

# Reversal of Hepatic Fibrosis — Fact or Fantasy?

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The prospect of reversing hepatic fibrosis has generated great interest now that basic science advances are being translated into promising new antifibrotic therapies. It is appropriate to recognize both the historical advances that created the framework for these successes, and the important role that HEPATOLOGY has played in disseminating them. A sense of urgency underlies this effort as the epidemics of HCV and NASH are becoming associated with advancing fibrosis. To maintain progress and minimize confusion among investigators and clinicians it is essential to standardize terms referring to fibrosis ‘reversal’ and ‘regression.’ There must also be rapid optimization of non-invasive markers of fibrosis to relieve this current bottleneck to conducting clinical trials. Progress in identifying genetic determinants of fibrosis could further refine patient selection for clinical trials and shorten their duration, as well as unearthing new directions of scientific inquiry. Realistic expectations for successful anti-fibrotic therapies reflect solid evidence of fibrosis regression in patients treated effectively for viral liver disease, as well as growing clarity in the understanding mechanisms of extracellular matrix production and degradation. The paradigms of stellate cell activation and apoptosis remain valuable frameworks for understanding pathways of hepatic fibrogenesis and fibrosis regression, respectively. Continued progress is essential in order to identify the determinants and dynamics of fibrosis reversibility, to discover additional targets for anti-fibrotic therapy, and to develop customized multi-drug regimens. These advances are sure to be captured in the next 25 years by HEPATOLOGY, and to profoundly impact the prognosis of patients with chronic liver disease. (HEPATOLOGY 2006;43:S82-S88.)

Few topics in hepatology spark as much excitement, anticipation, and controversy as the potential to reverse hepatic fibrosis and cirrhosis in patients with chronic liver disease. This 25th anniversary of HEPATOLOGY provides a timely opportunity to review progress in this area, mark the specific contributions of the journal to this rapidly advancing field, and point toward how this area is likely to evolve in the next 25 years. In doing so, we hope to establish the facts, which are accumulating rapidly, and to offer the fantasy of how these advances are likely to transform the care of patients with hepatic fibrosis.

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Abbreviations: MMP, matrix metalloproteinase; NASH, nonalcoholic steatohepatitis; HCV, hepatitis C virus; TIMP, tissue inhibitor of matrix metalloproteinase.

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## Treasures From the Past

With the tremendous progress in understanding hepatic cellular and molecular biology in the past 25 years, it is easy—but incorrect—to assume that the concept of fibrosis reversal is entirely a contemporary idea. Although in reviewing historical accounts of liver disease it is difficult to separate descriptions of the regenerative capacity of liver from the actual reversal of fibrosis (indeed the two are probably mechanistically linked), this omission likely reflects the silent nature of hepatic fibrosis, such that earlier descriptions of the fibrotic response were restricted only to autopsy findings of end-stage liver disease, for example, Mallory’s 1914 observation that “. . . cirrhosis is not a progressive chronic process, but an end-product.”<sup>1</sup> Thus, early changes associated with injury, which are accompanied by vigorous regeneration and matrix remodeling, were likely overlooked until liver biopsy became available.

However, several late 20th century publications specifically point to the capacity of the injured liver to resorb scar. For example, Popper and Udenfriend,<sup>2</sup> in a 1970 review, emphasized the importance of enzymatic processes to fibrosis regression, downplaying the role of cellular phagocytosis, and identifying potential determinants of irreversibil-

ity. This paper was followed by the first description of a physiological collagenase in liver in 1974 by Okazaki and Maruyama.<sup>3</sup> In this study, degradative activity of liver explants toward denatured collagen within a culture dish was present in rat liver from animals treated for 6 weeks with CCl<sub>4</sub>, whereas it was greatly reduced in livers with irreversible cirrhosis after more extended exposure. Although not clear to the authors at that time, this method was actually identifying gelatinase activity (*i.e.*, degradation of denatured rather than intact, triple helical collagen), which, based on current understanding, probably reflected activity of either matrix metalloproteinase-2 (MMP-2) and/or MMP-9. Finally, in a truly seminal 1979 review entitled “Cirrhosis of the liver: a reversible disease?”, Perez-Tamayo<sup>4</sup> catalogued evidence for reversibility of fibrosis and cirrhosis in both animal models and human disease, systematically enumerating key points that merit emphasis 27 years later, including the notions that “all experimental models of cirrhosis are reversible providing that the inciting agent is discontinued and sufficient time is allowed. . .”; and that “increased reticulum fibers are more easily resorbed than thick collagenous bundles.”<sup>4</sup>

