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Review

Emerging diagnostic and therapeutic challenges for skin fibrosis in systemic sclerosis

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A B S T R A C T

Systemic sclerosis (also called scleroderma, SSc) is a chronic autoimmune disorder characterized by excessive collagen deposition leading to skin fibrosis and various internal organ manifestations. The emergent diagnostics and therapeutic strategies for scleroderma focus on early detection and targeted interventions to improve patient outcomes and quality of life. Diagnostics for SSc have evolved significantly in recent years, driven by advancements in serological markers and imaging techniques. Autoantibody profiling, especially antinuclear antibodies (ANA) and specific scleroderma-associated autoantibodies, aids in identifying subsets of scleroderma and predicting disease progression. Furthermore, novel imaging modalities, such as high-frequency ultrasonography and optical coherence tomography, enable early detection of skin fibrosis and internal organ involvement, enhancing the diagnostic precision and allowing for tailored management. Therapeutic strategies for SSc are multifaceted, targeting immune dysregulation, vascular abnormalities, and fibrotic processes. Emerging biologic agents have shown promise in clinical trials, including monoclonal antibodies directed against key cytokines involved in fibrosis, such as transforming growth factor-β (TGF-β) and interleukin-6 (IL-6). Additionally, small-molecule inhibitors that disrupt fibrotic pathways, like tyrosine kinase inhibitors, have exhibited potential in limiting collagen deposition and preventing disease progression. Stem cell therapy, cell ablation and gene editing techniques hold great potential in regenerating damaged tissue and halting fibrotic processes. Early intervention remains crucial in managing SSc, as irreversible tissue damage often occurs in advanced stages. Novel diagnostic methods, such as biomarkers and gene expression profiling, are being explored to identify individuals at high risk for developing progressive severe disease and intervene proactively. Furthermore, patient-tailored therapeutic approaches, employing a combination of immunosuppressive agents and targeted anti-fibrotic therapies, are being investigated to improve treatment efficacy while minimizing adverse effects. The emergent diagnostics and therapeutic strategies in scleroderma are transforming the management of this challenging disease. Nevertheless, ongoing research and clinical trials are needed to optimize the efficacy and safety of these novel approaches in the complex and diverse spectrum of SSc manifestations.

1. Introduction

Systemic sclerosis (SSc) is an autoimmune disorder that causes endothelial cell damage, persistent myofibroblast activation, and persistent oversecretion of extracellular matrix, leading to progressive skin and organ fibrosis (Denton and Khanna, 2017). Since many of these feature are resistant to current therapies, SSc has a higher mortality rate than other rheumatic diseases, although recent data indicate some improvement in this trend especially for severe SSc (Domsic et al., 2014; Steen and Medsger, 1990). SSc is characterized by a high clinical heterogeneity, prevalence of organ involvement and SSc-related manifestation varying from one patient to the other. For individual patients this results in uncertainty regarding overall prognosis and makes stratification for potentially invasive therapies challenging (Nihtyanova et al., 2014; Liem et al., 2023). SSc is a rare disease but the inflammatory fibrotic process that it exemplifies is common to a range of resistant and prevalent conditions. Accordingly, SSc has a high level of unmet medical need, with no disease modifying drug adapted to all SSc patients.

Despite widespread knowledge and awareness of the disease amongst physicians, diagnosis in SSc is often delayed, adding to the potential progression of the disease pre-treatment (Pauling et al., 2021). Since seeing SSc patients is an uncommon occurrence for general physicians, the possible implications of a new diagnosis might be affected through lack of frequent experience of the condition. Even for specialists there remains uncertainty in diagnosing and determining the extent and severity of SSc at the time of presentation. A particular challenge is in the appropriate stratification of early-stage SSc patients for risk of progressive severe skin disease, or for the development of future complications and organ involvement. Involvement of specialist Centres is important in order to optimize evidence-based care and to facilitate inclusion in clinical trials and translational research programs.

The early clinical features of SSc can be varied and include symptoms such as Raynaud's and peripheral oedema. However, Raynaud's phenomenon and oedema are prevalent complaints amongst the general

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population. Strong positive findings for SSc diagnosis would include the presence of disease-specific autoantibodies as well as microvascular damage evident on nailfold capillaroscopy (Dinsdale et al., 2017).

Moreover, SSc patients at presentation often experience musculoskeletal symptoms and fatigue and when examined may have tendon crepitus due to fascial involvement, signs of interstitial lung disease (basal crepitations), or even have accelerated hypertension with acute kidney injury (renal crisis), as an early clinical manifestation (Penn et al., 2007).

Criteria for a very-early diagnosis of SSc (VEDOSS) have been recently validated, defining Raynaud's phenomenon, puffy fingers and positive ANA as entry criteria for SSc suspicion, and additional capillaroscopic findings or ANA specificity for SSc-related autoantibodies (anti-RNA polymerase III (ARA), anti-centromere (ACA) and anti-Topo-I (ATA)) as confirmation criteria, for a very early diagnosis of SSc (VEDOSS) (Lescoat, 2023).

