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Review Article

Pathogenesis of systemic sclerosis: an integrative review of recent advances

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Systemic sclerosis (SSc), or scleroderma, is a complex autoimmune connective tissue disease characterized by autoimmunity, vasculopathy, and progressive organ fibrosis, leading to severe organ dysfunction. The disease begins with a vascular injury triggered by autoimmune responses and environmental factors against a backdrop of genetic predisposition. This injury impairs angiogenesis and vasculogenesis, resulting in capillary loss and arteriolar constriction, which promotes immune cell infiltration and sustained inflammation within affected tissues. These vascular anomalies cause severe complications, including pulmonary artery hypertension, scleroderma renal crisis, and skin ulcers. Chronic inflammation fosters persistent fibroblast activation, resulting in extensive fibrosis that defines SSc. This review synthesizes the latest research on pathogenesis of SSc, highlighting the shift from fundamental research to a precision therapeutic approach. It explores the potential of technologies like flow cytometry and single-cell RNA sequencing to investigate pathogenic cell subtypes. These platforms integrate transcriptomic, genomic, proteomic, and epigenomic data to uncover insights into the underlying mechanisms of SSc pathogenesis. This review advocates for a multidisciplinary, patient-centric approach that harnesses recent scientific advances, directing future SSc research toward personalized and precise interventions.

Keywords: Systemic sclerosis, Pathogenesis, Vasculopathy, Immunity, Fibrosis

INTRODUCTION

Systemic sclerosis (SSc) is a chronic autoimmune connective tissue disease (CTD) marked by an unknown etiology, multifaceted mechanisms, and highly heterogeneous manifestations. SSc often progresses steadily, leading to significant morbidity and a heightened risk of mortality. Key features of the disease include fibroblast activation and excessive extracellular matrix (ECM) production. The interplay between vascular dysfunction, immune response, and fibrosis is critical to the diseases course.

The annual incidence of SSc ranges from 0.6 to 5.6 per 100,000 individuals, with a prevalence of 7.2 to 44.3 cases per 100,000 individuals, predominantly affecting females [1]. The condition poses a high mortality risk among CTDs, primarily due to pulmonary and cardiac complications. Furthermore, SSc impacts social and psychological well-being, reducing quality of life and imposing an economic burden.

Given the clinical heterogeneity manifestations and socioeconomic impact of SSc, early diagnosis and therapeutic interventions are essential. Furthermore, understanding its complex pathogenesis is crucial for developing targeted therapies to manage the varied clinical presentations effectively. Recent researches have uncovered numerous molecular and cellular pathways involved in SSc's onset and progression. This review aims to consolidate recent research efforts to unravel the pathogenesis of SSc, emphasizing the ongoing quest to understand this intricate

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condition. It highlights unmet needs and future research directions to further elucidate SSc's pathogenesis.

MAIN SUBJECTS

Pathophysiological framework of SSc

1) Overview of etiopathogenesis

In SSc, disruptions in immune regulation, endothelial function, and tissue repair are central to its pathogenesis. Environmental triggers induce epigenetic changes in genetically susceptible individuals (Figure 1). Endothelial damage is a critical preliminary stage in SSc, preceding clinical manifestations such as Raynaud's phenomenon, digital ulcers, and in severe cases, pulmonary artery hypertension (PAH). These vascular disturbances initiate immune reactions that drive fibrosis. The immune system plays a key role in promoting fibrosis and vasculopathy. Fibrosis, characterized by excessive ECM deposition in the skin and organs, lies at the core of SSc pathogenesis. This process is driven by cytokines, chemokines, and growth factors stimulating fibroblasts and myofibroblasts, which may also transdifferentiate from other cell types. Understanding the complex pathogenesis of SSc is paramount for developing targeted therapies that address the varied clinical manifestations.

2) Genetics of susceptibility to SSc

(1) Hereditary and ethnicity

The genetic predisposition to SSc varies by familial and ethnic factors. First-degree relatives of SSc patients have a 1.6% risk, significantly higher than the general population's 0.026%. However, the low concordance rate of 4.7% in twin studies indicates environmental factors also play a significant role in the development of SSc [2]. Epidemiological studies further reveal that SSc incidence and manifestations differ among ethnicities. For example, SSc prevalence is higher in European descent compared to Asians [3]. Additionally, genetic differences, such as the Human Leukocyte Antigen (HLA)-*B8DR3* haplotype, are linked to more severe symptoms in Europeans than in Asians [4]. These insights emphasize the importance of understanding the interplay between genetics and ethnicity to develop more tailored and effective therapeutic approaches.

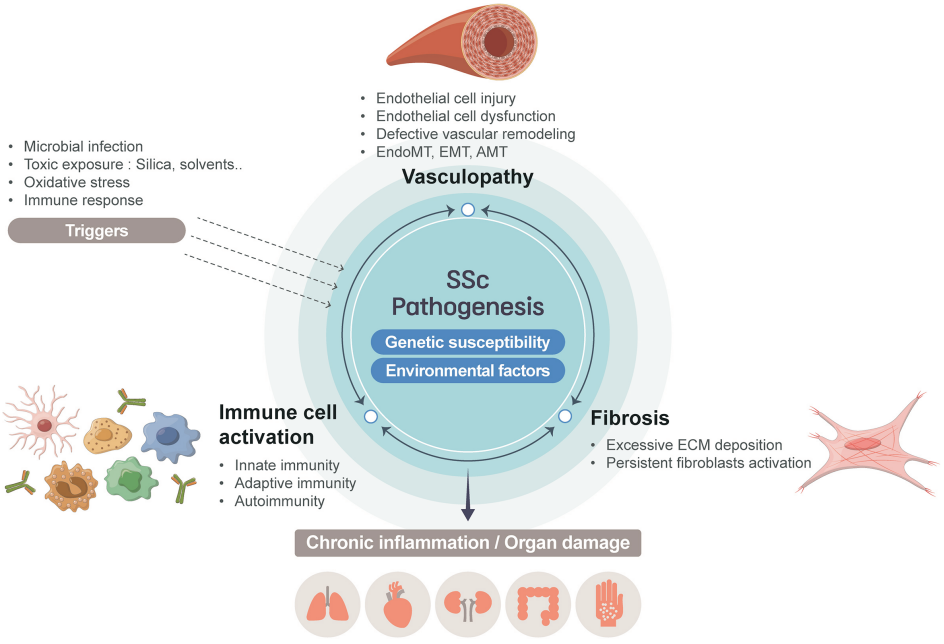

Figure 1. A comprehensive schematic diagram illustrating the etiopathogenesis of SSc, focusing on genetic susceptibility, environmental factors, and their impact on vasculopathy, immune activation, and fibrosis. Triggers such as microbial infection, silica exposure, and oxidative stress initiate disease progression. Key mechanisms include endothelial dysfunction, immune cell activation, and persistent fibroblast activation, leading to chronic inflammation and organ damage. SSc: systemic sclerosis, ECM: extracellular matrix, EndoMT: endothelial-to-mesenchymal transition, EMT: epithelial-to-mesenchymal transition, AMT: adipose-to-myofibroblast transition.

