Published in final edited form as:

Gastroenterology. 2014 June ; 146(7): 1823–1825. doi:10.1053/j.gastro.2014.04.010.

NOVEL FATE-TRACING STRATEGIES SHOW THAT HEPATIC STELLATE CELLS MEDIATE FIBROSIS IN VIVO

SHUANG LIANG,

Division of Gastroenterology Department of Medicine University of California San Diego La Jolla, California

ZHENYU ZHONG, and

Laboratory of Gene Regulation and Signal Transduction, Department of Pharmacology, School of Medicine, University of California San Diego, La Jolla, California

EKIHIRO SEKI

Division of Gastroenterology, Department of Medicine, University of California San Diego, La Jolla, California

Mederacke I, Hsu CC, Troeger JS, et al. Fate tracing reveals hepatic stellate cells as dominant contributors to liver fibrosis independent of its aetiology. *Nat Commun* 2013;4:2823.

Liver fibrosis often arises as a result of chronic liver damage and is characterized by the deposition of extracellular matrix (ECM) proteins, including collagen at injury sites, which can impair normal liver function and may lead to portal hypertension and hepatic failure (N Engl J Med 2004;350:1646–1654). Clinically, liver fibrosis leads to severe morbidity and mortality in patients (J Clin Invest 2005;115:209–218). Myofibroblast, a cell population that produce ECM proteins, has been shown as the mediator for liver fibrogenesis. However, the identity of cellular sources that give rise to myofibroblast remains elusive.

In a recent article published in *Nature Communications*, Mederacke et al discovered lecithin-retinol acyltransferase (Lrat) as a novel marker for hepatic stellate cells (HSCs). The expression of Lrat was distinctively high in HSCs, whereas other cells, such as hepatocytes, Kupffer cells, cholangiocytes, or endothelial cells, had undetectable Lrat expression. Consistent with its unique expression pattern, Lrat has been shown to play a pivotal role in regulating the formation of retinyl ester-containing lipid droplet, the signature for quiescent HSCs. With the help of this newly discovered marker for HSCs, Mederacke et al studied the precise contribution of HSCs in promoting liver fibrosis in vivo. Briefly, they designed a novel fate-tracing strategy by generating transgenic mice that have Cre recombinase expression cassette under the control of murine Lrat promoter. These animals were then crossed with floxed Zs-Green fluorescent mice; therefore, HSCs in the progeny mice would be labeled by Zs-Green fluorescence. Mederacke et al first confirmed that Lrat-Cre-labeled cells are mostly (>99%) HSCs, because Lrat-Cre expression and known markers of HSCs (ie, desmin and *Pdgfr β*) almost completely overlapped. To investigate the precise role of HSCs in promoting liver fibrogenesis in vivo, they performed fate-tracing experiments in several different fibrotic animal models, including toxin-induced liver fibrosis models (ie, carbon tetrachloride-induced and thioacetamide-induced liver fibrosis models) and biliary

liver fibrosis models (ie, bile duct ligation model, 3,5-diethoxycarbonyl-1,4-dihydrocollidine-containing diet model and Mdr2^{KO} mouse model). Strikingly, Mederacke et al found that HSCs are the dominant and universal cell population that gives rise to collagen-secreting myofibroblasts in various murine liver fibrosis models. This was also confirmed by the reduction of liver fibrosis in mice with depletion of HSCs using Lrat-induced diphtheria toxin receptor, which further strengthens the concept that HSCs are the responsible cell types in hepatic fibrosis in general.

Another important finding by Mederacke et al is that HSCs do not give rise to epithelial cells after liver damage. They found no increase in the numbers of Lrat-Cre and HNF4a (a hepatocyte marker) double-positive cells during liver regeneration after liver damage caused by 7 different hepatic injury models, including 70% partial hepatectomy model. In addition, by employing series of experiments using bone marrow transplantation, Mederacke et al further concluded that HSCs are endogenous to liver, rather than a bone marrow-derived cell population.

Comment

Although controversy exists, it is generally believed that different liver fibrosis-promoting agents (eg, alcohol abuse, obesity, viral infection, and cholestasis) may possess distinct mechanisms to induce fibrogenesis. Consistent with this notion, it is thought that depending on the cause of liver damage, different hepatic cell populations (eg, HSCs, hepatocytes, endothelial cells, portal fibroblasts, and bone marrow-derived fibrocytes) may be capable of giving rise to collagen-producing myofibroblasts to mediate liver fibrogenesis (Hepatology 2010;51:1438–1444; Gastrointest Liver Physiol 2013;304:G605–G614; Proc Natl Acad Sci U S A 1985;82:8681–8685; J Hepatol 2006;45:429–438). Because no Food and Drug Administration–approved anti-fibrotic drugs are available currently, the identification of cell population(s) that give rise to hepatic myofibroblasts is crucial for developing new therapeutic interventions for liver fibrosis. It has long been believed that HSCs are primary cell types to produce ECM proteins, such as collagen during liver fibrosis. Although quiescent HSCs store retinoid lipid droplets and do not produce collagen, they will, after activation by inflammatory and fibrogenic cytokines (eg, transforming growth factor- β , platelet-derived growth factor [PDGF]), lose lipid droplets, express α -smooth muscle actin and start to produce collagen, which contributes to the development of liver fibrosis. Although purified HSCs produce collagen after activation in vitro, the precise contribution of HSCs to liver fibrosis has not been convincingly determined in vivo, possibly owing to the lack of unique markers for HSCs (Proc Natl Acad Sci U S A 1985;82:8681–8685).

