Pathogenesis of Hepatic Fibrosis

Laura Smart

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- **Fibrosis is a wound healing response in which damaged regions are encapsulated by an extracellular matrix or scar.**
- **Fibrosis develops in almost all patients with chronic liver injury at variable rates depending in part upon the cause of liver disease and host factors.**
- **Fibrosis occurs earliest in regions where injury is most severe.**
- **While fibrosis is reversible in its initial stages, progressive fibrosis can lead to cirrhosis. The exact point when fibrosis becomes irreversible is incompletely understood.**
- **The development of fibrosis usually requires several months to years of ongoing injury.**

- A complex interplay among different hepatic cell types takes place during hepatic fibrogenesis

- Hepatocytes are targets for most hepatotoxic agents, including hepatitis viruses, alcohol metabolites, and bile acids

- Hepatic fibrosis is the result of the wound-healing response of the liver to repeated injury. After an acute liver injury, parenchymal cells regenerate and replace the necrotic or apoptotic cells. This process is associated with an inflammatory response and a limited deposition of extracellular matrix. If the hepatic injury persists, then eventually the liver regeneration fails, and hepatocytes are substituted with abundant extracellular matrix (ECM), including fibrillar collagen.

- Liver fibrosis is associated with major alterations in both the quantity and composition of ECM.

- In advanced stages, the liver contains approximately 6 times more ECM than normal, including collagens (I, III, and IV), fibronectin, undulin, elastin, laminin, hyaluronan, and proteoglycans. Accumulation of ECM results from both increased synthesis and decreased degradation.

- Matrix alterations observed during fibrogenesis alter cellular behavior by processes involving cell membrane receptors. One of the best characterized are integrins, which are a large family of homologous membrane linker proteins that control several cellular functions including gene expression, growth, and differentiation.

- Integrin signaling across the plasma membrane permits communication between the extracellular matrix and cytoskeleton. Signaling occurs in conjunction with the phosphorylation of several intracellular substrates.

- Stellate cells express integrin receptors for collagen, which may contribute to their activation and proliferation in response to deposition of these matrix components during injury.

- The extracellular matrix can also affect cell function indirectly by the release of cytokines.

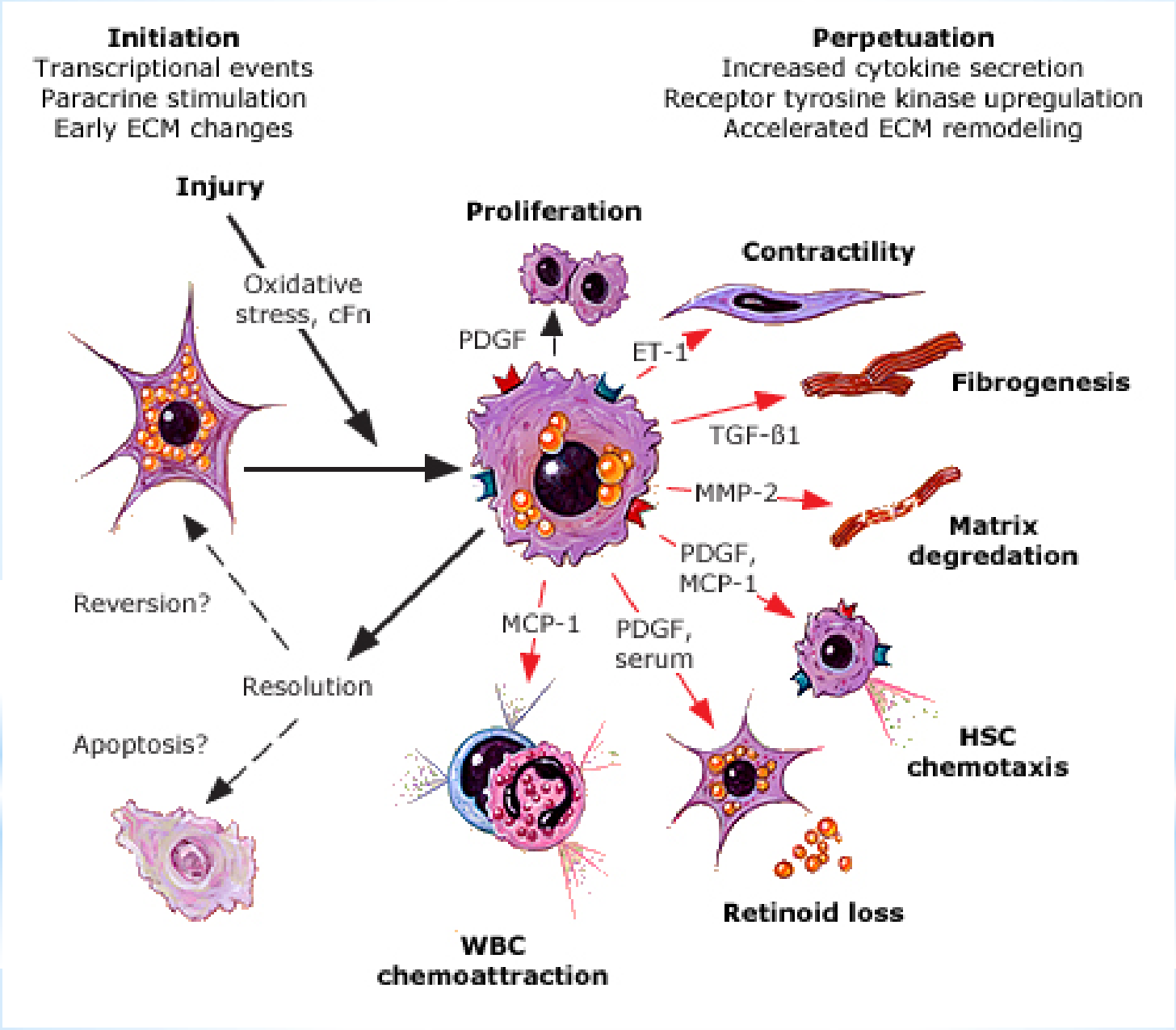
- The cytokines include PDGF, HGF, connective tissue growth factor (CTGF), TNFa, bFGF, and vascular endothelial cell growth factor