(2) Sex difference

SSc shows a sex difference, affecting female 3 to 4 times more than male, though male often face more severe complications like cardiac and pulmonary issues [5]. Sex hormones also influence this, with estrogen promoting autoimmunity, while androgens and progesterone offer protection. Differences in gut microbiota between sexes may also impact SSc susceptibility [6,7]. Epigenetic variations, especially in X chromosome inactivation (XCI), play a key role in female-dominated diseases like SSs. Abnormal XCI regulation can lead to overexpression of immune-related genes, with skewed XCI more common in female with SSc compared to healthy individuals [8], emphasizing the complexity of sex differences in SSc.

(3) HLA and non-HLA associations

The HLA system, known as the major histocompatibility complex on chromosome 6, is implicated in the autoimmune pathology of SSc. Specific HLA alleles, such as class I allele *B**08:01, class II alleles *DRB1**11:04, *DQB1**02:02, and *DPB1**13:01, are associated with SSc susceptibility across various ethnicities [9]. For example, HLA-*DRB1**11 increases SSc susceptibility in North India, while HLA-*DRB1**12 offers protective effects [10]. Genetic predisposition to SSc varies by ethnicity and is closely linked to distinct autoantibodies. For instance, European and Latino Americans frequently carry *DRB1**1104, *DQA1**0501, and *DQB1**0301 haplotypes, while African Americans show higher association with the *DRB1**0804 and HLA-*DRB1**1102 alleles [11]. HLA-*DPB1**1301 allele is linked to anti-topoisomerase antibodies across ethnicities [12].

Recent advancements in genotyping and genome-wide association studies (GWAS) have identified numerous genetic variants contributing to SSc susceptibility, such as *STAT4, IRF5*, and *CD247* [13]. A 2024 Japanese GWAS study has expanded these associations [14]. The genetic overlap between SSc and other autoimmune disorders like systemic lupus erythematosus suggests shared genetic etiology. Integrating genomic data, including transcriptomics and epigenetics, is crucial for a comprehensive understanding of SSc's genetic basis. Table 1 provides annotated loci associated with SSc and its related traits.

(4) Epigenetic regulation

Epigenetic regulation involves DNA methylation, histone modifications, and non-coding RNAs (ncRNAs), affecting gene expression and cell function. DNA methylation, primarily occurring on the cytosine bases within CpG dinucleotides, dynamically regulates chromatin structure along with histone modifications. The ncRNAs, particularly microRNAs (miRNAs), play key roles in SSc. Specific miRNAs are correlated with fibrosis and inflammation in SSc.

① DNA methylation

Recent studies have unveiled the epigenetic complexities of endothelial dysfunction in SSc. Genome-wide DNA methylation analysis in diffuse cutaneous SSc (dcSSc) endothelial cells (ECs) identified 2,455 differentially methylated CpG sites across 1,301 genes. Hypermethylation of genes such as *NOS1, DNMT3A/3B*, and *HDAC4* suggests epigenetic mechanisms of downregulation, whereas hypomethylated of *IL17RA, CTNNA3*, and *SDK1* implies a potential upregulation of these genes. Whole-genome bisulfite sequencing has revealed methylation variations in 340 genes within SSc $CD4^+$ T cells, with hypomethylation observed in genes related to the type I interferon (IFN) pathway [15]. In SSc fibroblasts, hypomethylation of collagen genes such as *COL8A1* and *COL29A1* in dcSSc and *COL1A1* and *COL12A1* in limited cutaneous SSc (lcSSc) has been identified. Additionally, hypermethylation causing downregulation of the *PARP-1* and *FLI1* genes contributes to sustained fibroblast activation [16,17].

② Histone modification

Histone modification enzymes, such as HDAC5 and EZH2, are upregulated in SSc ECs, altering angiogenesis through pathways like Notch. Assay for Transposase-Accessible Chromatin using sequencing revealed reduced chromatin accessibility in dcSSc dermal ECs and fibroblasts. Chromatin immunoprecipitation with sequencing and RNA sequencing studies identified distinct histone modifications in SSc monocytes influencing genes essential for IFN signaling through transcription factors (TFs) like IRF and STAT [18]. Transforming growth factor (TGF)-β induces autophagy in fibrotic diseases through SMAD3-dependent mechanism that downregulates MYST1, a key histone acetyltransferase. Inhibiting bromodomain and extra-terminal proteins, which regulate redox balance in activated myofibroblasts as epigenetic readers, has shown potential as a therapeutic approach [19].

③ Non-coding RNAs

Recent studies have highlighted key ncRNAs involved in SSc pathogenesis. Long non-coding RNA (lncRNA) negative

Table 1. The summary data of the annotated loci associated with SSc trait including all associations with p values below 8×10^{-8} according to the GWAS catalog (http://www.ebi.au.uk/gwas/)

