Proteomic analysis of scleroderma lesional skin reveals activated wound healing phenotype of epidermal cell layer

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Objective. To identify using proteomic analysis, proteins of altered abundance in the skin of patients with SSc.

Methods. 4 mm excision biopsies were obtained from the forearm involved skin of 12 diffuse SSc patients and 12 healthy controls. Two-dimensional electrophoresis was used to separate and define proteins in normal and SSc skin biopsy material. Proteins of altered abundance in the disease were formally identified by mass spectroscopy. Abnormalities of the epidermis were confirmed by immunohistochemistry.

Results. Proteomic analysis revealed altered abundance of proteins involved in extracellular matrix production, myofibroblast contractility, energy metabolism and response to oxidative stress. In addition, proteins specific to the epidermis and involved in epidermal cell differentiation were altered in abundance in the disease. SSc epidermis is thickened, has an expanded nucleated cell layer, and exhibits abnormal persistence of basal marker keratin 14, delayed expression of maturation markers keratin 1/10 and the induction of keratins 6 and 16, normally absent from interfollicular skin and induced following epidermal injury. These changes closely resemble the activated phenotype seen during wound healing.

Conclusions. Consistent with previous models of SSc pathogenesis these data are showing increased contractility, increased extracellular matrix and response to oxidative stress in the involved skin of recent onset SSc patients. In addition, we show that SSc epidermis has an activated, wound healing phenotype. These findings are important because epidermal cells activated by injury induce and regulate local fibroblasts during wound repair.

Key words: Scleroderma, Proteomics, Epidermis, Wound-healing.

Introduction

SSc is a severe systemic disease characterized by autoimmunity, vascular injury and fibrosis of the skin and internal organs [1]. Life expectancy is reduced in the disease due to organ fibrosis or vascular disease [2]. Persistent fibroblast activation is central to pathogenesis, and SSc fibroblasts have increased ability to secrete, adhere to and contract extracellular matrix [3]. The initiating factors leading to fibroblast activation in the disease are not completely understood, but endothelial cell injury, autoimmunity and persistent expression of fibrosis enhancing growth factors are involved [4–6]. Several growth factors and cytokines are implicated in SSc disease process including TGF-β, ET-1 and connective tissue growth factor (CTGF) [7–9].

Human epidermis, the outermost layer of the skin, is a stratified squamous epithelium consisting of several cell types including Langerhans cells, melanocytes and keratinocytes. The most dominant cell type is the keratinocyte. Keratins belong to the superfamily of intermediate filament proteins [10]. Epidermal keratinocytes have two alternative pathways open to them, terminal differentiation, or activation [11]. In normal healthy epidermis, keratinocytes enter terminal differentiation from basal layer to cornified layers, and survive through a process of self-renewal [12]. In contrast, following epidermal injury or in hyperproliferative disorders such as psoriasis or cancer, keratinocytes become activated and express activation markers, such as keratin 6, 16 and 17 [13–15]. Upon activation, keratinocytes produce and secrete a large numbers of cytokines, including ILs, growth factors, CSFs and chemokines [16, 17], which can influence local fibroblasts, endothelial cells and regulate the inflammatory response during wound healing.

Changes in the dermis have received much attention in SSc because overproduction of extracellular matrix by dermal fibroblasts accounts for much of the pathological skin thickening and scarring seen in the disease. Relatively, little is published about the epidermis in SSc, but changes in skin pigmentation which can be severe and widespread imply that the epidermis is also abnormal in the disease [18]. ET-1 [18], TGF-β [19], monocyte chemo-attractant protein-1 (MCP-1) [20], VEGF [21] and IL-21 receptor (IL-21R) [22] have all been shown to be up-regulated in the epidermis in SSc.

We performed a proteomic analysis of whole-skin biopsy material taken from the fibrotic skin lesions of patients with recent onset of SSc, drawing comparison with the array of proteins expressed in healthy control skin. Prominent among proteins of altered abundance in the disease are proteins specific to the epidermis and involved in keratinocyte differentiation. Furthermore, we show that in SSc, epidermal keratinocytes have deviated from the normal pattern of differentiation and are in an activated state, marked by the induction of keratin 6 and 16 in the disease.

