

Pathogenesis of systemic sclerosis—current concept and emerging treatments

Masutaka Furue^{1,2,3} · Chikage Mitoma^{1,2} · Hiroki Mitoma⁴ · Gaku Tsuji^{1,2} · Takahito Chiba¹ · Takeshi Nakahara^{1,3} · Hiroshi Uchi¹ · Takafumi Kadono⁵

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Abstract Systemic sclerosis (SSc) is an intractable multifaceted disease with high mortality. Although its pathogenesis is not fully understood, recent studies have advanced our knowledge on SSc. The cardinal pathological features of SSc are autoimmunity, vasculopathy, and fibrosis. The B cells in SSc are constitutively activated and lead to the production of a plethora of autoantibodies, such as anti-topoisomerase I and anti-centromere antibodies. In addition to these autoantibodies, which are valuable for diagnostic criteria or biomarkers, many other autoantibodies targeting endothelial cells, including endothelin type A receptor and angiotensin II type I receptor, are known to be functional and induce activation or apoptosis of endothelial cells. The autoantibody-mediated endothelial cell perturbation facilitates inflammatory cell infiltration, cytokine production, and myofibroblastic transformation of fibroblasts and endothelial cells. Profibrotic cytokines, such as transforming growth factor β , connective tissue growth factor, interleukin 4/interleukin 13, and interleukin 6, play a pivotal role in collagen production from myofibroblasts. Specific treatments targeting these

causative molecules may improve the clinical outcomes of patients with SSc. In this review, we summarize recent topics on the pathogenesis (autoantibodies, vasculopathy, and fibrosis), animal models, and emerging treatments for SSc.

Keywords Systemic sclerosis · Autoantibodies · Vasculopathy · Fibrosis

Introduction

The prevalence of systemic sclerosis (SSc) ranges from 0.7/100,000 to 53/100,000 depending on the different ethnicities, and there are higher numbers in the USA than in Europe or Japan [1]. In a nationwide, cross-sectional hospital-based study in Japan, patients with SSc comprised 0.92% of dermatological patients [2]. SSc has a high mortality rate due to the development of SSc-associated interstitial lung disease and pulmonary arterial hypertension, which are the most frequent causes of disability and mortality in SSc [3–5].

Depending on the extent of skin involvement, SSc is classified into the following two main subtypes: limited cutaneous SSc and diffuse cutaneous SSc. Given the heterogeneity of clinical symptoms and signs, the American College of Rheumatology/the European League against Rheumatism developed new classification criteria in 2013 [6–8].

SSc is not an inherited disease, therefore, twins show a low disease concordance rate (4.7%) that is similar between monozygotic and dizygotic twin pairs [9]. However, genetic factors contribute to its susceptibility, as shown by a 60-fold higher occurrence of the disease in families compared to the general population [10–12]. Genetic linkage studies and genome-wide association studies have identified many susceptible genes, including the major histocompatibility complex (MHC) class II as well as non-MHC genes related to the metabolism of

✉ Masutaka Furue
furue@dermatol.med.kyushu-u.ac.jp

¹ Department of Dermatology, Kyushu University, Maidashi 3-1-1, Higashiku, Fukuoka 812-8582, Japan

² Research and Clinical Center for Yusho and Dioxin, Kyushu University, Fukuoka, Japan

³ Division of Skin Surface Sensing, Department of Dermatology, Kyushu University, Fukuoka, Japan

⁴ Department of Clinical Immunology and Rheumatology/Infectious Disease, Kyushu University, Fukuoka, Japan

⁵ Department of Dermatology, St. Marianna University School of Medicine, Kawasaki, Japan

extracellular matrix molecules, innate immunity, macrophage activation, and T cell functions [10–12].

Systemic sclerosis (SSc) is a multisystem connective tissue disease characterized by three cardinal pathological features, including aberrant immune activation, vasculopathy, and tissue fibrosis with an unknown aetiology [3, 4, 13]. Although its pathogenesis is not fully understood, recent studies have advanced our knowledge on SSc. In this review, we summarize recent topics on the pathogenesis (autoantibodies, vasculopathy, and fibrosis), animal models, and emerging treatments for SSc.