Chromosome	Mapped gene	Reported trait	Ancestry	Chromosome	Mapped gene	Reported trait	Ancestry
1p34.3	KIAA0319L	SSc, SLE	EUR	7p15.2	JAZF1	SSc, SLE	EUR
1p31.3	IL12RB2	SSc, RA, IIM, SLE, CD	EUR	7q11.23	EIF4H, LIMK1	SSc, RA, IIM, SLE	EUR
1p13.2	PTPN22, AP4B1-AS1	SSc, RA, IIM, SLE	EUR	7q32.1	TNPO3	SSc, RA, IIM, SLE	EUR, AA, AC
1q21.3	FLG-AS1, FLG2	SSc	EAS		IRF5, KCP	SSc, RA, IIM, SLE	EUR, AA, AC
1q23.3	FCGR2B, FCGR3B	SSc	EUR, AA, AC		TPI1P2, CYCSP20	SSc, SLE	EUR, AA, AC, EAS
	Y_RNA, RPL31P11	SSc	EAS		RNU6-177P, LINC03072	SSc	EAS
1q24.2	CD247	SSc	EUR	8p23.1	FAM167A, BLK	SSc, RA, IIM, SLE	EUR, AA, AC, EAS
1q25.1	TNFSF4, PRDX6-AS1	SSc, RA, IIM, SLE	EUR, EAS	8q12.1	LINC01301	SSc	EUR, EAS
1q25.3	SMG7, NCF2	SSc, RA, IIM, SLE	EUR	8q12.2	CHD7, RAB2A	SSc	EUR
2q32.2	NAB1, GLS	SSc, RA, IIM, SLE	EUR	11p15.5	SCT, DRD4	SSc, RA, IIM, SLE	EUR
2q32.3	STAT4	SSc, RA, IIM, SLE	EUR, AA, EAS		RNU6-878P, CD81-AS1, CDHR5	SSc	EUR
3p24.1	LINC01967	SSc	EUR, EAS	11q23.3	CXCR5, Y_RNA	SSc	EUR
3p14.3	DNASE1L3	SSc, RA, IIM, SLE	EUR		DDX6	SSc	EUR, EAS
	PXK	SSc, SLE	EUR, EAS	12q13.2	ESYT1, ZC3H10	SSc	EUR
	FLNB	SSc	EUR, EAS	12q15	IFNG-AS1	SSc (ACA-positive)	EAS
3q13.33	ARHGAP31	SSc	EUR, EAS	12q24.13	PTPN11	SSc	EUR
3q25.33	IL12A-AS1	SSc	EUR, EAS	13q13.3	NBEA	SSc-ILD	EAS
	ARL14, RPL6P8	SSc, RA, IIM, SLE	EUR	14q32.33	AHNAK2, IGHM	SSc	EUR, EAS
4p16.3	DGKQ	SSc, RA, IIM, SLE	EUR, EAS	15q24.1	CSK	SSc	EUR, EAS
4q24	NFKB1	SSc	EUR, EAS	15q24.2	Metazoa_SRP, RPL36AP45	SSc, SLE	EUR
4q28.3	STMN1P2, RPS23P2	SSc (ACA-positive)	EAS	16p11.2	ITGAM	SSc, SLE	EUR
4q34.3	RNA5SP173, NDUFB5P1	SSc	EUR, EAS	16q24.1	IRF8-LINC01082, LINC02132	SSc, RA, IIM, SLE	EUR, AA, AC, EAS
4q35.1	UFSP2, ANKRD37	SSc	EAS	17q21.1	GSDMA, GSDMB	SSc	EUR, EAS
5q31.1	IRF1	SSc, CD	EUR		STAT3	SSc, CD	EUR
5q33.1	TNIP1	SSc, RA, IIM, SLE	EUR, EAS	17q25.1	NUP85	SSc	EUR, EAS
5q33.3	MIR3142HG	SSc, RA, IIM, SLE	EUR	19p13.2	TYK2	SSc, RA, IIM, SLE	EUR
6p21.32	HLA-region	SSc	EUR, EAS	19p13.11	IL12RB1	SSc	EUR, EAS
	MTCO3P1, NOTCH4, TSBP1-AS1	SSc	EUR	19q13.33	PRR12	SSc, RA, IIM, SLE	EUR
6p21.31	ZBTB9, GGNBP1	SSc, CD	EUR	20q13.12	SLC12A5	SSc	EUR, EAS
6q21	ATG5	SSc, RA, IIM, SLE	EUR, EAS	20q13.33	RTEL1-TNFRSF6B, RTEL1	SSc (ACA-positive)	EAS
6q23.3	TNFAIP3, SIMALR	SSc, RA, IIM, SLE	EUR, EAS	22q11.21	YDJC, CCDC116	SSc, RA, IIM, SLE	EUR
	LINC02539, WAKMAR2	SSc, SLE	EUR				

GWAS: genome-wide association study, SSc: systemic sclerosis, SLE: systemic lupus erythematosus, RA: rheumatoid arthritis, IIM: idiopathic inflammatory myopathies, CD: Crohn's disease, EUR: European, AA: African American, AC: Afro-Caribbean, EAS: East Asian.

regulator of the IFN response and miR-26a-2-ep are significant regulators of IFN signaling in SSc monocytes [20,21]. Elevated levels of miR-618, miR-126, and miR-139-5p in plasmacytoid dendritic cells (pDCs) are linked to persistent IFN release [22,23]. The role of ncRNAs in fibrosis regulation is evident in the miR-29 family, which targets collagen mRNA to reduce collagen production and promote apoptosis in dcSSc fibroblasts. MiR-155 is also associated with collagen production mediated by the NLRP3 inflammasome in SSc fibroblast [24]. LncRNAs like TSIX and HOTAIR induce fibroblast-to-myofibroblast transformation via Notch signaling [25,26], while suppression of lncRNA H19X reduces ECM synthesis and induces apoptosis in SSc fibroblasts [27]. These findings illustrate the complex epigenetic landscape in SSc, underscoring the interplay between epigenetic regulations and opening avenues for targeted therapeutic interventions.

3) Trigger: environmental factors

SSc is increasingly understood to be triggered by environmental factors such as silica, solvents, silicone breast implants, infections, and radiation. These factors highlight the diseases complex etiology involving both genetic predisposition and external stimuli.

(1) Occupational trigger: silica and organic solvents

A meta-analysis of studies from 1960 to 2014 identified occupational exposure to silica and solvents as significant risk factors for SSc, with silica exposure particularly associated with severe symptoms, including skin thickening, digital ulcers, and severe pulmonary and cardiac dysfunctions [28]. Silica exposure induces immune responses, triggering inflammation, autoantibody production, neutrophil apoptosis, and fibrosis activation in SSc.

Similarly, exposure to solvents, including white spirit, aromatic, and chlorinated solvents, and trichloroethylene, is associated with SSc development. Exposure to solvents induces immune dysfunction, characterized by elevated autoantibody levels and altered expression of genes associated with inflammation and apoptosis, indicating that environmental factors play a critical role in the development and progression of SSc.

(2) Infection

Research suggests that infectious agents like human cytomegalovirus (HCMV), human herpesvirus 6 (HHV-6), and Epstein-Barr virus (EBV) play significant roles in SSc pathogenesis. Recent studies confirm a high prevalence of beta-herpesvirus infection in SSc patients, evidenced by viral presence in tissue or blood and a significantly stronger immune response against HCMV and HHV-6 compared to controls [29]. This can lead to cellular damage through inflammatory cytokine production and molecular mimicry. CMV-specific $CD8^+$ T cells are correlated with disease severity [30]. These viral infections alter miRNA patterns in dermal fibroblasts, initiating fibrotic signaling [31].

Parvovirus B19 (B19V) infection contributes to fibrotic changes by inducing cellular senescence in human dermal fibroblasts, marked by increased β-galactosidase activity, cytokine secretion, and DNA damage [32]. The unique VP1 region of B19V exacerbates skin fibrosis in a murine model of SSc and increased inflammatory markers in macrophage [33], indicating the B19V's potential role in SSc pathophysiology.

EBV infection enhances toll-like receptor (TLR) signaling, increasing inflammatory cytokine production and promoting fibrotic transformation of fibroblasts. High EBV DNA levels and the presence of EBV lytic antigens in dermal vessels of SSc patients underscore its role in vascular injury [34].

(3) Microchemerism

Feto-maternal microchimerism (Mc), characterized by the exchange of cells between mother and fetus, may contribute to autoimmune responses. Female patients with SSc who have had sons exhibit male DNA higher than controls, indicating a role of Mc in SSc, independent of maternal-child HLA compatibility. Environmental exposures like vinyl chloride also seem to increase microchimeric cells, potentially triggering tissue fibrosis. Additionally, lower soluble HLA-G levels, linked to increased fetal Mc, may reduce immune tolerance and exacerbate SSc [35]. Female with lcSSc and dsSSc exhibit male Mc in distinct blood compartment, with lcSSc showing higher Mc in whole blood and dcSSc in PBMCs, suggesting a complex interplay of genetic, environmental, and immunological factors in SSc pathogenesis through the lens of Mc [36].