Materials and methods

Patients

SSc was defined according to internationally agreed guidelines [23]. Patients selected for inclusion in the study were from the diffuse subset and were within 2 yrs of the onset of skin changes. Ethical committee approval was obtained for the study from the ethical committee of the Royal Free Hospital, and all patients gave written informed consent to participate in the study. The 4 mm excision biopsies were obtained from the forearm involved skin of diffuse SSc patients, and from the forearm of healthy control subjects. Wound healing studies in healthy controls were performed by 4 mm excision biopsy at baseline and repeat biopsy on day 7. Biopsies were taken prior to commencement of
Proteomic analysis of SSc lesional skin

Immunoassays were performed using 5 μm paraffin sections. Staining was performed on six patients with diffuse SSc and six controls. Once section was de-waxed and rehydrated; antigen retrieval was performed using citrate buffer. Sections were blocked with normal serum in PBS for 30 min followed by incubation with primary antibodies for 1 h. Primary antibodies were used as follows: mouse monoclonal antibodies directed against human keratin 14 (Novoceastra Laboratories Newcastle # RTU-LL002), keratin 6 and keratin 16 (LL025) (Lab Vision Corporation, Freemont, CA, USA), integrin alpha 6 (Chemicon # CBL 458) and HSP-27(β1) (Cymbus Biotechnology, Southampton, UK). For some of the proteins studied, antibodies did not give effective staining on paraffin sections and therefore frozen sections were used. The 4 mm punch biopsies from patients and controls were embedded in Tissue-Tek optimum cutting temperature (OCT) compound (Sakura Finetek, Japan) fixation medium and frozen in isoprenalol cooled by liquid nitrogen and then stored at −70°C prior to analysis. Sections were cut at 5 μm using a cryostat and then fixed in ice-cold acetone. Primary antibodies were used as follows; anti-galectin 7 (kind gift Ichiro Kuwabara, Department of Dermatology, Davis School of Medicine, University of California), and anti-keratin 1/10 (Cymbus Biotechnology CBL 266).

Optimum dilution for the primary antibodies was determined by serial dilution. Bound primary antibodies were revealed using species-specific biotinylated secondary antibodies for 1 h. Binding determined using the avidin/biotin ABC system (Vector, Burlingame, CA, USA) and AEC substrate for peroxidase-red (Vector SK 4200). All subsequent steps were done at room temperature. Stained sections were imaged at 20× magnification using Zeiss Axioscope.


immunosuppressive or disease modifying treatments. Proteomic profiling was based on skin biopsy material from 12 SSc patients and 12 healthy control individuals. Skin biopsy material was frozen by immersion in liquid nitrogen and then stored in liquid nitrogen prior to analysis.

**Protein extraction**

The frozen skin biopsy samples were crushed to a fine powder between two cooled metal blocks. The powder was resuspended and solubilized in 200 μl lysis buffer [9.5 M urea, 1% DTT, 2% CHAPS, 0.8% Pharmalyte pH 3-10 (Pharmacia, Uppsala, Sweden), with complete protease inhibitor cocktail (Boehringer, Bracknell Berkshire, UK)]. The protein concentration of the samples was determined by a modified Bradford assay (Coomassie Plus, Pierce Protein). Preliminary studies established that 100 μg of protein is appropriate to obtain a good two-dimensional gel electrophoresis (2DE) profile, so this loading was used.

**First-dimension isoelectric focusing**

An aliquot of each sample [100 μg of protein for the initial proteomic profiling, and 400 μg of protein for the matrix-analysed light diffraction mass spectroscopy studies (MALDI-MS)] was added to re-swelling solution [8 M urea (Sigma-Aldrich, St Louis, MO), 0.5% CHAPS (Sigma-Aldrich), 0.2% DTT (BDH), 0.2% Pharmalyte 3-10 (Sigma-Aldrich)] to a final volume of 450 μl and used to re-swell isoelectric pH gradient (IPG) strips. Two different pH ranges were used for these experiments. A 18 cm IPG pH 3-10 non-linear strips (Amersham Pharmacia Biotech, Amersham, Bucks, UK) were used to give an overview of the majority of proteins present in the samples, and 18 cm IPG pH 6-9 linear strips (Amersham Pharmacia Biotech) were used to provide improved resolution of proteins in the basic region. Following overnight re-swelling, the IPG strips were focused for 60 kVh at 0.05 mA/strip at 20°C.

