TLR4 on the surface of scleroderma

By Lauren Martz, Staff Writer

BioLineRx Ltd.'s deal with the **University of Colorado** could give the biotech a disease-modifying therapy for cutaneous fibrosis in patients with diffuse systemic scleroderma.¹ It is still unclear whether the university's toll-like receptor 4 inhibitors will work in other tissues affected by the disease.

There are no marketed disease-modifying drugs for diffuse systemic scleroderma. The diffuse form of the autoimmune disease affects about a third of patients and involves an inflammatory and fibrotic skin component in addition to systemic inflammation and fibrosis of the lungs and other organs.

The diffuse form is generally more aggressive and progresses from inflammation to fibrosis more quickly than limited systemic scleroderma. Standard care includes immunosuppression and anti-inflammatory therapeutics as well as symptomatic treatments.

Scleroderma's etiology is unknown, although previous studies have provided a few hints. For example, a group including a member of the Northwestern team showed that transforming growth factor- β (TGF β ; TGF β) acts as a trigger for the fibroblast activation and cutaneous fibrosis that occurs in patients with scleroderma.²

Other groups have shown that the fibronectin extra domain A (FnEDA) splice variant is upregulated in skin lesions from patients with scleroderma³ and that toll-like receptor 4 (TLR4) ligands and signaling occur in skin and lung biopsies from patients with scleroderma.^{4,5} FnEDA typically is absent in healthy adult tissues and upregulated during injury.

With those clues in mind, John Varga and colleagues have built a clearer mechanistic picture of the disease and showed that FnEDA signaling through TLR4 plays a role in the fibrotic feed-forward loop in cutaneous scleroderma.

Varga is director of the Northwestern Scleroderma Program and a professor of medicine-rheumatology and dermatology at the **Northwestern University Feinberg School of Medicine**. The team also included a researcher from the **University of Michigan**.

In 48 patients with diffuse cutaneous scleroderma, serum FnEDA levels were 5-fold higher than those in 16 healthy controls. FnEDA mRNA levels in skin lesion biopsies from 20 patients were 3-fold higher than those in 8 healthy controls. In normal human foreskin fibroblasts, incubation with TGF β triggered fibrosis and induced 30-fold increases in FnEDA mRNA and 9-fold increases in protein levels.

In mouse models of cutaneous fibrosis, knockout of *Fneda* decreased dermal thickening and collagen accumulation compared with no alteration. These findings suggested that FnEDA is both increased and required for cutaneous fibrosis during scleroderma.

In fibroblast monolayers and human skin models, incubation with FnEDA increased collagen levels, wound healing and skin thickness, whereas pretreatment with a TLR4 inhibitor, siRNA targeting TLR4 or genetic knockout of the receptor prevented the profibrotic effects of FnEDA.

Finally, in a mouse model of cutaneous fibrosis, subcutaneous injection of a TLR4 inhibitor decreased skin thickness and collagen deposition compared with no treatment and prevented muscle necrosis.

Based on these findings, the researchers proposed that FnEDA signaling through TLR4 facilitates a loop that maintains the fibrotic state in the skin of patients with scleroderma.

The researchers proposed that FnEDA is upregulated upon a fibrotic trigger and is recognized by TLR4. Activation of TLR4 then stimulates an inflammatory reaction at the site, causing the release of more FnEDA.

Data were published in *Science Translational Medicine*.

Therapeutic development

Varga told *SciBX* that one of his next steps is developing TLR4 inhibitors.

"We are pursuing studies to identify specific, novel TLR4 inhibitors with maximal efficacy and optimal drug-like properties including stability and low toxicity," he said.

He noted that the TLR4 inhibitor used in the paper is no longer being pursued in the clinic, but his team is now working with a new class of orally available, highly selective TLR4 inhibitors with a distinct mechanism of inhibitory activity.

BioLineRx has an option to license lead compound T5342126. The molecule was developed by Varga's collaborator Hang Hubert Yin, an associate professor at the **University of Colorado at Boulder**.

"BioLineRx is currently in early stages with this compound, assessing dose regimens and safety in animal models. Next stages will include process development as well as safety and distribution requirements," said CSO Leah Klapper.

She added that the company is exploring

several potential indications for the inhibitor.

"TLR4 is involved in the actual fibrosis process and therefore specifically targets the problem and not only affects the immune system.

"This work provides insights into how we can break into the feed-forward loop that characterizes fibrosis. Once initiated, pathologic fibrosis progresses to a maintenance state, but there are hints that the body continues to run a process to resolve the fibrosis. Breaking into the feed-forward loop by blocking TLR4 signaling could allow the body to start resolving some of the pathology on its own."

**—Luke Evnin,
Scleroderma Research Foundation**

It is upstream in the cascade of fibrosis, enabling efficient modulation of the cascade,” said Klapper. “Most importantly, TLR4 is suggested to be a part of the mechanism responsible for the persistent chronic fibrosis as opposed to targets that only trigger fibrosis, suggesting a unique beneficial intervention.”

Luke Evnin, chairman of the **Scleroderma Research Foundation** and managing director of **MPM Capital**, agreed. “This work provides insights into how we can break into the feed-forward loop that characterizes fibrosis. Once initiated, pathologic fibrosis progresses to a maintenance state, but there are hints that the body continues to run a process to resolve the fibrosis. Breaking into the feed-forward loop by blocking TLR4 signaling could allow the body to start resolving some of the pathology on its own,” he said.

He added, “This is exciting because it suggests that intervening, even late once fibrosis has progressed, could have an impact for patients.”

Cutaneous limitations

An unanswered question is whether TLR4 also underlies fibrosis in other organs.

“Systemic scleroderma is not a local disease. It is accurate that part of the problem is localized to the skin, but there is also fibrosis of other organs that causes very serious complications for patients,” noted Evnin. Thus, he wanted to see the researchers study the role of TLR4 in other organs.

Varga told *SciBX* that his team is planning to evaluate the new compounds in a series of complementary models that recapitulate key features of scleroderma including fibrosis, autoimmunity and vasculopathy. He said that even if TLR4 is only at work in the skin, the program is well worth pursuing.

“Topical application of our compound is an extremely appealing strategy since it is expected to be able to hit the target—activated myofibroblasts within the fibrotic dermis—without systemic exposure,” said Varga. “We expect the topical approach to local therapy can avoid issues of immunomodulation and potential immunosuppression by a systemically absorbed TLR inhibitor.”

Varga told *SciBX* that in addition to designing new TLR4 inhibitors, his team is developing a companion diagnostic to identify patients most likely to benefit from TLR4 inhibitors.

“We have already succeeded in defining a provisional TLR signature based on genomewide microarray analysis of genes whose expression is modulated by TLR and have identified patient subsets with a strong TLR signature in skin biopsies,” he said.

Northwestern University and the University of Colorado have filed a patent application covering the use of TLR inhibitors to treat scleroderma. A separate patent covering the chemical structure of the lead TLR4 inhibitor has been issued to the University of Colorado, and BioLineRx has an exclusive option to license the IP.

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