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Autoantibodies against PDGF Receptor in Scleroderma

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Scleroderma (systemic sclerosis) is characterized by the deposition and accumulation of excessive amounts of collagen and extracellular-matrix molecules, the dysfunction of microvascular endothelial cells, and altered immune tolerance. These interacting and interdependent processes lead to chronic inflammation and tissue fibrosis.

To investigate complex mechanisms of disease pathogenesis, conventional wisdom dictates that a single aspect of disease be dissected out and examined in detail. The skin is a prominent target organ in scleroderma, and fibroblasts from affected patients are activated and display a variety of properties, including increased production of collagens and other extracellular-matrix proteins, abnormal growth patterns, and resistance to apoptosis. Unfortunately, such fibroblasts gradually lose their activated properties in culture. This phenomenon is an example of what systems biologists refer to as an "emergent property." Although such fibroblasts have a measurable, inherent biologic abnormality, the characteristics or emergent properties of these cells cannot be fully appreciated when they are isolated from other components that interact together in vivo with a resultant phenotype that is more complex than the sum of the individual parts.

What factors sustain the fibroblast phenotype in vivo in scleroderma? At the earliest stages, small-vessel abnormalities and lymphocyte activation and infiltration of target organs are present before the appearance of clinically apparent fibrosis. Thus, the sustaining factors include endothelial cells, fastidious subpopulations of aberrant fibroblasts or their precursors, and immune-system cells. Svegliati Baroni et al.¹ suggest in this issue of the *Journal* that autoantibodies could be one of the factors that sustain the profibrotic phenotype of such fibroblasts — an effect reminiscent of that of stimulating antibodies on the thyrotropin receptor in causing Graves' disease.

The existence of autoantibodies in scleroderma was described more than 40 years ago. Indeed, such autoantibodies, some highly disease-specific, are present in virtually all patients with scleroderma. Although the important autoantibodies are directed against nuclear components, it is clear that most patients with scleroderma also have antibodies against extracellular-matrix and cell-surface proteins, proteases, fibroblasts, and endothelial cells.² What would be needed to demonstrate that autoantibodies in scleroderma are pathogenic, not just epiphenomena resulting from

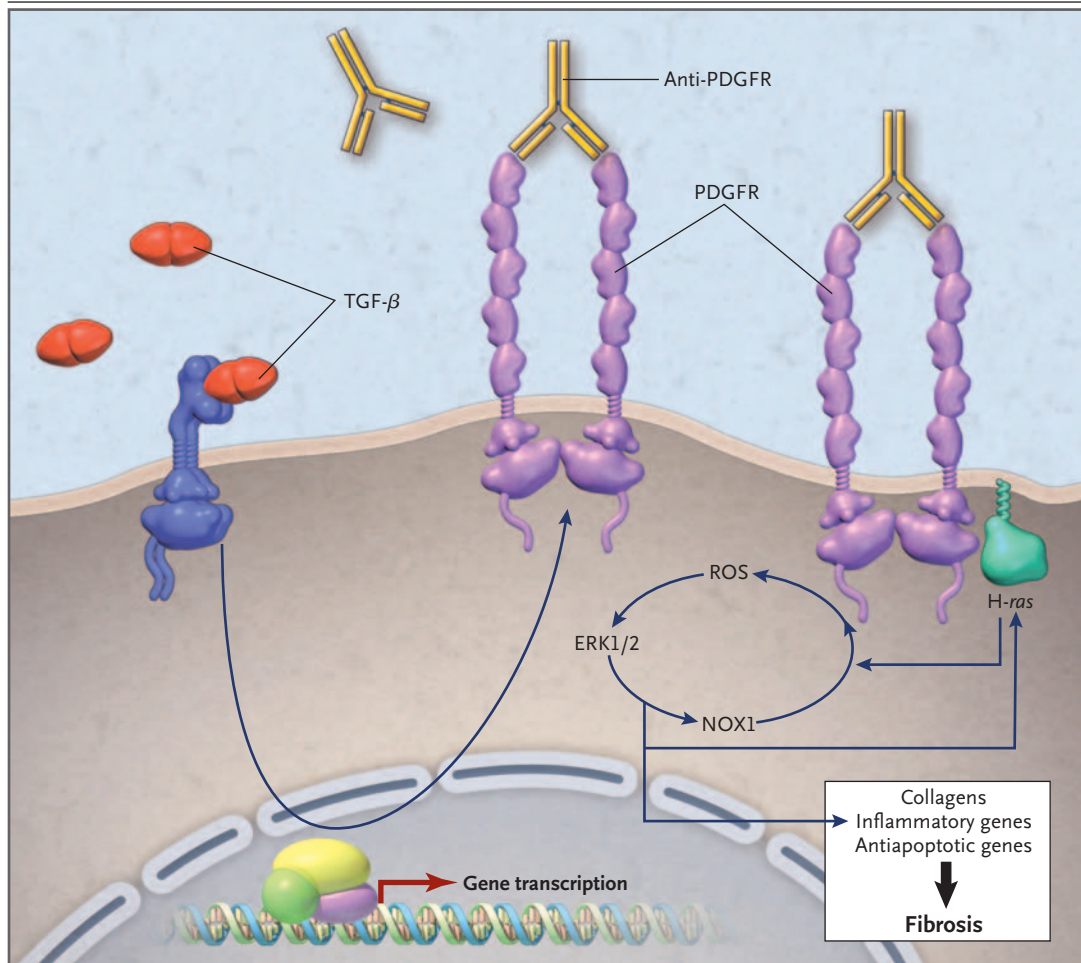


Figure 1. Selective Up-Regulation of PDGFR by Fibroblasts in Scleroderma.