Mederacke et al have made a key finding—the identification of a novel useful marker for HSCs, Lrat, that expresses exclusively in HSCs. This remarkable feature warrants the use of Cre driven by Lrat promoter as a faithful reporter for marking HSCs in vivo. Interestingly, a number of previous studies used Cre driven by human or murine glial fibrillary acidic protein promoter (GFAP-Cre) as a mean to study the contribution of HSCs in liver fibrosis (Stem Cells 2008;26:2104–2113; Gastroenterology 2012;142:938–946; Cell 2013;153:449–460). However, in contrast with these studies, Mederacke et al demonstrated that GFAP has rather limited expression in HSCs and, more important, is also expressed in cholangiocytes. Based

on the observations by Mederacke et al, we need to reevaluate the contribution of HSC to liver fibrogenesis described in the previous studies in which the GFAP-Cre strategy was deployed for labeling or depleting HSCs (Stem Cells 2008;26:2104–2113; Gastroenterology 2012;142:938–946; Cell 2013;153:449–460). These controversial results are likely owing to differences in the expression levels of Cre recombinase and/or floxed allele in the different reporter or mutant mice. Nonetheless, future study is needed to resolve the discrepancy.

To further confirm that Lrat-Cre–labeled Zs-Green positive cells are HSCs, Mederacke et al found that expression of Zs-Green fluorescence (proposed to mark HSCs) almost completely overlapped with the expressions of widely accepted HSC markers (ie, desmin and $\text{Pdgfr}\beta$), but not with the markers for other liver cell types, such as hepatocytes, Kupffer cells, and cholangiocytes. To further study the role of HSCs in promoting liver fibrogenesis in vivo, Mederacke et al sequentially investigated the involvement of HSCs in 6 different models of liver fibrosis, ranging from toxic and biliary liver fibrosis to fatty liver disease. Strikingly, in all of the fibrotic models tested, HSCs seemed to be the dominant and universal player that give rise to collagen-producing myofibroblasts, whereas other liver cells, such as cholangiocytes, hepatocytes, and endothelial cells, are not as important as HSCs. Mederacke et al also noted the existence of a small population of collagen-producing cells were not labeled by Lrat-Cre (they were defined as portal fibroblast-like cells), especially in cholestatic fibrosis models. These cells did not possess typical features for HSCs (ie, retinoid lipid droplets, and expression of L-rat, Lhx2 and HGF) and displayed distinct morphology compared with HSCs. Although these portal fibroblast-like cells were less abundant and had lower expression of fibrogenic genes than HSCs, the contribution of portal fibroblasts in cholestatic liver fibrosis has been extensively investigated (Hepatology 2010;51:1438–1444). Therefore, we cannot completely exclude their roles in promoting fibrosis in cholestatic liver diseases. Designing specific markers and further analyzing the importance of portal fibroblasts in mediating biliary fibrogenesis are required. Moreover, from a technical point of view, because Lrat-Cre can label >99% HSCs, this unique feature could facilitate HSC-specific functional study of genes of interest, including the ones that have been previously tested in HSCs using different reporter models, such as GFAP-Cre mice.

Another very important finding by Mederacke et al is that they provide evidence to exclude HSCs as the progenitors for liver epithelial cells. Fate-tracking studies performed previously by others using hGFAP-Cre and α -smooth muscle actin–CreERT2 mice indicate that HSCs might give rise to (24%) hepatocytes in liver fibrosis induced by methionine-choline–deficient ethionine-supplemented diet and bile duct ligation (Stem Cells 2008;26:2104–2113; J Clin Invest 2013;123:2380–2394). However, owing to the lack of specificity of hGFAP-Cre labeling as discussed, the data generated using such hGFAP-Cre reporter mice are inconclusive. Mederacke et al reexamined this question by employing Lrat-Cre fate-tracking strategy to determine if HSCs could give rise to hepatic epithelial cells under a number of liver injury and regeneration models. Strikingly, HSCs did not seem to give rise to hepatocytes after liver damage or partial hepatectomy. Similarly, Mederacke et al demonstrated that Lrat-Cre–labeled cells give rise to neither cytokeratin-positive liver progenitor cells nor cholangiocytes in the models of chronic liver injury including

cholestatic liver injury. These results provide solid support to a previously claimed notion that hepatocytes and bipotential progenitors are sufficient for hepatic epithelial regeneration (Gastroenterology 2012;143: 1564–1575; J Clin Invest 2011;121:4850–4860; Science 2008;322:1490–1494).

Although the study revealed that the majority of collagen producing cells are of HSC origin in mouse liver fibrosis models, it is uncertain whether HSCs would be a universal target for the treatment of human liver fibrosis because the pathogenesis of human liver fibrosis is more complicated than that of rodents. There is no suitable rodent model that resembles human hepatitis B and C, which limits the determination of the universal contribution of HSCs in all liver fibrosis. In addition, the study did not test for alcoholic liver fibrosis, which accounts for 44% of liver-related death in the United States. Currently, we do not have adequate animal models to study this type of liver fibrosis. Nonetheless, the study by Mederacke et al clearly demonstrated HSC as the dominant and universal player that produces collagen and other ECM contents to promote the mouse models of liver fibrosis. The technology using Lrat-Cre mice with floxed mutant mice will greatly improve more precise functional analysis of pathways or molecules specifically in HSCs in liver fibrosis. Based on the results generated by using Lrat-Cre mice, targeting specific pathways or molecules to induce HSC apoptosis or inhibit HSC activation or collagen production may be effective preventive/therapeutic approaches for human liver fibrosis.