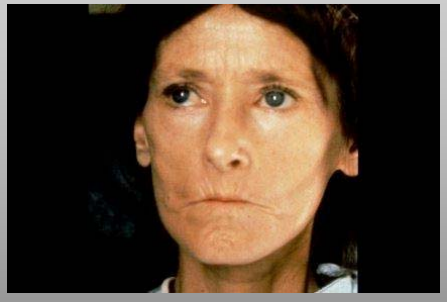
Scleroderma

- Chronic multisystemic disease characterized by vasculopathy, variable degree of inflammation, and fibrosis
- Incidence 3.7-22.8 cases/million
- Female:male 5:1
- Pulmonary fibrosis common, severe in 16%. Pulmonary hypertension occurs in 50% of cases and can lead to cor pulmonale. Pulmonary complications are the leading cause of death in this disease.

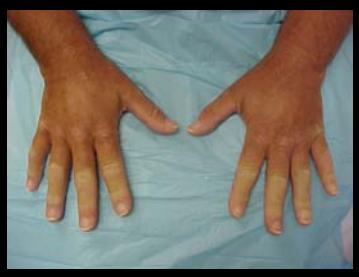
Clinical Features of Scleroderma (Systemic sclerosis; SSc)



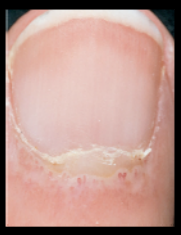
Typical facial features in advanced SSc

Digital ulceration from vascular damage

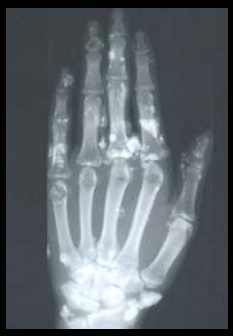
CREST: Calcinosis, Raynaud's phenomenon, Sclerodactyly, Esophageal dysmotility, Telangiectasias



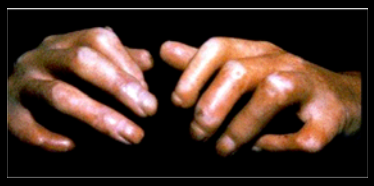
Ranaud's phenomenon



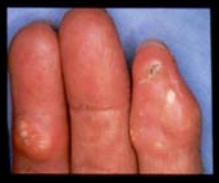
Telangiectasias



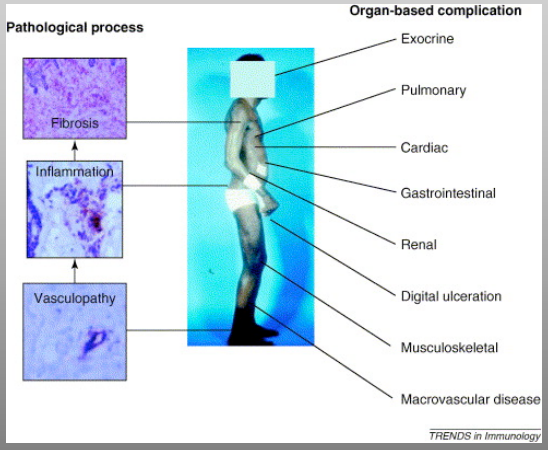
Calcinosis



Sclerodactyly



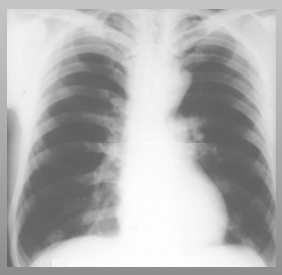
Organ Involvement in Scleroderma



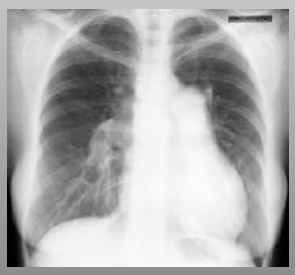
Differential organ involvement in SSc. The earliest pathological feature of SSc in the skin is vasculopathy with endothelial cell activation. Later, inflammation develops and finally fibrosis is prominent. Similar processes are likely to occur in all lesional tissues, leading to organ dysfunction. Cardiac, renal, lung and gut complications are the main causes of SSc-related mortality.

From: Denton and Black *Trends Immunol.* 26:596, 2005

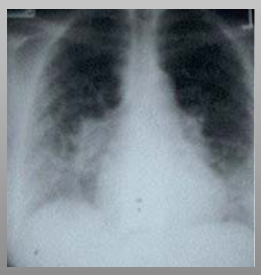
Pulmonary Manifestations of Scleroderma



Normal



Pulmonary Hypertension



Pulmonary Fibrosis

Major Clinical Sub-types of Scleroderma

Limited cutaneous SSc (60% of cases)

- No skin sclerosis proximal to knees or elbows.
- Longstanding Raynaud's phenomenon is typical.
- Most commonly associated autoantibody is anti-centromere Ab (ACA).
- A subgroup of these patients has manifestations of CREST (calcinosis, Raynaud's, esophageal dysmotility, telangiectasis).
- Isolated pulmonary hypertension and gastrointestinal tract dysmotility are the most common severe manifestations. Lung fibrosis, renal and cardiac involvement occur less often than in dcSSc.

Major Clinical Sub-types of Scleroderma

Diffuse cutaneous SSc (30% of cases)

• The cardinal feature is skin sclerosis proximal to the knees and elbows.

• Raynaud's phenomenon is universal but might manifest simultaneously or shortly after the development of skin sclerosis.

• Inflammatory features are prominent during the first 3 years of disease. Skin involvement often diminishes within two years.

• ACA is rarely present, anti-Scl-70 (topoisomease-1) or anti-RNA polymerase is typical.