HEPATOLOGY was launched within 2 years of that seminal review, which was perfectly timed to exploit the new knowledge encapsulated in Perez-Tamayo’s treatise. Remarkably, over the ensuing 25 years the journal has published almost exactly 250 papers dealing with hepatic fibrosis.\* The first paper of this type in the inaugural issue characterized collagen synthesis in murine schistosomiasis,<sup>5</sup> and the second was a description of a granuloma collagenase.<sup>6</sup> Also included in the very early years was a report on the use of cell culture to track collagen production by liver-derived cells,<sup>7</sup> a description of nonparenchymal smooth-muscle like cells from needle biopsies of fibrotic liver,<sup>8</sup> and one of the first characterizations of hepatic stellate cells (referred to as ‘fat-storing cells’) in primary culture.<sup>9</sup> Then, as now, HEPATOLOGY was a highly visible platform for the growth of the specialty in general, and the emergence of the field of hepatic fibrosis in particular.

### Cirrhosis “Reversal” and “Regression”—What’s in a Name?

A discussion of hepatic fibrosis reversal first requires a clarification of terms. The phrase “reversal of cirrhosis” is often used to imply a complete restoration of normal architecture after the establishment of cirrhosis, a concept that has provoked understandable skepticism by some ex-

perts in the field.<sup>10</sup> However, no accord has been reached over this term’s meaning, and thus confusion persists. A more palatable working definition would be to refer instead to “regression” of fibrosis or cirrhosis, indicating that the fibrosis content is less than earlier, without quantifying the extent of regression or suggesting that the histology has returned completely to normal. A concept of equal clinical importance is the potential to achieve “stasis” of fibrosis, or lack of progression in the face of continued liver injury. Such an outcome—in response to treatment of the underlying disease or to effective antifibrotic therapy—would be a great therapeutic advance in patients who are asymptomatic with well-preserved liver function, as it might ensure that such individuals eventually die of other causes “with” liver fibrosis rather than dying “of” cirrhosis.

### Diagnosis of Fibrosis, the Bottleneck to Progress in Clinical Trials

The proposal to use these terms raises the issues of how to quantify the regression of fibrosis, and whether methods for analyzing fibrosis regression should be the same as those used to assess progression. Currently, the lack of robust markers of fibrosis represents the single greatest factor limiting both the validation of progression or regression of fibrosis, and the testing of antifibrotic therapies in clinical trials. Percutaneous liver biopsy remains the gold standard for assessing fibrosis, but it is tainted by an increasingly sober recognition of its limitations. Three fibrosis scoring methods of biopsies are in widest use, the Ishak score,<sup>11</sup> the Metavir score,<sup>12</sup> and the Desmet/Scheuer staging system,<sup>13</sup> and all were developed before the idea of fibrosis regression gained traction. Each relies on progressive development of periportal, then septal fibrosis and finally nodule formation. The key distinguishing feature is the presence of two cirrhotic stages (5 and 6) in Ishak, and only one in the Metavir and Desmet/Scheuer systems. Inter-observer variability is low in all three systems; however, sampling error may be as great as 33% to 50%, as demonstrated by a study in which laparoscopic biopsy specimens were obtained from both lobes of patients with HCV, yet one third of 124 patients had differences of at least 1 stage between the 2 lobes<sup>14</sup>; follow-up reports from the same group suggest an even higher sampling error, with additional discrepancies between the gross appearance of the liver and the underlying pathological stage.<sup>15</sup> Sampling error is also emerging as a significant diagnostic obstacle in nonalcoholic steatohepatitis (NASH).<sup>16</sup>