Pathogenesis

Pathogenesis of SSc remains unknown, whereas it is commonly thought that autoimmunity and vasculopathy precede fibroblast activation and interstitial fibrosis [14, 15]. Autoantibodies to endothelial cells, ischemia–reperfusion injury following Raynaud’s phenomenon, generation of reactive oxygen species (ROS) with inflammatory cell infiltration, and subsequent cytokine production trigger myofibroblastic transformation of endothelial cells as well as fibroblasts and induce excessive production of collagens and other extracellular matrices [5, 8, 14, 15]. Various profibrotic cytokines, such as transforming growth factor β (TGF β), interleukin (IL)-4, IL-13, IL-6, and IL-33 are likely to be involved in the development of fibrosis [13, 14, 16–18].

Autoantibody and vasculopathy

The immune system activation is exemplified by the activation of B cells and the production of autoantibodies [19–23]. B cells from patients with SSc overexpress the B cell stimulatory receptor CD19 by 54% in patients with early SSc and by 28% in those with long-standing disease compared to normal controls [22–24]. Notably, a small increase (15–29%) in the CD19 expression of B cells in transgenic mice induced autoantibody production [25]. In contrast, the function of CD22, an inhibitory B cell molecule, is inhibited by anti-CD22 autoantibodies present in patients with SSc [22, 23, 25]. An increased CD19/CD22 ratio may facilitate the sustained activation of B cells and consequent overproduction of various autoantibodies [22, 23, 25, 26].

Anti-nuclear antibodies are found in the sera of the vast majority of SSc patients, and their antigenic specificity significantly correlates with the clinical characteristics of the disease [19–21]. Autoantibodies are currently the most reliable biomarkers for diagnosis, classification, and prediction of specific clinical features of SSc [19–21]. Certain autoantibodies, such as anti-topoisomerase I, anti-centromere, anti-RNAP III, anti-U3 RNP, anti-Th/To, and anti-U1 RNP antibodies, are closely associated with distinct clinical features and disease activities

of SSc [19–21, 27, 28] (Fig. 1). Other autoantibodies targeting a variety of cytoplasmic, cell membrane, and extracellular autoantigens were also detected in SSc [21, 23]. Some of them, such as anti-endothelial cell, anti-intercellular adhesion molecule-1 (ICAM-1), anti-endothelin type A receptor (ETAR), and anti-angiotensin II type I receptor (AT1R) antibodies, may be functional and pathogenic [21, 23]. In addition to autoantibodies, a plethora of biomarkers related to immune reactions [29, 30], endothelial cell function [31, 32], and the extracellular matrix [33, 34] have been reported recently [19].

Raynaud’s phenomenon and abnormal nailfold capillary changes, which are the representative vascular manifestations of SSc, often appear before the onset of sclerosis [8, 15, 35]. Raynaud’s phenomenon is the abnormal thermal regulation of blood flow that is probably triggered by endothelial injury [15]. The skin fibrosis tends to occur on locations, such as fingers, distal extremities, and face, which are frequently exposed to cold temperatures [15]. Nailfold capillaroscopy reveals structural alterations in capillaries that include dilatation, distortion, and microhemorrhages that lead to progressive loss of the capillaries (Fig. 1) [36]. Microscopically, capillary damage of SSc is characterized by endothelial apoptosis, intimal and medial fibrous thickening, and adventitial fibrosis with perivascular infiltration of the macrophages, B cells, and T cells [15]. Precapillary arterioles then show endothelial proliferation and mononuclear inflammatory infiltrates followed by intimal proliferation and luminal narrowing [37]. The origin of the cells that populate the intima and participate in collagen production remains unknown. However, activated resident fibroblasts, circulating fibroblast precursors (fibrocytes), the endothelial cells, and pericytes have all been implicated [15]. In line with these findings, an endothelial cell to mesenchymal transition is shown in the lesional vascular systems in SSc [5].