• There is a high frequency of interstitial lung disease, renal crisis and cardiac involvement.

Major Clinical Sub-types of Scleroderma

Scleroderma overlap syndromes

• Their features include those of limited or diffuse cutaneous SSc, together with those of one or more additional autoimmune rheumatic diseases, such as polyarthritis, myositis or SLE.

• Often associated with anti U1-ribonucleoprotein (U1-RNP), U3-RNP or polymyositis-scleroderma (PM-Scl) autoantibody reactivity.

Localized scleroderma

• Morphea scleroderma causes patches of hard skin that can persist for years.

• Linear scleroderma causes bands of hard skin across the face or an extremity, rarely involving muscle or bone

• Typically carries a good prognosis

Genetics of Scleroderma

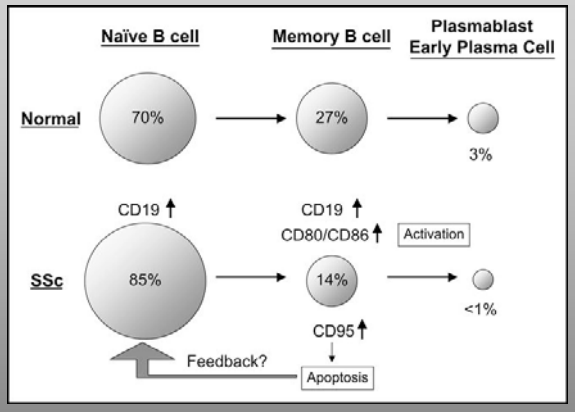
• Family history associated with increased risk of developing disease, but risk is only 1% for any individual.

• Concordance for both mono- and dizygotic twins is 5%. However, gene expression profiling of cultured dermal fibroblasts from monozygotic twins of an index case show a similar pro-fibrotic "signature" 46% of the time.

• Genetic studies indicate an association of scleroderma with polymorphisms in the promoters of the *TNF*, *MCP-1,* and *CD19* genes.

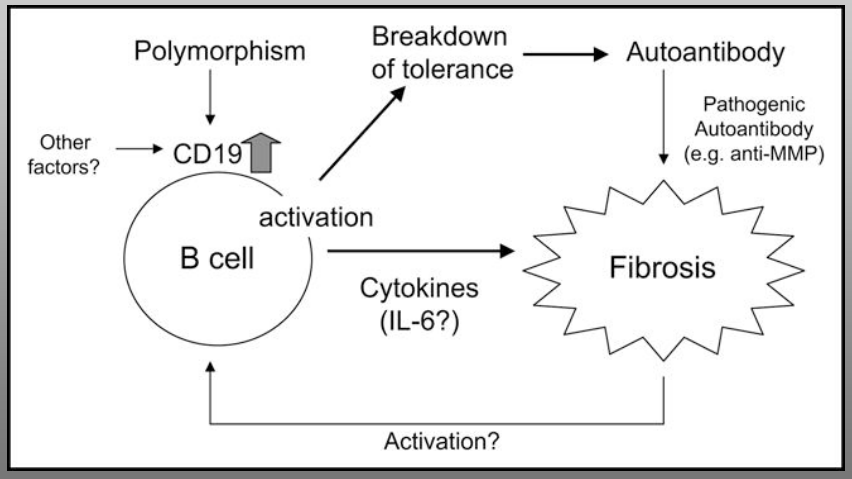
• Although genetic studies have suggested an association of the HLA-DRB1*01 allele with anti-centromere antibodies, association of ACA with polymorphisms in the *TNF* promoter was even stronger, suggesting linkage disequilibrium.

The Role of B Cells in Scleroderma



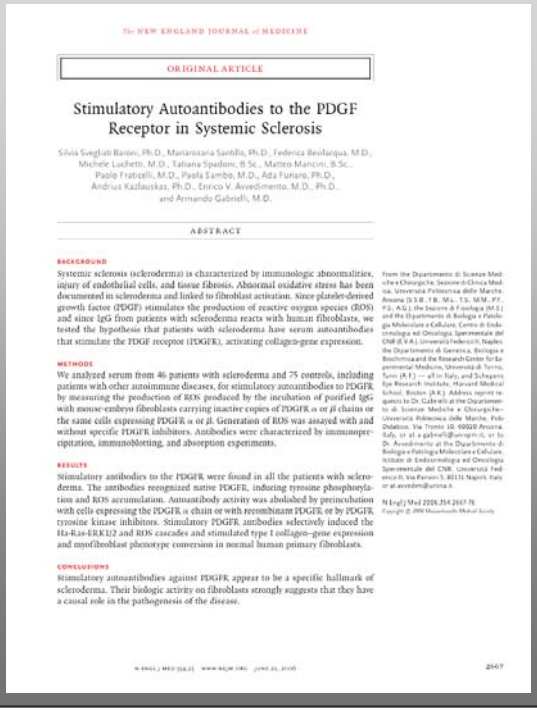
In the blood of patients with scleroderma, naive B cells are increased in number, while memory B cells and plasmablasts/early plasma cells are diminished. Memory B cells express higher levels of CD80 and CD86, and thus are chronically activated, possibly due to CD19 over-expression. CD95 expression is also increased on memory B cells, leading to augmented CD95-mediated apoptosis. The continuous loss of memory B cells and plasmablasts/early plasma cells may increase naive B-cell production in bone marrow to maintain the B-cell homeostasis in systemic sclerosis. From: Fujimoto and Sato: *Curr. Opin. Rheumatol.* 17:746, 2005.