- TGF-beta-1, derived from both paracrine and autocrine sources, remains the classic fibrogenic cytokine. It stimulates collagen transcription in stellate cells.

- Hepatic stellate cells are the main ECM-producing cells in the injured liver.
- In the normal liver, stellate cells reside in the space of Disse and are the major storage sites of vitamin A.
- Stellate cells are 15 percent of the total number of resident cells in normal liver.
- Following chronic injury, stellate cells activate or transdifferentiate into myofibroblast-like cells, acquiring contractile, proinflammatory, and fibrogenic properties.
- This change is characterized morphologically by enlargement of rough endoplasmic reticulum, diminution of vitamin A droplets, a ruffled nuclear membrane, appearance of contractile filaments, and proliferation.

- Activation consists of two major phases: Initiation and perpetuation
- Initiation (also called a "preinflammatory stage") refers to early changes in gene expression and phenotype that render the cells responsive to other cytokines and stimuli. Initiation results mostly from paracrine stimulation.
- Perpetuation results from the effects of these stimuli on maintaining the activated phenotype and generating fibrosis. Perpetuation involves autocrine as well as paracrine loops.
- The earliest changes observed during stellate activation result from paracrine stimulation by all neighboring cell types, including sinusoidal endothelium, Kupffer cells, hepatocytes, and platelets through release of cytokines.
- Kupffer cells stimulate matrix synthesis, cell proliferation, and release of retinoids by stellate cells through the actions of TGF-beta-1 and reactive oxygen intermediates.
- Hepatocyte apoptosis following injury also promotes stellate cell initiation through a process mediated by Fas, a protein involved in causing apoptosis.