Vasculopathy

Vasculopathy is central to the pathogenesis of SSc, including EC damage, dysfunction, and defective vascular remodeling. Vascular manifestations like Raynaud's phenomenon and nailfold capillaroscopic alterations are crucial for early diagnosis of SSc. Theses manifestations can progress to severe complications

like digital ulcers, PAH, and scleroderma renal crisis (SRC). While the exact initial triggers of SSc remain debated, many experts believe that microvascular damage is a crucial factor in the onset of the disease. Immunological factors, including autoantibodies and natural killer cells, contribute to EC injury, suggesting that vasculopathy may be secondary to both innate and adaptive immune responses [37]. Therefore, complex interactions between the immune system and EC function influence vessel integrity in the early stage of SSc development, preceding the onset of fibrosis.

1) Endothelial cell injury

EC integrity is critical for vascular function but is compromised by factors like infections and oxidative damage. In SSc, vascular pathology appears as fibroproliferative changes in small arteries and destructive capillary vasculopathy. The former thickens arterial walls, impacting permeability and tone, leading to tissue ischemia, while capillary damage induces EC apoptosis, resulting in micro-vessel loss and tissue fibrosis. These changes manifest in nailfold capillaries as initial enlargement and degradation, followed by reduction in number and the formation of aberrant vessels.

Autoantibodies such as anti-centromere, anti-topoisomerase I, and anti-RNA polymerase contribute to vascular dysfunction in SSc by enhancing endothelin-1 (ET-1), interleukin-6 (IL-6), IL-8, and TGF-β1 expression in ECs. Anti-endothelial cell antibodies disrupt vascular integrity through antibody-dependent cell-mediated cytotoxicity, correlating with increased circulating ECs, apoptotic microparticles and reduced endothelial progenitor cells (EPCs); thereby, impairing vascular repair. Antibodies against G-protein-coupled receptors, such as endothelin type A receptor (ETAR) and angiotensin II type 1 receptor (AT1R), mimic natural ligands to induce pro-inflammatory cytokine, VCAM-1 and CCL18 in ECs, promoting inflammation and fibrosis. Notably, specific AT1R and ETAR blockers can reduce these inflammatory effects, suggesting their therapeutic potential.

In early SSc, expanded $CD4^+$ and $CD8^+$ cytotoxic T cells near apoptotic ECs may result from impaired peripheral T cell tolerance [38]. $CD226^+CD8^+$ T cells in SSc exhibit heightened cytokine production, such as IL-13, and increased cytotoxicity toward human umbilical vascular endothelial cells (HUVECs). Conversely, neutralizing CD226 in these cells reduces cytokine production and cytolysis against HUVECs [39].

2) Defective vascular repair system

In SSc, vascular repair is impaired by disrupted angiogenesis and vasculogenesis. Despite elevated levels of vascular endothelial growth factor (VEGF), ET-1, and other growth factors like fibroblast growth factor (FGF)-2 and platelet-derived growth factor (PDGF), angiogenesis is hindered by insufficient pro-angiogenic mediators and the presence of inhibitors such as angiostatin and thrombospondin-1. VEGF receptor signaling impairment and the detrimental effects of anti-angiogenic VEGF165b isoform also hinder vascular repair [40]. Additionally, TFs such as FLI1 and desmoglein-2, which are crucial for EC adhesion and angiogenesis, are downregulated [41,42]. In SSc, vasculogenesis is impaired, with EPC counts inversely related to digital ulcer severity. EPCs face challenges in maturing into ECs due to an altered bone marrow environment and inhibitors like pentraxin-3 [43]. Additionally, immune-mediated apoptosis, driven by the Akt-FOXO3a-Bim pathway, further reduces EPC levels, exacerbating vascular repair impairment [44].

3) Mesenchymal transition

(1) Endothelial-to-mesenchymal transition

In SSc, endothelial-to-mesenchymal transition (EndoMT) transforms ECs into profibrotic myofibroblasts, disrupting vascular structure and function. During EndoMT, ECs lose endothelial markers and gain fibrotic markers, marking their shift to a fibrotic phenotype, driven by mediators such as TGF-β, ET-1, Wnt/β-catenin, and Notch pathway.

Disruptions in TF FLI1 and Snail1 drive EndoMT, with Snail1 being pivotal in TGF-β-induced mesenchymal transformation of ECs [45,46]. Single-cell ligand-receptor analysis has revealed that CXCL4 also promotes EndoMT by altering the EC phenotype, while leukotriene B4 signaling through BLT1 receptors triggers EndoMT via the PI3K/Akt/mTOR pathway independently of TGF-β [47]. Research suggests that M2 macrophages may play a therapeutic role, as their depletion worsens EndoMT.

Gut microbiota dysbiosis and its metabolite, trimethylamine N-oxide (TMAO), are linked to EndoMT, promoting myofibroblast differentiation from fibroblasts, ECs, and adipocytic progenitors. Elevated TMAO levels correlate with severe symptoms such as ILD and esophageal dysmotility, suggesting therapeutic potential in modulating gut microbiota to reduce EndoMT and its pathological consequences in SSc [48].

(2) Myofibroblast differentiation from diverse cell types

Pericytes play a key role in vascular integrity, interacting closely with ECs and capable of differentiating into various cell types, including myofibroblasts. A single-cell RNA sequencing (scRNA-seq) analysis revealed that overexpression of the Smad anchor for receptor activation in SSc mouse models blocks the pericyte-to-myofibroblast transition, with involvement from Th2 cells and macrophages [49]. Additionally, vascular smooth muscle cells in SSc become proliferative, producing excessive ECM, while adipocytic progenitors undergo an adipocyte-to-myofibroblast transition driven by TGF-β, FIZZ1, and Wnt/β-catenin signaling [50].

ScRNA-seq has also identified specific fibroblast populations driving fibrosis in SSc. In SSc-ILD, a large myofibroblast group was found to significantly contribute to lung fibrosis via elevated collagen expression [51]. In the skin, myofibroblasts differentiate in two stages from $SFRP2^{hi}$ progenitor fibroblasts, with key TFs like FOSL2 and SMAD3 regulating this process [52]. These insights point to fibroblast differentiation pathways as promising targets for anti-fibrotic therapies in SSc.

4) A pathogenic link between vasculopathy and fibrosis in SSc

EC dysfunction plays a pivotal role in the pathogenesis of SSc. The ECs apoptosis leads to capillary destruction, while EC activation exacerbates vascular injury, fostering chronic inflammation through modulating chemokines, cytokines, and adhesion molecules. ECs from SSc patients can activate fibroblasts and prime platelets and coagulation pathways, while increasing the release of pro-fibrotic mediators like TGF-β, PDGF, and FGF-2. Furthermore, EndoMT and the transdifferentiation of vascular wall-resident cells into myofibroblasts disrupt vascular integrity, contributing to fibroproliferative changes in SSc. Figure 2 illustrates the mechanisms and pathways involved in vasculopathy and fibrosis in SSc.