Two key features determining accuracy of liver biopsy are length and width, with a minimum of 2.5 cm generally required to achieve reproducible sampling.<sup>17</sup> At best,

\*Based on a PubMed search of HEPATOLOGY using the key words *fibrosis*, *stellate cells*, *collagen* or *extracellular matrix*.

a biopsy captures only 1/50,000 of the liver, and thus some sampling error seems inevitable. Morphometric and computerized systems<sup>17</sup> may yield data that is along a continuous rather than a discontinuous scale (*i.e.*, uninterrupted with no intermediate stages); however, if the tissue sample is not adequate, or there is uneven distribution of fibrosis, then these quantitative methods will not enhance the quality of the data obtained.

Moreover, there has been no validation of using biopsy to assess fibrosis regression rather than progression, and little is known about the speed or mechanisms of fibrosis regression, either pathologically or clinically. The latter is being addressed in a planned multicenter effort to characterize the dynamics of regression in a study termed REGRESS, which will follow a large cohort of patients who have successfully cleared hepatitis C virus (HCV) to determine how often fibrosis regresses, whether specific features either predict or correlate with regression, and whether regression correlates with improved clinical outcomes (N. Afdhal and J. McHutchinson, personal communication, 2005).

Based on the current limitations in diagnosing fibrosis and understanding the natural history of regression, drug developers are reluctant to invest resources in clinical trials when regression cannot be conclusively established. Moreover, a key requirement for future diagnostics will be the development of “biomarkers” rather than “surrogates.” Surrogates are endpoints that may correlate with fibrosis but do not directly reflect the underlying biology, whereas biomarkers directly reflect the biological process being measured. A simple example of the difference between surrogates and biomarkers is in a patient with a fever due to infection. In such a patient the use of antipyretics may normalize the temperature—a surrogate for infection—but they do not alter the underlying biology or directly reflect it. Biomarkers may additionally show evidence of changes in fibrogenic activity well before they are reflected in the absolute matrix content of liver tissue. An additional requirement will be the eventual correlation of biomarkers with clinical outcomes including death and complications, rather than simply the quantity of fibrosis.

### Why Is Fibrosis Assessment Suddenly So Important?

With the imminent development of meaningful antifibrotic therapies, the lack of robust noninvasive markers, combined with uncertainty about which patients are likely to progress, have, as noted above, become the most significant impediments to conducting clinical trials. Fortunately, we now recognize that the progression of fibrosis can be predicted and tracked, although it varies consider-

ably between patients. Risk factors for fibrosis progression are increasingly defined, especially in HCV and NASH. Many are easily identified, including alcohol consumption, age, sex, body mass index, lipid abnormalities, and immune dysregulation due to human immunodeficiency virus or immunosuppressant drugs. However, a sizable component of risk is attributable to host genes that have not been characterized. Based on this uncertainty, a sustained effort has begun to identify individual gene variants, or single nucleotide polymorphisms, that predict a more rapid rate of fibrosis progression, even in patients who may have little or no fibrosis at the time of analysis.<sup>18-20</sup> Such information will prove vital in designing meaningful clinical trials by ensuring that equal numbers of patients of a given risk are distributed in both control and treatment groups. Accurate assessment of fibrosis progression risk will thus be vital to establish drug efficacy.

The sense of urgency in developing diagnostics and antifibrotic therapies is also driven by the epidemiology of HCV and NASH. Earlier estimates of approximately 20% risk of progression to cirrhosis<sup>21</sup> may be too low, as these figures were based on prevalence studies at a time when many patients had only been infected for a relatively short time (*i.e.*, less than 10-15 years) and few had advanced disease, whereas we now recognize that although progression can be relatively indolent, it is inexorable in many patients.<sup>22,23</sup> Moreover, the entire cohort of patients with HCV, most of whom were infected before 1990, is aging, a feature that by itself confers accelerated fibrosis progression.<sup>24</sup> Furthermore, these patients are now carrying the infection for a considerable time and are entering a period of accelerated risk not only of fibrosis, but also of hepatocellular carcinoma. Similarly, the rising rate of obesity in the United States, Europe, and the Far East means that a growing number will harbor significant underlying liver disease because of NASH, with current estimates indicating a prevalence of approximately 30 million individuals in the United States alone<sup>25</sup> and far greater numbers worldwide.