**Second dimension SDS-PAGE**

Before the focused IPGs were run in the second dimension, the strips were equilibrated in buffer [1.5 M Tris (pH 8.8) (BDH), 6 M urea (Sigma-Aldrich), 30% glycerol (BDH), 2% SDS (Sigma-Aldrich), 0.01% Bromophenol Blue (LKB, Sweden), 1% DTT (BDH)] for 15 min, followed by another 15 min in buffer containing 4.8% iodoacetamide (Sigma-Aldrich). SDS-PAGE was performed overnight (20 mA/gel, 10°C) using 12%T/2.6%C separating gels in a DALT system (Amersham-Pharmacia Biotech).

**Visualization of 2DE protein profiles**

Once the second dimension had been run, gels were fixed (50% methanol, 10% acetic acid) overnight and then stained using a silver staining kit (Owl, Daiichi for proteomic profiling, and modified PlusOne, Amersham Biosciences for MALDI-MS) [24]. After silver staining, the gels were scanned at 100 μm resolution using Molecular Dynamics Personal Densitometer SI. Quantitative computer analysis was performed using PDQuest software (Bio-Rad, Hercules, CA, USA).

Normal and disease 2D gel images were subject to PDQuest analysis. In the case of IPG 3-10 gels, 821 protein spots were detected and the 2DE protein profiles showed good reproducibility, and in the case of IPG 6-9 gels, 321 protein spots were identified. A composite image representative of all of the normal gel images was made to provide a reference image of proteins present in normal skin for the two pH gradients. This reference image was calibrated in terms of protein isoelectric focus and molecular weight using the pH gradient shape for the IPG 3-10 and 6-9 strips provided by Amersham Pharmacia Biotech, and the molecular weight markers (Amersham) run in the second dimension of the SDS-PAGE gels.

**Statistical analysis**

Proteomic profiling was performed in two cycles. Following an initial round of profiling with five control and five scleroderma samples quantitative computer analysis was used to look for significant changes in abundance of proteins in the scleroderma samples when compared with healthy controls, using as a cut-off a 3-fold increase or decrease in protein level. This threshold was arbitrarily chosen as reflecting a large biologically important change in abundance of a protein. A second round of profiling was then performed using samples from a further seven patients and seven controls. In general, the second round of profiling confirmed the findings of the first round showing a 3-fold or near 3-fold difference in abundance in proteins identified during the first round. Student’s t-test was used for comparison of mean intensities between disease and healthy controls, using a threshold of P < 0.05 to establish the statistical significance of changes seen. Proteins present at altered abundance in scleroderma tissues were studied further by MALDI-TOF MS as detailed below.

**Peptide mass profiling by MALDI-TOF MS**

Protein spots of altered abundance in scleroderma tissues were cut from the gel, digested with trypsin and analysed by MALDI-TOF MS (Voyager DE-PRO), and the resulting peptide mass profiles used to identify the proteins by Swiss Protein database searching. Molecular function and biological process for the proteins of altered abundance were defined according to Gene Ontology database and by literature review.
Results

Proteins of altered abundance in scleroderma skin

Using IPG 3-10 strips 28 proteins were found to be increased >3-fold in SSc compared with control. Of the 28 protein spots examined, unequivocal identities were obtained for 16 proteins by MALDI-TOF MS, while the other 12 proteins could not be identified. These unidentified protein spots were present in low abundance on the 2D gels.

Using IPG 3-10 strips, five protein spots were identified as being of >3-fold decrease in abundance in the disease. Of these five protein spots, two could be formally identified by MALDI-TOF MS, whereas three spots which were present in low abundance could not be formally identified.

Further studies were performed to increase the separation of basic proteins using gradients in the pH 6–9 range. In these studies, 21 protein spots were identified showing >3-fold increase in the scleroderma samples. Of these 21 protein spots, unequivocal identities were obtained for only four of the proteins. Protein spots which could not be formally identified were generally present in low abundance on the gels.

Using the pH 6–9 gradients, only one protein was identified as decreased more than threefold in SSc biopsies, and this was successfully identified by MALDI-TOF MS.

Composite images representative of the normal gel images for the two pH gradients are shown in Fig. 1. The 23 protein spots for which unequivocal identities were obtained by MALDI-TOF MS are listed in Table 1 showing the number of peptides matched. We have attempted to classify proteins by functional groups according to gene ontology database and literature review.