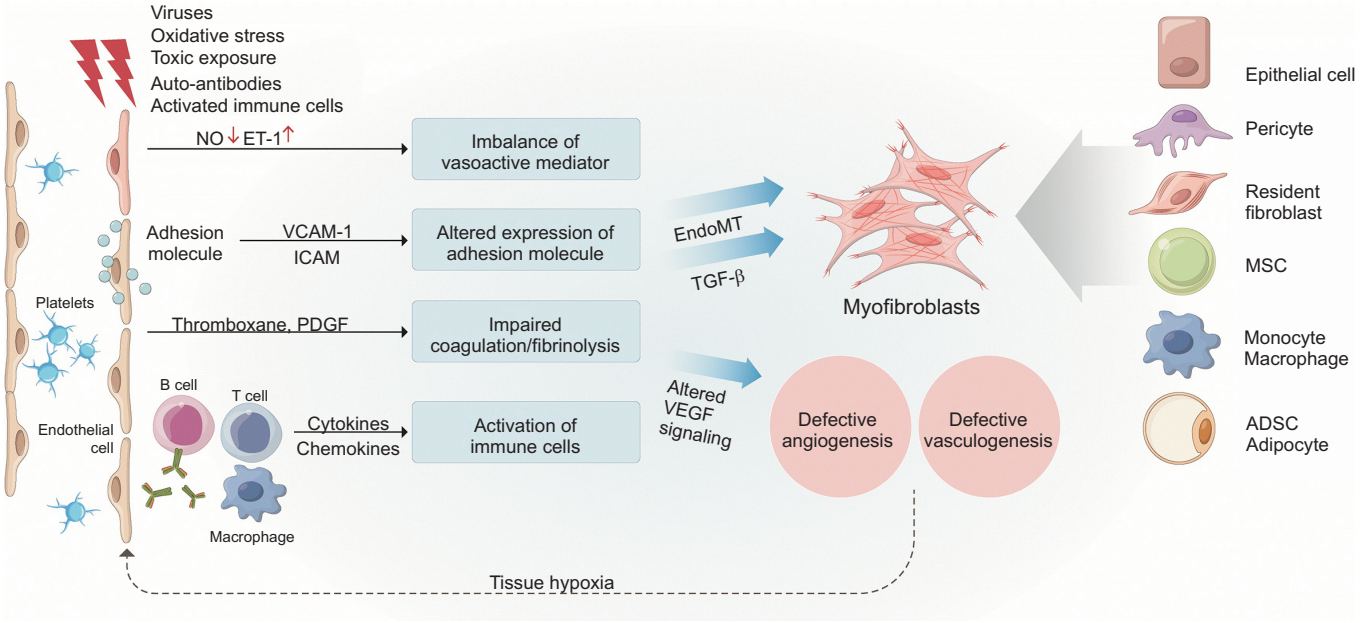

Figure 2. A pathologic association between vasculopathy and fibrosis in SSc. ECs are subjected to damage and dysfunction due to various stimuli, including viruses, oxidative stress, toxic materials, auto-antibodies, and activated immune cells. This dysfunction is characterized by an excess of vasoconstricting agents over vasodilating agents, altered expression of adhesion molecules, impaired coagulation pathways, and the activation of immune cells. Concurrently, enhanced fibroproliferative events within the vessel wall, coupled with reduced angiogenesis and vasculogenesis, are characteristic. Simultaneously, ECs undergo transformation into profibrotic myofibroblasts, further compromising both vascular structure and function. Moreover, the differentiation of myofibroblasts from various cell types, including pericytes, epithelial cells, MSCs, and adipocytes, also contributes to the development of fibroproliferative processes in SSc. NO: nitric oxide, ET-1: endothelin-1, VCAM-1: type 1 vascular cell adhesion molecules, ICAM: intercellular adhesion molecules, PDGF: platelet-derived growth factor, MSC: mesenchymal stem cell, EC: endothelial cell, SSc: systemic sclerosis, ADSC: adipose-derived stem cells, EndoMT: endothelial-to-mesenchymal transition, TGF-β: transforming growth factor-β, VEGF: vascular endothelial growth factor.

Immunologic contributions in SSc

1) Innate immunity in SSc

The innate immune system plays a key role in detecting homeostatic disruptions, and initiating inflammatory responses that drive fibrosis in SSc. Stress signals stimulate the release of pro-inflammatory cytokines like IL-1, IL-6, and tumor necrosis factor (TNF), activating macrophages and promoting TGF-β production, which further amplifies inflammation. Pattern recognition receptors (PRRs), including TLRs, detect damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns. Notably, PRRs are also present on stromal cells, such as ECs and fibroblasts, allowing these cells to produce and respond to inflammatory mediators and contribute to SSc pathogenesis. Dysregulated TLRs in stromal cells recognize DAMPs from damaged ECs, reinforcing their role in activating immune and stromal cells [53].

(1) Pattern recognition receptors

PRRs play a vital role in SSc through stimulating IFN-I and other cytokines, as well as activating mesenchymal cells like fibroblasts. TLR4 is notably overexpressed in SSc fibroblasts and inhibition of TLR4 has been shown to reduce tissue fibrosis, demonstrating therapeutic potential. Additionally, TLR8 is essential in pDCs for IFN-I production, while TLR7 and TLR9 recognize nucleic acids, enhancing the inflammatory cascade [54,55]. These TLRs interact with circulating nucleic acids, possibly complexed with autoantibodies or CXCL4, significantly enhance IFN-I production. Moreover, mitochondrial DNA activates immune pathways through TLR9 and the cytosolic GAS/STING pathways in SSc-ILD, impacting disease progression [56]. This underscores the complex role of PRRs in driving SSc pathogenesis through various molecular mechanisms.

(2) Monocytes

Monocytes are heterogeneous cell population categorized into three subsets based on CD14 and CD16 expression. They promote fibrosis by modulating inflammatory responses and differentiating into macrophages or fibroblast-like cells. $CD14^+$ monocytes exhibit disrupted cytoskeletal arrangements and ECM remodeling, with increased TGF-β signaling. These monocytes increase type I collagen and fibronectin production, particularly in co-culture with dermal fibroblasts [57]. Elevated $CD16^+$ monocytes correlate with severe symptoms like high modified Rodnan skin score (mRSS) and ILD [58]. Carcinoembryonic antigen-related cell adhesion molecule (CEACAM)-positive monocytes are prevalent in early SSc but decrease after immunosuppressive therapy, suggesting their role in early disease stages [59]. These findings highlight the critical role of monocyte subsets in the pathogenesis of SSc.