The Role of B Cells in Scleroderma



From: Fujimoto and Sato: *Curr. Opin. Rheumatol*. 17:746, 2005

Do B Cells and Autoantibodies Play a Causative Role in Scleroderma?



The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Stimulatory Autoantibodies to the PDGF Receptor in Systemic Sclerosis

Silvia Svegliati Baroni, Ph.D., Mariarosaria Santillo, Ph.D., Federica Bevilacqua, M.D., Michele Luchetti, M.D., Tatiana Spadoni, B.Sc., Matteo Mancini, B.Sc., Paolo Fraticelli, M.D., Paola Sambo, M.D., Ada Funaro, Ph.D., Andrius Kazlauskas, Ph.D., Enrico V. Avvedimento, M.D., Ph.D., and Armando Gabrielli, M.D.

ABSTRACT

BACKGROUND

Systemic sclerosis (scleroderma) is characterized by immunologic abnormalities, injury of endothelial cells, and tissue fibrosis. Abnormal oxidative stress has been documented in scleroderma and linked to fibroblast activation. Since platelet-derived growth factor (PDGF) stimulates the production of reactive oxygen species (ROS) and since IgG from patients with scleroderma reacts with human fibroblasts, we tested the hypothesis that patients with scleroderma have serum autoantibodies that stimulate the PDGF receptor (PDGFR), activating collagen-gene expression.

METHODS

We analyzed serum from 46 patients with scleroderma and 75 controls, including patients with other autoimmune diseases, for stimulatory autoantibodies to PDGFR by measuring the production of ROS produced by the incubation of purified IgG with mouse-embryo fibroblasts carrying inactive copies of PDGFR-α or -β chains or the same cells expressing PDGFR-α or -β. Generation of ROS was assayed with and without specific PDGFR inhibitors. Antibodies were characterized by immunoprecipitation, immunoblotting, and absorption experiments.

RESULTS

Stimulatory antibodies to the PDGFR were found in all the patients with scleroderma. The antibodies recognized native PDGFR, inducing tyrosine phosphorylation and ROS accumulation. Autoantibody activity was abolished by preincubation with cells expressing the PDGFR-α chain or with recombinant PDGFR, or by PDGFR tyrosine kinase inhibitors. Stimulatory PDGFR antibodies selectively induced the Ha-Ras–ERK1/2 and ROS cascades and stimulated type I collagen–gene expression and myofibroblast phenotype conversion in normal human primary fibroblasts.

CONCLUSIONS

Stimulatory autoantibodies against PDGFR appear to be a specific hallmark of scleroderma. Their biologic activity on fibroblasts strongly suggests that they have a causal role in the pathogenesis of the disease.

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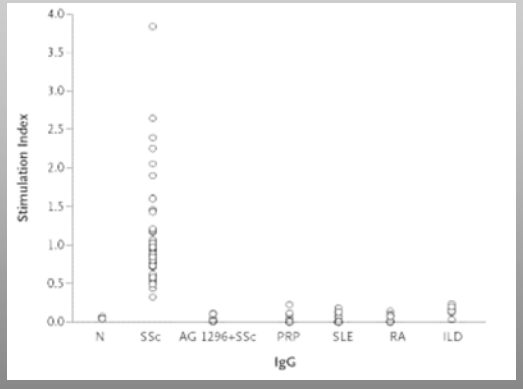
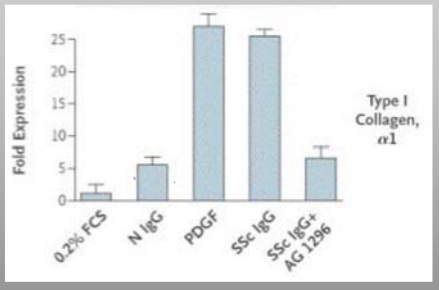
N Engl J Med 2006;354:2667-79.

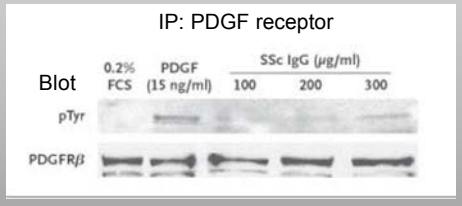
Autoantibodies to the PDGF Receptor Stimulates Production of Reactive Oxidants

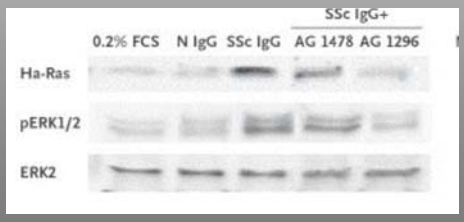


Oxidant production triggered by sera-derived IgG upon incubation with fibroblasts over-expressing the PDGF receptor. N, normal controls; SSc, scleroderma; PRP, primary Raynaud's phenomenon ; SLE, systemic lupus erythematosis; RA, rheumatoid arthritis; ILD, interstitial lung disease; AG 1296, PDGFR kinase inhibitor. From: Baroni et al., *New Engl. J. Med.* 354:2667, 2006.