Although SSc has the potential to involve multi-organ systems, the skin changes are characteristic, participating in early diagnosis, as well as being linked to overall prognosis and survival (Nihtyanova et al., 2014). In fact, the pattern of skin involvement is used as an important aspect of patient stratification. In cases of diffuse subset SSc (dcSSc), defined by skin involvement including distal and proximal changes, prominent oedema with skin tightness, arthropathy and generalised pruritus, are key features (Steen and Medsger, 1990) (Babulal Vadher et al., 2023). Presentation of such symptoms, especially if accompanied by Raynaud's and hand oedema, should prompt an urgent referral to initiate early treatment. Organ-based complications such as interstitial lung disease (ILD) or myocardial involvement will guide early treatment strategy, based on immunomodulatory drugs. Beyond dcSSc, which account for 30–40 % of SSc patients, the other major subset is limited cutaneous SSc (lcSSc) defined by distal cutaneous changes without proximal involvement and representing 50–60 % of the patients. SSc sine-scleroderma (ssSSc) is a third subset, accounting for 10 % of SSc patients and characterized by the absence of any skin fibrosis, but with SSc-related visceral manifestations and disease specific autoantibodies (Lescoat et al., 2023). LcSSc and ssSSc are considered less severe that dcSSc in terms of survival and mortality rate, but life-threatening manifestations can nonetheless occur in lcSSc or ssSSc. In all subsets, clinical examination allows major features of the external disease, as well as skin, vasculature, and musculoskeletal involvement, to be defined and assists in making a definite diagnosis. Organ-based complications require careful assessment in all patients through baseline and interval functional assessments including pulmonary function testing, high resolution chest CT, echocardiography and 6 min-walking test in all SSc patients, regardless of pattern of skin involvement (Akesson and Wollheim, 1989; Chan et al., 2019).

The relative importance of pathogenic mechanisms initiated by autoantibodies in SSc remains unclear, but in clinical practice SSc-specific autoantibodies are highly useful as predictive of disease outcome and the pattern of organ complications in SSc-patients, usually present on blood testing at the earliest stages of clinical disease (Burbelo et al., 2019; Hamaguchi, 2010). The three major SSc-specific autoantibodies routinely available are as follows; anti-centromere antibodies (ACA) present in 50 % of the cases, anti-topoisomerase-I antibodies (ATA) found in 20 % (ATA) and anti- RNA polymerase III antibodies in 10 % (ARA), generally seen as mutually exclusive non-overlapping groups. ATA are classically associated with clinical development of interstitial lung disease (ILD), ARA with scleroderma renal crisis, and ACA with better prognosis. ATA and ARA are also classically associated with dcSSc, although ATA positivity is not uncommon in patients with lcSSc. Interestingly, autoantibodies may also help predicting disease trajectory and natural history of skin involvement, dcSSc patients with ARA showing early rapidly increasing skin fibrosis with spontaneous improvement, whereas patients with ATA experience slower but progressive increase of skin fibrosis as assessed by the modified Rodnan skin score (mRSS).

To date, no single drug has shown efficacy on skin fibrosis in all SSc patients, confirming that skin fibrosis remains a hallmark of the disease which still needs effective therapy (Roofeh et al., 2020). Early active dcSSc has been identified as a window of opportunity for the treatment of skin fibrosis but enrichment strategies have failed to demonstrate clear and major efficacy of candidate therapies on skin fibrosis in phase III trials targeting this population and having the mRSS as their primary outcome measure (Khanna et al., 2018, 2020a, 2020b, 2020c). These results also suggest that despite recent progress in understanding disease pathogenesis and early mechanisms underlying SSc heterogeneity, key therapeutic targets are still to be defined to offer a game-changing therapeutic approach for all SSc patients (Lescoat et al., 2021a).

This narrative review will highlight the current understanding of main pathogenic mechanisms driving skin fibrosis and its heterogeneity in SSc patients and discuss a selection of promising therapeutic approaches that may help treating SSc-related skin involvement in the future.

1.1. Heterogeneity of skin involvement based on cellular signatures and differentially expressed genes: a first step towards precision medicine in SSc

Skin fibrosis in SSc includes a triad of interconnected pathogenic pathways; A. early endothelial damages with capillary leak due to disruption of endothelial junction and apoptosis of endothelial cells, B early inflammatory infiltrate of immune cells which participates in an oedematous phase of the disease (puffy hands and/or oedematous early dcSSc) and C unregulated activation of dermal fibroblasts with transition to myofibroblasts responsible for an uncontrolled extra-cellular matrix deposition as the hallmark of fibrosis (LeRoy, 1974). Cellular actors and mechanisms involved in the pathogenesis of SSc-related skin fibrosis i.e. early endothelial damages, oedema with inflammatory infiltrate and fibrosis at a later stage, are thus also involved in tissue repair. SSc is a prototypical disease in which such responses are disrupted and abnormal, although the trigger factor of such dysregulation is still to be determined in the majority of the cases. Identified triggers of SSc nonetheless include the exposure to crystalline silica as well as use of organic solvents (Muntyanu et al., 2023; Shivakumar et al., 2023).

As already alluded to, three main subsets of SSc patients are described based on the extent of skin fibrosis, i.e. SSc sine scleroderma (ssSSc), limited cutaneous subset (lcSSc) and diffuse cutaneous subset (dcSSc). The relevance of these subsets is supported by their prognostic value, survival decreasing from sine scleroderma to dcSSc (Lescoat et al., 2023). Recent works have also highlighted that autoantibodies were better predictors of survival than the cutaneous subsets, suggesting that a classification based on autoantibodies would be more relevant (Hamaguchi, 2010). Beyond these classification approaches, the analysis of differentially expressed genes and intrinsic signature in skin biopsies from SSc patients have also provided some insights into the pathogenesis of the skin fibrosis. Four main subsets were identified; 1) a "limited" signature mostly comprising samples from lcSSc patients and characterized by a low expression of cell proliferation pathway genes and T cell genes, and a dysregulation of cell adhesion, extracellular matrix, and vascular development-specific genes, 2) an "Inflammatory" subset characterized by high expression of genes related to the inflammatory lymphocyte infiltrates, including lymphocyte proliferation, humoral defense, and chemokine activity, 3) a "Diffuse-Proliferation" subset, mostly including dcSSc patients and defined by an increased expression of genes related to cell proliferation, including regulation of mitosis, cell cycle, and DNA replication, and 4) a "normal-like" subset with a gene expression signature resembling to normal skin, with prominent pathways of fatty acid metabolism (Milano et al., 2008; Keret et al., 2023). Interstingly these instrinsic pathways may facilitate prediction of treatment response, as in the phase II abatacept trial, only patients from the "inflammatory" subset showed treatment response, while other subsets showed disappointing results regarding treatment