(3) Macrophages

In SSc, macrophages are central to inflammation and fibrosis with TGF-β production. M1 macrophages, activated by IFN-γ and LPS, release pro-inflammatory cytokines, while M2 macrophages, stimulated by IL-4 and IL-13, support tissue repair and modulate inflammation. Abnormal M2 macrophage polarization is crucial in SSc, with these cells notably found in patient blood and skin as major sources of fibrosis-inducing cytokines. Researches showed that biological agents like nintedanib and apremilast have efficacy in reversing M2 polarization in SSc mouse models [60,61].

$CD14^+$ monocytes/macrophages, prevalent in SSc skin, migrate and differentiate into alveolar macrophages, exhibiting impaired efferocytosis capacities and resulting in sustained inflammation [62]. Their abnormal cytokine production, especially elevated levels of CCL18 and CXCL8 and reduced IL-10, significantly contributes to the progression of SSc [63].

(4) Dendritic cells

DCs include conventional and plasmacytoid subsets. cDCs are key antigen-presenting cells with TLRs that initiate adaptive immune responses. In contrast, pDCs excel in producing type I IFN and cytokines like B cell-activation factor (BAFF), essential for antibody production. The CXCL4-IFNα pathway in pDCs significantly influences SSc fibrosis, with high plasma CXCL4 levels correlating with severe symptoms like PAH and lung fibrosis [64]. A scRNA-seq study revealed a distinct type of $FCN1^+$ monocyte-derived dendritic cells in SSc skin, primarily located in perivascular areas and associated with disease severity [65].

(5) Neutrophils

Recent studies have elucidated the role of neutrophils in SSc. In SSc, neutrophils display impaired migration, defective extracellular trap formation, and compromised phagocytosis. These neutrophils also show decreased levels of CD16 and CD62L, along with weakened CXCR1 and CXCR2 chemokine recep-

tors. Additionally, the increased phosphorylation of STAT3 and STAT6 suggests disrupted signaling pathways [66,67]. These findings highlight the complex role of neutrophils in SSc and underscore potential targets for therapy.

2) Adaptive immunity in SSc

(1) T cells

In SSc, T cells, particularly $CD4^+$ and $CD8^+$ subsets, are significantly more abundant and active, especially in early-stage skin samples. $CD4^+$ T cells, often exhibiting a Th2 profile, are the major subtype driving the disease's progression. $CD8^+$ T cells produce IL-13 and IFN-γ, playing a critical role in early SSc by promoting severe cutaneous fibrosis and exhibiting cytotoxic activity. Altered Th1/Th2 ratios and increased Treg levels are noted, with IL-35 inhibiting $CD4^+$ T cell proliferation and promoting fibroblast functions through STAT1 pathways [68]. In SSc skin, Treg cells produce Th2 cytokines, IL-4 and IL-13, while blood Treg cells show receptors for skin migration. Exposure to IL-33 transforms skin Treg cells into a Th2-like phenotype, potentially compromising their regulatory functions [69,70]. Elevated circulating Th17 cells and $IFN\gamma^+$ $IL\text{-}17^+$ Th17 cells correlate with SSc activity, though their roles in fibrosis remains unclear. Additionally, B cell activation and autoantibody production, driven by interaction with Tfh cells, are significant in SSc [71]. Increased levels of Tfh cells, enhancing B cells activity, are associated with severe lung disease, indicating that these cells are potential therapeutic targets [72].

(2) B cells

B cells contribute to SSc pathogenesis by producing autoantibodies and enhancing fibrosis. SSc patients show an imbalance with elevated IL-6-producing effector B cells (Beff) and reduced IL-10-producing regulatory B cells (Breg), associated with severe skin symptoms and extensive ILD. This disrupted Beff/Breg ratio exacerbates inflammation and fibrosis, driven by pro-inflammatory cytokines from Beff cells and a lack of anti-inflammatory Breg cells [73]. The key B cells marker CD19, linked to fibrosis severity in SSc, offers a therapeutic target, as its inhibition lowers fibrosis and autoantibody levels. BAFF also enhances fibrosis by promoting B cell activity, with BAFF inhibitors showing promise in reducing fibrosis. The therapeutic use of IL-10 producing Breg cells decreases disease severity, suggesting potential targeted therapies to balance Beff and Breg activities for SSc management. Additionally, B cell depletion in SSc mouse model inhibits tissue fibrosis by suppressing the differentiation of profibrotic macrophages, providing a novel rationale for the therapeutic use of B cell depletion in SSc.

(3) Microbiome and SSc

Dysbiosis in SSc leads to significant immunological and metabolic changes, affecting the epithelial and mucosal barriers of the skin, lungs, and gastrointestinal tract (GIT). Th2 $CD4^+$ T cells activate fibroblasts and anti-muscarinic receptor autoantibodies, leading to neuropathic damage, ischemia, and collagen accumulation, which impair GIT motility and exacerbate conditions like small intestinal bacterial overgrowth. Moreover, SSc patients exhibit a distinct gut microbiota with fewer beneficial bacteria like *Faecalibacterium* and more pro-inflammatory bacteria such as *Fusobacterium and Lactobacillus*, suggesting microbial imbalances as a therapeutic target.

Similarly, dysbiosis in skin and lung microbiomes increases disease severity. Compromised skin barriers allow pathogens to increase inflammation, while decreased microbial diversity in the lungs correlates with reduced pulmonary function and higher mortality in idiopathic pulmonary fibrosis [74,75].

(4) Fibrosis

Fibrosis in SSc arises from chronic tissue injury, characterized by excessive ECM components and alteration of tissue structure. Unlike normal wound healing, myofibroblasts persist in the fibrosis process due to an imbalance of anti-apoptotic/pro-apoptotic mediators and tissue stiffness, which activates TGF-β and increases ECM production through mechanical sensors. Additionally, keratinocytes in SSc alter differentiation pathways and engage in TGF-β signaling, enhancing inflammatory and fibrotic responses in dermal fibroblasts. These keratinocytes produce factors like keratinocyte growth factor and matrix metalloproteinases, influencing fibroblast behavior and reinforcing a bidirectional interaction critical for skin integrity [76]. Under inflammatory cytokines such as IL-17A, IL-22, and TNF, keratinocytes modify responses through pathways like Wnt/β-catenin, which may mitigate TGF-β's effects, offering insights into potential therapeutic strategies targeting inflammation and fibrosis in SSc.

Pro-inflammatory and pro-fibrotic mediators in SSc

1) Type I interferon

IFN-1 is integral to the innate immune system and notably involved in SSc. Genetic polymorphisms in IFN-regulatory factors increase SSc risk and notable IFN excess is found in blood and skin samples of SSc patients [77]. A higher IFN signature in the plasma correlates with severe vascular and lung involvement and specific autoantibodies, such as anti-topoisomerase and anti-U1-RNP. This signature is linked to increased expression of IFN-stimulated genes in key affected organs. Although the precise role of IFN-1 in SSc pathogenesis is not fully elucidated, these findings highlight the critical role of IFN in the progression of SSc.

