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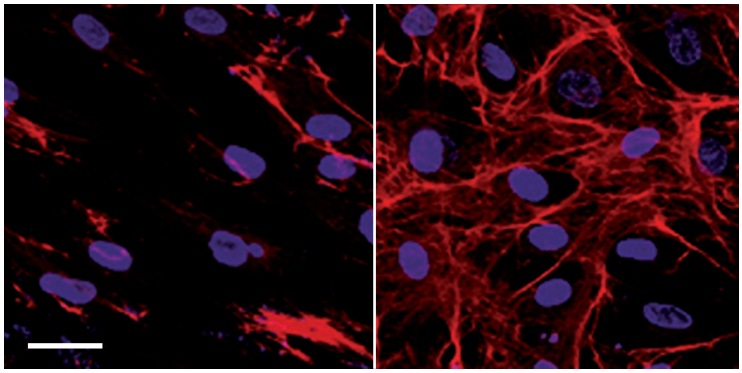
CONNECTIVE TISSUE DISEASES

TLR4 and fibronectin—persistent fibrogenesis in SSc



SSc fibroblasts make excess Fn^{EDA}. Fibroblasts from skin biopsies of a healthy adult (left) and a patient with SSc (right) immunostained with DAPI (blue) and antibodies to Fn^{EDA} (red). Bar = 25 μm. Images courtesy of J. Varga and S. Bhattacharyya.

Researchers in the USA are close to understanding the mechanism of chronic fibrosis associated with systemic sclerosis (SSc). John Varga and colleagues identified fibronectin extra domain A (Fn^{EDA}) as an endogenous damage-associated ligand for Toll-like receptor 4 (TLR4) and showed that skin fibroblasts in patients with SSc are chronically exposed to higher than normal levels of Fn^{EDA}. Varga says this chronic activation of fibroblasts, which involves transforming growth factor-β (TGF-β), "establishes a vicious cycle of persistent fibrogenesis."

In explanation of how the project began, Varga says "fibrosis is a major cause of morbidity and mortality in SSc. While we don't know what triggers fibrosis, we had even less understanding of why it persists, even when the initial injury is removed."

Fn^{EDA}, which is transiently expressed in extracellular matrix during tissue repair, is a multifunctional, alternative splice variant of fibronectin.

In *Science Translational Medicine*, the researchers, led by Swati Bhattacharyya, showed that Fn^{EDA} is 5-fold more abundant in serum from patients with diffuse cutaneous SSc ($n = 46$, 20.2 ± 1.6 μg/ml) than from healthy individuals ($n = 16$, 4.5 ± 0.8 μg/ml). With quantitative PCR and immunofluorescence they showed an increase in Fn^{EDA} expression in skin from biopsy samples from SSc patients, and *in vitro*, Fn^{EDA} RNA and protein production by foreskin fibroblasts was upregulated by SMAD-dependent TGF-β stimulation.

With TLR4-defective C3HeJ mice, siRNA knockdown or a small molecule inhibitor of TLR4, the researchers then showed that TLR4 is essential for Fn^{EDA}-induced collagen production by fibroblasts. Finally, TLR4 inhibition attenuated muscle necrosis, dermal thickening, cutaneous fibrosis and *COL1A1* and *ACAT2* expression in the skin of bleomycin-treated mice.

Varga predicts "these studies might open new doors for innovative treatments and explain chronic innate immunity in SSc." He wants to "identify patients who have the most active TLR signalling and develop novel drugs that selectively block fibroblast–Fn^{EDA} cross-talk, and thereby dampen chronic fibroblast activation."

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Original article Bhattacharyya, S. *et al.* Fibronectin^{EDA} promotes chronic cutaneous fibrosis through Toll-like receptor signaling. *Sci. Transl. Med.* doi:10.1126/scitranslmed.3008264