Stellate cell activation



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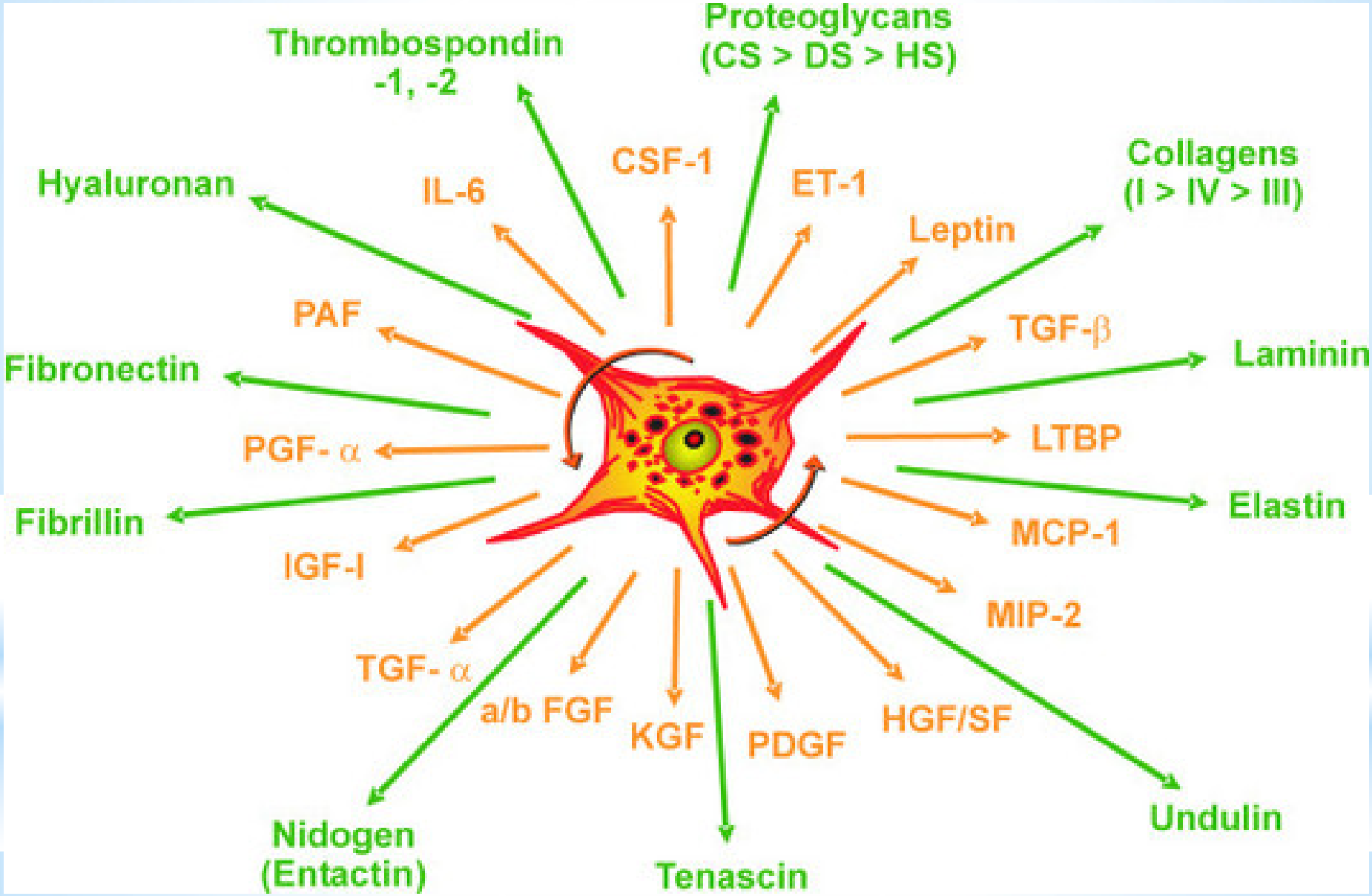
Activated Stellate Cell



•Damaged hepatocytes also induce the recruitment of white blood cells by inflammatory cells.

•Apoptosis of damaged hepatocytes stimulates the fibrogenic actions of liver myofibroblasts.

•Inflammatory cells, either lymphocytes or polymorphonuclear cells, activate stellate cells to secrete collagen. Activated stellate cells secrete inflammatory chemokines, express cell adhesion molecules, and modulate the activation of lymphocytes. Therefore, a vicious circle in which inflammatory and fibrogenic cells stimulate each other occurs.

•Activated stellate cells migrate and accumulate at the sites of tissue repair, secreting large amounts of ECM and regulating ECM degradation.

- Once stellate cells are activated, they release chemokines and other leukocyte chemoattractants while upregulating the expression of inflammatory receptors, chemokine receptors, and mediators of lipopolysaccharide signaling.

- Collagen synthesis in stellate cells is regulated at the transcriptional and posttranscriptional levels. Increased collagen mRNA stability mediates the increased collagen synthesis in activated stellate cells.

- Type IV collagen, fibrinogen, and urokinase type plasminogen activator stimulate resident stellate cells by activating latent cytokines such as TGF-β1. Fibrillar collagens can bind and stimulate stellate cells via integrins.

- The altered ECM can serve as a reservoir for growth factors and MMPs.

- Perpetuation of stellate cell activation involves changes in stellate cell behavior including proliferation, chemotaxis, fibrogenesis, contractility, matrix degradation, retinoid loss, and WBC chemoattractant and cytokine release.

- Platelet derived growth factor (PDGF) is the most potent stellate cell mitogen.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- Accumulation of ECM results from both increased synthesis and decreased degradation

- A critical element in matrix remodeling is a family of matrix-metalloproteinases (MMPs). These are calcium-dependent enzymes that specifically degrade collagens and non-collagenous substrates. Failure to degrade the increased interstitial, or scar matrix is a major determinant of progressive fibrosis.

