REVIEW ARTICLE

MECHANISMS OF DISEASE

Scleroderma

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Scleroderma (systemic sclerosis) is a complex disease in which extensive fibrosis, vascular alterations, and autoantibodies against various cellular antigens are among the principal features (Fig. 1 and 2).[1] There are two major subgroups in the commonly accepted classification of scleroderma: limited cutaneous scleroderma and diffuse cutaneous scleroderma.[2] In limited cutaneous scleroderma, fibrosis is mainly restricted to the hands, arms, and face. Raynaud's phenomenon is present for several years before fibrosis appears, pulmonary hypertension is frequent, and anticentromere antibodies occur in 50 to 90% of patients. Diffuse cutaneous scleroderma is a rapidly progressing disorder that affects a large area of the skin and compromises one or more internal organs.

We believe that the acronym CREST (calcinosis, Raynaud's phenomenon, esophageal motility dysfunction, sclerodactyly, and telangiectasia) is obsolete, since it cannot be assigned to only one subgroup of patients with the disease and does not sufficiently indicate the burden of internal-organ involvement. In rare cases, patients with scleroderma have no obvious skin involvement. Patients with scleroderma plus evidence of systemic lupus erythematosus, rheumatoid arthritis, polymyositis, or Sjögren's syndrome are considered to have an overlap syndrome. This classification can be useful, but none of the proposed classifications sufficiently reflect the heterogeneity of the clinical manifestations of scleroderma.

Scleroderma can lead to severe dysfunction and failure of almost any internal organ. Here, too, there is considerable heterogeneity (Table 1). Involvement of visceral organs is a major factor in determining the prognosis. The kidneys, esophagus, heart, and lungs are the most frequent targets. Renal involvement can be controlled by angiotensin-converting–enzyme inhibitors. Severely debilitating esophageal dysfunction is the most common visceral complication, and lung involvement is the leading cause of death.

The mechanisms underlying visceral involvement in scleroderma are unclear, despite progress in the treatment of these complications. Relevant data on mechanisms are limited, since most of the available information is derived from cross-sectional studies and from patients in various stages of the disease, often after treatment; moreover, there are no satisfactory animal models of scleroderma. Nevertheless, a critical evaluation of the available experimental and clinical data will help reduce ambiguity and may provide the basis for future studies of scleroderma.

EPIDEMIOLOGY AND GENETIC SUSCEPTIBILITY

The results of studies of the prevalence and incidence of scleroderma are conflicting because of methodologic variations in case ascertainment and geographic differences in these measurements. The available data indicate a prevalence ranging from 50 to 300 cases per 1 million persons and an incidence ranging from 2.3 to 22.8 cases per 1 million persons per year.[6] Women are at much higher risk for scleroderma than men, with a ratio ranging from 3:1 to 14:1. A slightly increased susceptibility

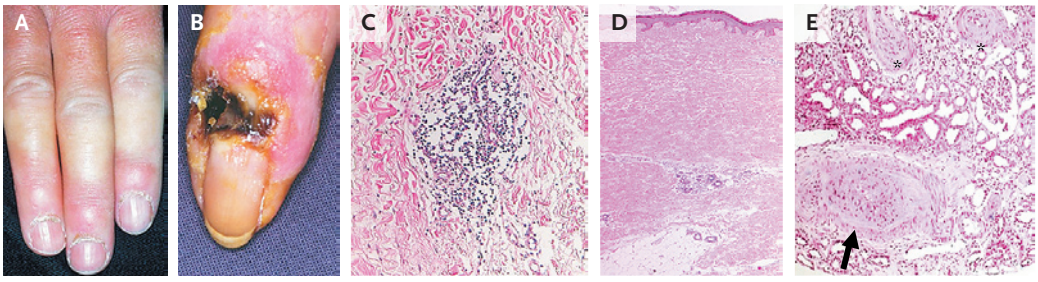


Figure 1. Clinical Signs and Histologic Features in Patients with Scleroderma.

Panel A shows hyperkeratosis of the nail folds of a patient in an edematous phase of limited cutaneous scleroderma. Panel B shows fingertip ulceration in a patient with limited cutaneous scleroderma. Panel C shows a lymphohistiocytic infiltrate around blood vessels in a skin specimen (hematoxylin and eosin). In Panel D, a skin-biopsy specimen from a patient with early diffuse disease shows intense deposition of collagenous matrix throughout the dermis, extending into the subcutaneous fat layer (hematoxylin and eosin). Panel E shows intimal and medial thickening in one interlobar artery (arrow) and two arcuate arteries (asterisks) in the kidney of a patient with scleroderma. The glomerular tuft is partially collapsed, and the tubular epithelium is atrophic. Fibrosis with mononuclear-cell infiltration is present in the interstitium (hematoxylin and eosin).

to scleroderma among blacks has been reported.[7,8] Familial clustering of the disease, the high frequency of other autoimmune disorders in families of patients with scleroderma, and differences in phenotypes among race and ethnic groups[8,9] all suggest that genetic factors contribute to scleroderma. Scleroderma-associated polymorphisms of genes encoding cytokines, cytokine receptors, chemokines, and extracellular proteins have been reported.[10] Many of these variants have been linked to cohorts of patients, but few have been independently confirmed. By contrast, there is strong evidence of linkage of certain HLA class II molecules to clinical phenotypes and particular autoantibodies.[11] The data provide support for the notion that scleroderma is not one clearly defined disease but a syndrome encompassing various phenotypes.

Environmental challenges (e.g., viruses, drugs, vinyl chloride, and silica) may induce clinical phenotypes that are similar or identical to scleroderma.[12] Moreover, several reports indicate that during pregnancy, fetal or maternal lymphocytes can cross the placenta and initiate a graft-versus-host reaction that culminates in scleroderma. There are clinical, serologic, and histopathological similarities between scleroderma and chronic graft-versus-host disease (GVHD), and allogeneic cells have been detected in peripheral-blood and skin-biopsy specimens obtained from patients with scleroderma.[13,14] However, rigorous evidence that these cells participate in the pathogenesis of scleroderma is lacking.