Unlike normal fibroblasts, fibroblasts in scleroderma increase the expression of PDGFR in response to transforming growth factor β (TGF- β), rendering the cells more sensitive to platelet-derived growth factor (PDGF).¹ 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 Harvey rat sarcoma (H-ras) gene. This signaling loop is present in normal fibroblasts but is relatively amplified in fibroblasts in patients with scleroderma.

a loss of tolerance? The presence of an autoantibody long before the onset of clinical disease would constitute circumstantial evidence of the causation of disease. This finding has been well documented in systemic lupus erythematosus and rheumatoid arthritis but has yet to be reported in scleroderma. If the autoantibody is central to disease development, it must be present in most, if not all, patients with scleroderma and must be disease-specific. The various scleroderma antinuclear antibodies correlate with certain disease features, but the individual subtypes are

mutually exclusive, with each present only in a minority of patients with scleroderma. Koch's third postulate would require that the autoantibody in question cause disease when introduced into a healthy animal, although the demonstration that the autoantibody affects key cellular processes that are important in disease pathogenesis in model systems is often used as a surrogate. Svegliati Baroni et al. describe a stimulatory autoantibody against platelet-derived growth factor receptor (PDGFR) that seems to fulfill some of these characteristics.

Yamakage et al.³ initially noted the selective up-regulation of PDGFR in scleroderma fibroblasts (Fig. 1), probably resulting from defects in the signaling of transforming growth factor β (TGF- β) that render the fibroblasts more sensitive to platelet-derived growth factor (PDGF). Later, Sambo et al. observed that fibroblasts constitutively produce a large amount of reactive oxygen species (ROS) through the NADPH oxidase (NOX1).⁴ ROS can mediate apoptosis and activate nuclear factor- κ B, which, in turn, activates the expression of inflammatory genes, including adhesion molecules and cytokines. The same group later reported the discovery of a signaling loop in normal fibroblasts in which PDGF triggered increased production of ROS by the activation of NOX1 (Fig. 1).⁵ ROS, in turn, activate the extracellular signal-regulated kinases 1 and 2 (ERK1/2) pathway, which induces the viral Harvey rat sarcoma (H-*ras*) gene.⁵ This gene can also further activate ERK1/2 through the v-*raf*-1 murine leukemia viral oncogene homologue 1 (Raf1) cascade. Activation of H-*ras* sets into motion a diverse set of intracellular pathways, including the anticancer target protein kinase B and nuclear factor- κ B, which influences apoptosis, inflammation, and collagen synthesis (Fig. 1).

As compared with normal cells, fibroblasts in scleroderma have a relatively amplified Ras-ERK1/2-ROS signaling loop, and disruption of the loop with various pharmacologic inhibitors reduced the transcription of collagen genes.⁵ Svegliati Baroni et al. now report the detection of autoantibodies in patients with scleroderma that stimulate PDGFR, triggering the Ras-ERK1/2-ROS signaling loop. In fact, all patients with scleroderma who were studied had anti-PDGFR-stimulating antibodies, whereas patients with systemic lupus erythematosus, rheumatoid arthritis, primary Raynaud's disease, or interstitial lung disease without scleroderma did not. The investigators also show that anti-PDGFR can be isolated from the serum of patients with scleroderma and that the autoantibody specifically binds to intact PDGFR. Finally, in partial fulfillment of Koch's third postulate, they show that purified immunoglobulins and clonal anti-PDGFR from patients with scleroderma activate the Ras-ERK1/2-ROS signaling loop in normal fibroblasts, increasing the expression of type I collagen and α -smooth-

muscle actin, two biochemical markers of scleroderma fibroblasts.

These findings will have to be verified in additional patient populations. Adoptive transfer studies in murine models and the demonstration that anti-PDGFR precedes the onset of disease should provide stronger evidence of the causation of scleroderma. In keeping with the paradigm of interacting systems, investigation of the effects of anti-PDGFR on endothelial and smooth-muscle cells would bring the microvascular component of scleroderma into the picture. The effects may be quite different in these cell types from those in fibroblasts, since the pathways activated by H-*ras* depend on the context of the cell type, the local microenvironment in which H-*ras* occurs, or both.

In summary, the present data, put in context, suggest that the profibrotic phenotype of fibroblasts in patients with scleroderma are maintained by at least three factors. First, abnormal TGF- β signaling results in an increased level of PDGFR. Second, there is relative amplification of the Ras-ERK1/2-ROS signaling loop, perhaps as a result of the up-regulation of PDGFR. Third, there is stimulation of autoantibodies against PDGFR that initiate and maintain the Ras-ERK1/2-ROS cascade. These observations provide an important step in the understanding of the mechanisms of interaction between autoimmunity and fibrosis in scleroderma and suggest many potential avenues, such as cytokine-receptor decoys, that could be explored as therapeutic targets for this disease.

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