### Regression of Cirrhosis and Fibrosis in Patients With Liver Disease—The Evidence Mounts

If one accepts that “regression” simply indicates a *bona fide* decrease in matrix content without necessarily returning the histology to normal, then there is little doubt of the capacity of the healing liver to resorb scar. From a practical perspective, experienced clinicians know that even patients with cirrhosis who are asymptomatic have a good short-term prognosis,<sup>26</sup> and thus individuals with lesser degrees of fibrosis are almost certain to remain sta-

ble, particularly if stasis or regression of fibrosis to precirrhotic stages can be achieved.

Evidence of either fibrotic or cirrhotic regression has now been documented in the entire spectrum of chronic liver diseases, including autoimmune hepatitis,<sup>27</sup> biliary obstruction,<sup>28</sup> iron overload,<sup>29</sup> NASH,<sup>30,31</sup> and viral hepatitis<sup>32-36</sup> (see references 37 and 38 for additional citations). Such evidence has begun to mount for at least 3 reasons: (1) More clinicians are aware of the potential for fibrosis reversal, and increasingly document this response in their patients and in clinical trials; (2) basic science advances provide a rational basis for understanding how fibrosis can reverse (see next section); and (3) most importantly, we are simply getting better at curing or suppressing liver disease, in particular through use of effective antivirals for HBV,<sup>32,33</sup> HCV,<sup>34,35</sup> and HDV<sup>36</sup> in large clinical trials, where reduction of fibrosis among significant numbers of patients cannot be explained solely by sampling error. Moreover, fibrosis reversal associated with antiviral therapy can also lead to meaningful improvement in liver function.<sup>39</sup> Whether such improvement will also ameliorate portal hypertension, decrease the incidence of hepatocellular carcinoma or improve survival is uncertain, but these endpoints will be important components of future long-term antifibrotic trials.

## Mechanisms of Extracellular Matrix Production and Degradation—The Facts

The mounting clinical evidence that fibrosis and even cirrhosis may regress is easier to digest now that we have begun establishing a rational scientific basis for these findings. A key to progress in the past two decades has been the identification of hepatic stellate cells and their myofibroblastic counterparts as the sources of the mediators, matrix molecules, proteases, and their inhibitors that orchestrate the wound-healing response in liver. The cardinal features of hepatic stellate cell behavior and the dominant cytokines in liver injury and fibrosis have been the subject of recent reviews<sup>40-44</sup> and are not presented in detail here.

Overall, however, the paradigm of stellate activation and resolution provides an important framework for understanding recent advances. *Activation* of hepatic stellate cells is the dominant event in hepatic fibrogenesis, and refers to the conversion of quiescent cell vitamin A-storing cells into proliferative, fibrogenic, and contractile “myofibroblasts.” Cellular *activation* proceeds along a continuum that involves progressive changes in cellular function, such that at any moment after injury, subpopulations of stellate and related cells exist with discrete cytoskeletal and phenotypic profiles.<sup>45</sup> The organization of stellate cell activation into a defined sequence provides a

helpful framework for exploring specific pathways. Early events have been termed *initiation* (also referred to as the “preinflammatory” stage). *Initiation* encompasses rapid changes in gene expression and phenotype that render the cells responsive to cytokines and other local stimuli. *Initiation* is associated with rapid gene induction resulting from paracrine stimulation by inflammatory cells and injured hepatocytes or bile duct cells, and from early changes in extracellular matrix composition. Cellular responses after initiation have been termed *perpetuation*, which encompasses those cellular events that amplify the activated phenotype through enhanced growth factor expression and responsiveness; this component of activation results from autocrine and paracrine stimulation, as well as from accelerated extracellular matrix remodeling. *Perpetuation* is a dynamic process, as illustrated by the sequential changes in transforming growth factor beta signaling occurring as stellate cells progressively activate in culture, for example.<sup>46,47</sup> Most importantly, *resolution* of stellate cell activation represents an essential step toward reversibility of fibrosis, as described in following sections.