Scleroderma-derived Antibodies to the PDGF Receptor Stimulate Cell Signaling and Collagen Production



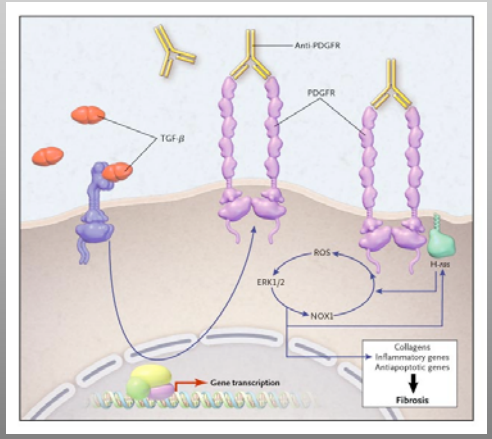




Legend: FCS, fetal calf serum; AG1478, EGFR tyrosine kinase inhibitor; AG1296, PDGFR kinase inhibitor

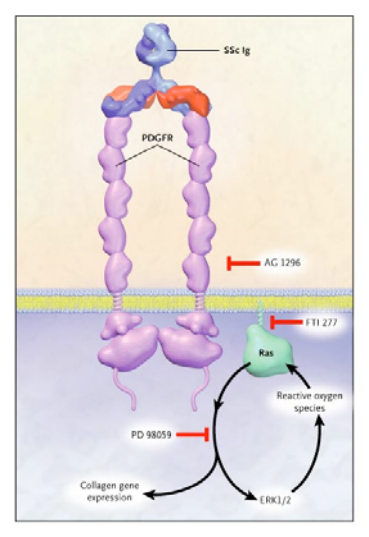
Adapted from: Baroni et al., *New Engl. J. Med.* 354:2667, 2006

Auto-antibodies to the PDGF Receptor: The Pathogenesis of Scleroderma Revealed?



Unlike normal fibroblasts, fibroblasts in scleroderma increase the expression of PDGFR in response to TGF-β, rendering the cells more sensitive to PDGF. The Ras–ERK1/2–ROS signaling pathway is triggered by PDGF or anti-PDGFR, which then activates NADPH oxidase (NOX1) to produce reactive oxygen species (ROS). These, in turn, activate extracellular signal-regulated kinases 1 and 2 (ERK1/2), which induce the H-*ras* gene. This signaling loop is present in normal fibroblasts but is relatively amplified in fibroblasts in patients with scleroderma. From: Tan, *New Engl. J. Med.* 354:2709, 2006.

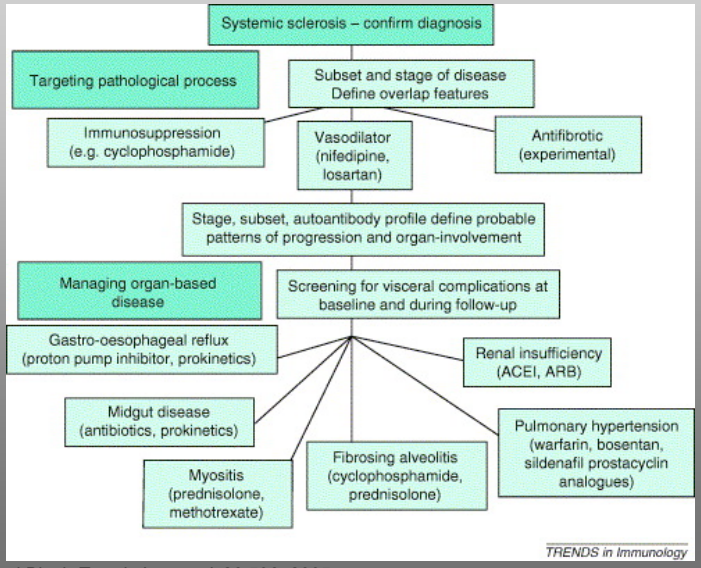
Potential Therapeutics for Scleroderma Based on Insight Into its Pathogenesis



If chronic over-stimulation of the PDGFR is required for the development and/or progression of scleroderma, then inhibition of PDGFR kinase activity (AG 1296), Ras post-translational processing (FTI 277), or ERK MAP kinase (PD 98059) may prove therapeutic.

From: Baroni et al., *New Engl. J. Med.* 354:2667, 2006.

Therapeutic Approach to the Treatment of Scleroderma



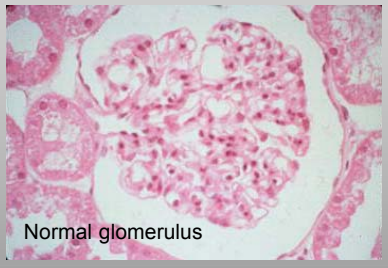
From: Denton and Black *Trends Immunol.* 26:596, 2005

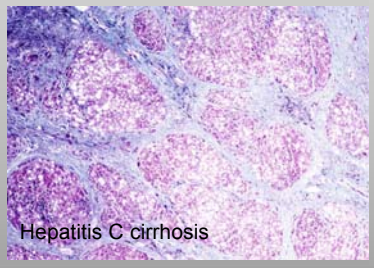
Fibrosis Can Occur in Any Organ and Can Lead to Irreversible Organ Damage

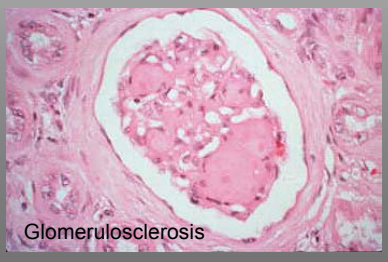
- Various theories have been proposed for the pathogenesis of fibrosing diseases. One view is that fibrosis represents a pathological variant of wound healing.
- An alternate view is that fibrosis is due to "unresolved inflammation."
- Another view is that fibrosis results from an "imbalance" in the activities of proteases and anti-proteases.

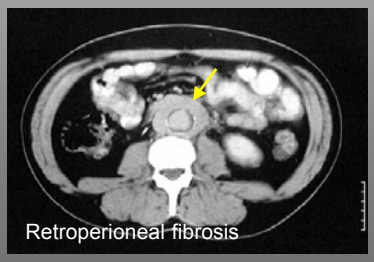
- Regardless of the precise etiology of fibrosis, the pathological deposition of collagen and other components of the extracellular matrix results from persistence of mesenchymal cells assuming a fibroblastic or myofibroblastic phenotype, producing copious amounts of components of the ECM.

