

Hepatic stellate cells are a major component of liver fibrosis and a target for the treatment of chronic liver disease

Alfonso Leija, Jorge Reyes, ✉ Lourdes Rodríguez

Facultad de Farmacia, Universidad Autónoma del Estado de Morelos
Av. Universidad 1001 Colonia Chamilpa, Cuernavaca, Morelos, México
E-mail: mlrodrig1@yahoo.com.mx

REVIEW

ABSTRACT

Fibrosis, defined as the excessive deposition of an extracellular matrix in an organ, is the main complication of chronic liver damage. Its endpoint is cirrhosis, which is responsible for significant morbidity and mortality. The accumulation of the extracellular matrix observed in fibrosis and cirrhosis is due to the activation of hepatic stellate cells. It is now widely accepted that various types of lesions (e.g., lesions caused by alcohol abuse and viral hepatitis) lead to liver fibrosis. The biological and biochemical characterization of hepatic stellate cells is thus essential (1) to understand the mechanisms underlying the progressive development of excessive scarring in the liver, and (2) to develop new treatments for specifically and efficiently targeting cells responsible for the development of fibrosis or cirrhosis. Here, we describe many of the molecular events that occur following hepatic stellate cell activation and its role in fibrogenesis, as well as providing a review of therapeutic strategies for treating this disease.

Key words: hepatic stellate cells, liver fibrosis, cytokines, growth factors

Biotecnología Aplicada 2007;24:19-25

RESUMEN

Las células estelares hepáticas como principal componente de la fibrosis y blanco para el tratamiento de la enfermedad hepática crónica. La fibrosis, definida como el depósito de matriz extracelular en un órgano, es la principal complicación del daño hepático crónico. El punto final de la fibrosis es la cirrosis, la cual es responsable de la alta morbilidad y mortalidad. La acumulación de matriz extracelular que se observa durante la fibrosis y cirrosis es debida a la activación de la célula estelar. Es ampliamente aceptado que diferentes tipos de lesiones (por ejemplo, aquellas causadas por el abuso del alcohol y la hepatitis viral) conducen a la fibrosis. La caracterización biológica y bioquímica de la célula estelar hepática es esencial para: (1) entender los mecanismos que llevan al desarrollo de la extensa cicatriz en el hígado, y (2) para desarrollar nuevos tratamientos dirigidos específica y eficientemente a las células responsables del desarrollo de la fibrosis/cirrosis. En esta revisión se describen muchos de los eventos moleculares que ocurren posterior a la activación de la célula estelar hepática y el papel que juega en la fibrogenesis, así como proporciona una revisión de las estrategias terapéuticas que se están implementando para tratar esta enfermedad.

Palabras claves: células estelares hepáticas, fibrosis, citocinas, factores de crecimiento

Introduction

The remarkable scientific advances in recent years on the molecular mechanisms involved in fibrogenesis have had little effect on new drugs for chronic liver diseases.

Liver fibrosis and its end stage, cirrhosis, are enormous healthcare problems worldwide. When fibrosis progresses to cirrhosis, life-threatening complications associated with liver disease occur, including variceal bleeding, formation of ascites, and hepatorenal syndrome. Liver fibrosis and cirrhosis are associated with an increased risk of developing hepatocellular carcinoma [1-3]. These complications create a substantial burden on healthcare resources and are expected to increase dramatically in the near future. The success of liver transplants and advances in the radiological and endoscopic management of portal hypertension have improved longevity and the quality of life of patients with liver cirrhosis. However, these interventions also highlight our current inability to alter the underlying fibrotic process in patients with liver disease [4-7].

Fibrosis is the liver's scarring response to an injury that occurs in most chronic inflammatory liver diseases. Liver fibrogenesis was traditionally considered a

«passive» and irreversible process, due to the collapse of the hepatic parenchyma and its substitution by a collagen-rich tissue [8-9]. However, a number of intensive clinical and experimental observations during the past 20 years indicate that it is a dynamic process of tissue repair that develops after repeated liver injury [10,11]. The onset of liver fibrosis is usually insidious, and most of the related morbidity and mortality occur after developing cirrhosis [12]. In most patients, progression to cirrhosis takes 15-20 years.

The role of Hepatic Stellate Cells in the Liver

Hepatic stellate cells (HSCs), formerly known as Ito cells, fat storing cells, or lipocytes, have been identified as the main extracellular matrix (ECM) producers in the injured liver. HSCs are located between parenchymal cell plates and endothelial linings and have different important functions: (1) retinoid storage and homeostasis; (2) remodeling of ECM by production of both components of ECM and matrix metalloproteinases (MMPs); (3) production of growth factors and cytokines; and (4) contraction and dilation of sinusoidal lumen in response to endothelin, angiotensin, or prostaglandins [13-17]. It

1. El-Serag HB, Mason AC. Rising incidence of hepatocellular carcinoma in the United States. *N Engl J Med* 1999;340:745-50.

2. Bataller R, Brenner DA. Liver fibrosis. *J Clin Invest* 2005;115:209-18.

3. Friedman SL. Mechanisms of disease: Mechanisms of hepatic fibrosis and therapeutic implications. *Nat Clin Pract Gastroenterol Hepatol* 2004;1:98-105.

4. Liang TJ, Rehermann B, Sep LB, Hoofnagle JH. NIH Conference, Pathogenesis, natural history, treatment and prevention of hepatitis C. *Ann Intern Med* 2000;132:296-305.

5. Albanis E, Friedman SL. Hepatic fibrosis. Pathogenesis and principles of therapy. *Clin Liver Dis* 2001;5:315-34.

6. Chander G, Sulkowski MS, Jenkes MW et al. Treatment of chronic hepatitis C: a systematic review. *Hepatology* 2002;36:S135-44.

7. Pinzani M, Rombouts K, Colagrande S. Fibrosis in chronic liver diseases: diagnosis and management. *J Hepato.* 2005;42:S22-36.

is well known that HSCs are involved in the development and regeneration of liver tissue, reorganization of hepatic ECM, development of hepatic fibrosis, and cancer cell invasiveness [18-21].