The endothelial apoptosis or activation is likely mediated by functional autoantibodies [23, 38]. Anti-endothelial cell antibodies cause endothelial cell apoptosis [39]. Anti-ICAM-1 antibodies induce the production of ROS and expression of vascular cell adhesion molecule-1 (VCAM-1) which may facilitate the attachment of immune cells [40]. Anti-ETAR and anti-AT1R autoantibodies, which are detected in most SSc patients, are agonistic antibodies that upregulate the expression of TGF- β , IL-8, and VCAM-1 of endothelial cells and cause fibrosis, vasoconstriction, and recruitment of immune cells [41–43]. Both AT1R and ETAR are expressed on cells of both the vascular and immune system [38]. Angiotensin II increased the production of TGF β and collagen by fibroblasts via AT1R [44]. Endothelin-1 also induced collagen production and vasoconstriction via ETAR [45]. The expressions of ETAR and AT1R are found to be highest in patients with early SSc [46]. The anti-ETAR and anti-AT1R antibodies from SSc patients induce obliterative vasculopathy when injected into mice [43]. The endothelial cell apoptosis has been demonstrated in the skin lesions of patients with SSc as well as in avian

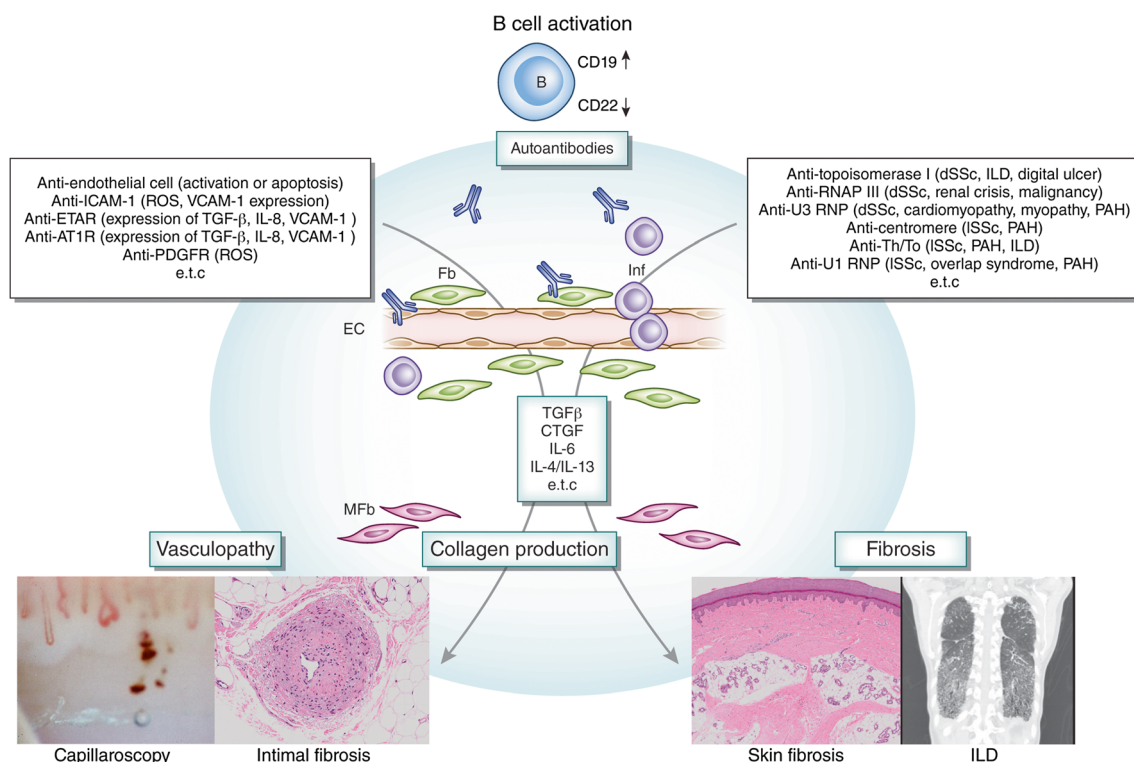


Fig. 1 Pathogenesis of systemic sclerosis. The cardinal pathological features of systemic sclerosis (SSc) are autoantibodies, vasculopathy, and fibrosis. B cells in SSc are constitutively activated with increased CD19 and decreased CD22 expression, which leads to the overproduction of a plethora of autoantibodies, such as anti-topoisomerase I, anti-RNAP III, anti-U3 RNP, anti-centromere, anti-Th/To, and anti-U1 RNP antibodies. These autoantibodies are associated with characteristic clinical features, such as diffuse SSc (dSSc), limited SSc (lSSc), interstitial lung disease (ILD), digital ulcer, renal crisis, malignancy, cardiomyopathy, myopathy, pulmonary artery hypertension (PAH), and overlap syndrome. Many other functional autoantibodies targeting endothelial cells, intercellular adhesion molecule 1 (ICAM-1), endothelin type A receptor (ETAR), and angiotensin II type I receptor