EARLY AND LATE LESIONS

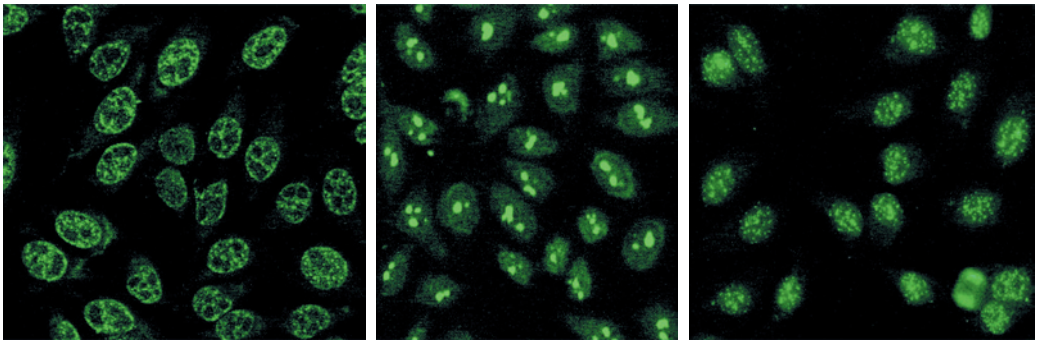
Important features of the tissue lesions in various stages of scleroderma are early microvascular damage, mononuclear-cell infiltrates, and slowly developing fibrosis (Fig. 1). In later stages of scleroderma, the main findings are very densely packed collagen in the dermis, loss of cells, and atrophy.

EARLY VASCULAR AND INFLAMMATORY ALTERATIONS

Vascular injury is an early event in scleroderma. It precedes fibrosis and involves small vessels, particularly the arterioles.[15,16] The vascular damage, which occurs in virtually all organs,[17,18] consists of large gaps between endothelial cells, loss of integrity of the endothelial lining, and vacuolization of endothelial-cell cytoplasm. In addition, there are several basal lamina-like layers, perivascular infiltrates of mononuclear immune cells (with rare lymphocytes) in the vessel wall, obliterative microvascular lesions, and rarefaction of capillaries.[15,16,19,20] The remarkable paucity of small blood vessels is a characteristic finding in later stages of scleroderma.

Notwithstanding the progressive loss of blood vessels and high plasma levels of vascular endothelial growth factor[21,22] caused by the adaptive response to hypoxia, there is a defect in vasculogenesis.[20,23,24] The molecular mechanism (or mechanisms) underlying this paradox is unknown: both angiogenic[21,22] and angiostatic[20,25,26] factors have been detected in early scleroderma. Notably,

A



B

Classic Autoantibodies	Clinical Features	New Autoantibodies	Role
Anti–topoisomerase I	Diffuse cutaneous scleroderma	Anti–endothelial cell	Induce apoptosis of endothelial cells
Anticentromere proteins	Limited cutaneous scleroderma, pulmonary hypertension	Anti–FBN 1	Activate normal human fibroblasts
Anti–RNA polymerase I/II	Diffuse cutaneous scleroderma, renal involvement	Anti–MMP 1 and 3	Prevent degradation of ECM proteins
Antipolymyositis, sclerosis	Polymyositis, calcinosis	Anti-PDGFR	Stimulate normal human fibroblasts through Ha-Ras-ERK1/2-ROS
Antifibrillarin (U3RNP)	Diffuse cutaneous scleroderma, internal-organ involvement	Anti–Nag-2	Induce endothelial-cell apoptosis
Anti-Th/To	Limited cutaneous scleroderma, pulmonary fibrosis		

Figure 2. Autoantibodies in Scleroderma.

Panel A shows antinuclear-antibody staining patterns. The speckled nuclear staining pattern (left) can be detected in 30% of patients with diffuse scleroderma and suggests the presence of anti–topoisomerase I antibodies. The homogeneous nucleolar staining pattern (center) is detected in 25 to 50% of patients with the myositis–scleroderma overlap syndrome. Unlike this homogeneous staining pattern, a pattern characterized by clumping of the nucleoli (not shown) is highly specific for diffuse scleroderma (in 5% of patients). Nucleolar antigens are RNA polymerases, fibrillarin, Th/To, or PM-Scl. The anticentromere-antibody staining pattern (right) can be detected in 70 to 80% of patients with limited cutaneous scleroderma and is associated with a high risk of pulmonary hypertension. The antigens are kinetochore proteins of the centromere regions of chromosomes. Panel B lists the classic and newly discovered autoantibodies in scleroderma. ECM denotes extracellular-matrix protein; ERK1/2 extracellular-signal–regulated kinases 1 and 2; FBN-1 fibrillin-1; MMP 1 and 3 matrix metalloproteinases 1 and 3; Nag-2 nonsteroidal anti-inflammatory drug–activated gene; PDGFR platelet-derived growth factor receptor; and ROS reactive oxygen species.

inflammatory cytokines such as tumor necrosis factor α can stimulate or inhibit angiogenesis depending on the duration of the stimulus.[27]

FIBROSIS

Fibrosis gradually replaces the vascular inflammatory phase of scleroderma and ultimately disrupts the architecture of the affected tissue. It is the cause of the main symptoms of the disease. Fibrosis in the skin begins in the lower dermis and upper subcutaneous layer and occurs together with loss of microvasculature, reduction of appendages, and loss of reticular structure and the rete ridges. The composition of accumulated matrix varies with the stage of the disease. A mixture of different collagen types, proteoglycans, and elastic fibers including fibrillin is typical of the early stages, whereas type I collagen accumulates in later stages.[28,29]