Examples of Fibrosis

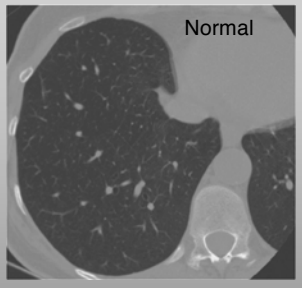

Normal glomerulus

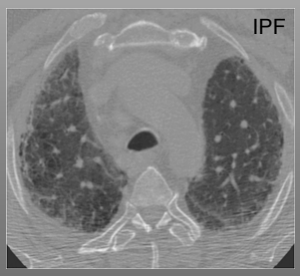

Hepatitis C cirrhosis

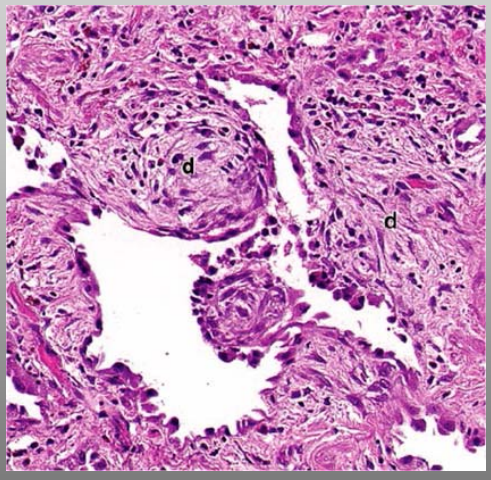

Glomerulosclerosis


Retroperioneal fibrosis

Idiopathic Pulmonary Fibrosis (IPF)







From: Best et al., *Radiology* 228:407, 2003;
Wittram et al. *Radiographics* 23:1057, 2003.

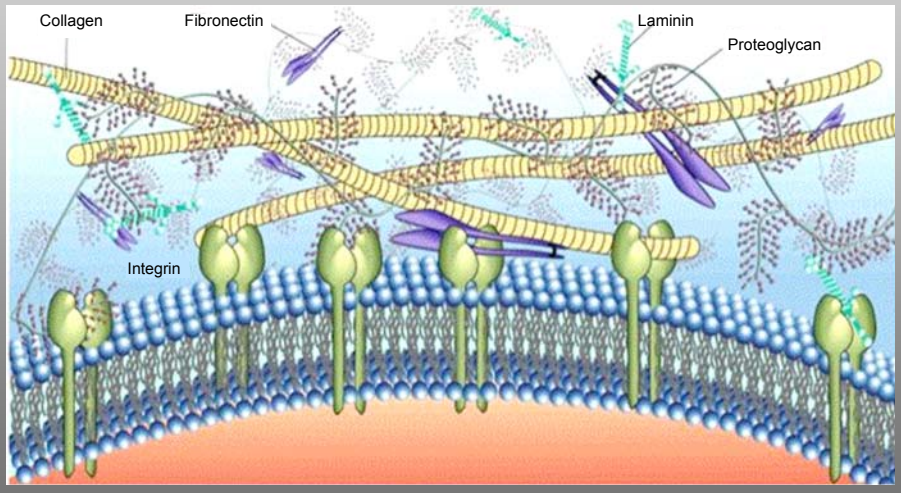
Three questions worth pondering…

1. What cells and molecules participate in fibrosis?

2. What is the pathogenesis of fibrosis?

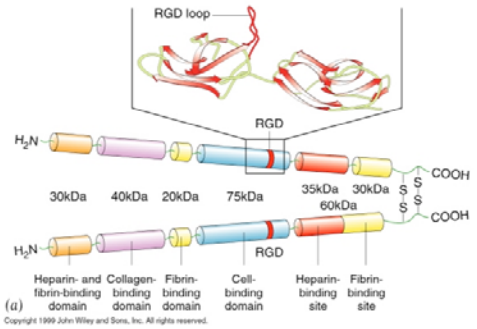
3. Can we intervene therapeutically and retard or reverse fibrosis?

Fibrosis Results from the Inappropriate Deposition of Extracellular Matrix

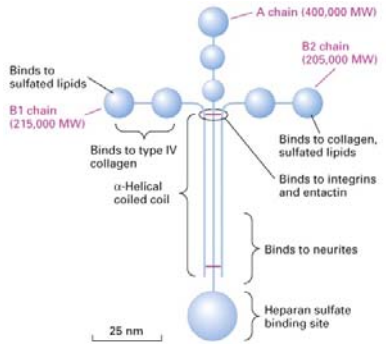


Two Engaging Members of the ECM

Fibronectin



Laminin



Diversity in Cells and Component of the ECM

Collagen	Anchor	Proteoglycan	Cell-Surface Receptor	Cells
I	fibronectin	chondroitin and dermatan sulfates	integrin	fibroblasts
II	fibronectin	chondroitin sulfate	integrin	chondrocytes
III	fibronectin	heparan sulfate and heparin	integrin	quiescent hepatocytes, epithelial; assoc. fibroblasts
IV	laminin	heparan sulfate and heparin	laminin receptors	all epithelial cells, endothelial cells, regenerating hepatocytes
V	fibronectin	heparan sulfate and heparin	integrin	quiescent fibroblasts
VI	fibronectin	heparan sulfate	litegrin	quiescent fibroblasts

Do not memorize this list

Fibrosis is triggered by growth factors, cytokines, and peptides

Important Pro-fibrotic Growth Factors

Growth factors

TGF-β, PDGF, FGF-2 (basic FGF), IGF-I, Connective tissue growth factor (CTGF), EGF