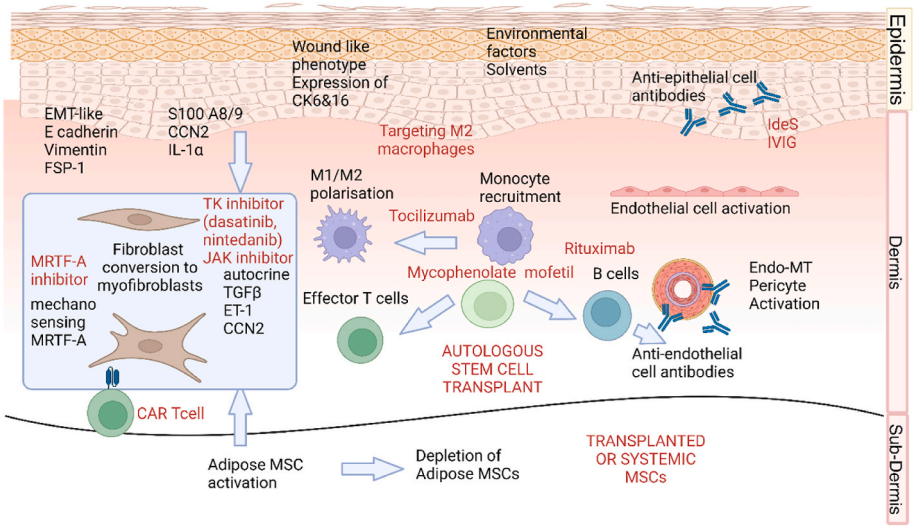

Fig. 1. Skin fibrosis in SSc: underlying biomechanisms and therapeutic approaches. Current and future possible therapies are highlighted in red.

efficacy on skin fibrosis (Khanna et al., 2020b) and furthermore, responses to tyrosine kinase inhibitors have been linked to the diffuse proliferative signature (Martyanov et al., 2017).

1.2. Key cell populations, pathways and mediators involved in skin fibrosis and related-potential therapeutic targets: fibroblasts as master-regulators of skin fibrosis in SSc

One advantage in studying the biomechanisms in SSc, through the superficial nature of the cutaneous lesions, is that disease tissue can be readily sample and biopsied to derive SSc dermal fibroblasts. These cells can be cultured from explants and multiple published studies have demonstrated persisting abnormalities *in vitro* such as enhanced expression of type I collagen and a smooth muscle actin (α-SMA), throughout several passages of tissue culture (LeRoy, 1974; Leroy, 1972). These model systems have been used to compare SSc lesional with healthy control cells to identify pathways and candidate factors upregulated in the fibrotic process, including enhanced autocrine stimulation, oversecretion of adhesive matricellular proteins and enhanced mechanosensing in the disease fibroblasts (Leask, 2011; Liu et al., 2011; Toyama et al., 2018; Chen et al., 2008). SSc skin derived fibroblasts persist as abnormally activated myofibroblasts *ex vivo* indicating epigenetic changes, oversecrete collagens I and III as well as fibronectin, and show dependence on enhanced mechano-transduction (LeRoy, 1974; Shiwen et al., 2015; Bergmann et al., 2018; Henderson et al., 2018). Moreover, these cells express markers of cellular senescence (Martyanov et al., 2019; Kizilay Mancini et al., 2022) and are resistant to apoptosis.

More recent data from single cell analysis of lesional and control tissue has given new insights into the dermal fibroblast populations involved in SSc-skin fibrosis. Published in Cell, data from over 90 patients at a single Centre has supported a model of recurrent activation and eventual depletion of a fibroblast precursor in the skin of patients (Gur et al., 2022). Immune cell dysfunction was mainly restricted to subpopulations of dcSSc patients (early inflammatory phase with immune activation). Diverse populations of activated fibroblasts were identified, including gene expression profiles indicating skin remodeling, metabolism, immune modulation, angiogenesis, coagulation, and neurogenesis roles. Also, they identified a discrete SSc associated fibroblast subset, termed ScAF with elongated extensions, and expressing LGR5. The authors are proposing a two step pathogenic mechanism of myofibroblast activtion, step 1 leading to lcSSc and step 2 leading to dcSSc, the latter step associated with more profound disturbances in ECM deposition pathways, plus induction of senescence associated genes and activation of profibrotic signaling pathways including JAK-STAT and IGF-1.

Furthermore, results from single cells studies in the United States have also highlighted distinct fibrolast populations involved in SSc skin fibrosis (Tabib et al., 2021). The authors highlight that myofibroblast numbers are increased in proportion to skin score in SSc. Using single cell RNA analysis of lesional skin, they profile different fibroast populations and draw comparison with healthy control samples. They infer a stepwise change in dermal fibroblast populations; step 1) SFRP2hi/DPP4-expressing progenitor fibroblast populations become activated and globally induce expression of activation markers such as PRSS23 and THBS1, step 2) a small subset of the SFRP2hi cells differentiate into myofibroblasts under the influence of a range of transcription factors including FOSL2, RUNX1, STAT1, FOXP1, IRF7 and CREB3L1, as well as SMAD3, leading to the pathogenic myofibroblast populations characterisitc of SSc skin fibrosis.

