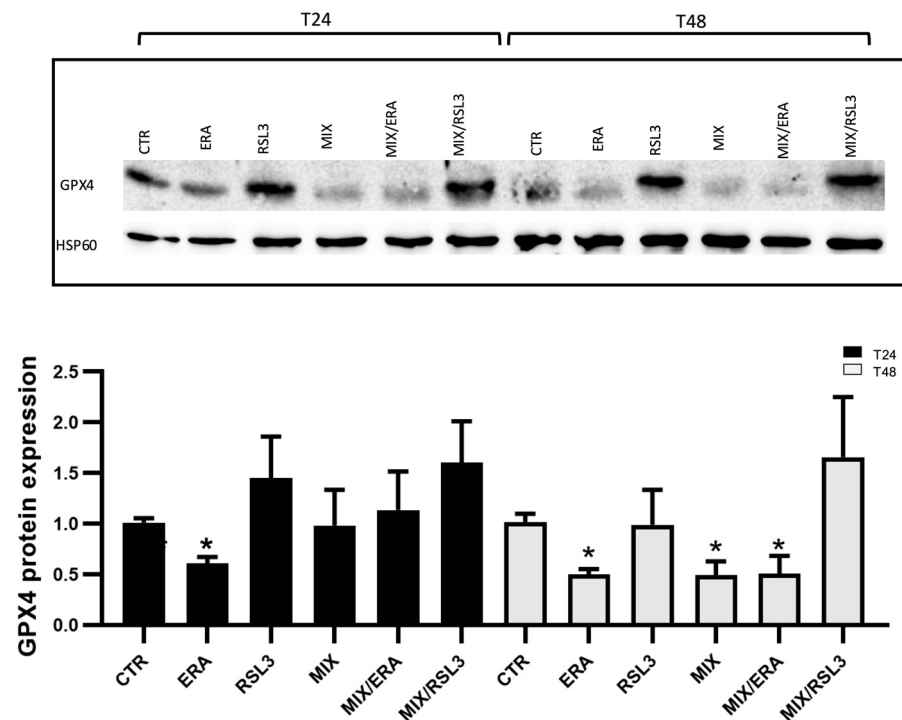


Figure 7. GPX4 protein expression in HaCaT cells. HaCaT cells after four days of differentiation with CaCl_2 were treated for T24 or T48 with erastin $20\mu\text{M}$ or RSL3 $5\mu\text{M}$ or MIX or with the combination MIX/erastin or MIX/RSL3. Quantification of immunoblot analysis of GPX4. Representative immunoblots are shown. The values were normalized to HSP60 and expressed as a fraction of CTR cells normalized to 1. Data are presented as mean values $\pm$ SEM. * $p < 0.05$. $n = 3$ independent experiments.

3.4. Psoriatic-like MIX Treatment Induced Ferroptosis-like Changes in HaCaT Cells

In HaCaT cells, after treatment with erastin, the levels of GSH significantly decreased at both T24 and T48, while, as expected, RSL3 treatment did not affect GSH levels (Figure 8A). The MIX significantly decreased GSH levels at T48 of treatment, suggesting an involvement of psoriatic-like cytokines in the induction of ferroptosis pathway. The treatment with the combination of MIX/erastin significantly reduced the GSH levels at both T24 and T48. To demonstrate the efficacy of the treatments on the induction of ferroptosis-like pathway we also analyzed the cells availability and cell toxicity. MTT analysis showed that erastin and RSL3 significantly reduced cell availability. Interestingly, treatments with the MIX significantly decreased cell availability and the combination treatments MIX/erastin or MIX/RSL3 further reduced it (Figure 8B). The cell toxicity analysis confirmed the data of cell availability (Figure 8C). Iron deposition assay further revealed elevated intracellular Fe^{2+} levels following treatment with RSL3, as expected (Figure 8D). Notably, MIX alone or in combination with RSL3 also induced an increase in intracellular iron levels comparable to those observed with RSL3 treatment (Figure 8D). These increases were more evident at T24 than T48. Altogether, these data (Figure 8A–D) strongly support the role of psoriatic-like cytokines MIX in inducing ferroptosis-associated modifications.

