Balancing act in liver fibrosis

By Michael J. Haas, Senior Writer

CXCR7, one of the few chemokine receptors against which there are virtually no drug development efforts, may be a novel target for liver fibrosis, according to new findings from a U.S. team.¹ The results also pointed to the well-trodden family member CXCR4 in the indication, and the next steps are to figure out whether to deliver systemic or liver-specific modulators of the targets.

Liver fibrosis is the result of aberrant healing processes in response to acute or chronic liver injury. Although liver endothelial cells are capable of triggering liver regeneration following acute injury,²⁻⁴ chronic injury can overactivate endothelial cells, thereby promoting fibrosis instead of regeneration for reasons that are poorly understood.

Because endothelial cells also are involved in organ development during embryogenesis, a U.S. team led by Shahin Rafii hypothesized that liver development and regeneration might share endothelial cell signaling pathways that could be targeted to prevent or treat liver fibrosis.

Rafii is a professor of genetic and regenerative medicine at **Weill Cornell Medical College** and an investigator at the **Howard Hughes Medical Institute**.

The team found clues supporting this hypothesis in published studies. The papers showed that signaling between chemokine CXC motif ligand 12 (Cxcl12; Sdf-1) and its receptors—Cxcr7 (CXC chemokine receptor 7) and Cxcr4 (Npy3r)—on endothelial cells was required for normal embryonic development of the GI tract and heart in mice and might be involved in pathological remodeling of those tissues after injury.⁵⁻⁷

To determine whether the SDF-1 signaling pathway plays a role in liver regeneration and fibrosis, the team began by examining the effects of acute and chronic liver injury on levels of Cxcr7 and Cxcr4 in mouse models.

The team found that acute liver injury increased Cxcr7 levels on liver endothelial cells compared with no injury but did not affect Cxcr4 levels. Chronic liver injury increased levels of Cxcr7, Cxcr4 and fibroblast growth factor receptor 1 (Fgfr1; Cd331) on liver endothelial cells compared with cells from uninjured controls.

Next, the team looked at mouse models of chronic liver injury that had endothelial cell-specific knockout of *Cxcr7*, *Cxcr4* or *Fgfr1*. *Cxcr7* deficiency decreased levels of inhibitor of DNA binding 1 (Id1)—a

transcription factor essential for liver regeneration²—and increased hepatic levels of collagen and other profibrotic factors compared with wild-type expression. Conversely, deficiency in *Cxcr4* or *Fgfr1* decreased hepatic levels of collagen and other profibrotic factors.

In wild-type mouse models of chronic liver injury, a CXCR7 agonist increased Id1 expression and decreased hepatic levels of profibrotic factors compared with vehicle.

Additional experiments in normal human liver endothelial cell lines showed that stimulation of FGFR1 signaling upregulated CXCR4 and downregulated CXCR7.

Lastly, the team showed that SDF-1 or a CXCR7 agonist upregulated proregenerative ID1 in wild-type human liver endothelial cell culture. SDF-1 did not upregulate ID1 in liver endothelial cells deficient in either CXCR4 or CXCR7—indicating that induction of ID1 requires cooperation between the two receptors, the team wrote in its report in *Nature*.

Seventh heaven

Collectively, the findings show that agonizing CXCR7, or antagonizing CXCR4 or FGFR1, might treat or prevent liver fibrosis, Rafii told SciBX. Of the three targets, he said that CXCR7 appears most promising.

In the mouse models “the CXCR7 agonist induced an endothelial cell response that not only prevented fibrosis but also preferentially stimulated regeneration,” said Rafii. “It’s conceivable that specific activation of CXCR7 would induce regeneration of functional liver tissue and cells while avoiding irreversible scar formation” after acute liver injury.

Bi-Sen Ding, first author on the study, said that CXCR4 and FGFR1 were less

promising because both proteins are involved in normal wound healing and angiogenesis in many tissues. Moreover, CXCR4 is required to stimulate proregenerative ID1 in liver endothelial cells.

“Inhibiting CXCR4 or FGFR1 might interfere with normal healing and regeneration processes that are required for liver repair,” he said. Ding is an assistant professor of genetic medicine at Weill Cornell.

The team included researchers from **Hofstra University**, **Yale School of Medicine**, **Angiocrine Bioscience Inc.** and **ChemoCentryx Inc.** Angiocrine isolated and cultured the mouse liver endothelial cells, and ChemoCentryx helped design the *Cxcr7* knockout experiments and interpreted the CXCR7 agonist data.

There are no disclosed CXCR7 agonists in development. ChemoCentryx’s CCX650 is the only disclosed CXCR7 antagonist. The small molecule is in preclinical testing to treat brain cancer.

Multiple companies are developing inhibitors of CXCR4 or FGFR1, although none is being tested in liver fibrosis.

Sanofi markets Mozobil plerixafor, a synthetic CXCR4 antagonist, to treat non-Hodgkin’s lymphoma (NHL) and multiple myeloma (MM). The drug also is in Phase I testing to treat acute myelogenous leukemia (AML). At least six other companies have CXCR4 antagonists

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TCM Biotech International Corp.

in preclinical to Phase II testing to treat cancer, cardiovascular diseases, ophthalmic disorders and/or hematological diseases.

At least seven companies have compounds that inhibit FGFR1 in Phase II to Phase III testing to treat hematological malignancies and solid tumors.

“The team’s results provide a therapeutic road map to achieve hepatic regeneration without provoking fibrosis following liver injury,” said Jim Chen, a project manager at **TCM Biotech International Corp.**

TCM’s TCM-808FB is in preclinical development to treat and prevent liver fibrosis. The company has not disclosed the compound’s target or therapeutic modality.

Chen said that the team also showed that activation of CXCR7 and CXCR4 on liver endothelial cells regulated the recruitment of macrophages and inflammatory molecules involved in liver regeneration and fibrosis. “This suggests that the balance between those two processes is not determined solely by the two chemokine receptors,” he said.

Thus, Chen wants to know more about how other immune system and inflammatory factors affect the CXCR7-ID1 and FGFR1-CXCR4 pathways following liver injury.

He also said that it is not yet clear whether systemic or liver-specific delivery of therapies targeting CXCR7 or CXCR4 would be safer and thus preferable.

Rafii and Ding agreed that further work needs to be done to explore the question of delivery modes but said that their findings already hint at the answer.

“Systemic therapies should work to a certain extent,” Rafii said. “But liver-specific delivery—or even liver endothelial cell-specific delivery—would be the optimal strategy, especially when devising approaches to inhibit CXCR4 or FGFR1,” in order to avoid interfering with their roles in healing and angiogenesis in other organs and tissues.

Alternatively, priming endothelial cells with SDF-1 or a CXCR7 agonist and then transplanting the cells into the liver “would be an ideal cell-based therapeutic strategy to stimulate regeneration without

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**—Shahin Rafii,
Weill Cornell Medical College**

causing fibrosis,” Ding said.

Nevertheless, identifying liver-specific or cell-based therapies for liver fibrosis is not the team’s top priority. “We first plan to test specific agonists of CXCR7 in the mouse models of liver injury to thoroughly investigate the potential therapeutic value of our findings,” Rafii said.

“In our ongoing work, we also want to delineate the differential functions of CXCR4 in stimulating regeneration and provoking

fibrosis,” Ding said. “We hope to identify a specific approach or mechanism that enables proregenerative cooperation between CXCR4 and CXCR7.”

According to Ding, Weill Cornell has filed a patent application covering the findings, and the IP is available for licensing.

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