The pieces of the puzzle underlying matrix degradation in liver have been partially assembled, but gaps remain in our understanding (Fig. 1). Several cell types can degrade matrix in liver, including neutrophils,<sup>48,49</sup> macrophages,<sup>50</sup> and stellate cells,<sup>51,52</sup> but their relative contribution is uncertain. Degradation occurs through the action of matrix metalloproteinases, enzymes that efficiently cleave collagens and other components of the extracellular matrix. In particular, matrix resorption during recovery from liver injury requires type I collagenase activity.<sup>53</sup> Remarkably, despite clear functional evidence of a type I collagenase *in vivo* for over 30 years, the structure and source of this activity in liver disease have remained elusive. Neither MMP-1, the most efficient type I collagenase *in vitro*, nor its rodent counterpart MMP-13, are increased during resolution of liver fibrosis. Although a “classical” type I collagenase is not induced during resolution of fibrosis, other enzymes may serve this function in liver, including membrane type I–matrix metalloproteinase (MT1-MMP), or MMP-2, a basement membrane collagenase/gelatinase that is markedly induced in stellate cells during injury.<sup>54,55</sup>

Perhaps more important than the source(s) and identify of proteases during regression of liver fibrosis, the inhibitors that regulate their activity play a vital role in orchestrating matrix degradation and stellate cell biology. In particular, tissue inhibitors of matrix metalloproteinases (TIMPs) are critical determinants of fibrosis reversal, because when fibrosis regresses, TIMP-1 levels are decreased associated with clearance of activated stellate cells

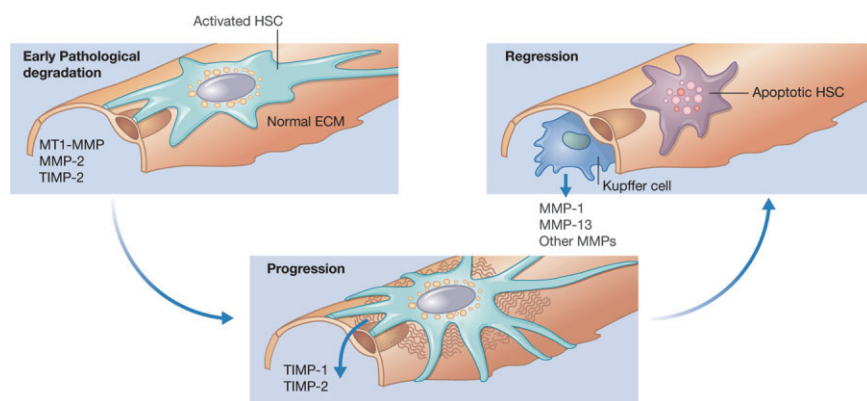


Fig. 1. Emerging mechanisms of early pathological matrix degradation, fibrosis progression, and fibrosis resolution in chronic liver disease. Activation of stellate cells (top left panel) is a key event in hepatic fibrosis, and is associated with pathological matrix degradation because of increased production of membrane type matrix metalloproteinase 1 (MT1-MMP), matrix metalloproteinase-2 (MMP-2), and tissue inhibitors of metalloproteinases (TIMPs), leading to replacement by interstitial collagen or scar matrix. As fibrosis progresses (middle panel), sustained expression of TIMPs prevents matrix degradation and apoptosis of activated stellate cells. Regression of fibrosis (upper right panel) is associated with increased apoptosis of activated stellate cells. Apoptosis requires decreased expression of tissue inhibitor of metalloproteinase-1 (TIMP-1), yielding a net increase in protease activity. These events may occur coincident with production of matrix metalloproteinases, which could include MMP-1 (in humans) or MMP-13 (in rodents), although cellular sources of these enzymes (possibly including Kupffer cells) and clear evidence of their induction *in vivo* are still lacking. Validation of these events and further elucidation of mechanisms underlying the regression of fibrosis represent key challenges for future studies. (Reprinted with permission from Zakim and Boyer, Textbook of Hepatology, 5th Edition, Elsevier, 2006).