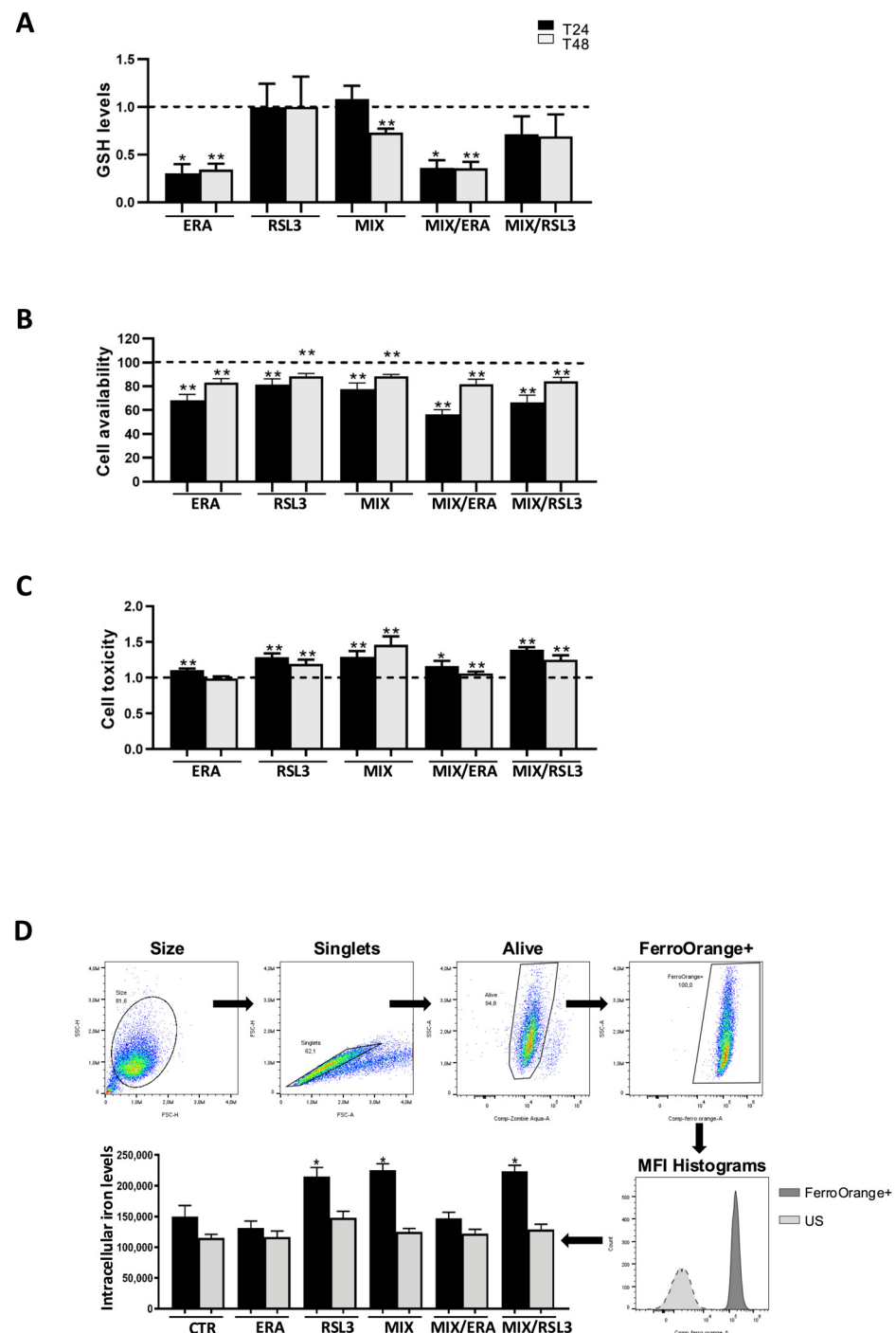


Figure 8. Analysis of ferroptosis induction in HaCaT cells. HaCaT cells after four days of differentiation with 1.8 mM CaCl_2 were treated for T24 or T48 with erastin 20 μM or RSL3 5 μM or the MIX or with the combination MIX/erastin or MIX/RSL3. **(A)** Evaluation of GSH levels. CTR: dotted line. Data as fold induction relative to CTR (CTR = 1). Mean values $\pm$ SEM ($n = 6$), * $p \leq 0.05$ vs. CTR, ** $p < 0.01$ vs. CTR. **(B)** Cell availability. CTR: dotted line. Data as percentage relative to CTR (CTR = 100%). Mean values $\pm$ SEM ($n = 6$), ** $p < 0.01$ vs. CTR. **(C)** Cell toxicity. CTR: dotted line. Data as fold induction relative to CTR (CTR = 1). Mean values $\pm$ SEM ($n = 6$), * $p \leq 0.05$ vs. CTR, ** $p < 0.01$ vs. CTR. All data are presented as fold induction normalized to 1 (mean CTR). **(D)** Intracellular iron levels. Representative FACS gating strategy and bar chart representing FACS quantitative analysis. In bar chart data represented as Relative Mean Fluorescence Intensity (MFI) = MFI samples with FerroOrange (FerroOrange+)—MFI samples containing only the Zombie Aqua™ dye (US). Mean values $\pm$ SEM ($n = 3$), * $p \leq 0.05$ vs. CTR.

To better understand whether the ferroptosis-inducers (erastin and RSL3) and MIX could induce mitochondrial damage we tested ATP levels and performed the ultrastructural analysis by transmission electron microscopy (Figure 9).

