A new angle in fibrosis

By Lauren Martz, Staff Writer

The cytokine CXCL10 has been shown to reduce lung fibrosis, but the mechanism by which this occurs remains unclear. **Duke University School of Medicine** researchers have now shown how CXCL10 interacts with syndecan 4, a heparan sulfate proteoglycan on the surface of lung fibroblasts that controls extracellular matrix dynamics.¹ But given the mouse model the researchers used, more work is needed to show that CXCL10 is actually affecting fibrosis rather than inflammation.

Pulmonary fibrosis is a condition of compromised lung function that results from excess production of extracellular matrix molecules such as collagen, fibronectin, tenascin and proteoglycans. During fibrosis, fibroblasts that produce the matrix proteins are recruited to the lungs.

Previous studies have shown that mice treated with chemokine CXC motif ligand 10 (CXCL10; IP-10) have reduced fibrosis in the lungs.² CXCL10 also inhibited fibroblast migration in lung fluid from injured animals.³ In addition, genetic knockout of *Cxcl10* in a mouse model of fibrosis exacerbated the disease.

Although CXCL10 has been shown to reduce lung fibrosis, the mechanism was mysterious because the chemokine's receptor, CXC chemokine receptor 3 (CXCR3), is not present on the cell surface of lung fibroblasts.

In the new paper published in *The Journal of Clinical Investigation*, Paul Noble and colleagues report that the interaction between CXCL10 and syndecan 4 (SDC4) on fibroblasts is what actually may prevent fibrosis.

Noble is professor of medicine and chief of the pulmonary, allergy and critical care division at Duke. The paper also included researchers from **Yale School of Medicine** and **Harvard Medical School**.

Based on previous work showing that SDC4 plays an essential role in injury and other stress situations, including kidney injury,⁴ wound healing⁵ and more recently in the lungs in a model of ozone exposure,⁶ Noble and his team decided to explore the role of SDC4 in lung fibrosis.

The researchers induced lung injury and fibrosis in mice using intratracheal administration of bleomycin, a generic cancer drug known to induce sterile lung injury. In *Sdc4*^{-/-} mice, bleomycin-induced fibrosis was significantly increased compared with that in wild-type littermate controls, suggesting that Sdc4 is necessary to protect against fibrosis.

In fibroblasts from *Sdc4* knockouts, Cxcl10 binding was reduced compared with that in wild-type cells, suggesting that Cxcl10 binds to fibroblasts through Sdc4.

The group then exposed cultured fibroblasts to fluid from the lungs of pulmonary fibrosis patients and observed fibroblast chemotaxis. Importantly, this migration was inhibited by treatment with CXCL10 lacking CXCR3 binding capacity, suggesting that it was indeed the interaction with SDC4 that triggered the antifibrotic effect.

CXCL10 did not stop the migration of *SDC4*^{-/-} fibroblasts, and mutation of CXCL10's heparan binding domain also prevented the cytokine's ability to inhibit migration of *SDC4*^{+/+} fibroblasts.

Altogether, the findings suggest CXCL10 binds to SDC4 and prevents fibroblast migration and fibrosis independent of its activity on CXCR3.

According to the paper's authors, therapeutically the findings could translate into treatments that employ CXCL10 devoid of CXCR3-binding capacity to boost SDC4 activity in fibroblasts of lung fibrosis patients. Avoiding binding to CXCR3, a T cell activator, would reduce potential side effects of using CXCL10 systemically.

Missing mechanism

Although the findings in *JCI* shed some light on the mystery of how CXCL10 exerts protective effects in fibrosis, it is still unknown exactly why this occurs. Indeed, the authors wrote that next steps include identifying the mechanism by which SDC4 regulates fibrosis.