Confirmation of altered epidermal differentiation in scleroderma skin

A number of proteins involved in epidermal cell differentiation and specific to the epidermis were of altered abundance in the disease (Table 1). Because of this we began to study epidermal cell differentiation in the disease. First, we confirmed that epidermal cell differentiation is altered in the disease (Fig. 2). We found a pattern of delayed and abnormal keratinocyte maturation with expansion of the nucleated cell layer and abnormal persistence of the basal cytokeratin 14 into suprabasal layers. These findings were confirming the proteomic analysis which showed elevated cytokeratin 14 in the disease specimens (Table 1). Also, we confirmed loss of galectin 7 in the disease, revealing strong expression of galectin 7 throughout the normal epidermis, which was depleted in the scleroderma sections, also confirming the proteomic analysis (Fig. 2). Heat-shock protein-27 (HSP-27) was confirmed to be predominantly epidermal in healthy control tissue and to be a maturation marker present in suprabasal and above layers. In scleroderma, HSP-27 was present throughout the epidermis including basal cell layer, and appeared generally increased due to expansion of the epidermis in the disease, again confirming the proteomic analysis showing increased HSP-27. In addition, we found altered expression of basal adhesion molecule α6-integrin which in healthy epidermis shows basal cell expression clearly demarcated adjacent to the basement membrane, but expression all around basal cells and into suprabasal layer in the disease epidermis (Fig. 2).

Scleroderma epidermis has an activated phenotype seen during wound healing

These findings lead us to draw comparison between the changes in scleroderma and those described during wound repair, where the epidermis becomes expanded and shows abnormal persistence of the basal cytokeratin 14 (Fig. 3).

Because of these findings, we went on to explore whether scleroderma epidermis is taking on an activated phenotype, staining for cytokeratins 6 and 16, markers of keratinocyte activation during wound healing. We confirmed that in scleroderma keratinocytes are abnormal, expressing cytokeratins 6 and 16, resembling the activated phenotype seen during excisional wound healing (Fig. 4).

Discussion

In this study, we have identified using proteomic analysis changes in the pattern of protein expression in SSc tissues vs controls. Using 2DE followed by PDQuest computer analysis we demonstrate proteins present in altered abundance in skin
<table>
<thead>
<tr>
<th>Spot number</th>
<th>Protein</th>
<th>Molecular weight (kDa)</th>
<th>pl</th>
<th>Swiss protein number</th>
<th>Number of peptides matched</th>
<th>Percent coverage (%)</th>
<th>Gene ontology molecular function</th>
<th>Gene ontology biological process</th>
<th>Abundance in control n = 12 mean (95% CI)</th>
<th>Abundance in scleroderma n = 12 mean (95% CI)</th>
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<td>11</td>
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<td>P04284</td>
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<td>29</td>
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<td>Epidermis development</td>
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<td>144 (108, 179)</td>
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<td>5.09</td>
<td>P02533</td>
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<td>50</td>
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<td>1814 (1236, 2392)</td>
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<td>P13647</td>
<td>13</td>
<td>21</td>
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<td>615 (409, 821)</td>
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<td>P31944</td>
<td>27</td>
<td>69</td>
<td>Caspase activity</td>
<td>Response to unfolded protein</td>
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<td>660 (479, 841)</td>
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<td>P47929</td>
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<td>Sugar binding</td>
<td>Heterophilic cell adhesion</td>
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<td>5.98</td>
<td>P04792</td>
<td>10</td>
<td>47</td>
<td>Protein binding</td>
<td>Response to unfolded protein</td>
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<td>1498 (865, 2131)</td>
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<td>4.79</td>
<td>P08670</td>
<td>7</td>
<td>33</td>
<td>Laminin receptor activity</td>
<td>Cell adhesion</td>
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<td>1753 (1359, 2147)</td>
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<td>P12324</td>
<td>11</td>
<td>37</td>
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<td>Cell motility</td>
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<td>1262 (1086, 1545)</td>
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<td>P08670</td>
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<td>Cell motility</td>
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<td>P27797</td>
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<td>8</td>
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<td>Protein folding</td>
<td>627 (366, 888)</td>
<td>1911 (1445, 2377)</td>
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<td>Serum amyloid P-component precursor</td>
<td>25</td>
<td>6.1</td>
<td>P02743</td>
<td>5</td>
<td>26</td>
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<td>3505 (2648, 4362)</td>
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<td>P01009</td>
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<td>31</td>
<td>Serine-type endopeptidase inhibitor activity</td>
<td>1253 (408, 2098)</td>
<td>4913 (4366, 5460)</td>
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<td>5.37</td>
<td>P01010</td>
<td>9</td>
<td>26</td>
<td>Serine-type endopeptidase inhibitor activity</td>
<td>849 (500, 1198)</td>
<td>4484 (3637, 5331)</td>
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<td>P06576</td>
<td>10</td>
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<td>Transporter activity</td>
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<td>3427 (1726, 5128)</td>
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<td>51</td>
<td>Fructose 1,6-biphosphate aldolase activity</td>
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<td>Carboxyl reductase activity</td>
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<td>884 (746, 1022)</td>
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<td>15</td>
<td>13</td>
<td>Extracellular matrix structural constituent</td>
<td>Extracellular matrix organization and biogenesis</td>
<td>446 (373, 519)</td>
<td>1457 (906, 2008)</td>
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<td>P12111</td>
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<td>627 (502, 752)</td>
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<td>38</td>
<td>7.56</td>
<td>P07355</td>
<td>26</td>
<td>61</td>
<td>Phospholipase inhibitor activity</td>
<td>Skeletal development</td>
<td>177 (112, 242)</td>
<td>736 (464, 1008)</td>
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<td>Carbonic anhydrase I</td>
<td>29</td>
<td>6.63</td>
<td>P00915</td>
<td>6</td>
<td>36</td>
<td>Carbonate dehydratase activity</td>
<td>27789 (17087, 38491)</td>
<td>9205 (5017, 13993)</td>
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biopsy material. By using peptide profiling with MALDI-TOF MS, we were able to formally identify 20 proteins elevated, and three proteins decreased in SSc tissue. Consistent with previous models of the disease, proteins of altered abundance include proteins involved in extra-cellular matrix production [3], myofibroblast contractility [25], and response to oxidative stress [26]. In addition, proteins specific to the epidermis and involved in keratinocyte differentiation were of altered abundance in the disease.