The findings of these technically impressive studies could be validated in focused candidate factor studies or in clinical trials with therapeis tailor-made to the identified cellular and molecular pathways.

1.3. Immune mechanisms feeding into the fibrosis: macrophages and macrophage polarization

Amongst the range of many cell types having a role in wound healing, macrophages have a critical regulatory role acting at all stages of tissue repair and undergoing reprogramming dependent on the cytokine and even mechanical properties of the wound environment. In particular, the polarization of macrophages towards reparative phenotype capable of stimulating myofibroblasts and ECM synthesis has received much attention in the field of fibrosis (Lescoat et al., 2021b).

Furthermore, macrophages have a critical role in innate and adaptive immune responses and are implicated in inflammatory autoimmne disorders. The heterogeneity of macrophage activation states is now well-established through a spectrum of responses to the local cytokine and growth factor levels. Granulocyte–macrophage colony stimulating factor (GM-CSF) and macrophage colony stimulating factor (M-CSF) have a notable role in the differentiation of macrophages from monocytes. GM–CSF– and M–CSF–generated macrophages share some common surface markers, but also differ significantly in gene expresion profiles (Lescoat et al., 2018). Further heterogeneity is induced downstream of these effects through exposure to PAMPS and cytokines. Classical or M1-like inflammatory activation is initiated by toll-like receptor (TLR) activation with or without IFNγ, whereas alternative or M2-like polarization is triggered by T-helper (Th)2 cytokines IL-4 or IL-13 (Gordon and

Table 1
Molecular targeted therapies in SSc skin fibrosis that have been studied in patients, clinical trials or emerging from pre-clinical models.

Drugs (small molecules, antagonists, bilogics)	Target Molecule	Known or Potential Impact on Skin Fibrosis
Imatinib	PDGFR, c-Abl kinase	Inhibits fibroblast activation and collagen production, reducing skin fibrosis.
Tocilizumab	IL-6 receptor	Blocks IL-6, reducing inflammation and fibrosis in the skin.
Pirfenidone	TGF-β, PDGF, TNF-α	Interferes with multiple profibrotic pathways, potentially decreasing collagen deposition and skin thickening.
Nintedanib	TGF-β, PDGF, FGFR	Inhibits multiple growth factors involved in fibrosis, potentially reducing skin thickening and scarring.
Methotrexate	Folate metabolism	Suppresses immune system overactivity, reducing inflammation and fibrosis in the skin.
Bosentan	Endothelin receptors	Blocks endothelin, potentially improving blood flow and reducing fibrosis in skin tissues.
Rituximab	CD20 antigen	Targets B-cells, reducing autoantibody production and possibly alleviating skin fibrosis.
Infliximab	TNF-α	Blocks TNF-α, decreasing inflammation and potentially improving skin fibrosis.
Abatacept	T-cell co-stimulation	Modulates T-cell activation, potentially reducing skin fibrosis.
Etanercept	TNF-α receptor	Binds to TNF-α, reducing its activity and potentially improving skin fibrosis.
Anakinra	IL-1 receptor antagonist	Inhibits IL-1, potentially reducing inflammation and fibrosis in the skin.
Secukinumab	IL-17 A	Blocks IL-17 A, potentially reducing inflammation and fibrosis in the skin.
Nilotinib	PDGFR, c-Abl kinase	Inhibits fibroblast activation and collagen production, potentially reducing skin fibrosis.
Mycophenolate Mofetil (MMF)	T- and B-cell proliferation	Suppresses immune system overactivity, reducing inflammation and fibrosis in the skin.
Fresolimumab	TGF-β	Blocks TGF-β, potentially reducing fibrosis and skin thickening.
Belimumab	B-lymphocyte stimulator (BLyS)	Inhibits BLyS, modulating B-cell function and potentially reducing skin fibrosis.
Anifrolumab	Type I interferon receptor	Targets Type I interferon receptor, potentially reducing inflammation and fibrosis.
Teprotumumab	IGF-1 receptor	Blocks IGF-1 receptor, potentially reducing skin thickening and fibrosis in scleroderma.
Brentuximab	CD30 antigen	Targets CD30, potentially reducing fibrosis by affecting immune responses.
Inebilizumab	CD19 antigen	Targets CD19, potentially modulating B-cell activity and reducing skin fibrosis.
Lanifibranor	PPAR agonist	Acts on PPAR receptors, potentially reducing inflammation and fibrosis in the skin.
Baricitinib/Tofacitinib	JAK1 and JAK2	Inhibits Janus kinases, potentially modulating immune responses and reducing skin fibrosis in scleroderma.
pravelimumab	CTGF	Blocks CTGF function reducing fibroblast activation and skin fibrosis
Romilkimab	IL-4/IL-13	Binds and neutralised IL-4 and IL-13 and impacts in skin fibrosis
Etaracizumab	integrin av/b3	Inhibit integrin-mediated cell adhesion and latent TGFβ activation.
Fasudil	RhoA/ROCK inhibitor	Inhibits myofibroblast activation and reduces innate immunity
SM04755	Wnt pathway inhibitor	Suppress inflammation and reduce fibrosis

Martinez, 2010). Further M2 subtypes that have been identified may be induced by the presence of immune complexes, IL-10 or glucocorticoids implicated in the M2b or M2c polarization states. In the classical inflammatory state, M1-like macropages secrete high levels of pro-inflammatory cytokines such as tumour necrosis factor (TNFa) or interleukin (IL-)12/23 representing an effector arm of the T-helper (Th) 1-type immune response. By way of contrast, M2-like cells are more adapted to production of anti-inflammatory cytokines such as IL-10 and transforming growth factor (TGFβ) and chemokines (CCL17 and CCL22) as part of Th2-type immune responses. JAK-STAT signaling play a crucial role in macrophage polarization, M1 polarization reliying on JAK1/2-STAT1 signaling, M2a JAK1/2-STAT6 signaling and M2c relying on JAK1-STAT3 signaling (Hart et al., 2011).

