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Reversion of hepatic stellate cell to a quiescent phenotype: From myth to reality?

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COMMENTARY ON:

Myofibroblasts revert to an inactive phenotype during regression of liver fibrosis. Kisseleva T, Cong M, Paik Y, Scholten D, Jiang C, Benner C, Iwaisako K, Moore-Morris T, Scott B, Tsukamoto H, Evans SM, Dillmann W, Glass CK, Brenner DA. Proc Natl Acad Sci U S A. 2012 Jun 12;109(24):9448-53. Copyright (2012). Abstract reprinted by permission from the National Academy of Sciences, USA.

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Abstract: *Myofibroblasts produce the fibrous scar in hepatic fibrosis. In the carbon tetrachloride (CCl(4)) model of liver fibrosis, quiescent hepatic stellate cells (HSC) are activated to become myofibroblasts. When the underlying etiological agent is removed, clinical and experimental fibrosis undergoes a remarkable regression with complete disappearance of these myofibroblasts. Although some myofibroblasts apoptose, it is unknown whether other myofibroblasts may revert to an inactive phenotype during regression of fibrosis. We elucidated the fate of HSCs/myofibroblasts during recovery from CCl(4)- and alcohol-induced liver fibrosis using Cre-LoxP-based genetic labeling of myofibroblasts. Here we demonstrate that half of the myofibroblasts escape apoptosis during regression of liver fibrosis, down-regulate fibrogenic genes, and acquire a phenotype similar to, but distinct from, quiescent HSCs in their ability to more rapidly reactivate into myofibroblasts in response to fibrogenic stimuli and strongly contribute to liver fibrosis. Inactivation of HSCs was associated with up-regulation of the anti-apoptotic genes Hspa1a/b, which participate in the survival of HSCs in culture and in vivo.*

AND

Deactivation of hepatic stellate cells during liver fibrosis resolution in mice. Troeger JS, Mederacke I, Gwak GY, Dapito DH, Mu X, Hsu CC, Pradere JP, Friedman RA, Schwabe RF. Gastroenterology. 2012 Oct;143(4):1073-83.e22. Copyright © 2012. Abstract reprinted by permission from the AGA Institute.

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Abstract: *Background & Aims: Activated hepatic stellate cells (HSCs), the main fibrogenic cell type in the liver, undergo apoptosis after cessation of liver injury, which contributes to resolution of fibrosis. In this study, we investigated whether HSC deactivation constitutes an additional mechanism of liver fibrosis resolution.*
Methods: HSC activation and deactivation were investigated by single-cell PCR and genetic tracking in transgenic mice that expressed a tamoxifen-inducible CreER under control of the endogenous vimentin promoter (Vimentin-CreER).
Results: Single-cell quantitative polymerase chain reaction demonstrated activation of almost the entire HSC population in fibrotic livers, and a gradual decrease of HSC activation during fibrosis resolution, indicating deactivation of HSCs. Vimentin-CreER marked activated HSCs, demonstrated by a 6- to 16-fold induction of a membrane-bound green fluorescent protein (mGFP) Cre-reporter after injection of carbon tetrachloride, in liver and isolated HSCs, and a shift in localization of mGFP-marked HSCs from peri-sinusoidal to fibrotic septa. Tracking of mGFP-positive HSCs revealed the persistence of 40%-45% of mGFP expression in livers and isolated HSCs 30-45 days after carbon tetrachloride was no longer administered, despite normalization of fibrogenesis parameters; these findings confirm reversal of HSC activation. After fibrosis resolution, mGFP expression was observed again in desmin-positive peri-sinusoidal HSCs; no mGFP expression was detected in hepatocytes or cholangiocytes, excluding mesenchymal-epithelial transition. Notably, reverted HSCs remained in a primed state, with higher levels of responsiveness to fibrogenic stimuli.
Conclusions: In mice, reversal of HSC activation contributes to termination of fibrogenesis during fibrosis resolution, but results in higher responsiveness of reverted HSCs to recurring fibrogenic stimulation.

Activation of hepatic stellate cells (HSC) in response to chronic liver injury is a key step in the pathogenesis of liver fibrosis. The activation process is characterized by a phenotypic switch from a quiescent vitamin A-rich to a myofibroblastic fibrogenic phenotype [1] (Fig. 1). Fibrosis has long been viewed as an irreversible process, but convincing clinical and experimental studies have demonstrated that fibrosis resolution may occur upon eradication of the liver insult [2]. Experimental models of fibrosis recovery consistently reported that elimination of activated HSC by apoptosis [3] or senescence [4] precedes restoration of fibrolytic pathways and regression of fibrosis, suggesting that clearance of activated HSC is a key step in the onset of fibrosis regression.