CELL TYPES IN LESIONS

ENDOTHELIAL CELLS

Endothelial cells are affected early in scleroderma.[30] In early lesions there is endothelial-cell apoptosis, or changes of the endothelial phenotype in the absence of endothelial-cell proliferation or precursor differentiation.[20,31,32] The mobilization of endothelial precursors from bone marrow is related to disease severity, but recruitment of such cells to peripheral vasculature has not been shown.[33] The interaction of progenitor endothelial cells with platelets and platelet-derived growth factor (PDGF) is essential for the matura-

Table 1. Clinical Findings in Patients with Scleroderma in Four Countries.*

Finding	Diffuse Cutaneous Scleroderma				Limited Cutaneous Scleroderma			
	United States (N=119)	France (N=30)	Germany (N=484)	Italy (N=177)	United States (N=128)	France (N=97)	Germany (N=674)	Italy (N=565)
	percentage of patients				*percentage of patients*			
Calcinosis	23	16	NR	20	42	36	NR	22
Raynaud's phenomenon	97	100	94.2	94	99	99	96.3	96
Articular involvement	98	70	56.6†	22	78	65	44.9†	16
Esophageal dysmotility	67	79	69.3	69	67	63	59.2	55
Lung fibrosis	30	57	56.1	71‡	37	30	20.8	53‡
Isolated pulmonary arterial hypertension	2	12	27.7	NR	31	9	60.0	NR
Heart involvement	11§	10§	23.0¶	32‖	19§	14§	12.0¶	23‖
Reduced LVEF	20**	15**	NR	NR	6**	12**	NR	NR
Renal crisis	17	7	15.9	12	2	0	9.1	6

* Data are from Meyer et al.,[3] Hunzelmann et al.,[4] and Ferri et al.[5] The study in Italy included a third subgroup of patients who were said to have intermediate cutaneous scleroderma; data on these patients are not shown in the table. LVEF denotes left ventricular ejection fraction, and NR not reported.
† This value includes patients with both muscle and articular involvement.
‡ This value includes patients who also had isolated pulmonary hypertension.
§ In the country listed, heart involvement was defined by the presence of arrhythmia requiring treatment.
¶ This value includes patients with one of the following: palpitations, a conduction disturbance, or diastolic dysfunction.
‖ This value includes patients with one of the following: pericarditis, congestive heart failure, severe arrhythmia, or a conduction disturbance.
** The LVEF was less than 50% on echocardiography or there was diastolic dysfunction.

tion and recruitment of endothelial precursors.[34,35] The perivascular space is a preferred site of early lesions in scleroderma. Progressive wall thickening and perivascular infiltrates are features of the vascular lesions in this compartment, indicating the involvement of vascular smooth-muscle cells and pericytes.

PERICYTES AND SMOOTH-MUSCLE CELLS

Small vessels contain vascular smooth-muscle cells and pericytes. Pericytes have the potential to differentiate into vascular smooth-muscle cells, fibroblasts, and myofibroblasts (specialized contractile cells expressing α–smooth-muscle actin and the ED-A splice variant of fibronectin)[36-38] and to influence endothelial-cell proliferation.[39]

Increased thickness of the vascular wall, caused by the proliferation of vascular smooth-muscle cells, indicates that these cells are responding to scleroderma-induced injury. Pericytes in the lesion overexpress several cytokine receptors, including PDGF receptor (PDGFR),[40] but this occurs only in early lesions and in patients with Raynaud's phenomenon and antinuclear antibodies. These cells proliferate and contribute to increased wall thickness.[41] Collectively, the cellular changes in early lesions are loss of endothelial cells, proliferating pericytes and vascular smooth-muscle cells, and immune cells in the perivascular space. Endothelial cells are the only mesenchymal cell type that undergo apoptosis in early scleroderma, whereas vascular smooth-muscle cells and pericytes proliferate vigorously.

FIBROBLASTS

Fibroblasts appear to orchestrate the production, deposition, and remodeling of collagens and other extracellular-matrix components. Fibroblasts in scleroderma are heterogeneous in terms of collagen synthesis.[42] Overproduction of collagen is due to enhanced transcription or increased stability of collagen-specific messenger RNA.[43] Up-regulated transcription of collagen genes in scleroderma cells is autonomous and maintained in vitro over several passages.[44] Fibroblasts in scleroderma can convert to myofibroblasts,[38] and they overexpress several cytokines (e.g., transforming growth factor β [TGF-β] and monocyte chemoattractant protein 1) and TGF-β receptors.[45] These findings underscore the role of autocrine loops in sustaining the fibrotic reaction. In addition, fibroblasts in patients with scleroderma contain an excess of reactive oxygen species. The origin of activated fibroblasts in the skin and internal organs of patients with scleroderma is still debated. Fibroblasts may undergo local activation or originate from resident pericytes, mesenchymal stem cells, or progenitor cells (e.g., fibrocytes) recruited from the circulation.[46]

MONONUCLEAR CELLS

The cellular infiltrates in the early lesions of scleroderma consist mostly of T cells, macrophages, B cells, and mast cells.[47-49] T cells in skin lesions are predominantly CD4+ cells,[49] display markers of activation,[50] exhibit oligoclonal expansion,[51] and are predominantly type 2 helper T (Th2) cells.[52] These characteristics parallel the increased serum levels of cytokines derived from Th2 cells in scleroderma.[53,54] CD20-positive B cells are also found in skin lesions.[48] They may contribute to the pathogenesis of fibrosis through the secretion of interleukin-6 and TGF-β[55,56] and the production of autoantibodies.