Dysregulated macrophage polarization has been identified in the fibrotic tissues and blood of patients with SSc (reviewed in (Lescoat et al., 2018)). An increased circulating myeloid population co-expressing M2 markers CD204, CD163, CD206 as well as M1 markers CD80, CD86, TLR4 was identified in SSc patients indicating both M1 and M2-like properties (Soldano et al., 2018). Also, gene expression analysis of affected SSc skin in dcSSc has shown highly upregulated macrophage signatures that combine both M1 and M2 like signatures and associated with severity of disease (Skaug et al., 2020). M1 macrophages were more specifically up-regulated in patients with very early disease, suggesting that the very early edematous phase of SSc skin disease is supported by a pro-inflammatory signature driven by macrophages while later definite fibrosis is largely driven by the interaction between M2 profibrotic macrophages and fibroblasts and/or myofibroblasts. These results on M1/M2 polarization profile in SSc patients is also in accordance with data from SSc mouse models, such as the HOCl systemic model of SSc, in which gene expression profile in the skin showed an up-regulation of both M1 and M2 markers (Lescoat et al., 2020). Beyond these M1 and M2 populations, single cell transcriptome analysis from dcSSc skin biopsies also identified new population of macrophages including FCGR3A + macrophages (i.e. $CD16^{+}$ macrophages), characterized by the expression of profibrotic cytokines and chemokines such as IL-6 or CCL18 (Xue et al., 2022). With respect to their polarization state the FCGR3A + macrophages expressed M2 markers including CD204 (MSR1), CD163 or MS4A4 A but also upregulated signaling pathways including 'response to lipopolysaccharide' usually associated with M1 polarization, again supporting the notion of combined pro-inflammatory and profibrotic phenotype. These data confirmed that the polarization state of macrophages in SSc cannot be fully captured by the dichotomic M1/M2 polarization profile and that more subtle identification of key macrophages sub populations is needed.

Other physiological properties of macrophages are also disrupted in patients with SSc, including the ability of macrophages to efferocytose and clear apoptotic cells (Ballerie et al., 2019). Efferocytosis is supposed to play a key role in the prevention of autoimmunity, through the release of regulatory cytokines such as IL-10 once efferocytosis is efficiently performed by macrophages (Keret et al., 2023). The defect of efferocytosis in SSc patients might partly be explained by the excess of M1 polarization in SSc as such pro-inflammatory macrophages are associated with decreased efferocytosis capacities. The defect of efferocytosis

might also participate in the accumulation of apoptotic debris in the fibrotic tissue of SSc patients and experimental mice (Maehara et al., 2020; Yamamoto and Nishioka, 2004). This accumulation of apoptotic bodies with subsequent formation of immune complexes composed of ANA and nuclear autoantigens may favor the production of osteopontin by myeloids cells such as monocytes, which in return can then promote fibroblast migration and activation with pro-fibrotic effects (Gao et al., 2020). Such a pathogenic loop driven by immune complexes may link autoantibodies and macrophages, identifying both innate and adaptive immunity as key drivers of SSc-related fibrosis. The interplay between macrophages and adaptive immunity and SSc-related autoimmune process is also supported by mouse models, since B-cells participate in the differentiation and polarization of M2 macrophages in the bleomycin mouse models. In this systemic mouse model of SSc, B-cell depletion decreased the expression of the M2 marker CD206, in the lung and skin, demonstrating the important role of the cross talk between B-cells and macrophages in the pathogenesis of SSc (Numajiri et al., 2021). *In vitro*, co-culture of B-cells from bleomycin mice with macrophages from untreated mice also induced M2 polarization in an IL-6 dependent manner.

1.4. Mannose receptor as a target for therapy in SSc

Since high expression of the CD206 the mannose receptor, is near to specific for the M2-like profibrotic cells, it is a potnetial target for tailor-made therapies in SSc. Accordingly, CD206 expression is known to be upregulated in SSc macrophages (Mohamed et al., 2021) and induced in macropages by SSc fibroblast derived exosomes (Bhandari et al., 2023), and therefore considered a rational target for therapies designed to suppress the active M2-like signature. Such treatments could include CAR T cells (see below), monoclonal antibodies or small molecule inhibitors of CD206 downstream signaling. In the cancer field, where CD206 positive macrophages are known to promote tumour environment stroma formation, peptides which bind CD206 have been shown to reprogramme the tumour associated macrophages to a more M1-like inflammatory signature, suppressing the M2 like polarization. Certain peptide sequences were identified having immunomodulatory effects including capable of reprogramming pathogenic macrophages. RP-182, a 10 amino acid peptide, was shown to induce a conformational change in CD206 inducing endocytosis, phagosome-lysosome formation, and triggering apoptosis in these cells. These effects led to a shift in phenotype away from M2-like macrophages toward an M1 phenotype, enhancing antitumor immune responses in mouse cancer models (Jaynes et al., 2020).