Cytokines

Th2 cytokines (IL-4, IL-13); IL-1, IL-6

Peptides

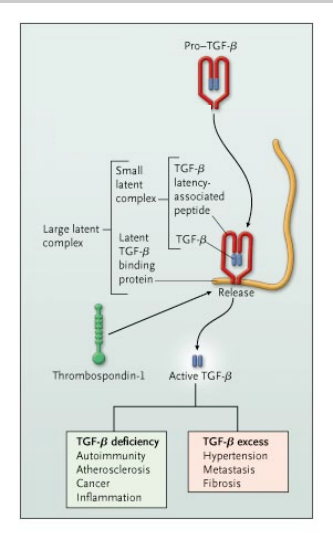
Endothelin I, Ang-II

Chemokines

C-C chemokines

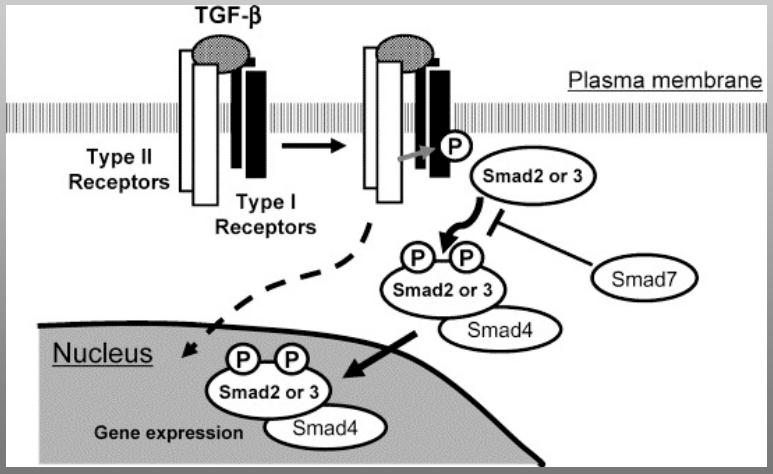
Do memorize this list

Activation of Latent TGF-β: Not So Simple



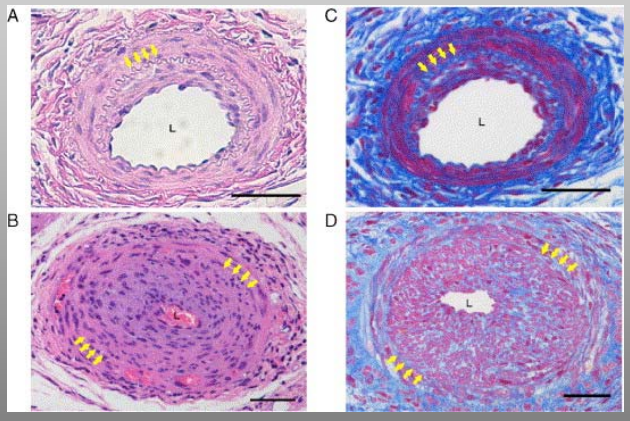
TGF-β is synthesized by virtually all types of cells, in the form of an inactive homodimeric propeptide, pro-TGF-β. After TGF-β is cleaved from the pro-peptide, additional downstream events control the extent to which TGF-β is available. The small latent complex, composed of TGF-β and its latency-associated peptide, and the large latent complex, composed of TGF-β, latency-associated peptide, and the latent TGF-β binding protein control where and when active TGF-β is made available. The matrix protein **thrombospondin-1** binds to the latency-associated peptide, which results in the activation of TGF-β, an essential step for the binding of TGF-β to its receptors. Plasmin, some **MMPs**, and two members of the **integrin** family also activate the latent complexes of TGF-β. From: Ugust and Suthanthiran *New Engl. J. Med.* 354: 2721, 2006.

Basic Paradigm of TGF-β Signal Transduction



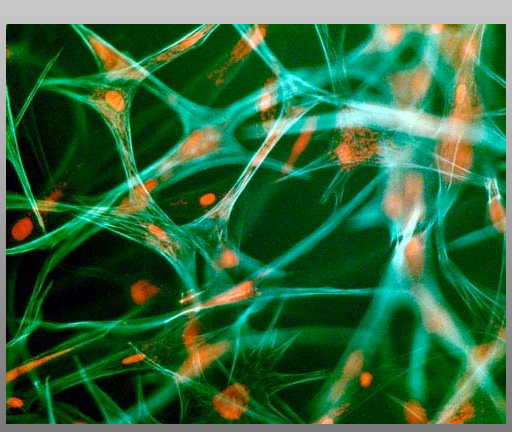
From: Yokote et al., *Trends Cardiovasc. Med.* 16:240, 2006

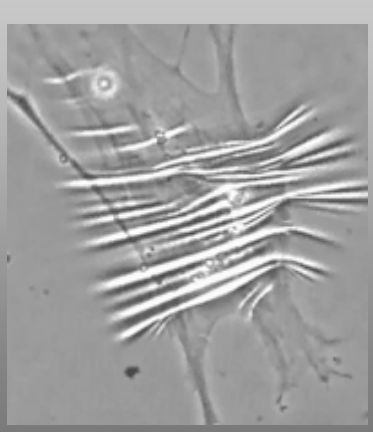
Anti-proliferative and Pro-fibrotic Properties of TGF-β Signaling Revealed by Gene Targeting in Mice



Enhanced neointimal hyperplasia and reduced matrix deposition in the arteries of Smad3-null mice upon injury. Photomicrographs showing representative cross sections of hematoxylin/eosin-stained (**A** and **B**) and Masson's trichrome-stained (**C** and **D**) femoral arteries from wild-type (**A** and **C**) and Smad3-null (**B** and **D**) mice 3 weeks after endothelial injury by photochemically induced thrombosis method. *Arrows* indicate the positions of the internal elastic lamina. L, vascular lumen. Frrom: *Circ Res* 96:904, 2005.