Previous studies of SSc have focused on changes in the dermal layer because of the excessive collagen deposition and vascular dysfunction. The epidermis on the other hand has received little attention, although epithelial–fibroblast interaction have been shown to play crucial role in the regulation of tissue development, homeostasis and repair [27, 28], and in the onset of fibrotic diseases for example in pulmonary fibrosis [29, 30], keloids [31] and hypertrophic scarring [32], and now appear to be relevant to the pathogenesis of SSc.

The maturation of epidermis is a complex process and keratinocytes play major role in this process. Keratinocytes synthesize structural components of the epidermal barrier through a programmed process of differentiation [33]. In normal epidermis, proliferative cells are located in the basal layer where they strongly express cytokeratins 5 and 14, and as they withdraw from the cell cycle and migrate, keratinocytes differentiate and exhibit keratin 1 and keratin 10 expression [34]. In SSc, the pattern of keratin expression is altered. Keratin 14 is seen to persist into subrabasal and above layers and this is accompanied by delay in the expression of terminal differentiation marker keratins 1 and 10, suggesting a disruption in terminal differentiation which results in a delay in maturation in the disorder. Integrins are transmembrane receptors, which play a crucial role in forming bonds between the cells of the basal layer with the basement membrane and stabilize cell-to-cell interaction and cell matrix adhesion in the epidermis [35]. The hemidesmosome-associated integrin-α6 which forms a complex with β4-subunit is sharply localized at the basal surface of basal cells. However, in the disease, the expression of the α6-integrin was no longer confined to the basal membrane zone and this was accompanied by loss of basal intensity. Furthermore, we found increase expression of 27 kDa heat-shock protein (HSP-27), a member of small heat-shock protein family. Hsp-27 is involved in the regulation in epidermal cell growth and differentiation [36, 37]. Staining for single-stranded DNA using 4', 6-diamidino-2-phenylindole