HSCs mainly exhibit two phenotypes: the «quiescent» state and the «activated» or «trans-differentiated» state. Cytoplasmic lipid droplets containing retinyl esters and long cytoplasmic processes with fine branching are characteristic of quiescent HSCs, as seen in normal liver tissue. In their activated state, HSCs lack the lipid droplets and long processes and display proliferative and fibrogenic myofibroblast-like phenotypes [22, 23]. The activation or trans-differentiation process of HSCs is regulated by paracrine and autocrine loops of growth factors in association with fibrosis in pathological conditions such as liver injury and fibrosis [24-26]. HSC activation is accompanied by changes in gene expression and phenotype, and finally leads to enhanced cytokine expression and responsiveness, and the accelerated production of ECM components, MMPs, and tissue inhibitors of metalloproteinase (TIMPs) to promote remodeling [2, 27, 28]. The mechanisms mediating activation of HSCs are not completely clear. However, it is well known that there is a complex interplay among different hepatic cell types during hepatic fibrogenesis. Parenchymal cells (hepatocytes) and non-parenchymal cells (HSCs, endothelial sinusoidal cells, Kupffer cells, and biliary cells), as well as inflammatory cells, are involved in this process [11].

Cellular Interactions Involved in the Activation of HSCs

The cellular basis of hepatic fibrogenesis has recently been outlined. Hepatic fibrosis is the result of the wound-healing response of liver cells to chronic or iterative injury. After an acute liver injury (e.g., viral hepatitis), parenchymal cells regenerate and replace the necrotic or apoptotic cells. This process is

associated with an *inflammatory response* and a limited deposition of ECM. The first lines of defense are provided by NK (natural killer) and NKT cells, of which populations are relatively increased in the liver compared to the periphery. These cells are activated in the liver, where the expression of interferon α (IFN- α) and IFN-inducible genes is extremely high during the early phase of the hepatitis virus infection. Activated NK and NKT cells secrete IFN- γ , which inhibits the replication of HCV through a non-cytolytic process. During HBV infection, those cells contribute significantly to the suppression of viral replication. Dendritic cells (DCs) or resident macrophages in the liver are capable of taking up viral antigens, and processing and presenting them to other immune cells. DCs develop into a mature phenotype and migrate to lymphoid tissues, where they stimulate effectors, including T cells and B cells. Following the encounter of DCs with other cells, DCs secrete various cytokines (interleukin-12 (IL-12), tumor necrosis factor α (TNF- α), IFN- α , and IL-10) instructing or regulating the functions of the adjacent cells. Helper T cells have an immunoregulatory function mediated by the secretion of cytokines that support either cytotoxic T lymphocyte (CTLs) generation (Th1 with secretion of IL-2, IFN- γ , and TNF- α) or B cell function and antibody production (Th2 with secretion of IL-4, IL-5, IL-10, and IL-13). There are two plausible mechanisms for the CTL-mediated HCV clearance from the liver: by inducing apoptosis in infected hepatocytes (*cytolytic mechanism*) or by releasing IFN- γ to suppress HCV replication (*non-cytolytic mechanism*) (Figure 1) [29-31]. If the hepatic injury persists, then eventually liver regeneration fails, and hepatocytes are substituted by abundant ECM, including fibrillar collagen and other matrix proteins that disrupt the architecture of the liver [32, 33].

The distribution of the fibrous material depends on

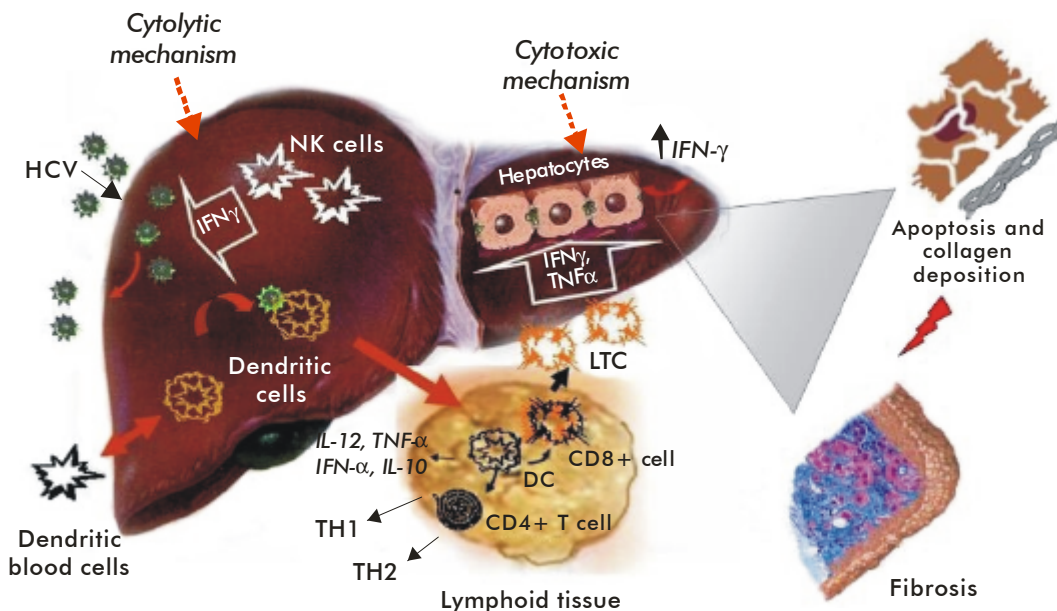


Figure 1. Immunological response during viral hepatitis. CTL, cytotoxic T Lymphocyte; DC, dendritic cell; HCV, hepatitis C virus; NK, natural killer cell; Th, helper T cell.