SOLUBLE MEDIATORS

CYTOKINES AND GROWTH FACTORS

Genomewide transcription profiles of skin-biopsy specimens obtained from patients with scleroderma have provided direct evidence of the involvement of cytokines in the activation of fibroblasts. Within the limitations of such an approach (e.g., variations according to the site of the biopsy, mixed cell populations, and post-transcriptional regulation), the data indicate systemic changes of gene transcription in endothelial cells, fibroblasts, and B and T lymphocytes in scleroderma. These studies have shown transcriptional changes in clinically affected and unaffected skin.[48]

TGF-β

TGF-β is a potent profibrotic cytokine.[57] DNA microarray analysis indicates that a group of TGF-β–dependent genes are overexpressed in biopsy specimens from skin lesions in patients with scleroderma.[48] TGF-β is also the strongest inducer of myofibroblasts, and it modulates the expression of various cytokine receptors, including receptors for TGF-β and PDGF.[45,58] In scleroderma fibroblasts, TGF-β further up-regulates connective-tissue growth factor (CTGF), a cysteine-rich modular protein belonging to the CCN family of

matricellular growth factors (CYR61, CTGF, and NOV [nephroblastoma overexpressed])[59] that has biologic activities similar to those of TGF-β. Enhanced TGF-β and CTGF expression has been detected in scleroderma lesions, and enhanced TGF-β signaling in fibroblasts causes skin fibrosis in a mouse model that appears to recapitulate the clinical and histologic features of scleroderma.[60]

Smad-dependent or Smad-independent signaling downstream of TGF-β has been extensively characterized in scleroderma cells (Fig. 3).[61] Inhibition of protein kinase C delta, geranyl transferase 1, or stress-activated protein kinase p38 eliminates the expression of collagen I and III in scleroderma cells.[62,63] TGF-β, produced as inactive precursor, can be activated by thrombospondin and by $\alpha_v\beta_3$ integrin, underscoring the interaction among cytokines, extracellular matrix, and integrins. The expression of all these molecules is induced in scleroderma.[64,65]

PDGF

PDGF, which is linked to wound healing and fibrosis, may have a role in scleroderma. The presence of stimulatory antibodies to PDGFR in serum from patients with scleroderma, the strong stimulation by PDGF of the pericyte-to-fibroblast transition,[38] the presence of high levels of PDGF and its beta receptor in skin lesions from patients with scleroderma,[66,67] and the beneficial effects of selective inhibitors of PDGF signaling on dermal fibrosis[68] all indicate the importance of PDGF in scleroderma. PDGF inhibitors may thus have a therapeutic benefit in fibrosis.

Other Cytokines and Biologically Active Substances

Endothelin-1 acts in concert with TGF-β to convert fibroblasts into myofibroblasts.[69] The beneficial effect of endothelin-1–receptor inhibitors on pulmonary hypertension in patients with scleroderma indicates that endothelin-1 is an important signaling molecule in this disease. Inhibition of endothelin signaling may alleviate the overstimulation of TGF-β in scleroderma.[70] Many other cytokines have been implicated in the angiogenesis, angiostasis, fibrosis, and localized inflammation in scleroderma. To date, there is no compelling evidence linking the levels and activity of these cytokines to one or more specific pathogenic events in this condition (Table 2).

EXTRACELLULAR-MATRIX COMPONENTS AND THEIR RECEPTORS

The hallmark of scleroderma is excessive deposition of extracellular-matrix components, caused by overproduction of collagen and other glycoproteins (e.g., fibronectin and fibrillin).[42,43] The macromolecular arrangement of collagens in scleroderma is altered by cross-links that are normally seen in bone but not skin collagen matrix; these cross-links are formed by lysyl hydroxylase 2, the level of which is increased in scleroderma.[71]

Extracellular-matrix molecules modulate cellular responses by regulating the activity of cytokines and growth factors. For example, TGF-β–fibrillin interaction is required for fibroblast activation in scleroderma. The extracellular matrix also provides points of adhesion, which are bound by integrins, transmembrane receptors connecting the extracellular-matrix environment to the cytoskeleton, thereby mediating outside-in and inside-out signaling.[72] Integrin $\alpha_1\beta_1$ elicits signals to down-regulate collagen synthesis by fibroblasts; $\alpha_1\beta_1$-knockout mice have enhanced collagen synthesis in wounds.[73] Fibroblasts in patients with scleroderma have reduced surface levels of $\alpha_1\beta_1$ integrin, resulting in the failure of integrin to down-regulate collagen synthesis.[74] Impairment of integrin signaling may amplify fibrosis in scleroderma. There is accumulating evidence that crosstalk between different integrins and extracellular-matrix molecules determines the activity of many cytokines and growth factors that interact directly with responding target cells.[64,65] Overall, the altered extracellular matrix in scleroderma probably provides an environment that amplifies receptor-mediated cell activation.

AUTOANTIBODIES

Scleroderma is associated with several autoantibodies, some of which are important diagnostic markers. Tests for autoantibodies against topoisomerase I (Scl-70), centromere-associated proteins, and nucleolar antigens can be useful in facilitating the diagnosis and formulating a prognosis. Although the autoantibodies correlate with disease severity and the risk of specific organ complications, their pathogenetic relevance is unclear. Recently, autoantibodies against nonnuclear antigens have been described (Fig. 2), including antibodies against cell-surface antigens. Antibodies against PDGFR appear to be agonistic, since they stimu-