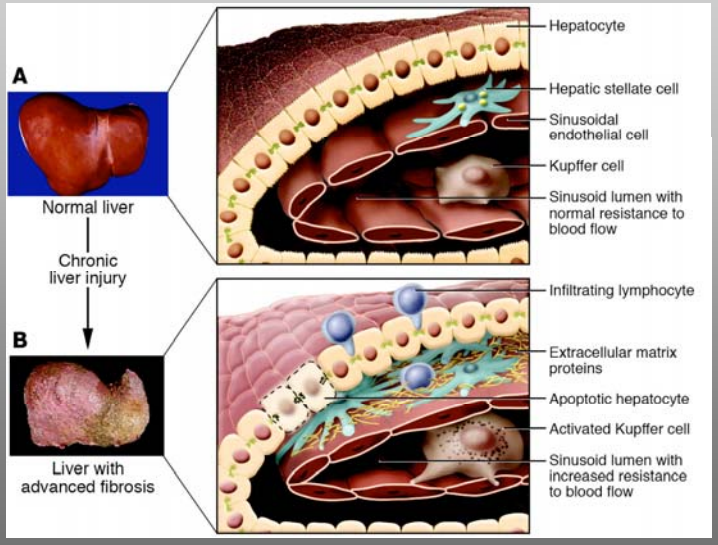
The Myofibroblast, a Collagen-producing Contractile Cell Involved in Wound Healing





α-smooth muscle actin

The Hepatic Stellate Cell is the "Myofibroblast-equivalent" in Cirrhosis

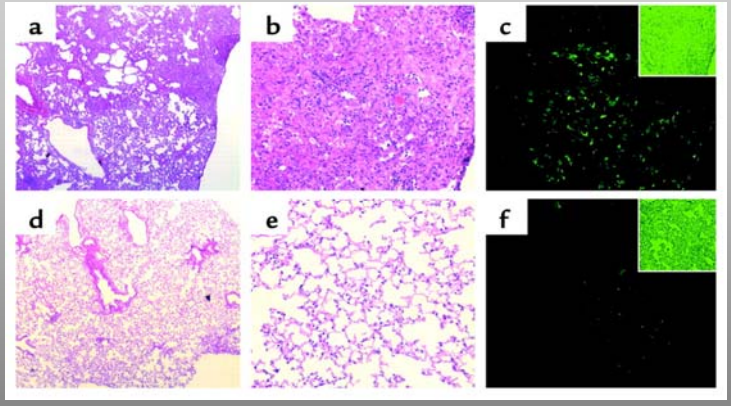


From Bataller and Brenner, *J. Clin. Invest*. 115:209, 2005

What are the cellular origins of fibroblasts and myofibroblasts?

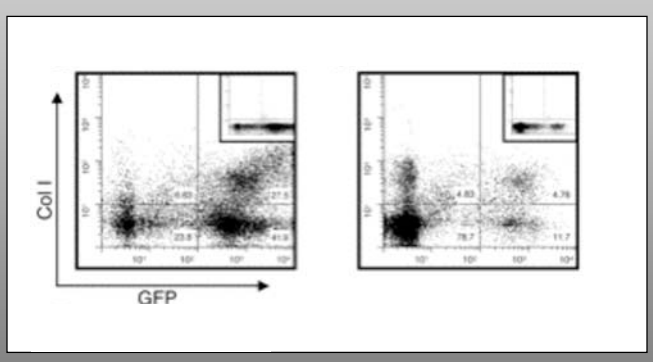
- Local proliferation of mesenchymal cells
- Influx of blood-borne fibroblast precursors (often termed “fibrocytes”)
- Epithelial-mesenchymal transformation (EMT)

Use of Bone Marrow Chimeras to Determine the Cellular Origin of Fibroblasts



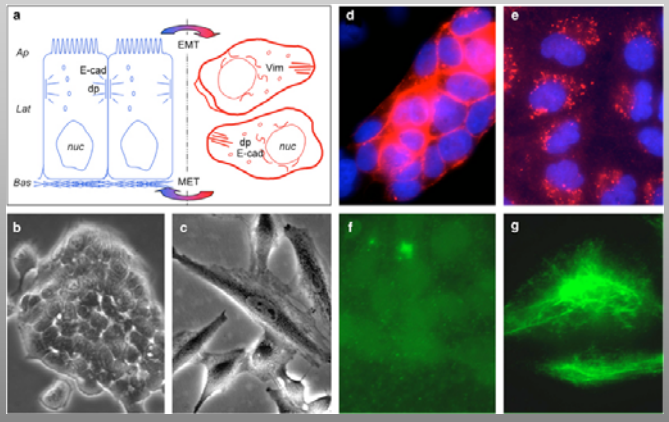
Representative lung sections from bleomycin (BLM)-treated (**a**–**c**) or saline-treated (**d**–**f**) GFP BM chimera mice were evaluated at day 28 after BLM or saline treatment. The H&E-stained sections revealed severe distortion of lung alveolar architecture due to extensive fibrosis (**a** and **b**, at x40 and x200, respectively). (**c**) Numerous GFP^+ cells in densely cellular fibrotic lesions were easily identifiable by fluorescence microscopy in BLM-treated lungs. x200. In contrast, H&E-stained lung sections from saline-treated GFP BM chimera mice showed normal lung architecture (**d** and **e**, at x40 and x200, respectively), with a few scattered GFP^+ cells visualized by fluorescence microscopy (**f**, at x200). Insets in **c** and **f** showed the light-microscopic appearance of the respective sections examined by fluorescence microscopy. x200. From: Hashimoto et al., *J. Clin. Invest.* 113:243, 2004.