(AT1R) as well as platelet-derived growth factor receptor (PDGFR) are known to induce activation or apoptosis of endothelial cell (EC). The stimulated ECs express reactive oxygen species (ROS), transforming growth factor β (TGF β), interleukin-8 (IL-8), and vascular endothelial cell adhesion molecule 1 (VCAM-1). The autoantibody-mediated endothelial cell perturbation facilitates inflammatory cell (Inf) infiltration, cytokine production, and myofibroblastic transformation of fibroblasts (Fb) and ECs. Profibrotic cytokines, such as TGF β , connective tissue growth factor (CTGF), IL-6, and IL-4/IL-13, play a pivotal role in collagen production from myofibroblasts (MFb). Perturbation of ECs and consequent profibrotic processes result in vasculopathy (including capillaroscopic abnormalities and intimal fibrosis) and tissue fibrosis (including skin fibrosis and ILD)

SSc models [47]. Moreover, many SSc patients have antibodies against a human cytomegalovirus-derived UL94 protein that shares homology with NAG-2 (tetraspan novel antigen-2), which is expressed on the surface of human endothelial cell and fibroblast [48, 49]. The anti-UL94 peptide antibodies bind to NAG-2 and induce “scleroderma-like” gene expression in endothelial cells and fibroblasts [48, 49]. Thus, the molecular mimicry mechanism links antibodies against the human-cytomegalovirus-derived protein UL94 to the pathogenesis of systemic sclerosis [48, 49].

Fibrosis

The degree of cell infiltration correlates with both the degree and progression of skin thickening, which results from excessive accumulation of type 1 collagen and extracellular matrix proteins [3, 8, 50, 51]. These infiltrated cells, especially endothelial cells and fibroblasts, are potential candidates for

producing various cytokines, chemokines, and growth factors that induce fibrosis [3, 8, 50, 51]. Despite the differences in the extent and distribution of skin involvement, both limited and diffuse type SSc produce marked fibrosis followed by the preceded autoimmunity, vasculopathy, and perivascular inflammatory cell infiltration [8, 50, 51].

Representative profibrotic growth factors and cytokines encompass TGF β , connective tissue growth factor (CTGF), platelet-derived growth factor (PDGF), IL-6, and IL-4/IL-13 (Fig. 1). After ligation with TGF β receptor type II (TGF β RII) and TGF β RI, TGF β induces gene transcription of type I collagen, α smooth muscle actin (α SMA), and CTGF via SMAD2/SMAD3 phosphorylation [3]. An increased TGF β RI/TGF β RII ratio was found in the SSc fibroblasts, which was implicated in the overactivation of TGF β signaling [52]. The expression of α SMA is a hallmark of myofibroblastic transformation of activated fibroblasts, which is also frequently detected in SSc [53, 54]. CTGF is a cysteine-rich modular protein belonging to the

CCN family of matricellular growth factors, all of which function as adaptor molecules connecting the cell surface to the extracellular matrix [3, 55]. The expression of CTGF is induced by TGF- β , ET-1, and hypoxia [3, 55]. The levels of CTGF are markedly elevated in the lesional skin of SSc patients [56] and in mouse models of scleroderma [57].