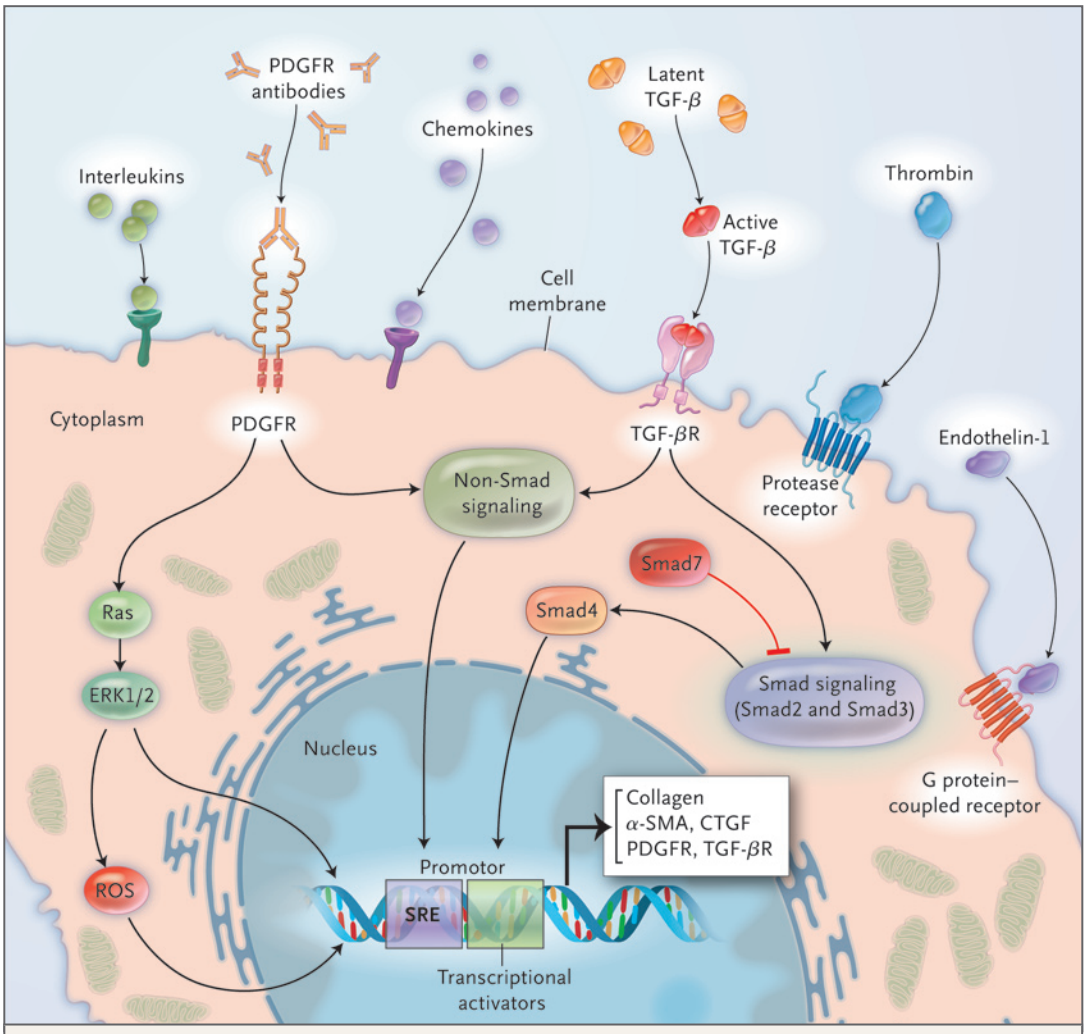


Figure 3. Activation of Fibroblasts in Scleroderma.

External factors such as interleukins, chemokines, thrombin, endothelin-1, growth factors, reactive oxygen species (ROS), and activating antibodies trigger signaling cascades in fibroblasts. For example, the phosphorylation of Smad2 triggers a signaling cascade from Smad3 to Smad1, which interacts with Smad4 and regulates gene transcription in the nucleus. Activation of transforming growth factor β (TGF-β) receptors (TGF-βR) also results in the activation of pathways not involving Smad proteins,[61] modulating transcription factors. These pathways intersect with pathways induced by activation of platelet-derived growth factor receptors (PDGFR), leading to a complex intracellular signaling network. Production of extracellular-matrix protein, cytoskeleton, cytokines, and cytokine receptors is thereby stimulated; these participate in regulatory loops to sustained fibroblast activation. CTGF denotes connective-tissue growth factor, ERK1/2 extracellular-signal–regulated kinases 1 and 2, α-SMA α–smooth-muscle actin, and SRE serum-responsive element.

late a specific signaling cascade.[75] However, the specificity of these stimulatory autoantibodies remains to be established. The same type of autoantibodies with PDGF agonistic activity has been detected in crude immunoglobulin derived from the serum of patients with sclerodermatous GVHD, and a significant beneficial effect of PDGFR-signaling inhibitors has been reported in resistant cases of sclerodermatous GVHD.[76]

REACTIVE OXYGEN SPECIES

High levels of reactive oxygen species and oxidative stress have been directly or indirectly impli-

Table 2. Cytokines, Growth Factors, and Biologically Active Substances Involved in the Pathogenesis of Scleroderma.*