2) Damage-associated molecular patterns

DAMPs, also known as alarmins, have a crucial role in initiating immune responses after cell injury. These molecules, including HMGB-1 and S100 proteins, activate both innate immune cells and non-immune cells like epithelial cells and fibroblasts. Studies have explored the role of DAMPs in fibrosis through interaction with TLR4 [78]. Elevated serum levels of HMGB-1 correlate with increased SSc severity. Furthermore, high S100A4 levels were observed in both the serum and skin fibroblast cultures of SSc patients compared to healthy controls and were associated with SSc complications like ILD and SRC [79].

3) Interleukin-1 family

The IL-1 cytokine family, including IL-1α, IL-1β, IL-18, and IL-33, promotes the differentiation and persistence of myofibroblasts in SS. IL-1α and IL-1β enhance fibroblast proliferation and activation by upregulating IL-6, PDGF, and TGF-β1. IL-1β further contributes to EndoMT and Th17 cell differentiation, which are critical in SSc inflammation and fibrosis [80,81].

IL-33 promotes Th2 differentiation and cytokine production, contributing to fibrosis in SSc. Elevated serum IL-33 levels in SSc correlate with more severe skin sclerosis and pulmonary fibrosis [82].

4) IL-4, IL-5, and IL-13

Lymphocytic infiltrates with a Th2-predominant profile, characterized by increased levels of IL-4 and IL-13, have been observed in the skin and lungs of SSc patients before fibrosis development. $CD4^+$ $CD8^+$ double-positive T cells in lesional skin produce high levels of IL-4, promoting myofibroblast maturation and collagen gene expression in SSc [83].

Recent studies underscore the importance of Th2 cytokines in SSc fibrosis. A Mendelian randomization study suggests a protective role for IL-5, with lower levels potentially increasing SSc risk [84]. Additionally, elevated IL-13 in SSc serum and tissues is linked to fibrosis severity, as IL-13-producing $CD8^+$ T cells drive dermal fibrosis and ECM production [85]. These insights highlight Th2 cytokines as key contributors to fibrosis in SSc progression.

5) IL-6 family

The IL-6 family plays a significant role in modulating immune responses relevant to SSc. Elevated IL-6 levels in SSc contribute to Th2 differentiation and collagen production in fibroblasts, correlating with more severe skin involvement. IL-11 is upregulated in fibroblasts of SSc-ILD patients, influencing myofibroblast formation, with tocilizumab trials and mRNA studies further confirming its role [86]. Targeting IL-11, as demonstrated by the experimental treatment TJ301, offers therapeutic potential, though the precise impact requires further study [87,88]. Additionally, IL-31, produced primarily by Th2 cells, is overexpressed in SSc fibroblasts; blocking IL-31/IL-31RA reduces fibrosis and Th2 polarization in SSc models, highlighting it as a promising therapeutic target [89].

6) IL-17

Elevated levels of IL-17, primarily produced by Th17 cells, are implicated in SSc pathogenesis. IL-17A, particularly impactful in SSc models, drives inflammation by inducing cytokines and chemokines (e.g., CCL-2, IL-8) in fibroblasts and ECs. It also promotes vascular inflammation and fibroblast activation by upregulating chemokines like CCL-20 and adhesion molecules ICAM-1 and VCAM-1 in ECs. While IL-17's role in human fibrosis remains debated, the anti-IL-17 receptor A antibody brodalumab has shown clinical benefits, reducing skin thickening (mRSS) and improving pulmonary function in SSc patients [90].

7) Transforming growth factor-β

TGF-β is a key pleiotropic cytokine in SSc. It initiates fibrosis through pathways such as SMAD, ERK, JNK, and PI3K/Akt. These pathways lead to fibroblast activation and tissue remodeling. In SSc, TGF-β upregulates genes such as *CTGF, THBS1,*

SERPINE1, and *COMP*, exacerbating fibrosis. The high-affinity antibody fresolimumab, targeting all three TGF-β isoforms, has shown efficacy in reducing myofibroblast infiltration and improving skin fibrosis in SSc [91]. Epigenetic mechanisms further amplify TGF-β's profibrotic effects, with SSc fibroblasts maintaining autocrine TGF-β signaling that promotes survival and resistance to apoptosis [92]. Additionally, TGF-β modulates immune responses by promoting Treg and Th17 cell differentiation while inhibiting Th1 and Th2 cell differentiation, thereby fostering fibroblast heterogeneity. These multifaceted roles underscore TGF-β's significance in SSc pathogenesis and therapeutic potential.