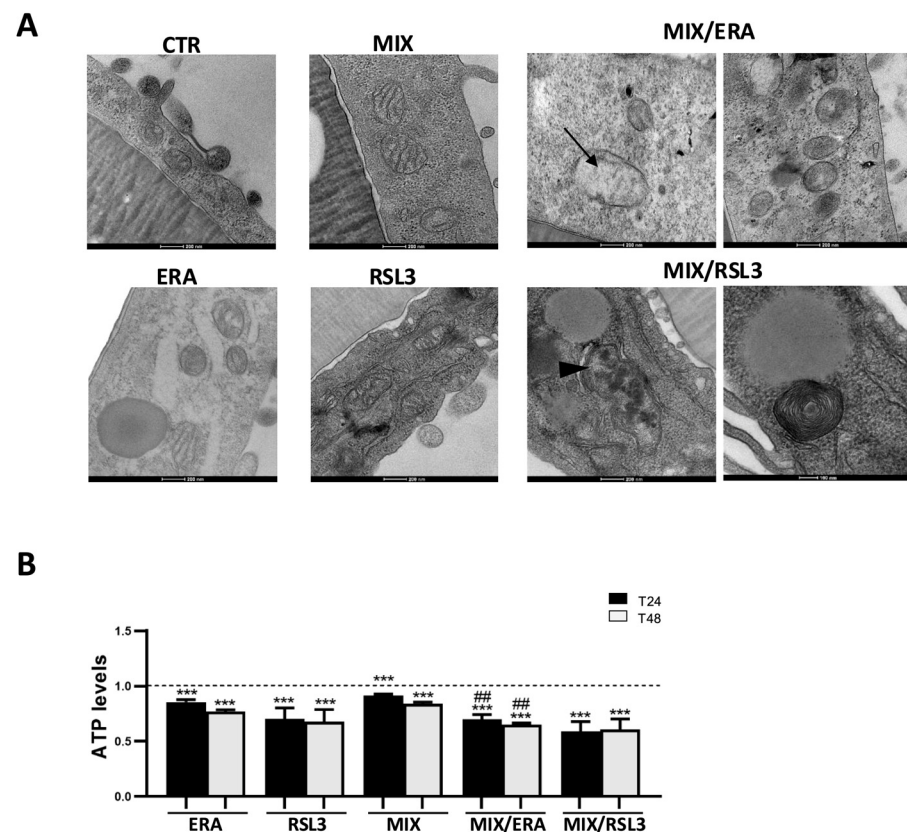


Figure 9. Mitochondrial ferroptosis-induced alterations in HaCaT cells. HaCaT cells after four days of differentiation with 1.8 mM CaCl_2 were treated for T24 or T48 with erastin 20 μM or RSL3 5 μM or the MIX or with the combination MIX/erastin or MIX/RSL3. **(A)** Transmission electron microscopy analysis of araldite ultrathin sections. Scale bar: 200 nm. Arrow and arrowhead indicate the ultrastructural mitochondrial modifications (see Results). **(B)** Evaluation of ATP levels. Mean values $\pm$ SEM ($n = 6$), *** $p < 0.001$ vs. CTR, ## $p < 0.01$ vs. erastin. Data are presented as fold induction relative to CTR (CTR = 1).

By TEM analysis, no morphological alterations were detected in any sample regarding the mitochondrial fine structure at T24. At T48, both erastin and RSL3 alone induced only a slight disarrangement of the cristae if compared with control cells and MIX-incubated cells (Figure 9A). On the other hand, the combined incubation with one inducer of ferroptosis and the MIX, profoundly affected the HaCaT ultrastructure. The mitochondrial membrane was always well-preserved, but in the case of MIX/erastin treatment mitochondrial cristae were completely absent (Figure 9A, arrow), while when cells were incubated with MIX/RSL3 the mitochondrial matrix exhibited electron-dense aggregates (Figure 9A, arrowhead, MIX/RSL3 representative images, left). Moreover, abundant lamellar bodies were evident in the cytoplasm of MIX/RSL3-treated cells (Figure 9A, MIX/RSL3 representative images, right).

Accordingly, ATP levels were decreased after treatments with erastin, RSL3 as well as MIX and the combination treatments. The combination treatment MIX/erastin further reduced it significantly after T48. These data suggest that MIX has a direct involvement in the alteration of the activity of the mitochondria (Figure 9B). In presence of an inducer of ferroptosis, not only the activity but also the structural integrity of mitochondria was affected (Figure 9A).

4. Discussion

The early pathogenetic mechanisms underlying psoriatic lesion formation remain incompletely understood, particularly concerning the initial responses of epidermal keratinocytes to a complex inflammatory environment. For this reason, in this study we investigated the effects of a complex proinflammatory psoriatic cytokine milieu, focusing on the IL-17/23 axis, on differentiated HaCaT keratinocytes. We focused on three key biological processes characterizing psoriatic plaque formation—proliferation, differentiation, and ferroptosis—taking into account the additive/synergistic effect upon a specific cytokinic milieu.

As a study model, we used HaCaT cells, spontaneously transformed keratinocytes derived from human epidermis. These cells have a wide range of applications due to their ability to efficiently proliferate and, under the presence of calcium, to differentiate *in vitro*, making them a valuable tool for approaching keratinocyte differentiation *in vitro*. In our experiments HaCaT cells were treated with a cytokines MIX reproducing a psoriatic-like inflammatory milieu.

Previous studies have shown that exposure of keratinocytes to individual cytokines exerts distinct effects on proliferation and differentiation. In HaCaT cells exposed to IL-17 alone, the expression of the differentiation marker filaggrin and of many genes involved in cellular adhesion was significantly decreased [45]. IL-22 alone significantly inhibited differentiation, but not proliferation, in NHEK [46]. Conversely, the combination of IL-22 and IL-17 induced proinflammatory cytokine production without affecting keratinocyte differentiation [47]. Two recent studies investigated the effects of a psoriasis-like inflammation with a mixture composed of IL-17, IL-22, TNF- α , IFN- γ , and KGF [48] or IL-17, IL-22, TNF- α , oncostatin, and IL-1 α [5] on keratinocytes. However, we trust that the co-presence of IL-23, the master psoriatic cytokine [26,27], with the effector psoriatic cytokines has not been fully explored strictly considering the differentiative keratinocyte process and hence should be considered, thereby adding valuable new insights. To the best of our knowledge, this is the first study investigating a complete psoriatic milieu on differentiated human keratinocytes, to highlight the current established knowledge.

A first relevant observation concerns keratinocyte proliferation. Control HaCaT cells exhibited a time-dependent increase in proliferative activity. In contrast, the exposure to the cytokine MIX significantly reduced proliferation at T48 (Figure 6) and decreased cell availability at both time points examined (Figure 8). These findings regarding cell proliferation are in line with previous observations obtained in 3D organotypic cultures of human skin exposed to proinflammatory cytokines [35] and support the concept that the known suprabasal hyperproliferation detected in advanced stages of the psoriatic lesions could represent a late response to early cytokine-induced injury affecting epidermal keratinocytes.