1.5. Role of the epidermis in the pathogenesis of SSc

There has been significant research into the immune abnormalities and the role of endothelial dysfunction and fibroblast abnormalities in SSc, but the potential contribution of epithelial cells has received less attention. Within the fibrotic areas the myofibroblasts are derived from multiple sources including local tissue-resident fibroblasts, infiltrating monocyte derived cells (“fibrocytes”) (Grieb and Bucala, 2012) subcutaneous fat derived mesenchymal stem cells (MSCs) (Marangoni et al., 2015; Horsley, 2022), perivascular stem cells (pericytes) (Rajkumar et al., 1999), and other transdifferentiating tissue resident cells such as endothelial cells undergoing endothelial to mesenchymal transition (endoMT) (Good et al., 2015), (Nikitorowicz-Buniak et al., 2015). Moreover, there is now emerging evidence showing that dysregulation of the epidermis may contribute to the pro-fibrotic response of SSc (Nikitorowicz-Buniak et al., 2014, 2015; Aden et al., 2008, 2010; Berkowitz et al., 2023; Russo et al., 2021). Epithelial dysfunction is already implicated in other fibrotic disease such as idiopathic lung fibrosis (Wolters et al., 2018; Parimon et al., 2020), and renal fibrosis (Zeisberg and Kalluri, 2004). Moreover, epithelial-fibroblast interactions are well established as being pivotal in restoring tissue homeostasis after wounding (Werner et al., 2007).

The epidermis is a specialised self-regenerating stratified squamous epithelium (reviewed in (Watt, 2014)) within which the keratinocytes are polar cells, whose function is directed by micro-environment factors, including cell-cell contact, cell-ECM interactions, mechanosensing and metabolic factors such as O2 level as well as pH determine differentiation and activation state (Louis et al., 2022). In health the basal keratinocyte express certain characteristic cytokeratins; cytokeratin 5 (K5) and 14 (K14), which are lost as the keratinocytes commit to differentiation and migrate upwards, switching to K1 and K10 (Fuchs and Green, 1980). This process is markedly altered in SSc where there is abnormal persistence of the basal K14 into the spinous and granular suprabasal layers, combined with delayed expression of K10 (Aden et al., 2008; Berkowitz et al., 2023). Moreover, involucrin and loricrin, which have roles in keratinocyte maturation and formation of the keratinocyte protein envelop, are altered in the SSc epidermis (Nikitorowicz-Buniak et al., 2014; Russo et al., 2021). Keratinocyte maturation, size and epidermal thickness are consistently abnormal in biopsies from SSc patients (Nikitorowicz-Buniak et al., 2014). This pattern is recognised as a wound healing phenotype leading to further investigation of other changes characteristic of repair epithelial layers, such as thickening, and expression of cytokeratins usually restricted to wound environments (Singer and Clark, 1999; Snyder et al., 2016).

In the proliferative phase, or re-epithelialisation of wound healing, keratinocytes become activated and they begin to express wound specific cytokeratins, K6 and K16, which facilitate keratinocyte migration and enables the cells to withstand wound environments and cover the wound defects (Watanabe et al., 1995). In SSc, abnormal expression of K6 and K16 in epidermal keratinocytes (Aden et al., 2010) has been confirmed by several teams (Berkowitz et al., 2023; Russo et al., 2021) and is consistent with an activated wound healing, pro-fibrotic phenotype occurring at the level of the epidermis. Dysregulation of the keratinocytes and their switch to a wound healing phenotype are not fully explained by current data. Abnormal expression of homeobox genes has been demonstrated via unbiased profiling (Russo et al., 2021). Epidermal changes could be explained by persistence of activated wound healing responses in the underlying dermis or else via the effects of enhanced mechanical stiffness of the skin, as a hallmark of fibrotic tissue. Moreover, we recently identified that SSc IgG autoantibodies are capable of binding to and activating keratinocytes, potentially inducing their activation phenotype.

Pigmentary changes in the skin, both adaptive physiological and pathological, generally reflect changes occurring in the epidermal layer where melanocytes release pigmentary granules into basal layer keratinocytes. Several studies have focused on pigmentary changes and their relevance for altered biomechanisms in SSc (Tabata et al., 2000). Marked pigmentary changes are seen in around half of SSc patients, and the two well-characterized patterns are vitiligo-like with perifollicular hyperpigmentation (salt and pepper pattern) and diffuse hyperpigmentation pattern. The diffuse hyperpigmentation pattern is seen more in dcSSc with higher skin scores, and moreover, such patients are more likely to develop digital ulcers consistent with some link to the vasculopathy. Melanocyte numbers were increased in SSc patients of disease duration less than 5 years, decreasing thereafter in late-stage disease (Leroy et al., 2019). Loss of CCN3 (Nov) by melanocytes was found to be an observed molecular change in the hyper pigmentary areas (Henrot et al., 2020). The pigment changes have a higher prevalence in Hispanic and African American patients than in whites (Nusbaum et al., 2016). Mechanistically, the increased melanocyte content as described above could be due to increase chemotaxis of melanocytes or enhanced proliferation in the SSc epidermis microenvironment. Our own studies point towards elevated stem cell factor (SCF, Kit-ligand) in the fibrotic skin, which is capable of attracting melanocytes via their expression of c-Kit (Ahmed Abdi et al., 2017).

1.6. Release of pro-inflammatory and pro-fibrotic factors by the SSc epidermis

The importance of keratinocytes as immune cells is increasingly recognised (reviewed in (Piipponen et al., 2020)) and as such keratinocytes are a source of chemokines, anti-microbial peptides, inflammatory cytokines and DAMPS and may influence immune and endothelial cells via release of exosomes. In their role as first line of defence keratinocytes respond to pathogen associated molecular patterns including LPS, endotoxins and viral nucleic acids. These functions are medicated by expression of Toll-like receptors, lectin receptors and other pathogen sensing mechanisms such as the NOD-like receptors. The exposure to pathogen related material leads to induction of innate immune responses in keratinocytes leading to release of factors such as IL-1α, IL-8, and type I interferons. The activated keratinocytes secrete IL-1α, which in an autocrine fashion increase keratinocyte proliferation and migration. IL-1α also acts in paracrine manner to stimulate KGF release by underlying local fibroblasts, which in turn feeds back to promote keratinocyte proliferation and migration (Werner et al., 2007; Werner and Smola, 2001). These effects are recapitulated in the SSc epidermis. An essential role for IL-1α in epithelial-fibroblast cross talk has been shown in several studies (Aden et al., 2010; Russo et al., 2021). Furthermore, feedback via SSc dermal fibroblast overexpression of KGF has been indicate as causing feedback activation of keratinocytes (Canady et al., 2013).