8. Bassell DM. Hepatic fibrosis as wound repair a progress report. *J Gastroenterol* 1998;33:295-302.

9. Afdhal NH, Nunes D. Evaluation of liver fibrosis: a concise review. *Am. J. Gastroenterol.* 2004;99:1160-74.

10. Bataller R, Brenner DA. Hepatic stellate cells as a target for the treatment of liver fibrosis. *Semin Liver Dis* 2001;21:437-51.

11. Friedman SL. Molecular regulation of hepatic fibrosis, an integrated cellular response to tissue injury. *J Biol Chem* 2000;275:2247-50.

12. Par A, Par G. Liver fibrosis: pathophysiology, diagnosis and treatment. *Orv Hetil* 2005;146:3-13.

13. Rockey DC. The cell and molecular biology of hepatic fibrogenesis. Clinical and therapeutics implications. *Clin Liver Dis* 2000;4:319-55.

14. Neubauer K, Saile B, Ramadori G. Liver fibrosis and altered matrix synthesis. *Can J Gastroenterol* 2001;15:187-93.

15. Pinzani M, Gentilini P. Biology of hepatic stellate cells and their possible relevance in the pathogenesis of portal hypertension in cirrhosis. *Semin Liver Dis* 1999;19:397-410.

16. Geerts, A. History, heterogeneity, developmental biology, and functions of quiescent hepatic stellate cells. *Semin. Liver Dis* 2001;21:311-35.

17. Safadi R, Friedman SL. Hepatic fibrosis – Role of hepatic stellate cell activation. *Med Gen Med* 2002;15(4):27-38.

18. Mabuchi A, Mullaney I, Sheard PA, Zimmermann A, Senoo H, Wheatley AM. Role of hepatic stellate cells in the early phase of liver regeneration in rat: formation of tight adhesion to parenchymal cells. *Comp Hepatol* 2003;3:S29-37.

the origin of the liver injury. In chronic viral hepatitis and chronic cholestatic disorders, the fibrotic tissue is initially located around portal tracts, while in alcohol-induced liver disease it is located in pericentral and perisinusoidal areas [34, 35]. As fibrotic liver diseases advance, there is a progression from collagen bands to bridging fibrosis and finally to cirrhosis.

Hepatocytes are targets for a number of hepatotoxic agents, including hepatitis viruses, alcohol metabolites, and bile acids [36]. Moreover, the apoptosis of damaged hepatocytes releases fibrogenic substances and stimulates the immune response leading to infiltration by inflammatory cells [37, 38]. Inflammatory cells found in the areas of hepatocellular necrosis, either lymphocytes or polymorphonuclear cells, attract fibrogenic cell types such as HSCs. The damage of hepatocytes and the inflammatory cells induce the release of chemical mediators that influence HSCs and induce their activation [39] (Figure 2). On the other hand, activated HSCs also secrete inflammatory chemokines and express cell adhesion molecules that activate leukocytes. Therefore, a vicious circle in which inflammatory and fibrogenic cells stimulate each other is likely to occur. Kupffer cells are resident macrophages that play a major role in liver inflammation by releasing reactive oxygen species and cytokines [31, 40, 41]. The role of sinusoidal endothelial cells in liver

fibrogenesis is not well known. *In vitro* studies indicate that sinusoidal cells secrete inflammatory and fibrogenic factors such as leptin and stimulate the proliferation of lymphocytes [42]. Several experimental results support the hypothesis that HSCs mediate the final common pathway of fibrosis [43]. Trans-differentiation of HSCs *in situ* is also a tissue phenomenon, which is mediated by paracrine acting mediators released from genuine liver cells (hepatocytes, Kupffer cells, sinusoidal endothelial cells) and from invading, non-hepatic cells such as platelets, lymphocytes, and leukocyte subtypes [12, 24].

Production of HSCs and Regulation by Growth Factors and Cytokines

In most tissues, including the liver, constitutive production of cytokines is absent or low. However, as physiologic and pathologic stimuli activate cells, the production of these autocrine, paracrine, and endocrine effector molecules increases, and they in turn orchestrate the tissue's response to the stimulus [44, 45].

There is increasing evidence that certain cytokines play a major role in inflammatory liver diseases and liver tissue repair. To date, it is well known that cytokines, growth factors, and other soluble mediators are involved in this complex mechanism that

19. Sato M, Suzuki S, Senoo H. Hepatic stellate cells: unique characteristics in cell biology and phenotype. *Cell Struct. Funct* 2003;28:105-12.

20. Vinas O. Human hepatic stellate cells show features of antigen-presenting cells and stimulate lymphocyte proliferation. *Hepatology* 2003;38:919-29.

21. Bachem MG, Schmid-Kotsas A, Schuenemann M, Fundel M, Adler G, Menke A, Siech M, Buck A, Gruenert A. The increased deposition of connective tissue in pancreas carcinoma is the result of a paracrine stimulation of pancreatic stellate cells by cancer cells. *Mol Biol Cell* 2002;13:4a-11a.

22. Reeves HL, Friedman SL. Activation of hepatic stellate cells - a key issue in liver fibrosis. *Front Biosci.* 2002;7:d808-26.

23. Senoo H. Structure and function of hepatic stellate cells. *Med Electron Microsc.* 2004;37:3-15.

24. Yang C, Zeisberg M, Mosterman B, Sudhakar A, Yerramalla U, Holthaus K, Xu L, Eng F, Afdhal N, Kalluri R. Liver fibrosis: insights into migration of hepatic stellate cells in response to extracellular matrix and growth factors. *Gastroenterology* 2003;124:147-59.

25. Rockey DC. Hepatic blood flow regulation by stellate cells in normal and injured liver. *Semin in Liver Dis* 2001;21:337-50.