Regarding intercellular adhesion, control HaCaT cells showed a progressive increase in CLDN-1 and ZO-1 immunofluorescence over time, reflecting ongoing calcium-induced differentiation. Conversely, the cytokine MIX selectively impaired membrane-associated CLDN-1 immunostaining, while total CLDN-1 protein levels remained unchanged (Figure 4), with an apparent discrepancy between immunofluorescence analysis and total protein content of CLDN1. We can hypothesize that our experimental proinflammatory microenvironment induced a CLDN1 mislocalization and/or disassociation without affecting the total protein amount, as previously reported in stimulated HaCaT cells [49–51]. In parallel, the MIX affected ZO-1 distribution and, to a lesser extent, ZO-1 total protein expression (Figure 4).

These observations are in accordance with evidence describing tight junction remodelling in early psoriatic plaques within the suprabasal layers [17]. The different effect exerted by the MIX on the two TJ proteins can be explained considering that the role of

CLDN-1 is exclusively involved in intercellular adhesion, while ZO-1 plays a broader role, contributing not only to TJs formation during differentiation but also to cell proliferation [52,53]. A similar correlation between ZO-1 and cell proliferation induced by psoriatic cytokines MIX exists in the 3D setting [35].

We next evaluated keratin expression as a marker of cell differentiation. Consistent with the enhanced proliferation activity herein reported (Figure 6) and with previous reports describing the coexistence of proliferation and early differentiation in calcium-treated HaCaT cells [38], cytoplasmatic K14 displayed an increasing expression trend in control samples at T48 as evidenced by immunofluorescence analysis, although same results are not reflected by total K14 protein levels (Figure 5). This apparent discrepancy likely reflects changes in K14 filament organization rather than overall expression and is compatible with the intermediate differentiation state of our HaCaT model. Notably, following 24 h of exposure to the psoriatic cytokine MIX, an increase in K14 levels was observed (Figure 5A,B). These results mirror some observations in psoriatic epidermal lesions reporting enhanced and more widespread expression in the suprabasal layers of the basal/proliferative marker K14 [54]. At T48, however, no statistically significant differences in K14 protein levels were detected between control and MIX-treated cells (Figure 5A,B).

Both Western blot and quantitative immunofluorescence analyses were instead agreed in revealing relatively stable K10 expression levels (Figure 5C,D). These findings suggest that, during the early phases of exposure to a psoriatic cytokine environment, K10 expression is not markedly affected. This interpretation does not contradict previous reports demonstrating cytokine-dependent modulation of K10/K14 expression during keratinocyte differentiation and stress responses [55,56], but rather indicates that such changes may become more pronounced at later experimental time points or under different experimental conditions.

Interestingly, in our experimental conditions, keratinocyte proliferation persisted during the differentiation process (Figure 6), as previously reported by Wilson et al. [57]. Up to now, the correlation between HaCaT cell proliferation and differentiation has been studied in other different experimental conditions [56,58,59]. In our setting, HaCaT cells cultured in the presence of calcium for a short time represent a 2D cell model mimicking an initial phase of epidermal differentiation, similar to that detected in the lowest layers of the spinous epidermal compartment where the calcium gradient progressively increases from the basal layer towards the most differentiated granular cells [60].

Beyond proliferation and differentiation, our study provides new insight into the potential involvement of ferroptosis-related pathways in early psoriatic keratinocyte responses. Exposure to the cytokine MIX significantly decreased GPX4 expression (Figure 7), in accordance with recent experimental and clinical studies reporting reduced GPX4 expression, enhanced lipid peroxidation, and mitochondrial alterations in psoriatic lesions [30,33,61–63]. However, the functional role of ferroptosis in psoriasis remains debated, with conflicting evidence suggesting either a pathogenic contribution or a compensatory, potentially protective response. Our data support a model in which inflammation and ferroptosis are not mutually exclusive but rather represent interconnected processes. Specifically, cytokine-induced oxidative stress may prime keratinocytes for ferroptosis, while ferroptosis-associated cellular damage may, in turn, amplify inflammatory signalling. Consistently, the cytokine MIX depleted intracellular GSH levels, impaired ATP production (Figures 8 and 9), and sensitized keratinocytes to ferroptosis inducers such as erastin and RSL3 (Figures 7–9). Consistently, cytokine MIX also induced an increase in intracellular iron levels at T24, and to a lesser extent at T48 (Figure 8). These findings indicate that a proinflammatory psoriatic environment can lower the antioxidant capacity of keratinocytes, thereby facilitating ferroptosis-like changes. Notably, treatment with the cytokine MIX

alone did not induce overt mitochondrial ultrastructural damage, although it significantly reduced ATP levels, suggesting an early functional mitochondrial impairment. Structural mitochondrial alterations became evident only in presence of ferroptosis inducers combined with the cytokine MIX (Figure 9), indicating that the psoriatic inflammatory milieu acts as a sensitizing factor rather than a direct trigger of terminal ferroptotic damage. This distinction supports the use of the term “ferroptosis-like changes” to describe our findings.

Ultrastructural analyses further revealed differential mitochondrial responses to erastin and RSL3, consistent with their distinct molecular targets within the ferroptotic cascade (Figure 1). In the case of the MIX/erastin incubation, the disappearance of the mitochondrial cristae suggested a direct and profound effect on these organelles, while the treatment with MIX/RSL3 induced an uneven disarrangement of the subcellular mitochondrial organization accompanied by abundant cytoplasmic multilamellar bodies. These structures may represent storage compartments for oxidized material normally eliminated by autophagy, a process that may be impaired under our experimental proinflammatory conditions. Overall, these subcellular observations add new insights into the cellular/molecular events triggered by these two ferroptotic agents in the presence of a psoriatic proinflammatory milieu.