Variable	Main Cell Source	Pathogenic Relevance	Effect in Scleroderma
Interleukin-1	Macrophages, monocytes	Has a role in production of interleukin-6 and PDGF-α by fibroblasts	Constitutively expressed in skin fibroblasts
Interleukin-4	Th2 lymphocytes	Stimulates fibroblast proliferation, chemotaxis, and collagen synthesis; stimulates production of TGF-β, CTGF, and TIMP-1; up-regulates expression of adhesion molecules by endothelial cells	Increased levels in serum; increased protein and gene expression in skin and in cultured fibroblasts; increased number of interleukin-4–producing T lymphocytes
Interleukin-6	Fibroblasts, macrophages, endothelial cells, B cells, T cells	Stimulates collagen and TIMP-1 synthesis; promotes a Th2-polarized immune response	Increased levels in tissue and serum; enhanced production in vitro by PBMC and cultured fibroblasts
Interleukin-8	Alveolar macrophages, lung fibroblasts, skin fibroblasts	Serves as a potent chemoattractant and activator of neutrophils; promotes fibroblast chemotaxis	Elevated levels in serum, skin specimens, and bronchoalveolar-lavage fluids
Interleukin-10	Activated B cells, monocytes	Promotes a predominant Th2 immune response that induces collagen synthesis	Increased levels in serum
Interleukin-13	Th2 lymphocytes	Induces fibrosis through a TGF-β–dependent and TGF-β–independent mechanism	Increased levels in serum
Interleukin-17	Th1 and Th2 lymphocytes	Induces proliferation of fibroblasts; stimulates fibroblast production of collagen, interleukin-6, and PDGF by stimulating macrophage production of TNF-α and interleukin-1; induces endothelial-cell production of interleukin-1 and increased expression of interleukin-6, ICAM-1, and VCAM-1	Increased levels in serum; overexpressed in skin
TGF-β	Macrophages, fibroblasts, T cells, B cells, platelets, endothelial cells	Induces proliferation of fibroblasts and production of CTGF and endothelin-1; stimulates synthesis of collagens, fibronectin, proteoglycans; inhibits extracellular-matrix degradation by reduced synthesis of MMP and induction of TIMP-1; stimulates expression of TGF-β and PDGF receptors	Elevated levels of TβRI in vivo; increased levels of TGF-β in skin in some studies; elevated expression and phosphorylation levels of Smad2 or Smad3 effectors of TGF-β–signaling pathway
CTGF (CCN2)	Fibroblasts, endothelial cells, smooth-muscle cells	Induced by TGF-β, interleukin-4, and VEGF; induces proliferation and chemotaxis of fibroblasts and stimulates production of extracellular matrix	Elevated levels in serum; increased gene expression in skin and in fibroblasts in vitro
TNF-α	Macrophages, T cells, B cells, endothelial cells, fibroblasts, vascular smooth-muscle cells	Stimulates a profibrotic or antifibrotic response, depending on experimental conditions	Contradictory outcomes in patients with scleroderma treated with TNF-α antagonists
MCP-1/CCL2	Macrophages, fibroblasts, endothelial cells	Stimulates collagen production in part through TGF-β; regulates migration of monocytes and Th2 cells	Elevated levels in serum; increased spontaneous production by PBMC; increased expression in lesional skin
MCP-3	Mononuclear cells, skin fibroblasts	Promotes leukocyte movement; activates proa2(I) collagen promoter–reporter gene constructs	Increased expression in skin-biopsy specimens from patients with early scleroderma and in fibroblasts cultured from skin-biopsy specimens

PDGF	Platelets, macrophages, endothelial cells, fibroblasts	Serves as mitogen and chemoattractant for fibroblasts; induces synthesis of collagen, fibronectin, proteoglycans; stimulates secretion of TGF-β type I, MCP-1, interleukin-6	Elevated expression of PDGF and PDGF in skin; increased levels in bronchoalveolar-lavage biologic fluids
Endothelin-1	Endothelial cells, fibroblasts, vascular smooth-muscle cells	Activates vascular smooth-muscle cells; induces proliferation and chemotaxis of macrophages and vascular smooth-muscle cells; differentiates fibroblasts into myofibroblasts; increases extracellular-matrix production by fibroblasts	Increased levels in serum and bronchoalveolar-lavage biologic fluids; increased expression in tissues
IGF-II	Fetal cells	Stimulates production of type I collagen and fibronectin in scleroderma lung fibroblasts in vitro	Increased gene and protein expression in lung fibroblasts; increased immunostaining in scleroderma-related lung disease
Angiotensin II	Skin fibroblasts	Increases production of type I collagen	Increased levels in serum; increased gene expression in cultured fibroblasts; increased expression in skin-biopsy specimens from patients with limited cutaneous scleroderma

* CCL2 denotes chemokine ligand 2, CTGF connective-tissue growth factor (also known as CCN2), ICAM-1 intercellular adhesion molecule 1, IGF-II insulin-like growth factor II, MCP-1 monocyte chemoattractant protein 1, MCP-3 monocyte chemoattractant protein 3, MMP matrix metalloproteinases, PBMC peripheral-blood mononuclear cells, PDGF platelet-derived growth factor, TβRI transforming growth factor β (TGF-β) receptor type I, Th1 type 1 helper T cells, Th2 type 2 helper T cells, TIMP-1 tissue inhibitor of MMP 1, TNF-α tumor necrosis factor α, VCAM-1 vascular-cell adhesion molecule 1, and VEGF vascular endothelial growth factor.

cated in scleroderma.[77-79] The origin and the perturbation of cellular reactive oxygen species appear to be specific for scleroderma. In almost all inflammatory diseases, the increase in levels of cellular reactive oxygen species is a direct consequence of the activation of mononuclear blood cells.[80] In scleroderma, the high levels of reactive oxygen species in mesenchymal cells are relatively independent of the inflammatory status; they persist in vitro in the absence of growth factors and cytokines, render cells sensitive to stress, and induce DNA damage.[81] The source of reactive oxygen species is the membrane NADPH oxidase system, which is stimulated in all cell types within or surrounding the vessel wall in response to injury.[82-84] Furthermore, free radicals have direct profibrogenic effects on fibroblasts,[77-85] and they contribute to the release of mediators implicated in fibrosis.[86,87]

THE IMMUNE SYSTEM, OXIDATIVE STRESS, AND FIBROSIS

The hierarchy and relevance of the cells and soluble mediators described above in the pathogenesis of scleroderma are not clear. We present a plausible series of events that lead to scleroderma, based on links among the immune system, oxidative stress, and fibrosis.