through apoptosis.<sup>56,57</sup> In contrast, sustained TIMP-1 expression inhibits protease activity and blocks apoptosis of activated stellate cells,<sup>55,56,58</sup> with activated nuclear factor kappaB signaling providing a molecular signal to preserve this activated state of stellate cells.<sup>59,60</sup> Cytokines, including nerve growth factor, may drive stellate cell apoptosis through these same pathways,<sup>61</sup> whereas survival signals, some of which are bound to surrounding extracellular matrix, can antagonize the apoptotic signals.<sup>53</sup>

**When Does Fibrosis Become Truly Irreversible and Why?** Animal models have begun to address this question by defining the temporal events accompanying fibrosis resolution. Sustained experimental liver injury in rodents by CCl<sub>4</sub> leads to delayed reversibility due to thick collagen bands, high levels of TIMP-1, and significant collagen cross-linking by tissue transglutaminase,<sup>62</sup> much of which was predicted by Perez-Tamayo in 1979.<sup>4</sup> Moreover, transglutaminase expression is increased in activated stellate cells.<sup>63</sup> In fact, the role of collagen cross-linking was also raised decades ago, and explains earlier efforts to characterize and develop inhibitors of lysyl oxidase, another enzyme regulating collagen cross-linking.<sup>64,65</sup> Also resurrected has been the concept of “micronodular” versus “macronodular” cirrhosis. Conversion from micro- to macro-nodular cirrhosis was reported in HEPATOLOGY in 1983,<sup>66</sup> and later was reinforced by studies defining “incomplete septal fibrosis” as a consequence of cirrhotic regression.<sup>67</sup> Remarkably, this “micro- to macro-” transition is exactly what is also observed in animal models of regression.<sup>62</sup>

## When Will We Be Able to Treat Cirrhosis? The Fantasy

In this 25th anniversary year of HEPATOLOGY, identifying priorities for future basic research and clinical trials is appropriate, while acknowledging successes of the past. Based on these recent findings in animals, the molecular determinants of fibrosis regression in animals and humans must be more comprehensively defined. We need to characterize the mechanisms and sources regulating matrix protease activity and cross-linking in the fibrotic milieu. These findings will not only uncover novel approaches to antifibrotic therapy but also help customize these strategies by linking them with the extent of fibrosis accumulation or cross-linking.

Conversely, the progress in understanding hepatic fibrosis made since the seminal observations by authors in early issues of HEPATOLOGY has been very inspiring. Although the prospect of treating fibrosis is not new, the foundation upon which current strategies of antifibrotic therapies are built is far sturdier and more realistic. Studies of gene therapy using protease delivery,<sup>48,68</sup> for example, establish proof-of-principle that even dense scar can be resorbed. A trove of studies in animal models have defined dozens of potential antifibrotics worthy of clinical trials.<sup>38,40,69,70</sup> These observations, combined with solid evidence of fibrosis regression in humans after successful treatment of the underlying liver disease, augur well for accelerating progress. Therapies will be increasingly tailored to host genotype and disease-specific features, and

given in combination. Methods of defining the risk of fibrosis progression and the likelihood of treatment response will be established. The two most immediate hurdles—the development of better diagnostics to clarify end points in clinical trials, and the need to establish that an antifibrotic can halt or regress fibrosis even when the underlying disease is unchecked—will be surmounted. Considering how far the field has advanced in 25 years, these fantasies are sure to become fact before HEPATOLOGY celebrates its 50th anniversary.

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