5. Conclusions

Despite certain limitations of the present study, such as the lack of functional rescue experiments using ferroptosis inhibitors, our data demonstrate that exposure of differentiated keratinocytes to a complete psoriatic cytokine microenvironment is sufficient to affect early differentiation and intercellular adhesion while simultaneously priming ferroptosis-related pathways. This experimental model provides a useful platform for investigating early pathogenic events in psoriasis and for exploring the interplay between inflammation, oxidative stress, and regulated cell death, potentially opening new perspectives for targeted therapeutic strategies. Some small molecules, such as JAK inhibitors, which are part of the armamentarium for psoriasis treatment, can interfere—at a diverse level—with ferroptosis; elucidating the relationship between the inflammatory psoriatic milieu and ferroptosis may further support the potential clinical relevance of ferroptosis-modulating approaches in psoriasis management.

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Abbreviations

The following abbreviations are used in this manuscript:

CLDN-1	Claudin 1
ZO-1	Zonula Occludens 1
CTR	Control
MIX	Psoriatic cytokine mixture
BrdU	5-bromo-2'-deoxyuridine
K	Keratin
IL	Interleukin
GSH	Glutathione
GPX4	Glutathione peroxidase 4
ERA	Erastin
TEM	Transmission Electron Microscopy
BSA	Bovine Serum Albumin.
PBS	Phosphate-Buffered Saline solution

References

1. Wong, R.; Geyer, S.; Weninger, W.; Guimbeteau, J.C.; Wong, J.K. The dynamic anatomy and patterning of skin. *Exp. Dermatol.* **2016**, *25*, 92–98. [\[CrossRef\]](#)
2. D'Orazio, J.; Jarrett, S.; Amaro-Ortiz, A.; Scott, T. UV radiation and the skin. *Int. J. Mol. Sci.* **2013**, *14*, 12222–12248. [\[CrossRef\]](#)
3. Sun, L.; Liu, W.; Zhang, L.J. The Role of Toll-Like Receptors in Skin Host Defense, Psoriasis, and Atopic Dermatitis. *J. Immunol. Res.* **2019**, *2019*, 1824624. [\[CrossRef\]](#)
4. Yin, J.; Zhang, S.; Yang, C.; Wang, Y.; Shi, B.; Zheng, Q.; Zeng, N.; Huang, H. Mechanotransduction in skin wound healing and scar formation: Potential therapeutic targets for controlling hypertrophic scarring. *Front. Immunol.* **2022**, *13*, 1028410. [\[CrossRef\]](#) [\[PubMed\]](#)
5. Zhu, Q.; Zhao, L.; Ding, H.; Song, J.; Zhang, Q.; Yu, S.; Wang, Y.; Wang, H. Interleukins and Psoriasis. *J. Cutan. Med. Surg.* **2024**, *28*, NP19–NP35. [\[CrossRef\]](#)
6. Kabashima, K.; Honda, T.; Ginhoux, F.; Egawa, G. The immunological anatomy of the skin. *Nat. Rev. Immunol.* **2019**, *19*, 19–30. [\[CrossRef\]](#)
7. Nestle, F.O.; Kaplan, D.H.; Barker, J. Psoriasis. *N. Engl. J. Med.* **2009**, *361*, 496–509. [\[CrossRef\]](#)
8. Grine, L.; Dejager, L.; Libert, C.; Vandenbroucke, R.E. An inflammatory triangle in psoriasis: TNF, type I IFNs and IL-17. *Cytokine Growth Factor. Rev.* **2015**, *26*, 25–33. [\[CrossRef\]](#) [\[PubMed\]](#)
9. Prignano, F.; Donetti, E. Looking at Interleukin-22 from a New Dermatological Perspective: From Epidermal Homeostasis to Its Role in Chronic Skin Diseases. *Dermatology* **2022**, *238*, 829–836. [\[CrossRef\]](#) [\[PubMed\]](#)
10. Johnson-Huang, L.M.; Lowes, M.A.; Krueger, J.G. Putting together the psoriasis puzzle: An update on developing targeted therapies. *Dis. Model. Mech.* **2012**, *5*, 423–433. [\[CrossRef\]](#)
11. Prignano, F.; Lombardo, G.; Indino, S.; Ricceri, F.; Cornaghi, L.; Donetti, E.B. Evaluation of expression of Toll-Like Receptors 7 and 9, proliferation, and cytoskeletal biomarkers in plaque and guttate psoriasis: A pilot morphological study. *Eur. J. Histochem.* **2021**, *65*, 3218. [\[CrossRef\]](#)
12. Karantza, V. Keratins in health and cancer: More than mere epithelial cell markers. *Oncogene* **2011**, *30*, 127–138. [\[CrossRef\]](#)
13. Bouchard, C.; Grenier, A.; Cardinal, S.; Bélanger, S.; Voyer, N.; Pouliot, R. Antipsoriatic Potential of Quebecol and Its Derivatives. *Pharmaceutics* **2022**, *14*, 1129. [\[CrossRef\]](#) [\[PubMed\]](#)
14. Roth, W.; Hatzfeld, M.; Friedrich, M.; Thiering, S.; Magin, T.M. Keratin function and regulation in tissue homeostasis and pathogenesis. *Biomol. Concepts* **2012**, *3*, 161–173. [\[CrossRef\]](#)
15. Van Itallie, C.M.; Anderson, J.M. Architecture of tight junctions and principles of molecular composition. *Semin. Cell Dev. Biol.* **2014**, *36*, 157–165. [\[CrossRef\]](#)