We do not know the primary triggering event in scleroderma. It is probably an autoimmune process against mesenchymal cells.[88] Whatever the primary trigger, at the cellular level, a slight increase in reactive oxygen species generates mild oxidative stress early in the disease, coinciding with endothelial-cell abnormalities and initial perivascular inflammation.[15,16,89] These abnormalities, which are likely to be mild, are responsible for subtle vascular dysfunction that is not clinically manifested (Fig. 4A). Low and persistent levels of superoxide, converted to hydrogen peroxide, can traverse lipid membranes. High levels of hydrogen peroxide in a single cell are sufficient to activate neighboring normal cells and to generate an inflammatory focus releasing a large array of mediators (Fig. 4). Low levels of reactive oxygen species are responsible for the down-regulation of proteasome activity in primary cells, mimicking the slow decay of proteasome activity seen in senescent cells.[92] Several proteins are stabilized by impaired proteasome function,[81,93] and the increase in levels of Ras

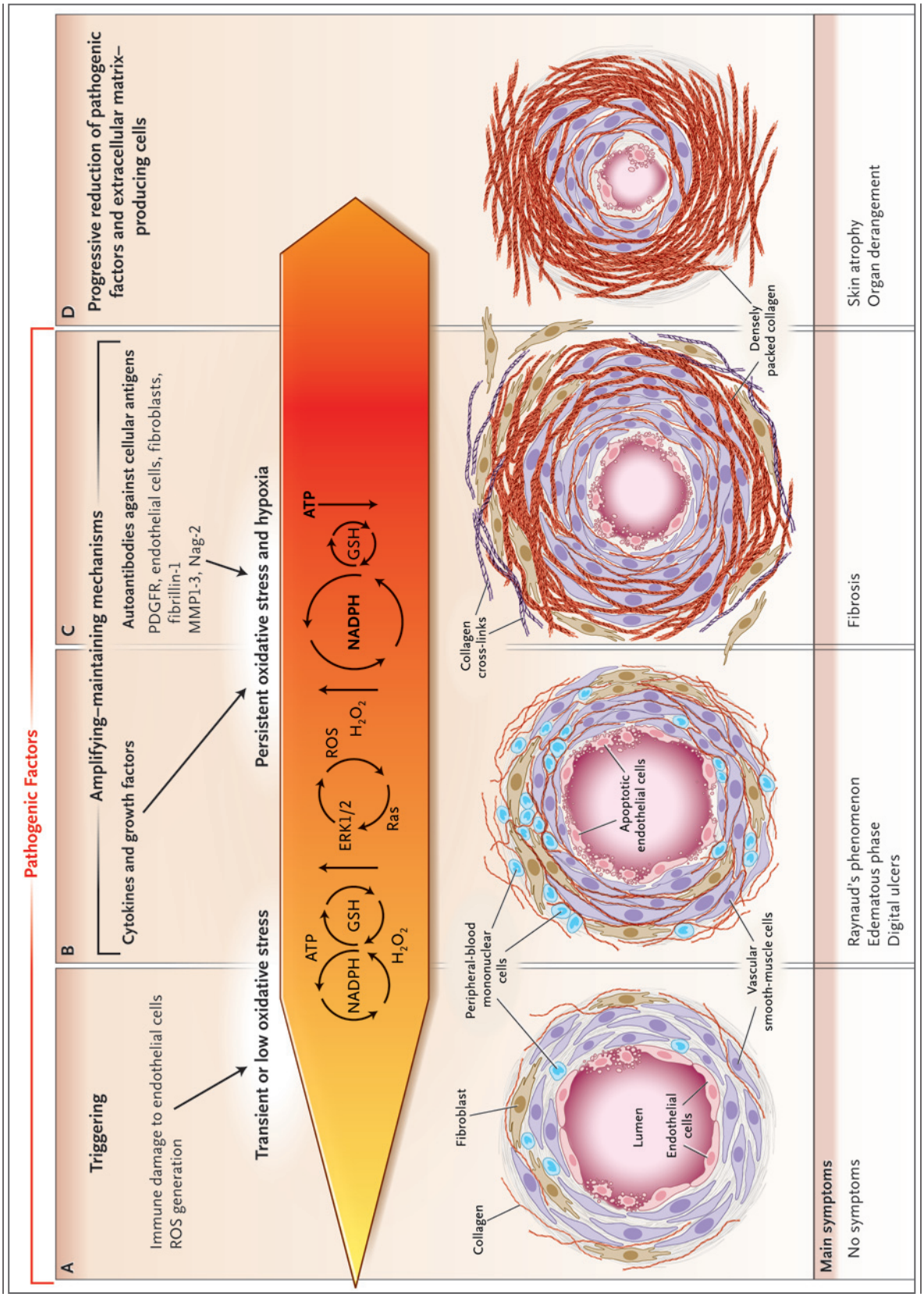


Figure 4 (facing page). Lesions in Different Stages of Scleroderma.

As shown in Panel A, microvascular injury is one of the early events in the pathogenesis of scleroderma and is characterized by endothelial-cell damage, the proliferation of basal-lamina layers, occasional entrapment of peripheral-blood mononuclear cells in the vessel wall, and initial perivascular mononuclear-cell infiltrates. Endothelial cells show signs of increased programmed cell death. One or more reactive oxygen species (ROS)–generating triggering agents could be responsible for this stage. ROS may be generated inside the vascular lumen by peripheral-blood cells[47,78] or within the vessel wall by macrophages, endothelial cells, vascular smooth-muscle cells, or adventitial fibroblasts in response to one or more noxious agents. Although low levels of ROS are necessary for normal vascular function, excessive production is responsible for functional and structural damage. As shown in Panel B, uncontrolled production of ROS activates local mesenchymal cells, inducing chemotaxis, proliferation, extracellular-matrix production, and the release of cytokines and growth factors that amplify the inflammatory focus.[90] An autocrine circuitry (Ha-Ras–extracellular-signal–regulated kinases 1 and 2 [ERK1/2]/ROS) maintains ROS at levels that are high because of the reduced turnover of cytokine receptors. Structural and functional abnormalities of vessel walls and intravascular changes occur, leading to overt clinical symptoms. As shown in Panel C, the next stage is dominated by fibrosis, derangement of visceral-organ architecture, rarefaction of blood vessels, and consequently, hypoxia,[91] which contributes to the maintenance of fibrosis. As shown in Panel D, once the single or multiple mechanisms responsible for mesenchymal-cell activation subside or recede or mesenchymal cells themselves undergo senescence or apoptosis,[81] the disease burns out. The clinical picture is dominated by internal-organ derangement. Triggering, amplifying, and maintenance factors are not necessarily confined to a single stage. Environmental, local, and genetic factors can influence the disease progression. In the inset, coupling of the NADPH oxidase to the glutathione (GSH) cycle is shown. Glucose metabolism, in particular G6PD, generates NADPH/H^+, which is rapidly oxidized by NADPH oxidase enzymes to NADP+ H^+-e-. H^+ enters the GSH cycle: oxidized GSH (GSSG) is reduced by GSH reductase (GRH) to GSH, which is oxidized back to GSSG by GSH peroxidase. This enzyme uses as a preferred substrate H_2O_2 ($2GSH + H_2O_2 \rightarrow GS–SG + 2H_2O$), produced by SOD and superoxide generated by the NADPH oxidase cycle. GSH is synthesized from amino acids by the enzyme γ-glutamyl-cysteine synthetase, a rate-limiting reaction, which is tightly dependent on ATP. ATP depletion reduces GSH synthesis, increases peroxides, and unleashes the NADPH oxidase cycle, which generates a large excess of ROS, unbuffered by GSH.