GFP+ Col I+ Cells Derived from the Bone Marrow Populate Lungs During Experimental Pulmonary Fibrosis in Mice



Representative flow cytometry tracings of cells isolated from lungs of bleomycin- and control saline-treated mice and stained with antibodies that detect Col I. Insets show staining of cells using an isotype-matched control antibody. From: Hashimoto et al., *J. Clin. Invest.* 113:243, 2004.

Epithelial-mesenchymal Transformation (EMT)

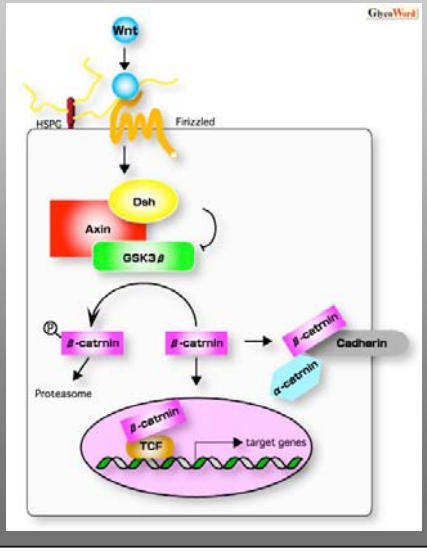


(**a**) Schematic of EMT. Epithelial cells (in **blue**) adhere to each other through adherens junctions, using **E-cadherin** (E-cad), and their desmosomes (tight junctions) constituted by various proteins such as desmoplakin (dp). Mesenchymal cells (in **red**) are neither adherent nor apically polarized and have low expression of E-cadherin and desmoplakin. The intermediate filament protein vimentin (vim) is induced in mesenchymal cells. (**b–g**) EMT in a human squamous cell carcinoma line. Prior to EMT (**b**) and after EMT (**c**). E-cadherin (**red** in **d** and **e**) and vimentin (**f** and **g**) immunolocalization in epithelial (**d** and **f**) and mesenchymal cells following EMT (**e** and **g**). Adapted from Larue and Bellacosa, *Oncogene* 24:7443, 2005.

E-Cadherin Appears to be a Critical Regulator of EMT

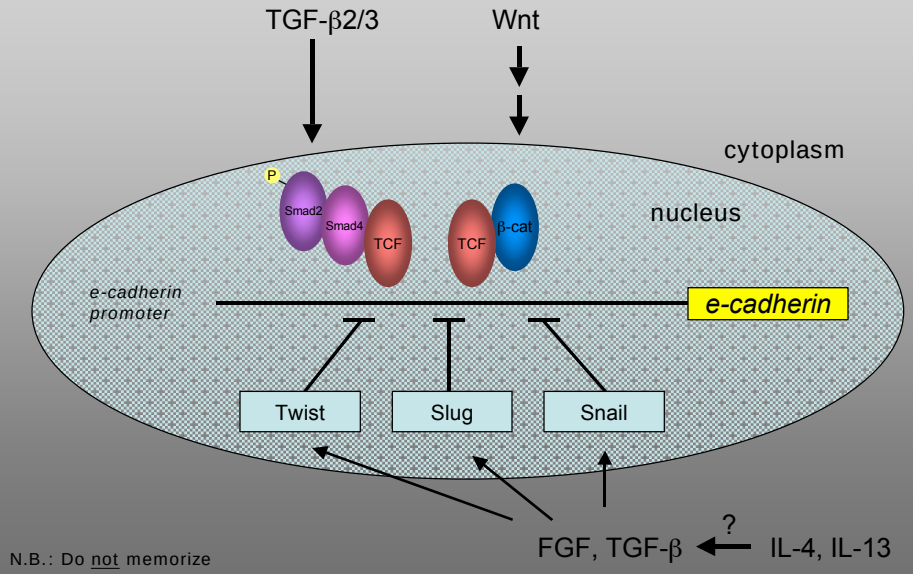
Is repression/disruption of E-cadherin expression sufficient to induce EMT?

The "Canonical" Wnt Pathway Leads to Activation of a Subset of Genes Important in Development



N.B.: Do not memorize

Repressing E-Cadherin Triggers EMT



N.B.: Do not memorize

Summary

1. Scleroderma is an autoimmune disease involving multiple organs. It is characterized by a vasculopathy, varying degrees of inflammation, and fibrosis. Several major clinical variants of scleroderma have been described.

2. Pulmonary complications of scleroderma are common and severe in substantial minority. Death is due to severe pulmonary hypertension, pulmonary fibrosis, or both, ultimately leading to cor pulmonale.

3. Autoantibodies play an important role in the pathogenesis to scleroderma. Agonistic autoantibodies against the PDGFR have been isolated from patients with scleroderma.

4. Various theories of fibrogenesis have been proposed. Regardless of the precise etiology, fibrosis is due to the excessive deposition of ECM by the major collagen-producing cells in the body, fibroblasts and myofibroblasts.

5. Fibroblasts arise from a combination of local proliferation of resident mesenchymal cells, EMT, and influx of fibroblast precursors from the bone marrow.

6. Among the various pro-fibrogenic growth factors, TGF-β usually plays an important role in fibrosis. TGF-β triggers increased production of ECM proteins, induces EMT, and typically induces proliferation (rather than cell cycle arrest) in fibroblasts.