16. Volksdorf, T.; Heilmann, J.; Eming, S.A.; Schawjinski, K.; Zorn-Kruppa, M.; Ueck, C.; Vidal, Y.S.S.; Windhorst, S.; Jucker, M.; Moll, I.; et al. Tight Junction Proteins Claudin-1 and Occludin Are Important for Cutaneous Wound Healing. *Am. J. Pathol.* **2017**, *187*, 1301–1312. [[CrossRef](#)]
17. Kirschner, N.; Poetzel, C.; von den Driesch, P.; Wladykowski, E.; Moll, I.; Behne, M.J.; Brandner, J.M. Alteration of tight junction proteins is an early event in psoriasis: Putative involvement of proinflammatory cytokines. *Am. J. Pathol.* **2009**, *175*, 1095–1106. [[CrossRef](#)]
18. Brotas, A.M.; Cunha, J.M.; Lago, E.H.; Machado, C.C.; Carneiro, S.C. Tumor necrosis factor-alpha and the cytokine network in psoriasis. *An. Bras. Dermatol.* **2012**, *87*, 673–681; quiz 682–673. [[CrossRef](#)]
19. Furue, M.; Kadono, T. The contribution of IL-17 to the development of autoimmunity in psoriasis. *Innate Immun.* **2019**, *25*, 337–343. [[CrossRef](#)] [[PubMed](#)]
20. Voglis, S.; Moos, S.; Kloos, L.; Wanke, F.; Zayoud, M.; Pelczar, P.; Giannou, A.D.; Pezer, S.; Albers, M.; Luessi, F.; et al. Regulation of IL-22BP in psoriasis. *Sci. Rep.* **2018**, *8*, 5085. [[CrossRef](#)]
21. Abdo, A.I.K.; Tye, G.J. Interleukin 23 and autoimmune diseases: Current and possible future therapies. *Inflamm. Res.* **2020**, *69*, 463–480. [[CrossRef](#)]
22. Guilloteau, K.; Paris, I.; Pedretti, N.; Boniface, K.; Juchaux, F.; Huguier, V.; Guillet, G.; Bernard, F.X.; Lecron, J.C.; Morel, F. Skin Inflammation Induced by the Synergistic Action of IL-17A, IL-22, Oncostatin M, IL-1{alpha}, and TNF-{alpha} Recapitulates Some Features of Psoriasis. *J. Immunol.* **2010**, *184*, 5263–5270. [[CrossRef](#)]
23. Rabeony, H.; Petit-Paris, I.; Garnier, J.; Barrault, C.; Pedretti, N.; Guilloteau, K.; Jegou, J.F.; Guillet, G.; Huguier, V.; Lecron, J.C.; et al. Inhibition of keratinocyte differentiation by the synergistic effect of IL-17A, IL-22, IL-1 α , TNF α and oncostatin M. *PLoS ONE* **2014**, *9*, e101937. [[CrossRef](#)] [[PubMed](#)]
24. Zheng, H.; Gu, L.; Wang, Z.; Zhou, H.; Zhang, C.; Teng, X.; Hu, Z.; Wei, X.; Liu, X.; Zeng, F.; et al. Establishing Transcription Profile of Psoriasiform Cutaneous In Vitro using HaCaT Cells Stimulated with Combination of Cytokines. *J. Vis. Exp.* **2021**, *169*, e61537. [[CrossRef](#)]
25. Zima, K.; Purzycka-Bohdan, D.; Szczerkowska-Dobosz, A.; Gabig-Cimińska, M. Keratinocyte-Mediated Antigen Presentation in Psoriasis: Preliminary Insights from In Vitro Studies. *Int. J. Mol. Sci.* **2024**, *25*, 13387. [[CrossRef](#)]
26. Sharma, A.; Dhiman, S.; Singh, T.G.; Bhatia, R.; Awasthi, A. Psoriasis unveiled: The cellular ballet, molecular symphony, and genetic puzzle. *Int. Immunopharmacol.* **2025**, *161*, 115021. [[CrossRef](#)]
27. Chiricozzi, A.; Saraceno, R.; Chimenti, M.S.; Guttman-Yassky, E.; Krueger, J.G. Role of IL-23 in the pathogenesis of psoriasis: A novel potential therapeutic target? *Expert. Opin. Ther. Targets* **2014**, *18*, 513–525. [[CrossRef](#)]
28. Li, S.; Luo, X.; Zhang, S.; Su, Y.; Deng, M.; Zhu, Y.; Zhang, P.; Wu, R.; Zhao, M. Ferroptosis Activation Contributes to the Formation of Skin Lesions in Psoriasis Vulgaris. *Antioxidants* **2023**, *12*, 310. [[CrossRef](#)]
29. Vats, K.; Tian, H.; Singh, K.; Tyurina, Y.Y.; Sparvero, L.J.; Tyurin, V.A.; Kruglov, O.; Chang, A.; Wang, J.; Green, F.; et al. Ferroptosis of select skin epithelial cells initiates and maintains chronic systemic immune-mediated psoriatic disease. *J. Clin. Investig.* **2024**, *135*, e183219. [[CrossRef](#)] [[PubMed](#)]
30. Shou, Y.; Yang, L.; Yang, Y.; Xu, J. Inhibition of keratinocyte ferroptosis suppresses psoriatic inflammation. *Cell Death Dis.* **2021**, *12*, 1009. [[CrossRef](#)] [[PubMed](#)]
31. Dixon, S.J.; Lemberg, K.M.; Lamprecht, M.R.; Skouta, R.; Zaitsev, E.M.; Gleason, C.E.; Patel, D.N.; Bauer, A.J.; Cantley, A.M.; Yang, W.S.; et al. Ferroptosis: An iron-dependent form of nonapoptotic cell death. *Cell* **2012**, *149*, 1060–1072. [[CrossRef](#)]
32. Ursini, F.; Maiorino, M. Lipid peroxidation and ferroptosis: The role of GSH and GPx4. *Free Radic. Biol. Med.* **2020**, *152*, 175–185. [[CrossRef](#)]
33. Wu, N.; Hu, Q.; Fu, Z.; Tong, X.; Gao, L.; Tan, L.; Yan, S.; Wang, D.; Zeng, J.; Lu, J.; et al. Pilot study of the role of ferroptosis in abnormal biological behaviour of keratinocytes in psoriasis vulgaris. *Arch. Dermatol. Res.* **2024**, *316*, 604. [[CrossRef](#)]
34. Cornaghi, L.; Gagliano, N.; Preis, F.W.B.; Prignano, F.; Donetti, E. Inside-out and outside-in organotypic normal human skin culture: JAK-STAT pathway is activated after pro-inflammatory psoriatic cytokine exposure. *Tissue Cell* **2022**, *74*, 101675. [[CrossRef](#)] [[PubMed](#)]
35. Donetti, E.; Lombardo, G.; Baruffaldi Preis, F.; Cornaghi, L.B.; Pescitelli, L.; Prignano, F. 3D skin model to investigate the early epidermal morphological psoriatic features. *J. Transl. Sci.* **2019**, *6*, 4. [[CrossRef](#)]
36. Du, Y.; Guo, Z. Recent progress in ferroptosis: Inducers and inhibitors. *Cell Death Discov.* **2022**, *8*, 501. [[CrossRef](#)] [[PubMed](#)]
37. Yang, W.S.; SriRamaratnam, R.; Welsch, M.E.; Shimada, K.; Skouta, R.; Viswanathan, V.S.; Cheah, J.H.; Clemons, P.A.; Shamji, A.F.; Clish, C.B.; et al. Regulation of ferroptotic cancer cell death by GPX4. *Cell* **2014**, *156*, 317–331. [[CrossRef](#)]
38. Fujisaki, H.; Futaki, S.; Yamada, M.; Sekiguchi, K.; Hayashi, T.; Ikejima, T.; Hattori, S. Respective optimal calcium concentrations for proliferation on type I collagen fibrils in two keratinocyte line cells, HaCaT and FEPE1L-8. *Regen. Ther.* **2018**, *8*, 73–79. [[CrossRef](#)]
39. Boniface, K.; Bernard, F.X.; Garcia, M.; Gurney, A.L.; Lecron, J.C.; Morel, F. IL-22 inhibits epidermal differentiation and induces proinflammatory gene expression and migration of human keratinocytes. *J. Immunol.* **2005**, *174*, 3695–3702. [[CrossRef](#)]