Aberrant expression of both pro-inflammatory and pro-fibrotic factors has been demonstrated in and adjacent to the SSc epidermis. Demonstrated by profiling of candidate secreted factors, SSc epidermis synthesises the matricellular protein CCN2 which is found deposited at the epidermal-dermal interface (Nikitorowicz-Buniak et al., 2014). Over-expression of CCN2 is thought to be a hallmark of fibrotic processes, acting as a modifier of cell-matrix interactions (Liu et al., 2011). Through their work on mouse models (Wang et al., 2011)31) demonstrated that mice over-expressing CCN2 undergo accelerated skin, lung and renal fibrosis. Conversely mice which are CCN2 knock outs have relative resistance to bleomycin induced skin fibrosis. The evidence points towards CCN2 mediating fibroblast migration, proliferation, increased ECM synthesis and reduced ECM breakdown through up-regulation of TIMPs. TGFβ is a pro-fibrotic cytokine, central to SSc pathogenesis (Lafyatis, 2014). However, total and LAP associated TGFβ do not appear elevated in SSc epidermis (Aden et al., 2010), also supported by McCoy et al., who demonstrated keratinocyte activation independent of TGF-β (McCoy et al., 2017),

Moreover, several pro-inflammatory factors have been found to be upregulated in SSc epidermis, which include, as already mentioned IL-1α. Furthermore, IL1-α polymorphisms are known to be linked to increased risk and severity of SSc in certain populations (Kawaguchi et al., 2003). There is evidence showing increased expression of IL-1R in the SSc fibroblasts, and that blocking IL-1R is associated with decreased levels of IL-6 and PDGF-A in those fibroblasts (Kawaguchi et al., 1993). Russo et al., 2021 have demonstrated *in vitro*, that SSc keratinocytes are able to stimulate fibroblast production of IL-6 and IL-8, and that this is mediated through IL-1α, as blockade of the IL-1R inhibited the production of those cytokines from fibroblasts in the presence of SSc epidermal conditioned media (Russo et al., 2021). Despite the obvious implication of IL-1α in epidermal-dermal crosstalk and promotion of fibrosis, in a small clinical trial the blockade of IL-1α using rilonacept did not reduce skin score or alter IL-6 expression in the skin of SSc patients (Mantero et al., 2018).

In terms of damage associated molecular patterns (DAMPS) being released in the SSc epidermis, S100A9 is calcium binding protein existing mostly as a stable heterodimer with S100A8 and overexpressed by activated epithelial cells as well as neutrophils and monocytes, where it plays a pro-inflammatory role by promoting leucocyte recruitment and cytokine release (Foell et al., 2007) via RAGE and TLR-4 respectively. In SSc, overexpression of S100A9 has been demonstrated throughout the epidermis, not seen in healthy controls, additionally SSc fibroblasts, through TLR-4, produce excess CCN-2 in response to stimulation by S100A9 (Nikitorowicz-Buniak et al., 2014). Consistent with this model, a study by McCoy et al. was able to demonstrate increased expression of COL1A1 and α-SMA in healthy fibroblasts incubated with conditioned media. From SSc keratinocytes (McCoy et al., 2017). Moreover, based on single cell profiling of lesional skin, prominent changes in keratinocyte signatures have been confirmed, including expression of wound cytokeratins and in this instance high levels of S100A8 were identified in the epidermis (Berkowitz et al., 2023).

1.7. Transition between epithelial cells and mesenchymal cells in SSc

Epithelial to mesenchymal transition (EMT) refers to the process in which an epithelial cell loses its polarity to become a mesenchymal cell (Kalluri, 2009). There are three types of EMT; type 1 is seen in embryogenesis, type 2 is seen with tissue regeneration and pathological organ fibrosis, type 3 is seen with malignancy and metastases. Biomarkers exist to identify the different subtypes of EMT.

The role of TGFβ as a driver of EMT is well known, with studies showing stimulation of epithelial cells with TFGβ is enough to induce EMT in tissue culture (Chen et al., 2008). The transcriptional changes in EMT are due to TFGβ mediated phosphorylation of smad2/3, which bind smad4 and translocate to the nucleus to suppress or activate target gene transcription (Xu et al., 2009).

Previous work has demonstrated SSc epidermis staining for FSP-1 and vimentin (Nikitorowicz-Buniak et al., 2015), they also demonstrated an increase in SNAI1 copies, but not SNAI2 – raising the idea of a partial EMT, supported further as they were unable to demonstrate loss of E-cadherin or decrease in collagen IV, that would lead to fully evoked EMT process with migration into the dermis.

More recently secreted frizzled-related protein (SFRP4), a known Wnt signaling modulator, has been shown to be increased in skin samples in those with SSc (Bayle et al., 2008) were able to show increased SFRP4 staining in the SSc basal epidermal cells, in their work SFRP4 expressing epidermal cells co expressed vimectin but lacked caveolin (Tinazzi et al., 2021). This was replicated in their EMT model: where lung cancer epithelial cells induced by TGFβ, had increased SFRP4 expression, vimectin, SNAI1, N-cahedrin and loss of calveollin and E-cahedrin. It is possible that EMT in the epidermis is responsible for some of the activated fibroblast population in SSc, especially given the persistence of K14 in the suprabasal layers (Bergmann et al., 2018), in a process mediated by TFGβ and Wnt signaling.