The expression of PDGF and its receptors is elevated in SSc fibroblasts and in the lesional skin [58]. Antibodies that stimulate the PDGF receptor (PDGFR) were frequently found in SSc patients [59]. The autoantibodies recognize native PDGFR and selectively activate Ha-Ras-ERK1/2 and ROS cascades to induce type I collagen gene expression and myofibroblast phenotype conversion in normal human fibroblasts [59]. The activated B cells secreted IL-6, which directly stimulates fibroblasts [60–62]. IL-6 induces the type I collagen expression via enhancing TGF β -Smad3 signalling pathway [62]. Both IL-4 and IL-13 likely activate fibroblasts and induce type I collagen synthesis via a TGF β -independent approach [63–65]. Serum levels of IL-4 and IL-13 were significantly higher in patients with SSc compared to normal controls [66]. It is likely that IL-13, rather than IL-4, plays a more dominant role in fibrosis [17, 67]. Notably, CD8+ T cells producing IL-13 have been shown to have skin-homing receptors in SSc skin and they upregulate type I collagen production when incubated with healthy dermal fibroblasts [68].

Animal models of systemic sclerosis

Inducible fibrotic models

Among inducible animal models, the most widely used and established ones are a bleomycin-induced SSc model and a sclerodermatous graft-versus-host disease model in mice [13, 69–71]. Recently, three new inducible murine models of SSc have been established by utilizing ROS [72], DNA topoisomerase I antigen with complete Freund's adjuvant [73], and angiotensin II [74]. Basically, the focus of these inducible models was put on the induction of fibrosis tissue and, to a lesser extent, on the immunological aspects of SSc, instead of its vascular pathology [13].

Genetic animal models

The best-characterized genetic animal models of SSc are tight skin-1 (Tsk-1) mice, in which an in-frame tandem partial reduplication of the *Fbn1* gene encoding fibrillin-1, which is a major component of microfibrils mediating elastic fiber assembly, is responsible for the phenotype [75]. Although the Tsk-1 mice exhibit TGF β upregulation with the aberrant activation of B cells and autoantibody production, the fibrosis of Tsk-1 mice occurs in the hypodermis, which is not observed in human SSc [13]. Mice with constitutively active TGF β RI

recapitulate clinical, histological, and biochemical features of human SSc [76]. In addition, mice expressing the *Ctgf* gene in fibroblasts [77] or *Wnt10b* in adipocytes [78] exhibit extensive fibrosis in the dermis and internal organs.

New genetic animal models

Fra-2 is one of the components of Fos (c-Fos, Fra-1, Fra-2, FosB) which dimerize with Jun (c-Jun, JunB, JunD) subunits to make AP-1 transcription factor [13]. The *Fra2* transgenic mice developed dermal and pulmonary fibrosis following the apoptosis of endothelial cells [79, 80]. Urokinase-type plasminogen activator receptor (uPAR) is a glycosylphosphatidylinositol-anchored cell surface receptor expressed by several cell types, including lymphohaematopoietic cells, fibroblasts, and endothelial cells [13]. A major role of uPAR is to concentrate its ligand, uPA, at the cell-matrix interface. uPA has a serine protease activity that induces the conversion of plasminogen to plasmin and the activation of growth factors and pro-enzymes, such as matrix metalloproteinases [13]. Interestingly, the cleavage and/or inactivation of uPA/uPAR is associated with the transition of fibroblasts to myofibroblasts and subsequent fibrosis as well as with the functional and structural abnormalities of microvasculature in SSc [81–84]. In line with these findings, uPAR-deficient mice recapitulate the fibrotic and vascular features of SSc [85]. However, both *Fra2* transgenic mice and uPAR-null mice totally lack immunological aspects, specifically autoantibody production, in SSc (Table 1) [13].

In contrast to these animal models, *Klf5*^{+/-}; *Fli1*^{+/-} mice develop immune activation, vasculopathy, and fibrosis, which are the three cardinal features of SSc [13, 86]. Fli1 is a member of the Ets transcription factor family expressed in endothelial and hematopoietic cells under physiological conditions, and to a lesser extent in dermal fibroblasts [13]. Fli1 exerts a potent repressor of the *COL1A1* and *COL1A2* gene expression in dermal fibroblasts [87, 88]. The expression of Fli1 was decreased in dermal fibroblasts, dermal microvascular endothelial cells, and perivascular inflammatory cells in the lesional and non-lesional skin of SSc patients, especially in the lesional skin [88]. Fli1 haploinsufficiency is enough to reduce the