40. Donetti, E.; Cornaghi, L.; Gualerzi, A.; Baruffaldi Preis, F.W.; Prignano, F. An innovative three-dimensional model of normal human skin to study the proinflammatory psoriatic effects of tumor necrosis factor- α and interleukin-17. *Cytokine* **2014**, *68*, 1–8. [\[CrossRef\]](#)
41. Riva, F.; Casasco, A.; Casasco, M.; Calligaro, A.; Cornaglia, A.I. Growth and stratification of epithelial cells in minimal culture conditions. In *Epidermal Cells: Methods in Molecular Biology*; Humana Press: Totowa, NJ, USA, 2010; Volume 585, pp. 25–43. [\[CrossRef\]](#)
42. Hennings, H.; Holbrook, K.A. Calcium regulation of cell-cell contact and differentiation of epidermal cells in culture. An ultrastructural study. *Exp. Cell Res.* **1983**, *143*, 127–142. [\[CrossRef\]](#)
43. Capone, A.; Visco, V.; Belleudi, F.; Marchese, C.; Cardinali, G.; Bellocchi, M.; Picardo, M.; Frati, L.; Torrisi, M.R. Up-modulation of the expression of functional keratinocyte growth factor receptors induced by high cell density in the human keratinocyte HaCaT cell line. *Cell Growth Differ.* **2000**, *11*, 607–614. [\[PubMed\]](#)
44. Ishida-Yamamoto, A.; Igawa, S.; Kishibe, M. Molecular basis of the skin barrier structures revealed by electron microscopy. *Exp. Dermatol.* **2018**, *27*, 841–846. [\[CrossRef\]](#) [\[PubMed\]](#)
45. Gutowska-Owsiak, D.; Schaupp, A.L.; Salimi, M.; Selvakumar, T.A.; McPherson, T.; Taylor, S.; Ogg, G.S. IL-17 downregulates filaggrin and affects keratinocyte expression of genes associated with cellular adhesion. *Exp. Dermatol.* **2012**, *21*, 104–110. [\[CrossRef\]](#) [\[PubMed\]](#)
46. Zhuang, L.; Ma, W.; Yan, J.; Zhong, H. Evaluation of the effects of IL-22 on the proliferation and differentiation of keratinocytes in vitro. *Mol. Med. Rep.* **2020**, *22*, 2715–2722. [\[CrossRef\]](#)
47. Cho, K.A.; Kim, J.Y.; Woo, S.Y.; Park, H.J.; Lee, K.H.; Pae, C.U. Interleukin-17 and Interleukin-22 Induced Proinflammatory Cytokine Production in Keratinocytes via Inhibitor of Nuclear Factor κ B Kinase- α Expression. *Ann. Dermatol.* **2012**, *24*, 398–405. [\[CrossRef\]](#)
48. Ghaffarinia, A.; Poliska, S.; Ayaydin, F.; Goblos, A.; Parvaneh, S.; Manczinger, M.; Balogh, F.; Erdei, L.; Vereb, Z.; Szabo, K.; et al. Unraveling Transcriptome Profile, Epigenetic Dynamics, and Morphological Changes in Psoriasis-like Keratinocytes: “Insights into Similarity with Psoriatic Lesional Epidermis”. *Cells* **2023**, *12*, 2825. [\[CrossRef\]](#)
49. Kobayashi, M.; Shu, S.; Marunaka, K.; Matsunaga, T.; Ikari, A. Weak Ultraviolet B Enhances the Mislocalization of Claudin-1 Mediated by Nitric Oxide and Peroxynitrite Production in Human Keratinocyte-Derived HaCaT Cells. *Int. J. Mol. Sci.* **2020**, *21*, 7138. [\[CrossRef\]](#)
50. Marunaka, K.; Kobayashi, M.; Shu, S.; Matsunaga, T.; Ikari, A. Brazilian Green Propolis Rescues Oxidative Stress-Induced Mislocalization of Claudin-1 in Human Keratinocyte-Derived HaCaT Cells. *Int. J. Mol. Sci.* **2019**, *20*, 3869. [\[CrossRef\]](#)
51. Ohnemus, U.; Kohrmeyer, K.; Houdek, P.; Rohde, H.; Wladykowski, E.; Vidal, S.; Horstkotte, M.A.; Aepfelbacher, M.; Kirschner, N.; Behne, M.J.; et al. Regulation of epidermal tight-junctions (TJ) during infection with exfoliative toxin-negative Staphylococcus strains. *J. Investig. Dermatol.* **2008**, *128*, 906–916. [\[CrossRef\]](#)