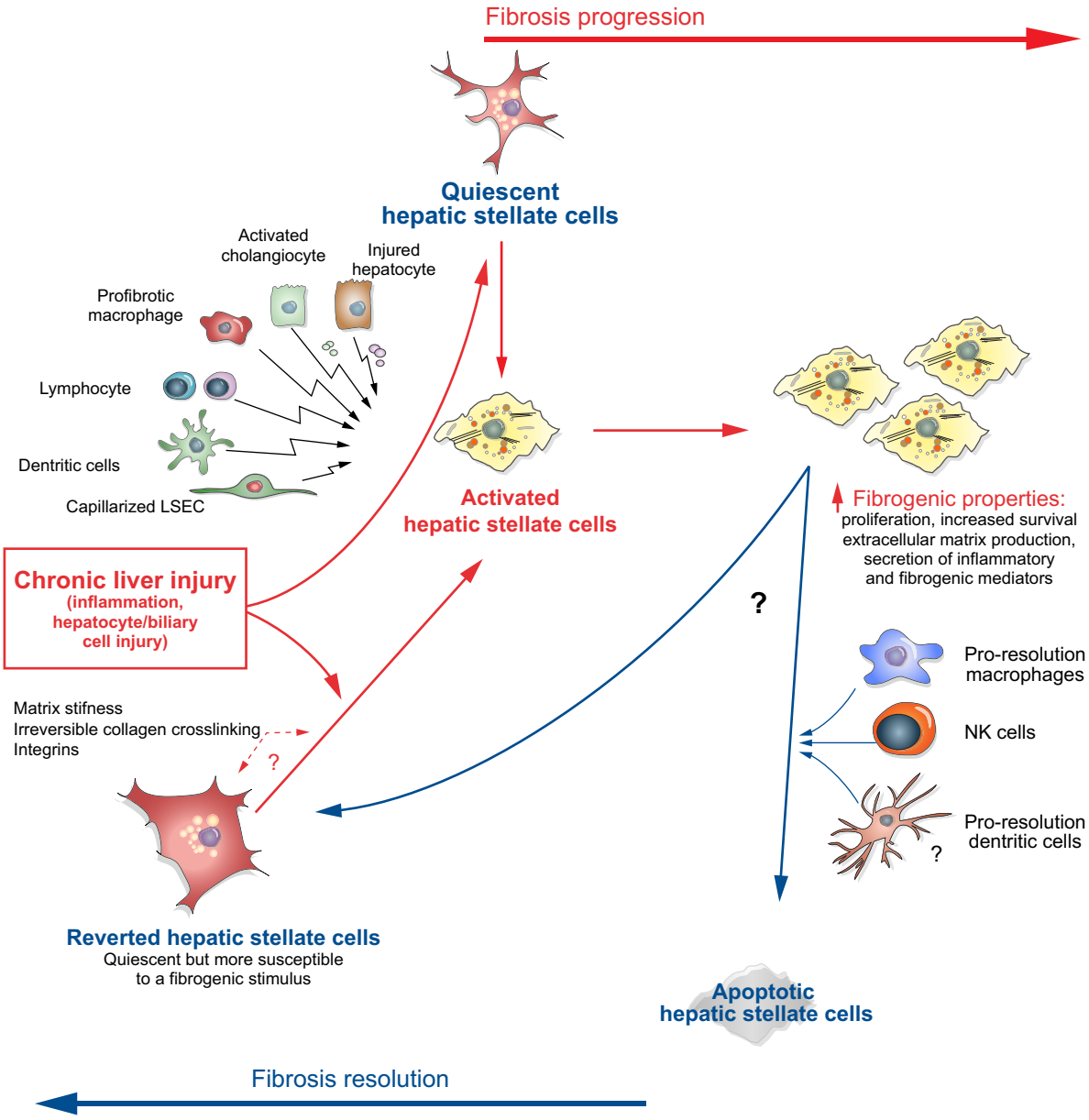

Fig. 1. Fate of hepatic stellate cells during liver fibrosis progression and resolution. Upon liver injury, HSCs undergo transdifferentiation from a quiescent to a myofibroblastic phenotype. The process is orchestrated by interactions with neighboring parenchymal and non-parenchymal cells (immune cells, capillarized liver sinusoidal endothelial cells), and by cell matrix components, interactions mediated through integrins, and matrix stiffness. Activated HSCs are characterized by enhanced capacity to proliferate and produce proinflammatory and profibrogenic mediators and are responsible for secreting extracellular matrix that characterizes liver fibrosis. Clearance of activated HSC is a key step in fibrosis regression, and may result from apoptosis, promoted by NK cells, dendritic cells, and proresolution macrophages, or from reversion to a quiescent phenotype. The studies of Troeger and Kisseleva demonstrate significant reversion/inactivation of myofibroblastic HSC in animals undergoing fibrosis recovery. However, deactivated HSC do not fully revert to a quiescent state, but retain a preactivated intermediate state and show enhanced susceptibility to subsequent insult.

Although culture studies suggested that disappearance of activated HSC may also result from reversal to a quiescent phenotype, whether deactivation may occur *in vivo* during fibrosis regression remained unsolved. In two recent reports, Kisseleva *et al.* [5] and Troeger *et al.* [6] elegantly address this issue, using a combination of genetic cell fate tracking approach [5,6], single cell PCR [6], and microarray analysis [5,6].