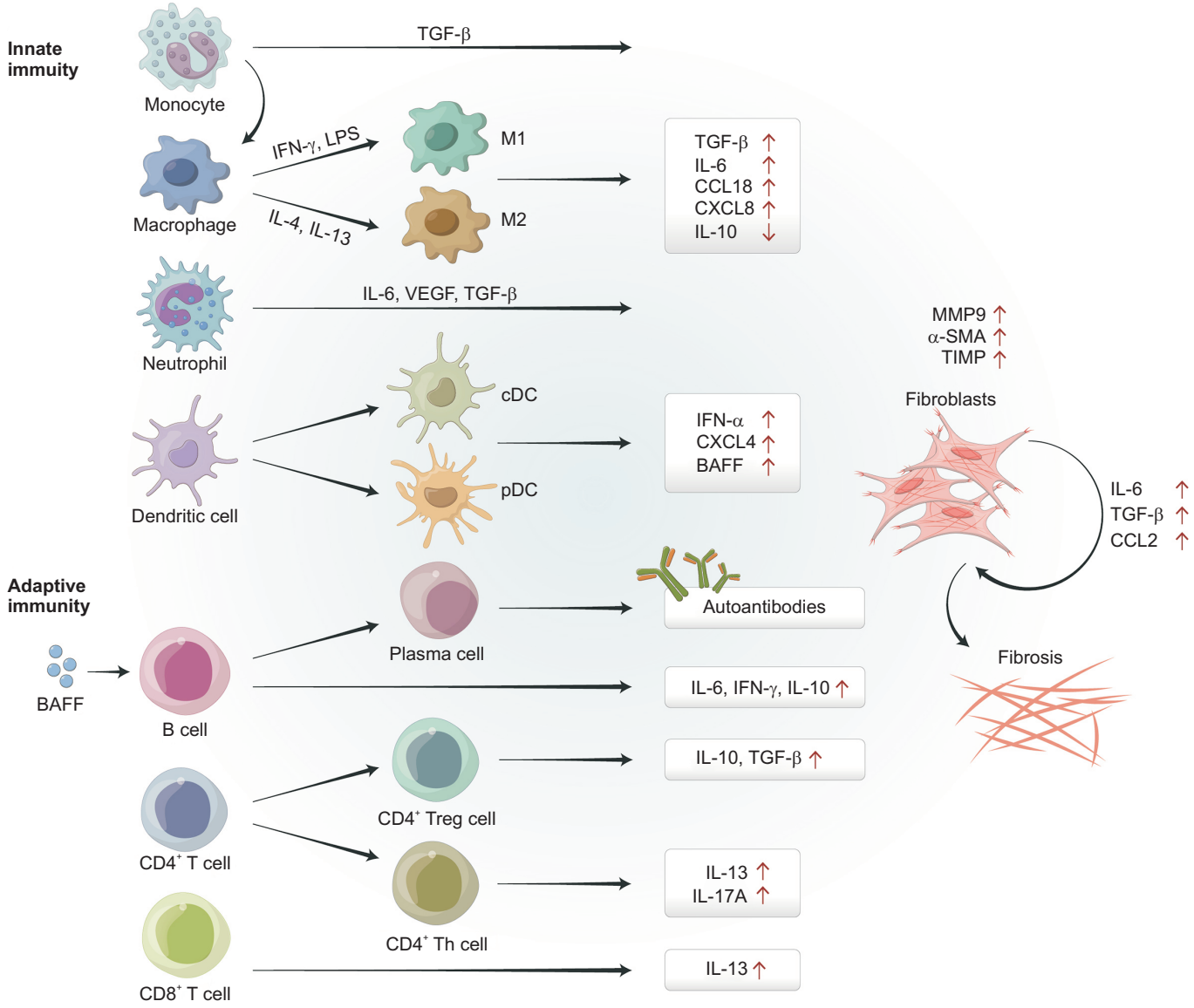

Figure 3. The pro-inflammatory and profibrotic network involved in the pathogenesis of SSc. This figure highlights the intricate interactions between various immune cells and their mediators. Monocytes differentiate into macrophages under TGF-β signaling, with M1 macrophages releasing pro-inflammatory cytokines, while abnormally polarized M2 macrophages produce pro-fibrotic TGF-β. Neutrophils in SSc exhibit impaired migration and function. pDCs secrete IFN-α, CXCL4, and BAFF, which are linked to severe fibrosis. BAFF-activated B cells produce autoantibodies, further promoting inflammation. $CD4^+$ T cells release cytokines (e.g., IL-6, TGF-β, IL-13), which activate fibroblasts. These activated fibroblasts increase collagen and fibrosis marker production, amplifying IL-6, TGF-β, and CCL2 signaling, thereby driving fibrosis in SSc. SSc: systemic sclerosis, EC: endothelial cell, TGF-β: transforming growth factor beta, VEGF: vascular endothelial growth factor, MMP: matrix metalloproteinase, α-SMA: alpha-smooth muscle actin, TIMP: tissue inhibitor of metalloproteinases, IFN-γ: interferon gamma, LPS: lipopolysaccharides, IL: interleukin, BAFF: B cell activating factor, cDC: conventional dendritic cell, pDC: plasmacytoid dendritic cell, EPC: endothelial progenitor cell.

8) Cellular communication network factors family

The cellular communication network factors (CCN) family, particularly CCN2 and CCN3, is important in fibrosis within SSc. CCN2, also known as connective tissue growth factor, is elevated in SSc skin and serum, correlating with the severity of skin and lung fibrosis. It enhances fibroblast adhesion to ECM components and responses to TGF-β and ET-1, forming an autocrine profibrotic loop [93]. In mouse models, CCN2 loss reduces skin thickness and myofibroblast presence [94]. Conversely, CCN3 exhibits anti-fibrotic properties by inhibiting fibrous protein assembly and supporting angiogenesis, counterbalancing CCN2's effects, and is thus a promising therapeutic target for SSc.

9) Platelet-derived growth factor

PDGF is crucial in SSc progression, particularly through the PDGF/PDGFR axis, which supports fibroblast and smooth muscle cell activity. Elevated PDGF isoforms are observed in SSc skin and bronchoalveolar lavage fluid, enhancing fibrosis. Inhibiting PDGFR signaling through RNA interference or downregulation of miR-30b reduces fibrotic markers like α-SMA and Col1A2 [95]. Additionally, PDGFRα-targeting autoantibodies exacerbate fibrosis by increasing ECM and vascular cell production, suggesting that the PDGF pathway holds potential as a diagnostic and therapeutic target in SSc [96].

10) Reactive oxygen species

Oxidative stress, marked by elevated reactive oxygen species (ROS) like superoxide and nitric oxide (NO), is a significant factor in SSc. An imbalance between NO and ET-1 leads to ischemic events and further oxidative stress. Increased oxidative markers, such as malondialdehyde and advanced oxidation protein products, correlate with disease severity and fibrosis. ROS also facilitate fibrosis by promoting M2-like monocyte polarization and fibroblast activation [97]. Furthermore, ROS activation of the NLRP3 inflammasome contributes to fibrosis, presenting a potential therapeutic target in SSc [98]. Figure 3 illustrates the pro-inflammatory and profibrotic network involved in the pathogenesis of SSc.

Navigating the future of SSc research

Recent advancements in SSc research are paving the way toward precision medicine, with multi-omic platforms revealing connections between autoantibodies, clinical symptoms, and organ involvement. Multi-omic analyses, integrating data from transcriptomics, genomics, proteomics, and more, have illuminated aspects of SSc pathogenesis. Techniques like cytometry by time of flight (CyTOF) and extended polydimensional immunome characterization have identified key biomarkers for diagnosis and treatment, and recent CyTOF studies have found a potentially protective T cell subset in SSc [99,100]. Additionally, scRNA-seq has highlighted distinct fibroblast subsets with pathogenic roles, contributing to a better understanding of SSc's cellular diversity [100].

The decreasing cost of sequencing and advances in bioinformatics are expected to expand the use of these technologies, underscoring the importance of collaborative networks in advancing SSc diagnostics and therapies. Targeted treatments, inspired by insights into inflammatory pathways, include emerging approaches like CD19 CAR T-cell therapy, warranting careful monitoring for associated risks [101,102].

Future research aims to merge clinical characteristics with multi-omic data to support personalized, data-driven treatments that consider the genetic profile of each patient. Integrating new technologies with patient-reported outcomes may foster a more comprehensive, patient-centered approach to SSc management.

CONCLUSION

In conclusion, this review underscores significant advancements in SSc research and the shift toward precision medicine. Multi-omic platforms have unveiled intricate correlations between genetic, epigenetic, and environmental factors that drive SSc, offering essential insights for identifying biomarkers and therapeutic targets for personalized treatments. Advanced technologies like CyTOF and scRNA-seq have illuminated the diverse cellular landscape in SSc, deepening our understanding of its underlying mechanisms.

Recognizing the complex interplay between genetic predispositions, immune dysregulation, and environmental triggers is crucial for developing innovative therapies. This review emphasizes the importance of a multidisciplinary, patient-centered approach, with ongoing collaboration across scientific fields needed to refine diagnostics and treatments. By harnessing leading-edge research and technology, we move closer to achieving personalized, effective SSc therapies, marking meaningful progress in managing this complex condition.

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CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

AUTHOR CONTRIBUTIONS

HHS and SJM contributed to the study conception, design, and data acquisition. All authors contributed to the analysis and/or interpretation of data. HHS and SJM contributed to drafting the manuscript. HHS and SJM contributed to revising the manuscript. All authors reviewed and approved the final version of the manuscript.

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