"They state in the paper that CXCL10 has no effect on collagen synthesis, so that brings up the question: What is the mechanism? They say that it has an effect on fibroblasts, but it is unclear how that leads to collagen synthesis," said Peter Van Vlasselaer, president and CEO of **Arresto BioSciences**. "Before making a statement about fibrosis, they will need to get some fuel for the role of syndecan 4 and CXCL10 in other models of fibrosis in general. It is considered that other types of fibrosis, such as liver fibrosis, arise by the same mechanisms, so intervention with CXCL10 should be effective in them as well."

Arresto has an undisclosed compound in preclinical testing to treat fibrosis.

Thomas Pap, professor of experimental medicine and director of the Institute of Experimental Musculoskeletal Medicine at the **University Hospital Muenster**, agreed. "The problem is that the mechanism of how syndecan 4 and its lack of binding to CXCL10 affect fibrosis is unknown," he said.

Van Vlasselaer further pointed out that although CXCL10 is induced by interferon- γ (IFNG; IFN- γ), Phase III studies of **InterMune Inc.**'s Actimmune interferon- γ -1b failed to meet the primary endpoint of reduced mortality in idiopathic pulmonary fibrosis (IPF), and development for the indication was discontinued in 2007. Thus, asked Van Vlasselaer, if IFN- γ was ineffective at treating pulmonary fibrosis, why would a product induced by IFN- γ be effective?

Model behavior

Without a clear connection linking SDC4-induced signaling to CXCL10, Pap suggested it is possible the fibrotic effects described in the *JCI* article were at least in part a consequence of the knockout model itself. "It needs to be excluded that the knockout of *Sdc4* in the mice altered lung development in such a way that fibrotic phenotype occurred," he said.

Indeed, Pap said it is possible that CXCL10 might be reducing inflammation in the lung to a greater extent than reducing fibrosis.

"One major concern is that the authors use a bleomycin model. The issue is that for the first several days, this model is driven by inflammation, after which fibrosis actually develops. The researchers began treatment with CXCL10 immediately after administration of bleomycin, suggesting that they were treating the inflammatory phase of the disease," noted Van Vlasselaer. "This brings up an unanswered question: Does CXCL10 treat fibrosis? Or is it actually acting on the inflammation? This point is so important because it is already known that treating fibrosis with anti-inflammatory agents is largely unsuccessful."

Pap did note that if the consequence of enhancing the interaction between CXCL10 and SDC4 is reducing inflammation, the therapeutic strategy proposed by the authors could be particularly effective in inflammatory forms of the disease, such as fibrosis that results from systemic sclerosis.

However, he added that "successes with this model in the past have not translated strongly to therapeutic effect in man," suggesting that the findings should be confirmed in additional models of fibrosis.

Van Vlasselaer's final concern was that the bleomycin model only shows preventative—and not therapeutic—effects in pulmonary fibrosis.

"This is a preventative model that certainly has interesting scientific and academic merits, but before this goes into the drug development path, we would like to see therapeutic effect. They would need to treat animals with established fibrosis and see an impact on disease progression and possibly cause disease regression," he said. "One key piece of data required will be that treatment with CXCL10 can provide a survival benefit."

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Arresto BioSciences**

Drug properties

If CXCL10 is taken forward into the clinic, the molecule might have to be modified to improve its *in vivo* properties.

CXCL10 is a small peptide—only 77 amino acids in length—which could lead to half-life issues. "Small peptides generally provide only a few minutes of action if you inject them intravenously. This could explain why the Duke team injected the compound intramuscularly in the mice," Van Vlasselaer told *SciBX*. "Because the researchers had to inject it intramuscularly, daily, this is probably not a

very good starting compound for drug development."

Pap said it isn't yet clear which molecule, CXCL10 or SDC4, should be upregulated.

"While there are a lot of issues to address with this potential method, there is certainly potential if the team could modify the compound to have a good pharmacokinetic profile, which would allow subcutaneous and less frequent dosing," concluded Van Vlasselaer.

The authors of the *JCI* paper declined requests for interviews. The patenting and licensing statuses were not available.

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