Both groups used mouse models in which quiescent and activated HSC can be permanently stamped and tracked with a fluorescent label at the onset of fibrosis and during resolution. Kisseleva *et al.* [5] generated a type I collagen promoter-driven Cre-mediated removal of a STOP cassette in a reporter (YFP) mouse strain. Because only activated hepatic myofibroblasts express collagen I, they can be identified by YFP labeling. Liver

fibrosis was induced by chronic carbon tetrachloride administration or intragastric alcohol feeding in *Cola1(1)*$^{Cre\text{-}YFP}$ or *Cola2(1)*$^{Cre\text{-}YFP}$ mice, and the fate of YFP-labeled HSC was followed in mice that were allowed to recover for 4–7 weeks. As previously reported, recovery was associated with a marked decrease in the density of α-smooth muscle actin (α-SMA)-positive cells and a parallel normalization of fibrosis parameters. More surprisingly, few apoptotic YFP positive cells were detected (2.6%) whereas hepatic YFP labeling persisted in 50% of cells expressing vitamin A. The low rate of apoptosis compared to previous studies [3] might be due to the ephemeral nature of apoptosis, but nevertheless, these findings argue for the reversion of a significant number of myofibroblastic HSC to a quiescent state during the recovery phase. Similar findings were obtained by Troeger et al [6]. They followed the fate of activated hepatic stellate cells using a bacterial artificial chromososme transgenic mice expressing a tamoxifen-inducible CreER driven by the endogenous promoter for vimentin (an intermediate filament protein highly expressed in myofibroblasts), that were crossed to a fluorescent (mGFP) mouse reporter mice. Activated HSC were tracked in mice exposed to carbon tetrachloride, thioacetamide or bile duct ligation during fibrosis progression and recovery. In addition, FACS sorting and single cell PCR analysis of mGFP labeled cells, isolated at various times after cessation of injury, indicated that the expression of characteristic fibrogenic markers of activated HSC (collagen I, TIMP1, a-SMA, TGF-bR) gradually decreases over time during the recovery period.

Intriguingly, comparative gene analysis showed that although reverted HSC reacquire several features of quiescence, they also display specific characteristics with respect to quiescent stellate cells that had never been exposed to a fibrogenic stimulus. Reverted HSC in culture displayed enhanced susceptibility to subsequent activation by TGF-β1 or mitogens [5,6]. These data indicated that reverted HSC do not fully revert to a quiescent state, but rather retain a preactivated intermediate state (Fig. 1). Interestingly, administration of a second round of carbon tetrachloride after a 6-month recovery period induced more severe fibrosis, compared to mice that received one round of the hepatotoxin, suggesting that reverted HSC may account for more rapid and more severe fibrosis progression upon recurrence of liver injury. Characterization of the mechanisms that maintain HSC in an "intermediate" phenotype is required to fully understand why activated HSC only partially deactivate upon cessation of the insult, but this issue was not addressed in either study. In this respect, whether irreversible changes in the hepatic microenvironment within the injured liver underlie partial phenotype reversion needs to be considered. Indeed, matrix stiffness, cell matrix components such as collagen 1 and integrins, are known to drive and maintain HSC in the activated state [1,7], and it has been reported that irreversible collagen cross-linking persists even after long-term recovery from fibrosis [8] (Fig. 1). In addition, the role of neighboring cells also warrants further investigation, in particular the liver sinusoidal endothelial cells, which control HSC activation/deactivation [9].

Altogether, the findings of Kisseleva and Troeger broaden our current view of fibrogenic cell heterogeneity based on their diverse origin, including HSC, portal myofibroblasts, bone marrow-derived fibroblasts and fibrocytes. Indeed, these studies identify hepatic stellate cells as a plastic population that can adopt a wide range of phenotypes, ranging from a fully quiescent to an activated myofibroblastic state, as described for myeloid cells. Nevertheless, a number of issues remain unresolved. In particular, whether the inactivation process is specific to HSC or also affects other liver fibrogenic cells remains to be determined. This is an important point, given the predominant role of specific liver fibrogenic cell populations according to the cause of liver injury, as illustrated by the major contribution of portal myofibroblasts to biliary cirrhosis [10]. Another major question relates to the characterization of the mechanisms regulating elimination of activated HSC by apoptosis or phenotype reversion, and the functional consequences of the two pathways on fibrosis resolution. In this respect, the study of Kisseleva *et al.* [5] suggests that anti-apoptotic signals such as HSPa1a/b are specifically expressed by HSC undergoing inactivation and participate in the survival of HSCs in culture and *in vivo*. Although Troger *et al.* did not confirm these findings [6], these data suggest that the balance between pro and anti-apoptotic proteins may represent one of the key mechanisms that govern the fate of activated HSC. Finally, determining whether the reverted phenotype represents an ultimate state or if inactivated cells can further evolve towards a quiescent phenotype will also be a critical issue to be addressed.

These two convergent studies provide compelling experimental evidence supporting *in vivo* HSC reversion during fibrosis recovery (Fig. 1). The question as to the causal relationship between HSC reversion and fibrosis resolution still remains open as well as to the respective roles of apoptotic *vs.* reverted HSCs in the recovery process. Determination of the clinical relevance of the current findings is another major issue that might prove challenging, given the current lack of assays available for HSC fate tracking in humans.

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Conflict of interest

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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