Table 1 Phenotypic differences of new genetic animal models for systemic sclerosis

	<i>Fra2</i> tg mice	uPAR ^{-/-} mice	<i>Klf5</i> ^{+/-} ; <i>Fli1</i> ^{+/-} mice
Dermal fibrosis	+	+	+
Vasculopathy in the skin	+	+	+
Pulmonary fibrosis	+	+	+
Pulmonary arterial occlusion	+	-	+
Autoantibody production	-	-	+

production of type I collagen [87, 88]. However, *Fli1* haploinsufficiency had no effect on CTGF expression [86]. Therefore, some additional factors are mandatory to upregulate the CTGF expression that is also the hallmark feature of dermal fibroblasts derived from SSc patients [13, 56]. Kruppel-like factor 5 (KLF5) is a potent repressor of the *Ctgf* gene [86]. Moreover, KLF5 was downregulated in the lesional skin of SSc [89]. Thus, *Klf5*^{+/-}; *Fli1*^{+/-} double haploinsufficiency mice spontaneously develop remarkable dermal fibrosis, which is characterized by the increase in dermal thickness, the amount of collagen content, and the mRNA of the *Colla1*, *Colla2*, and *Ctgf* genes, at the age of 3 months [86]. In addition, the pathological cascade from vasculopathy (stenosis of arterioles and bushy capillaries) to tissue fibrosis was also present in these mice [86]. Furthermore, the *Klf5*^{+/-}; *Fli1*^{+/-} mice mimicked the immunological aspects of human SSc, including increased CD19 expression in B cells, upregulated production of IL-6, and the production of autoantibodies (Table 1) [86].

Treatments and therapeutic perspectives

Although the treatment outcomes are not satisfactory, immunosuppressive agents, such as corticosteroids [8], methotrexate [90, 91], cyclophosphamide [92, 93], mycophenolate mofetil [94, 95], and intravenous immunoglobulin [96, 97], are currently being used [98]. ETAR antagonist and angiotensin-converting enzyme blockade have been used to treat clinical complications related to vasculopathies of patients with SSc [99, 100]. Injection of botulinum toxin is useful for preventing Raynaud's phenomenon [101].

B cell depletion by rituximab (anti-CD20 antibody) improves lung function and skin thickening in patients with SSc [102–104]. Fresolimumab, a neutralizing antibody that targets all three isoforms of TGFβ, also decreases skin fibrosis with histological reduction of dermal myofibroblasts [105]. As for the anti-IL-6 receptor α antibody, a randomized controlled trial revealed that tocilizumab tended to reduce the skin thickening compared to placebo, but not significantly [106, 107]. Rapamycin binds to the FK-506 binding protein 12 and inhibits the function of mammalian target of rapamycin. Rapamycin prevents fibrosis of the skin and lungs as well as autoantibody production in a murine model of SSc [108]. In a phase I, single-blinded, randomized, parallel trial of rapamycin versus methotrexate, rapamycin was just as effective as methotrexate for skin sclerosis [109]. Pirfenidone, a pyridine with a simple chemical structure, is an antifibrotic agent, and it has been approved for the treatment of idiopathic pulmonary fibrosis worldwide [110, 111]. Although the molecular mechanisms underlying the antifibrotic effects of pirfenidone are not completely understood, it is thought to work predominantly by modulating TGFβ and TNFα

signaling [8, 112]. Further clinical studies are needed to prove the effectiveness of pirfenidone on the skin and pulmonary fibrosis in SSc.

Conclusion

Pathogenesis of SSc is dominated by complex interactions between vascular, immunological, and fibrotic processes, but it is still poorly understood. SSc is an intractable disease with high mortality. Research efforts towards understanding the cellular and molecular basis of scleroderma have aimed to reveal novel molecular targets and diagnostic agents, which has led to early and accurate diagnosis as well as innovative therapies against this disease [10, 112]. The development of preclinical models, including animal models that accurately recapitulate human disease, will be essential tools for the ultimate goal of finding a cure for this disease. The list of potential molecular targets for the treatment of fibrosis is growing. Several of those studies to target pathogenetic molecules have direct translational implications for treating SSc in the very near future [6, 11, 111].

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