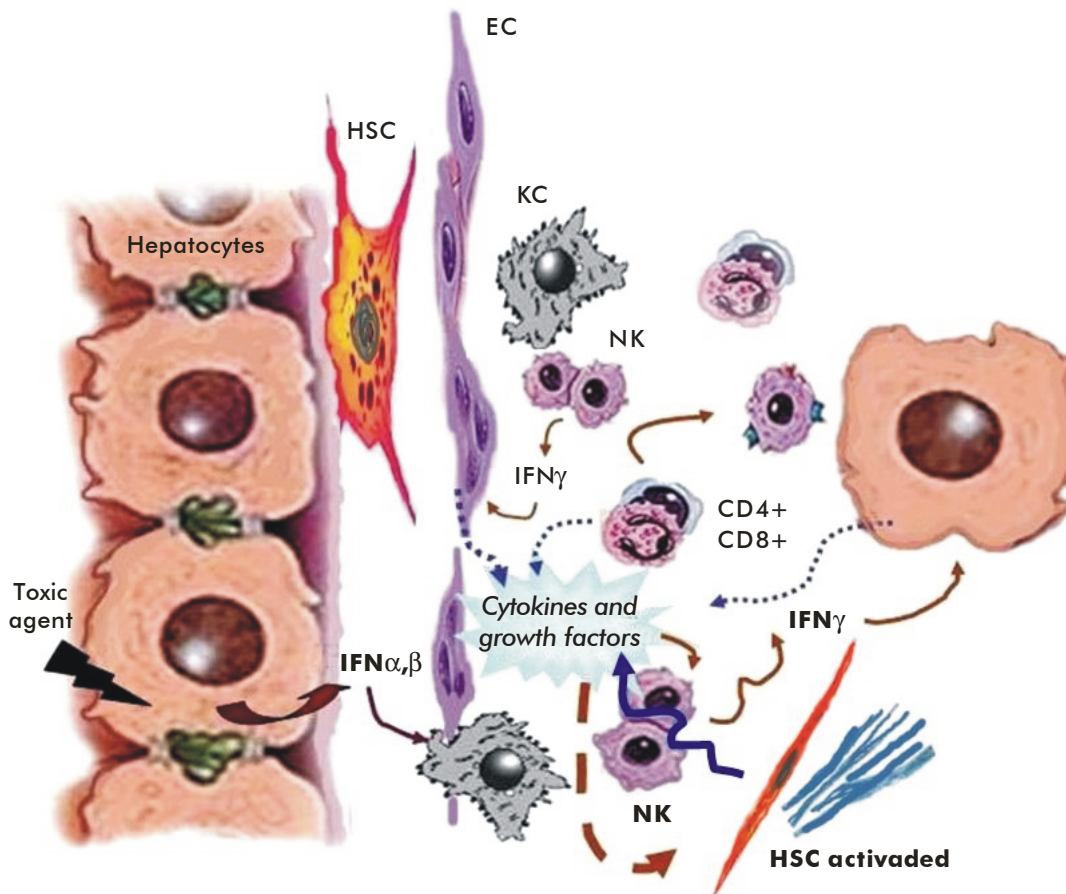


Figure 2. Molecular and cellular crosstalk involved in the activation of HSC (For details see text). HSC, hepatic stellate cells; EC, endothelial cells; KC, Kupffer cells; NK natural killer; IFNs, interferon.

orchestrates the wound-healing response in the liver [46]. These mediators are either present locally and stored in an inactive form bound to the ECM, or are produced by different cell types during the acute phase of aggression as a part of the inflammatory reaction.

At least three cytokines from different cytokine families, namely the pro-inflammatory molecule TNF- α and the anti-inflammatory cytokine IL-10, have been found to be key factors in various aspects of liver diseases. This does not mean that other cytokines are not important in fibrogenesis. TNF- α production is one of the earliest events in many types of liver injury, triggering the production of other cytokines that together recruit inflammatory cells, kill hepatocytes, and initiate a hepatic healing response that includes fibrogenesis [47].

IL-10 is secreted by several cell populations including T-helper cells, monocytes and macrophages, DCs, B cells, and keratinocytes. This cytokine suppresses inflammation through several mechanisms, including the reduction of HLA class II expression, decreasing T-cell secretion of IL-2, and diminishing the production of IL-1 α , IL-1 β , TNF- α , and IL-8 by activated monocytes and macrophages [26, 45, 46, 48].

The transforming growth factor- β 1 (TGF- β 1) and the platelet-derived growth factor (PDGF) are fibrogenic and proliferative cytokines, respectively, for HSCs. TGF- β 1 was found to be increased in experimental and human hepatic fibrosis, and TGF- β 1 induces the production of ECM by HSCs [49, 50]. TGF- β 1 is produced by hepatocytes, Kupffer cells, platelets, and sinusoidal cells. Hepatic injury is associated with the increased production of PDGF by platelets and up regulation of the PDGF receptor in HSCs [51-53].

Secretion of *cytokines* by resident or incoming lymphocytes in the context of the inflammatory response also contributes to the regulation of fibrogenesis. According to their cytokine profiles, CD-4 T cells can promote either cell-mediated immunity, through the secretion of IFN- γ , IL-2, or TNF (Th1 cells), or humoral immunity (Th2 cells) through IL4, IL5, or IL13. Experimental evidence suggests that different Th profiles might favor or limit fibrosis [54, 55].

HSCs are also important in the synthesis of several soluble factors needed for the establishment of paracrine and autocrine loops within the liver. They synthesize a broad spectrum of cytokines and growth factor-binding proteins including the epidermal growth factor, insulin-like growth factor-I and -II, insulin-like growth factor-binding proteins, interleukins, macrophage colony-stimulating factor (M-CSF), latent TGF- β -binding proteins, CTGF, chemokines, and many substances and proteins controlling the activity of these factors and allowing them to interact with all hepatic and non hepatic cell types [13, 15, 24, 56]. Furthermore, different components of the plasminogen activation system and different metalloproteases were identified in HSCs [57-59], again demonstrating that the biological functions of this hepatic cell fraction are only partially understood and are not solely restricted to the liver.