Accordingly, it is possible that in certain SSc patients the fibrotic process begins at the level of the epidermis, where activated keratinocytes promote differentiation of underlying dermal fibroblasts into myofibroblasts. Targeting the epithelial cell-fibroblast interactions might benefit in patients with an active keratinocyte signature.

1.8. Novel and emerging treatments: cellular therapy in the treatment of SSc

Although there is no disease modifying therapy available for all SSc patients, myeloablative chemotherapy followed by rescuing hematopoiesis stem cell transplantation has proven to improve survival in patients with severe dcSSc, despite procedure related-mortality (van Laar et al., 2013; Sullivan et al., 2018). Although the main therapeutic effects are thought to be related to chemotherapy, stems cells may exert immunomodulatory effects. Mesenchymal stromal cells (MSC) are adherent polyclonal cells with fibroblast-like morphology able to differentiate *in vitro* into osteoblasts, adipocytes or chondroblasts with potential immunomodulatory properties that could be utilized for the treatment of SSc (Farge et al., 2021). MSC can be derived from bone marrow, adipose tissue, umbilical cord and additional sources. In pre-clinical models of SSc, including HOCl or bleomycin mouse models, MSC of various origin depending of the study, were able to limit lung

and skin fibrosis (Maria et al., 2016; Suzuka et al., 2022). In a phase I trial, heterologous BM-MSC showed to limit skin fibrosis and stabilize ILD in patients with refractory dcSSc (Farge et al., 2021). High serum levels of TGFβ signature in the serum was associated with poor treatment response, suggesting that a careful selection of patients based on biomarkers will be needed to successfully implement MSC in the therapeutic strategy of SSc (Loisel et al., 2023).

Beyond MSC, other cell-based therapies are currently considered for the treatment of SSc. Among them, Chimeric antigen receptor (CAR)-based approaches may be especially promising (Ellebrecht et al., 2016). CARs consist of an extracellular domain which binds a specific antigen expressed on target cells, and intracellular domain that mediates signal transduction upon antigen binding. CAR-T are thus autologous engineered T-cells that can specifically suppress cells expressing the antigen recognised by their chimeric receptors. CD19-CAR-T cells have shown promising results in refractory systemic lupus, a some case reports have been published in SSc, suggesting that such approach could also be relevant in SSc (Mackensen et al., 2022; Bergmann et al., 2023). Beyond CD19 and lympho-ablation, CAR-T cells could also be used to target senescent cells or activated myofibroblasts. Other cellular therapies, including T cells engineered to express a chimeric autoantibody receptor (CAAR-T) are under consideration for the treatment of autoimmune disease with identified auto-antigens playing an important role in the pathogenesis of these diseases (Aghajanian et al., 2022). A summary of the above underlying biomechanisms and the rational approaches to therapy is shown in the schematic (Fig. 1).

1.9. Senescence as a therapeutic target in SSc

In cellular senescence there is an irreversible exit of cells from the cell cycle accompanied by a failure of apoptotic cell death, leading to persistence of degenerative and potentially pathogenic cells. (Tsou et al., 2022). In SSc-related fibrosis, a disorder with genetic prediscpossition but onset triggered in mid-lief, there is a persisting excess population of senescent cells in the involved tissues, belived to have a harmful effect worsening the exces ECM secretion and contributing to inflammation. Through a secretome called senescence-associated secretory pattern (SASP), these populations of cells release factors including IL-6 and TGFβ, as well as expressing β galactosidase. Two types of therapeutic drugs have emerged in this field as potential strategies to selectively target senescent cells (senolytics) or those that block or interfere with the senescence-related signals and the influence of SASP on tagets cells (senomorphics) (Bergmann et al., 2023). The senolytic dasatinib, a tyrosine kinase inhibitor, used in combination with a plant flavanol quercetin, can specifically target senescent cells in a range of cellular and animal models. Dasatanib has already een evaluated in treating the fibrotic skin and ILD changes in SSc (Martyanov et al., 2017, 2019). In a small open-label trial in SSc patients, dasatanib responsive individuals had higher senescence gene expression profile pre-treatment, compared to non-responders and decreasing SASP signature was associate with responsive skin sore and stable lung involvement (Martyanov et al., 2017).

1.10. Future perspectives: tailor-made specific therapies for SSc

As presented above, SSc represents a heterogeneous disease process resulting from multiple cellulr and molecular biomechanisms, which is resistant to current therapies. It is likely that resistance to treatment and difficulties with therapeutic trials originates in part due to the multiple overlapping andnetworking biomechanisms which my by [ass specific interventions or even evole to do so. Approaches to tackle this challenging prolem include deep phenotyping of new patients. As well as careful cliical and antibody profiling, tissue sampling for single cell, proteomic and ATACseq analysis could become routine at baseline and repeated after a period of therapy. This active disease associated pathways could be identifed on an individual patient basis as well as carefull evaluation to determine engagement of the therpeutic target. The cost of these approaches cpould be offset against the likely high cost of caring dfor chronic disabling disease and are justifiable given the likely market value of certain of the therapeutics. Current molecular targetted therapies that have been evaluated in clinical trials or based on pre-clinical data are summarised in Table 1.

The future landscape of this challenging clinical and fascinating diisorder might shift emphasis from rescuing organ based complications to delivering tailor-made therapeutics at the earliest stages of disease.

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