protein accounts for the sensitivity of cells to growth factors.[81,93] Reactive oxygen species also inhibit tyrosine phosphatases[94] and maintain MEK (MAP–extracellular-signal–regulated kinase [ERK]) 1 and ERK 2 (ERK1/2) (protein kinases that are important in cell proliferation) in the phosphorylated, active state. The NADPH oxidase subunits p67 and p47 undergo phosphorylation by ERK1/2 and stimulate the production of reactive oxygen species.[95] These events generate an autoamplification circuit linking Ras with ERK1/2 and reactive oxygen species,[81] which in turn amplifies and maintains the cytokines and growth factors and their cognate receptors in an autocrine loop (Fig. 4B).[94] These events have been detected in primary scleroderma fibroblasts, which generate reactive oxygen species–Ras–ERK1/2 when cultured in low serum and after several passages in vitro. Inhibition of any component of this loop abolished reactive oxygen species, DNA damage, and collagen synthesis.[81] Under normal conditions, overstimulation of receptors is prevented by receptor down-regulation and desensitization. In scleroderma, the initial signal is long-lasting, persistent, and not subjected to down-regulation, because it is less intense than under normal conditions and continuous.

In vivo, the reactive oxygen species–Ras–ERK1/2 circuitry can be induced and maintained in vascular smooth-muscle cells and fibroblasts by the diffusion of hydrogen peroxide from fibroblasts,[77] migration of monocytes through endothelial-cell gaps,[47,78] and exposure of membrane-bound antibodies in lymphocytes to specific cellular antigens (Fig. 4A). In this context, endothelial cells may succumb to the stress induced by reactive oxygen species that are produced by lymphocyte–mesenchymal-cell interactions, while in the same area, pericytes, fibroblasts, and smooth-muscle cells proliferate in a Ras-dependent manner, leading to vessel-wall thickening.[96] This crucial event exacerbates hypoxia under conditions of stress (e.g., cold) and depletes ATP. In normal conditions, in the presence of ATP, the NADPH-oxidase system is coupled to glutathione (GSH) synthesis. Even partial loss of ATP uncouples the system and reduces cellular GSH (Fig. 4B and 4C).[97] Under these conditions, reactive oxygen species cannot be buffered, and they cause further damage to endothelial cells and persistent activation of vascular smooth-muscle cells, pericytes, and fibroblasts. The process is further amplified by the nonspecific stabilization of several cytokine receptors by reactive oxygen species.[92]

This step probably corresponds to the first symptom of scleroderma. Recurrent Raynaud's phenomenon could be the direct consequence of

the structural changes of the vessel and the perturbed control of vascular tone due to an imbalance between vasodilatory and vasoconstrictive mediators. At this stage, the patient may have early signs of skin and visceral fibrosis (Fig. 4B).

Mesenchymal cells become progressively hypersensitive to cytokines induced by local reactive oxygen species.[98] Cytokines activate mesenchymal precursor cells and lead to the transformation of fibroblasts to myofibroblasts.

The continuous synthesis of collagen and other extracellular-matrix components causes fibrosis in skin and visceral organs. Profound disruption of visceral-organ architecture and the important microvascular alterations are responsible for tissue hypoxia, which becomes the leading mechanism in maintaining the production of reactive oxygen species,[99] and for the fibrotic process, which occurs through some mechanisms that are dependent on and others that are independent of hypoxia-inducible factor isoform 1α (Fig. 4C).[100-102]

Once the inflammatory reaction subsides, the disease burns out. Atrophy is now the main dermatologic feature, and the extent of internal-organ derangement determines the ultimate prognosis (Fig. 4D). Long-term remodeling involving modified matrix-metalloproteinase profiles stimulated by T lymphocytes[103] may resolve tissue fibrosis.

CONCLUSIONS

Several aspects of the pathogenesis of scleroderma still await elucidation. Transcription profiling has revealed a systemic signature of the disease that is the same in both affected and unaffected areas. Many genes can be induced by TGF-β, Ras, and reactive oxygen species, and an amplification loop linking tyrosine kinase receptors (Ras, reactive oxygen species, and ERK1/2) with receptors of TGF-β and CTGF has been found. These circuits activate fibroblasts.[81,90]

Targeted inhibition of signaling pathways by tyrosine kinase inhibitors such as PDGFR, serine–threonine kinase inhibitors such as TGF-β receptors, and farnesyl tranferase inhibitors such as Ras could interfere with the disease process. If autoantibodies turn out to be of functional relevance in some patients, combinatorial trials with B-cell–depleting antibodies may also be feasible. The identification of biomarkers of disease severity, such as transcription patterns, cellular reactive oxygen species, DNA damage signatures, and levels of collagen and α–smooth-muscle actin in peripheral monocytes or bioptic fibroblasts will pave the way toward the development of disease-specific and stage-specific targeted therapies and the identification of well-defined end points for clinical trials.

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