HSCs also contribute to the formation of inflammatory granuloma with the synthesis and extracellular release of polymorphonuclear and macrophage-monocyte chemoattractants or differentiation factors such as monocyte chemoattractant protein-1 and M-CSF. M-CSF, a member of the CC class of chemokines, which actively participates in the influx of peripheral monocytes and their differentiation to phagocytic cells at the site of the injury [60]. Moreover, activated human HSCs express receptors for a number of chemokines; these cytokines activate various intracellular pathways involved in the inflammatory reaction [45, 61]. These mediators compete with anti-inflammatory cytokines such as IL-10, which are also produced by HSCs (62).

The pathways for perpetuating the activated HSC phenotype include the acquisition and maintenance of new functions such as proliferation, release of pro-inflammatory cytokines and chemokines, matrix degradation, and, of course, fibrogenesis. Most of these new functions are sustained by an autocrine loop characterized by the production of several major mediators and the enhancement of cell response to these factors through both the up-regulation of their own membrane receptor and the enhancement of intracellular signaling.

Hepatic Stellate Cells as the Major Mediators of Fibrosis

HSCs play a crucial role in offense mechanisms, regeneration, and fibrosis in the hepatic tissue. They also participate in the maintenance of the ECM and of the space of Disse. HSCs acquire a particularly important function when the liver is submitted to a harmful process. HSCs receive numerous paracrine stimuli provided by harmed hepatocytes, endothelial cells, Kupffer cells, and ECM altered by the aggression [20]. Subsequently, HSCs become more responsive to cytokines. HSCs begin to produce cytokines that attract and stimulate themselves and leukocytes (autocrine and paracrine mechanisms), they start to proliferate, lose their vitamin A deposits, acquire contractile capacity and the synthesis of α -smooth muscle actin (α -SMA) and large amounts of the main components of the ECM, including collagen types I, III, and IV, fibronectin, laminin, and proteoglycans (Figure 3) [17, 22].

Collagen types I and III are the main framework of the so-called «fibrillar matrix», which gradually substitutes the basal membrane-like structures found along the sinusoids and in between the hepatocellular plates. The space of Disse, where HSCs are located, is a virtual space constituted by an ECM network composed of type IV collagen, associated with non-collagenous components, such as laminin. In advanced active fibrosis, the deposition of ECM is dense enough to resemble a vascular basement membrane, an appearance termed «*capillarization of the sinusoids*». The large majority of collagen types III and IV and laminin are synthesized by HSCs and sinusoidal endothelial cells, whereas all cell types synthesize small amounts of collagen type I [59].

During active hepatofibrogenesis, however, HSCs become the major ECM-producing cell type, with a

26. Issa R, Williams E, Trim N, Kendall T, Arthur MJ, Reichen J, Benyon RC, Iredale JP. Apoptosis of hepatic stellate cells: involvement in resolution of biliary fibrosis and regulation by soluble growth factors. *Gut* 2001;48:548-57.

27. Benyon D, Arthur MJ. Extracellular matrix degradation and the role of stellate cells. *Semin Liver Dis* 2001;21:373-84.

28. Schuppan D, Ruehl M, Somasundaram R, Hahn EG. Matrix as a modulator of hepatic fibrogenesis. *Semin Liver Dis* 2001;21:351-72.

29. Bertoletti A, Ferrari C. Kinetics of the immune response during HBV and HCV infection. *Hepatology* 2003;38:4-13.

30. Lieber CS. Hepatic and metabolic effects of alcohol (1966 to 1973). *Gastroenterology* 1973;65:821-46.

31. Liu TZ, Lee KT, Chern CL, Cheng JT, Stern A, and Tsai LY. Free radical-triggered hepatic injury of experimental obstructive jaundice of rats involves overproduction of proinflammatory cytokines and enhanced activation of nuclear factor kappaB. *Ann Clin Lab Sci* 2001;31:383-90.

32. Bataller R, Gabele E, Parsons CJ, Morris T, Yang L, Schoonhoven R, Brenner DA, Rippe RA. Systemic infusion of angiotensin II exacerbates liver fibrosis in bile duct-ligated rats. *Hepatology* 2005;41:1046-55.

33. Schuppan D, Krebs A, Bauer M, Hahn EG. Hepatitis C and liver fibrosis. *Cell Death Differ* 2003;10:S59-67.

34. Aguilera V, Berenguer M. Hepatitis C and fibrosis. *Rev Esp Enf Dig* 2004;96:402-14.

35. Iredale JP. Cirrhosis: new research provides a basis for rational and targeted treatments. *BMJ* 2003;327:143-7.

36. Wu J, Danielsson A, Zern MA. Toxicity of hepatotoxins: new insights into mechanisms and therapy. *Expert Opin Investig Drugs* 1999;8:585-607.

37. Canbay A, Friedman S, Gores GJ. Apoptosis: the nexus of liver injury and fibrosis. *Hepatology* 2004;39:273-8.

38. Canbay A, Higuchi H, Bronk SF, Taniuchi M, Sebo TJ, Gores GJ. Fas enhances fibrogenesis in the bile duct ligated mouse: a link between apoptosis and fibrosis. *Gastroenterology* 2002;123:1323-30.

39. Choi S, Mae A. Role of inflammation in nonalcoholic steatohepatitis. *Curr Opin Gastroenterol* 2005;21:702-7.

40. Marra F. Hepatic stellate cells and the regulation of liver inflammation. *J. Hepatol* 1999;31:1120-30.

41. Naito M, Hasegawa G, Ebe Y, Yamamoto T. Differentiation and function of Kupffer cells. *Med Electron Microsc* 2004;37:16-28.

42. Arii S, Imamura M. Physiological role of sinusoidal endothelial cells and Kupffer cells and their implication in the pathogenesis of liver injury. *J Hepatobiliary Pancreat Surg* 2000;7:40-8.

43. Poynard T, Mathurin P, Lai CL, Guyader D, Poupon R, Tainturier MH, Myers RP, Muntenau M, Ratziu V, Manns M, Vogel A, Capron F, Chedid A, Bedossa P. A comparison of fibrosis progression in chronic liver diseases. *J Hepatol* 2003;38:257-65.