52. Matter, K.; Balda, M.S. Signalling to and from tight junctions. *Nat. Rev. Mol. Cell Biol.* **2003**, *4*, 225–236. [\[CrossRef\]](#)
53. Imafuku, K.; Iwata, H.; Natsuga, K.; Okumura, M.; Kobayashi, Y.; Kitahata, H.; Kubo, A.; Nagayama, M.; Ujiie, H. Zonula occludens-1 distribution and barrier functions are affected by epithelial proliferation and turnover rates. *Cell Prolif.* **2023**, *56*, e13441. [\[CrossRef\]](#)
54. Thewes, M.; Stadler, R.; Korge, B.; Mischke, D. Normal psoriatic epidermis expression of hyperproliferation-associated keratins. *Arch. Dermatol. Res.* **1991**, *283*, 465–471. [\[CrossRef\]](#)
55. Sakaguchi, M.; Miyazaki, M.; Takaishi, M.; Sakaguchi, Y.; Makino, E.; Kataoka, N.; Yamada, H.; Namba, M.; Huh, N.H. S100C/A11 is a key mediator of Ca(2+)-induced growth inhibition of human epidermal keratinocytes. *J. Cell Biol.* **2003**, *163*, 825–835. [\[CrossRef\]](#)
56. Colombo, I.; Sangiovanni, E.; Maggio, R.; Mattozzi, C.; Zava, S.; Corbett, Y.; Fumagalli, M.; Carlino, C.; Corsetto, P.A.; Scacabarozzi, D.; et al. HaCaT Cells as a Reliable In Vitro Differentiation Model to Dissect the Inflammatory/Repair Response of Human Keratinocytes. *Mediators Inflamm.* **2017**, *2017*, 7435621. [\[CrossRef\]](#) [\[PubMed\]](#)
57. Wilson, V.G. Growth and differentiation of HaCaT keratinocytes. In *Epidermal Cells: Methods in Molecular Biology*; Springer: New York, NY, USA, 2014; Volume 1195, pp. 33–41. [\[CrossRef\]](#)
58. Micallef, L.; Belaubre, F.; Pinon, A.; Jayat-Vignoles, C.; Delage, C.; Charveron, M.; Simon, A. Effects of extracellular calcium on the growth-differentiation switch in immortalized keratinocyte HaCaT cells compared with normal human keratinocytes. *Exp. Dermatol.* **2009**, *18*, 143–151. [\[CrossRef\]](#)
59. Seo, M.D.; Kang, T.J.; Lee, C.H.; Lee, A.Y.; Noh, M. HaCaT Keratinocytes and Primary Epidermal Keratinocytes Have Different Transcriptional Profiles of Cornified Envelope-Associated Genes to T Helper Cell Cytokines. *Biomol. Ther.* **2012**, *20*, 171–176. [\[CrossRef\]](#) [\[PubMed\]](#)
60. Bollag, W.B. Down-Regulated Calcium-Sensing Receptor in Keratinocytes and Skin from Aged Mice and Humans Impairs Function. *J. Investig. Dermatol.* **2021**, *141*, 2558–2561. [\[CrossRef\]](#)

61. Kato, C.; Kusumoto, I.; Kato, S.; Otoki, Y.; Ito, J.; Totsuka, H.; Rajgopal, A.; Hong, J.; Nakagawa, K. Induction of ferroptosis in human keratinocyte HaCaT cells by squalene hydroperoxide: Possible prevention of skin ferroptosis by botanical extracts. *Biochem. Biophys. Res. Commun.* **2024**, *698*, 149553. [[CrossRef](#)] [[PubMed](#)]
62. Arbiser, J.L.; Bonner, M.Y.; Ward, N.; Elsey, J.; Rao, S. Selenium unmasks protective iron armor: A possible defense against cutaneous inflammation and cancer. *Biochim. Biophys. Acta Gen. Subj.* **2018**, *1862*, 2518–2527. [[CrossRef](#)]
63. Benhadou, F.; Mintoff, D.; Del Marmol, V. Psoriasis: Keratinocytes or Immune Cells—Which Is the Trigger? *Dermatology* **2019**, *235*, 91–100. [[CrossRef](#)] [[PubMed](#)]

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