![Image](https://academic.oup.com/rheumatology/article-abstract/47/12/1754/1785523/1758)
basal cells. The membrane laminin-5, which coincides with loss of contact with adjacent keratinocytes and reduction in basement migration of mitotic cells in the basal layer is stimulated by lack of and changes to cell adhesion and cell fate [42, 43]. Upon wounding include cell migration, proliferation, cytoskeletal re-organization during re-epithelialization, keratinocytes undergo changes which proliferation, re-epithelialization and tissue remodelling [41]. Wound healing is divided into four stages: inflammation, from a persistent dysregulated wound healing response [40]. and it has been previously suggested that scleroderma may result scleroderma epidermis and those described during wound healing, chosen to draw comparison between the changes in the associated with wound healing [39]. For this reason, we have characteristic differences in the epidermis of scleroderma patients, showing increased epidermal thickness, increased basal cell number and elevated melanin content [38]. Changes in the pattern of keratin gene expression, loss of differentiation and increase in epidermal thickness are features associated with wound healing [39]. For this reason, we have chosen to draw comparison between the changes in the scleroderma epidermis and those described during wound healing, and it has been previously suggested that scleroderma may result from a persistent dysregulated wound healing response [40]. Wound healing is divided into four stages: inflammation, proliferation, re-epithelialization and tissue remodelling [41]. During re-epithelialization, keratinocytes undergo changes which include cell migration, proliferation, cytoskeletal re-organization and changes to cell adhesion and cell fate [42, 43]. Upon wounding migration of mitotic cells in the basal layer is stimulated by lack of contact with adjacent keratinocytes and reduction in basement membrane laminin-5, which coincides with loss of α6β4 integrin in basal cells. The α6β4-integrin then participates in specific aspect of

(DAPI) showed expansion of the nucleated cell layers in the disease compared with control. Consistent with this finding, an in vivo study using confocal laser scanning microscopy revealed characteristic differences in the epidermis of scleroderma patients, showing increased epidermal thickness, increased basal cell number and elevated melanin content [38].

Changes in the pattern of keratin gene expression, loss of differentiation and increase in epidermal thickness are features associated with wound healing [39]. For this reason, we have chosen to draw comparison between the changes in the scleroderma epidermis and those described during wound healing, and it has been previously suggested that scleroderma may result from a persistent dysregulated wound healing response [40]. Wound healing is divided into four stages: inflammation, proliferation, re-epithelialization and tissue remodelling [41]. During re-epithelialization, keratinocytes undergo changes which include cell migration, proliferation, cytoskeletal re-organization and changes to cell adhesion and cell fate [42, 43]. Upon wounding migration of mitotic cells in the basal layer is stimulated by lack of contact with adjacent keratinocytes and reduction in basement membrane laminin-5, which coincides with loss of α6β4 integrin in basal cells. The α6β4-integrin then participates in specific aspect of

the migration process, the formation and the stabilization of actin-associated motility [44, 45]. In the differentiation layer, differentiation-specific keratin 1/10 is down-regulated and this is substituted by the induction of keratin-6, 16 and 17 [46–48]. In addition, increase in expression of HSP-27 during regenerating tissue is said to serve both a regulatory function in mediating keratinocyte migration and protective function against the wound stressful environment [49]. Positive immunostaining for the presence of keratin 6 and 16 in scleroderma epidermis provides evidence of activation of keratinocyte in scleroderma epidermis, which further supports our view that scleroderma epidermis resembles those changes described during wound healing.

Following tissue injury, keratinocytes are able to produce and secrete cytokines and chemokines that regulate fibroblast, endothelial cell and inflammatory responses [16, 17]. We suggest that activated epidermal cells contribute to the fibrotic response in scleroderma skin lesions via their interaction with dermal fibroblasts. Consistent with this idea increased levels of ET-1 [18], MCP-1 [20], TGF-β [19], VEGF [21] and IL-21R [22] have been previously reported in scleroderma epidermis.

In conclusion, based on our findings, we suggest that epidermal keratinocytes in scleroderma are in an activated state, and in part contribute in an amplifying pro-fibrotic loop leading to scleroderma tissue remodelling. In unpublished studies, we have found that SSc epidermal sheets are able to activate fibroblasts in vitro, leading to fibroblast contractility and CTGF expression. Disruption of epithelial–fibroblast paracrine signalling is a potential therapeutic approach to the treatment of established fibrosis.

**Rheumatology key messages**

- Scleroderma epidermis is activated via wound healing pathway of differentiation.
- Activated epidermal cells are important regulators of local fibroblasts through growth factors and cytokines.
- Epithelial–fibroblast interactions may contribute to scleroderma fibrosis.

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