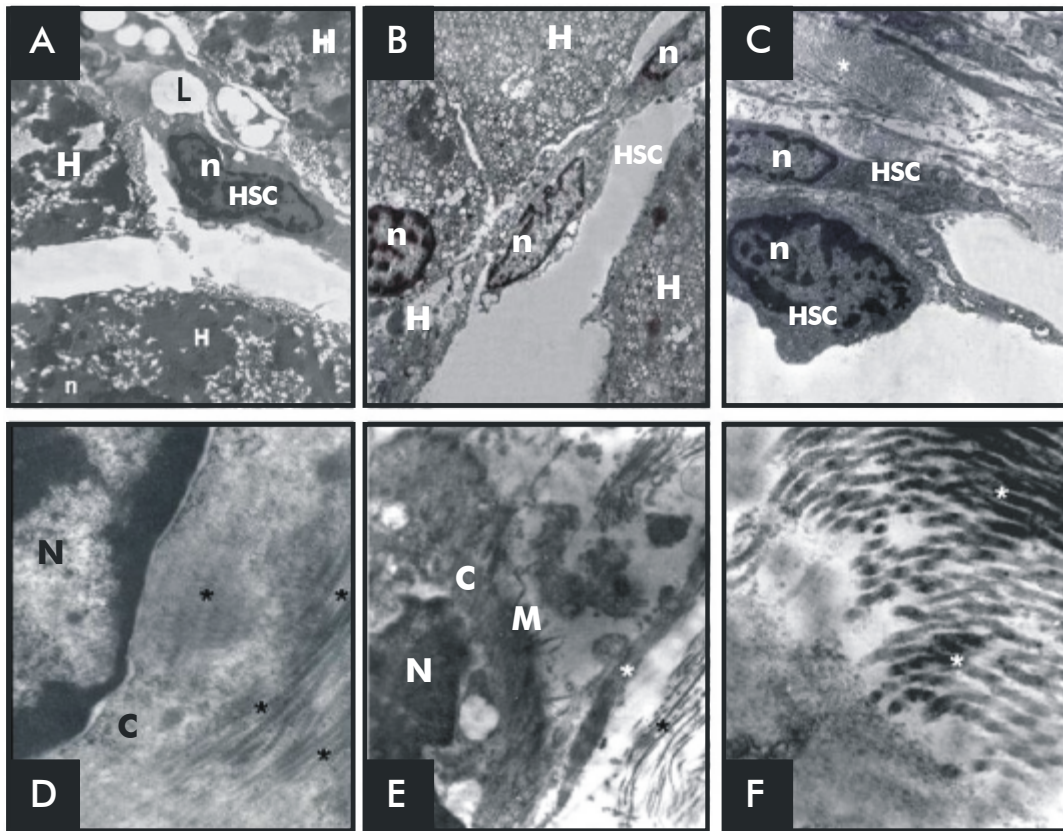


Figure 3. Transmission electron micrographs of liver tissue. (A), hepatic stellate cells in the normal tissue (M5000); (B), Fibrosis induced by chronic administration of CCl_4 , HSCs lost its lipid droplet (M3000); (C) Morphological changes observed during HSC activation, presence of abundant amount of ECM (M7000); (D) Collagen newly synthesized in HSC cytoplasm (M20000); (E) Exocytosis of collagen from HSC (M17000); (F) Abundant collagen fibers in extracellular space (M25000). H, hepatocyte; HSC, hepatic stellate cell; n, nucleus; L, lipid droplet; *, fibrillar matrix; c, collagen.

predominant production of collagen type I [29]. The net result of injury is fibrogenesis caused by HSC activation and this is typically viewed as a lesion with progressive accumulation of excess ECM. It is clear that fibrogenesis involves the dynamic interplay of matrix synthesis and degradation. Under normal circumstances, there is a balance between matrix synthesis and degradation. In contrast, under abnormal circumstances, the balance is disrupted and this leads to fibrosis and cirrhosis. The degradation portion of the remodeling process is coordinated by MMPs and TIMPs [63, 64].

It is unclear what happens to HSCs during fibrosis and cirrhosis resolution. There is a decrease in the number of activated HSCs as fibrosis resolves, but it is uncertain if HSCs return to a quiescent state or undergo apoptosis. Cell culture has demonstrated that the HSCs can revert to a quiescent cell type, but it is unknown if this occurs *in vivo*.

Therapeutic Approaches to Liver Fibrosis

Several tools may be potentially effective in the treatment of fibrosis. They may target any of the following biological mechanisms: (1) removal of the initial fibrotic stimulus; (2) inhibition of HSC activation or induction of apoptosis; (3) targeting liver

inflammation; (4) removal of excess fibrous tissue; (5) stimulation of liver regeneration; and (6) removal of the initial fibrotic stimulus.

Removing the causal agent is presumably the most efficient way to prevent liver fibrosis progression and stimulate regression. In chronic hepatitis C, several randomized clinical trials of patients with chronic HCV infection treated either with IFN- α alone or combined with ribavirin have shown stabilization or even a partial decrease in liver fibrosis in repeated liver biopsies, mainly when there is a virological response [65, 66]. The decrease in the inflammatory reaction, which is often observed by antiviral treatment even in the absence of virus eradication, may play a major role, but a direct anti fibrotic effect of IFN is also supported by *in vitro* and experimental evidence [67, 68].

Inhibition of HSC Activation

Owing to the new functions acquired by activated HSCs, substances that inhibit either activation or accumulation of HSCs might be of major importance in attenuating fibrogenic response. Because of the complexity and multiplicity of pathways involved or associated with initial phases of HSC activation and the maintenance phase, there are multiple therapeutic targets. Different strategies for targeting these pathways have been developed, such as neutralizing

44. Crews FT, Bechara R, Brown LA, Guidot DM, Mandrekar P, Oak S, Qin L, Szabo G, Wheeler M, Zou J. Cytokines and Alcohol. *Alcoholism: Clin & Exp Res* 2006;30:195-230.

45. Tilg H, Kaser A, Moschen AR. How to modulate inflammatory cytokines in liver diseases. *Liver International* 2006;26:1029-39.

46. Marra F. Chemokines in liver inflammation and fibrosis. *Front Biosci* 2002;7:d1899-1914.

47. Varela-Rey M, Montiel-Duarte C, Osés-Prieto JA et al. p38 MAPK mediates the regulation of alpha1(I): procollagen mRNA levels by TNF-alpha and TGF-beta in a cell line of rat hepatic stellate cells (1). *FEBS Lett.* 2002;528:133-8.

48. Kowala-Piaskowska A, Mozer-Lisewska I, Figlerowicz M, Machowska L, Sluzewski W. Interleukin 6 and 12, Alanine Aminotransferase Activity, and HCV Viral Load in Children with Chronic Hepatitis C Treated with Interferon and Ribavirin. *Inflammation* 2004;2(6):319-25.

49. Dooley S, Delvoux B, Lahme B, Mangasser-Stephan K, Gressner AM. Modulation of transforming growth factor beta response and signaling during transdifferentiation of rat hepatic stellate cells to myofibroblasts. *Hepatology* 2000;31:1094-106.

anti-TGF- β antibodies, soluble or truncated receptors [69]. These strategies have been shown to be efficient in preventing fibrosis in different rat models of liver fibrogenesis [70, 71].

Different therapeutic options targeting the proliferation of activated HSCs have also been developed; they act either by blocking PDGF receptors or through intracellular pathways required for cell proliferation. It has been shown that the tyrosine kinase inhibitor specific for PDGF-R reduces the proliferative response of HSCs [72]. Similarly, IFN- β and IFN- γ , but not IFN- α , inhibits rat HSC proliferation [73].

Regardless of the etiology of the disease, antifibrotic therapy may be targeted at inducing the apoptosis of HSCs, increasing the activity of MMP, or eliminating profibrogenic signals. Thus, activation of the death receptor-mediated pathway results in HSC apoptosis. However, the Fas receptor is expressed ubiquitously on HSCs and hepatocytes and causes apoptosis of both cell types [74]. Several cytokines can induce HSC apoptosis. For example, IFN- γ successfully blocks HSC proliferation and induces HSC apoptosis [75]. Tumor necrosis factor- α also has an anti-proliferative effect on HSCs and induces HSC apoptosis in the presence of cycloheximide, an inhibitor of protein synthesis [47].

Several substances with antioxidant properties have been tested, with contrasting results. They may protect or restore the defenses against oxidation, or scavenge free radicals such as vitamin E (α -tocopherol), resveratrol, silymarin, and dimethylsulphoxide. Most have shown conclusive effects in various *in vitro* and experimental models of liver fibrogenesis (CCl₄-induced fibrosis or bile duct-ligated models) (76).

Targeting Liver Inflammation

The association between liver inflammation and

fibrosis is very strong in chronic hepatitis. Promising experimental results suggested that IL-10, an anti-inflammatory and antifibrotic cytokine synthesized by activated HSCs, may be efficient at reversing fibrosis in experimental models mainly by down-regulating the Th1 proinflammatory response (61, 62).

Destruction of Excess Fibrous Tissue

It has recently been suggested that the modulation of MMP and TIMP activity is an efficient tool for liver fibrosis regression, and there are several potential targets for interrupting or stimulating this pathway, since MMPs are regulated mainly at a post-translational level (77, 78). Collagen biosynthesis involves several post-translational modifications leading to the production of highly stable cross-linked molecules. Prolyl-4-hydroxylase is critical in this process since it catalyses the synthesis of the hydroxyproline residues that are critical for the stability of the triple helix collagen. They have shown striking efficacy in animal models by decreasing the amount of collagen accumulation. They may also combine with other mechanisms such as the inhibition of HSC activation (79).

Since fibrogenesis is a slow process requiring years or decades, the assessment of changes during short time intervals is problematic. Furthermore, prospective human clinical trials of potential anti-fibrotic agents may be costly because of their length.

Conclusions

Significant progress has been made in understanding the role of HSCs in fibrogenesis, which has led to many potential therapies for improving fibrosis. It is clear that HSC trans-differentiation *in vivo* is a complex and multistep process influenced by many factors. The mechanisms whereby these factors affect fibrosis *in vivo* are not well understood but are under active research in many laboratories.

50. Dooley S, Delvoux B, Streckert M, Bonzel L, Stopa M, Ten Dijke P, Gressner AM. Transforming growth beta signal transduction in hepatic stellate cells via Sma 2/3 phosphorylation, a pathway that is abrogated during *in vitro* progression to myofibroblast. TGF beta signal transduction during transdifferentiation of hepatic stellate cells. *FEBS Lett* 2001;502:4-10.

51. Bonner JC. Regulation of PDGF and its receptors in fibrotic diseases. *Cytokine Growth Factor Rev.* 2004;15:255-73.

52. Campbell JS, Hughes SD, Gilbertson DG, Palmer TE, Holdren MS, Haran AC, Odell MM, Bauer RL, Ren HP, Haugen HS, Yeh MM, Fausto N. Platelet-derived growth factor C induces liver fibrosis, steatosis, and hepatocellular carcinoma. *Proc Natl Acad Sci USA* 2005;102:3389-94.

53. Kordes C, Brookmann S, Haussinger D, Klonowski-Stumpe H. Differential and synergistic effects of platelet-derived growth factor-BB and transforming growth factor- β 1 on activated pancreatic stellate cells. *Pancreas* 2005;31:156-67.

54. de Lalla C, Galli G, Aldrighetti L, et al. Production of profibrotic cytokines by invariant NKT cells characterizes cirrhosis progression in chronic viral hepatitis. *J Immunol* 2004; 173:1417-25.

55. Torok N, Wu J, Zern MA, Halsted C, French S, Friedman SL, Zhan SS. Phagocytosis of apoptotic bodies by hepatic stellate cells occurs *in vivo* and is an important mechanism in liver fibrogenesis. *Gastroenterology* 2005;125:4(S2):A15.

56. Schiemann U, Glas J, Török P, Simperl C, Martin K, König A, Schmidt F, Schaefer M, Folwaczny C. Response to Combination Therapy with Interferon Alfa-2a and Ribavirin in Chronic Hepatitis C According to a TNF- α Promoter Polymorphism. *Digestion* 2003;68:1-4.

57. Leyland H, Gentry J, Arthur MJ, Benyon RC. The plasminogen-activating system in hepatic stellate cells. *Hepatology* 1996;24:1172-8.

58. Iorite M, Bh evitt D, Benitez J. The ADAMs: a molecular protein family with metalloproteinase and proteoglycan degrading activities expressed by the hepatic stellate cells. *Hepatology* 2001;32:345-68.

59. Giannelli G, Quaranta V, Antonaci S. Tissue remodelling in liver diseases. *Histol. Histopathol.* 2003;18:1267-74.

60. Sprenger H, Kaufmann A, Garn H, Lahme B, Gerns D, Gressner AM. Differential expression of monocyte chemotactic protein

-1 (MCP-1) in transforming rat hepatic stellate cells. *J Hepatol* 1999;30:88-94.

61. Louis H, Le Moine O, Goldman M, Deviere J. Modulation of liver injury by interleukin-10. *Acta Gastroenterol Belg.* 2003;66:7-14.

62. Li-Juan Z, Wei-Da Z, Mei-Na S, Xiao-Zhong W. Effects of interleukin-10 on activation and apoptosis of hepatic stellate cells in fibrotic rat liver. *World J Gastroenterol* 2006;12:1918-23.

63. Han YP, Zhou L, Wang J, Xiong S, Garner WL, French SW, Tsukamoto H. Essential role of matrix metalloproteinases in interleukin-1 induced myofibroblastic activation of hepatic stellate cellin collagen. *J Biol Chem* 2004;279:4820-8.

64. Ferracioli D, Zambelli LN, Silva F, Zucoloto S, Candolo AL, de Castro O. Liver cirrhosis and hepatic stellate cells. *Acta Cir Bras* 2006;21:234-56.

65. Derbala M, Amer A, Bener A, et al. Pegylated interferon-alpha 2b-ribavirin combination in Egyptian patients with genotype 4 chronic hepatitis. *J Viral Hepat* 2005;12:380-5.

66. Kamal SM, El Tawil AA, Nakano T, et al. Peginterferon {alpha}-2b and ribavirin therapy in chronic hepatitis C genotype 4:

- impact of treatment duration and viral kinetics on sustained virological response. *Gut* 2005;54:858-66.
67. Wong T, Lee S. Hepatitis C: a review for primary care physicians. *CMAJ* 2006;174:649-59.
68. Chandan G, Sung-W L, Roy N, Roy J. Cell Culture Models and Animal Models of Viral Hepatitis. Part II: Hepatitis C. *Lab Animal* 2005;34:39-47.
69. Yata Y, Gotwals P, Koteliensky V, Rockey DC. Dose-dependent inhibition of hepatic fibrosis in mice by a TGF-beta soluble receptor: implications for antifibrotic therapy. *Hepatology* 2002;35:1022-30.
70. McGaha TL, Phelps RG, Spiera H, Bona C. Halofuginone, an inhibitor of type-I collagen synthesis and skin sclerosis, blocks transforming-growth-factor-beta-mediated Smad3 activation in fibroblasts. *J Invest Dermatol* 2002;118:461-70.
71. Mei-Na S, Yue-Hong H, Wei-Da Z, Li-Juan Z, Zhi-Xin C, Xiao-Zhong W. Relationship between transforming growth factor β 1 and anti-fibrotic effect of interleukin-10. *World J Gastroenterol* 2006;12:2357-62.
72. Iwamoto H, Nakamuta N, Tada S, Sugimoto R, Enoji N, Nawata H. Platelet-derived growth factor receptor tyrosine kinase inhibitor AG1295 attenuates rat hepatic stellate cell growth. *J Lab Clin Med* 2000;135:406-12.
73. Poynard T, McHutchison J, Trepo C, Lindsay K, Goodman Z, Mei-Hsiu L, *et al.* Impact of pegylated Interferon α -2-b and ribavirin on liver fibrosis in patients with chronic hepatitis C. *Gastroenterology* 2002;122:1303-13.
74. Taimr P, Higuchi H, Kocova E, Rippe RA, Friedman S, Gores GJ. Activated stellate cells express the TRAIL receptor-2/death receptor-5 and undergo TRAIL-mediated apoptosis. *Hepatology* 2003;37:87-95.
75. Saile B, Eisenbach C, Dudas J, El-Armouche H, Ramadori G. Interferon-gamma acts proapoptotic on hepatic stellate cells (HSC) and abrogates the antiapoptotic effect of interferon-alpha by an HSP70-dependent pathway. *Eur J Cell Biol* 2004;83:469-76.
76. Ichiro S. Antifibrogenic Therapies in Chronic HCV Infection. *Current Drug Targets Infectious Disorders* 2001;1:227-40.
77. Parsons CJ, Bradford BU, Pan CQ *et al.* Antifibrotic effects of a tissue inhibitor of metalloproteinase-1 antibody on established liver fibrosis in rats. *Hepatology* 2004;40:1106-15.
78. Fallowfield JA, Iredale JP. Targeted treatments for cirrhosis. *Expert Opin Ther Targets* 2004;8:423-35.
79. Rockey DC. Antifibrotic therapy in chronic liver disease. *Clin Gastroenterol Hepatol*. 2005;3:95-107.

Received in december, 2006 Accepted
for publication in april, 2007.