- Matrix metalloproteinase-1 (MMP-1) is the main protease that can degrade type I collagen, the principal collagen in fibrotic liver.

- Decreased activity of ECM-removing MMPs is mainly due to an overexpression of their specific inhibitors (TIMPs).

- Progressive fibrosis is associated with marked increases in TIMP-1 and TIMP-2, leading to a net decrease in protease activity, and therefore more unopposed matrix accumulation.

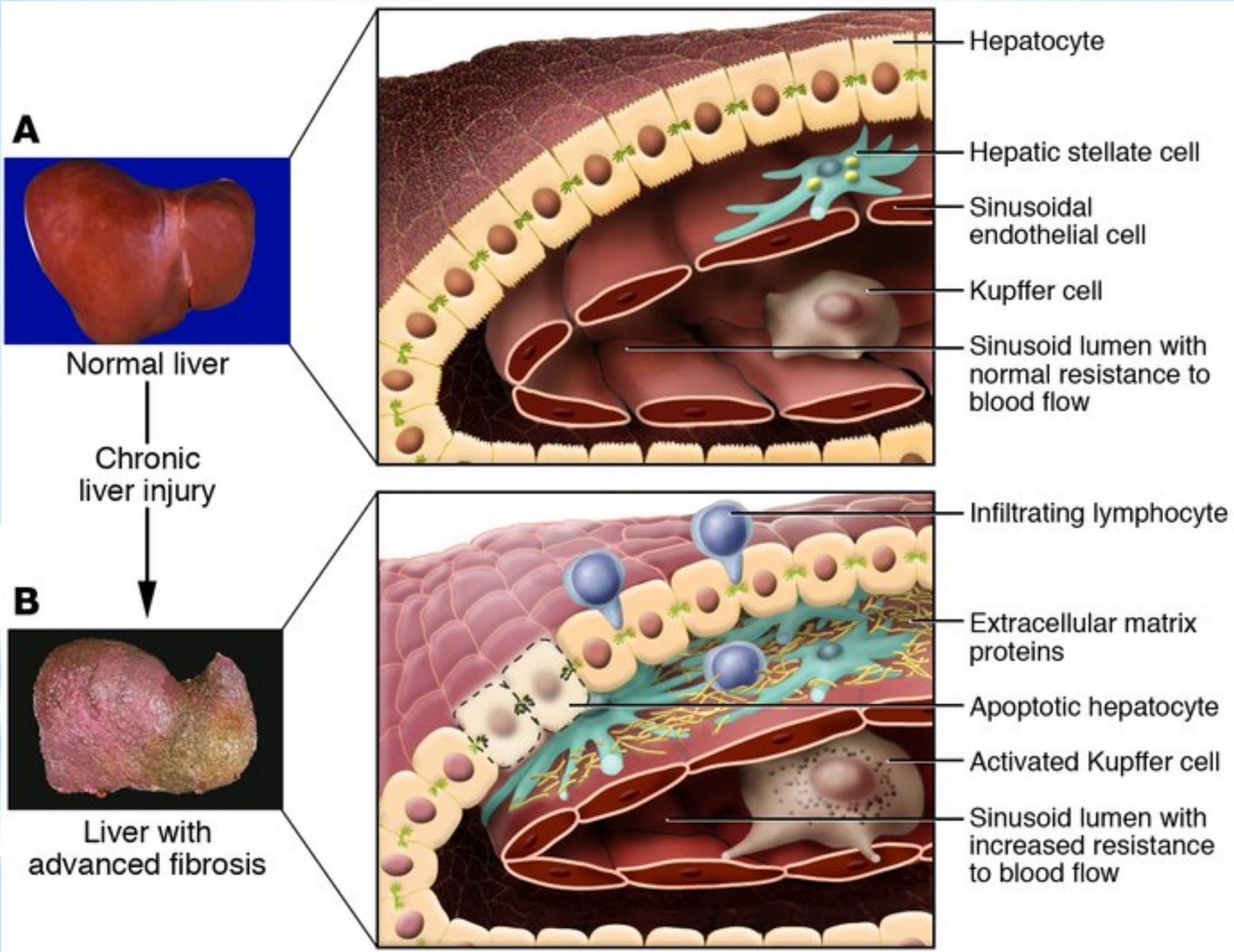
- Stellate cells are the major source of TIMPs.

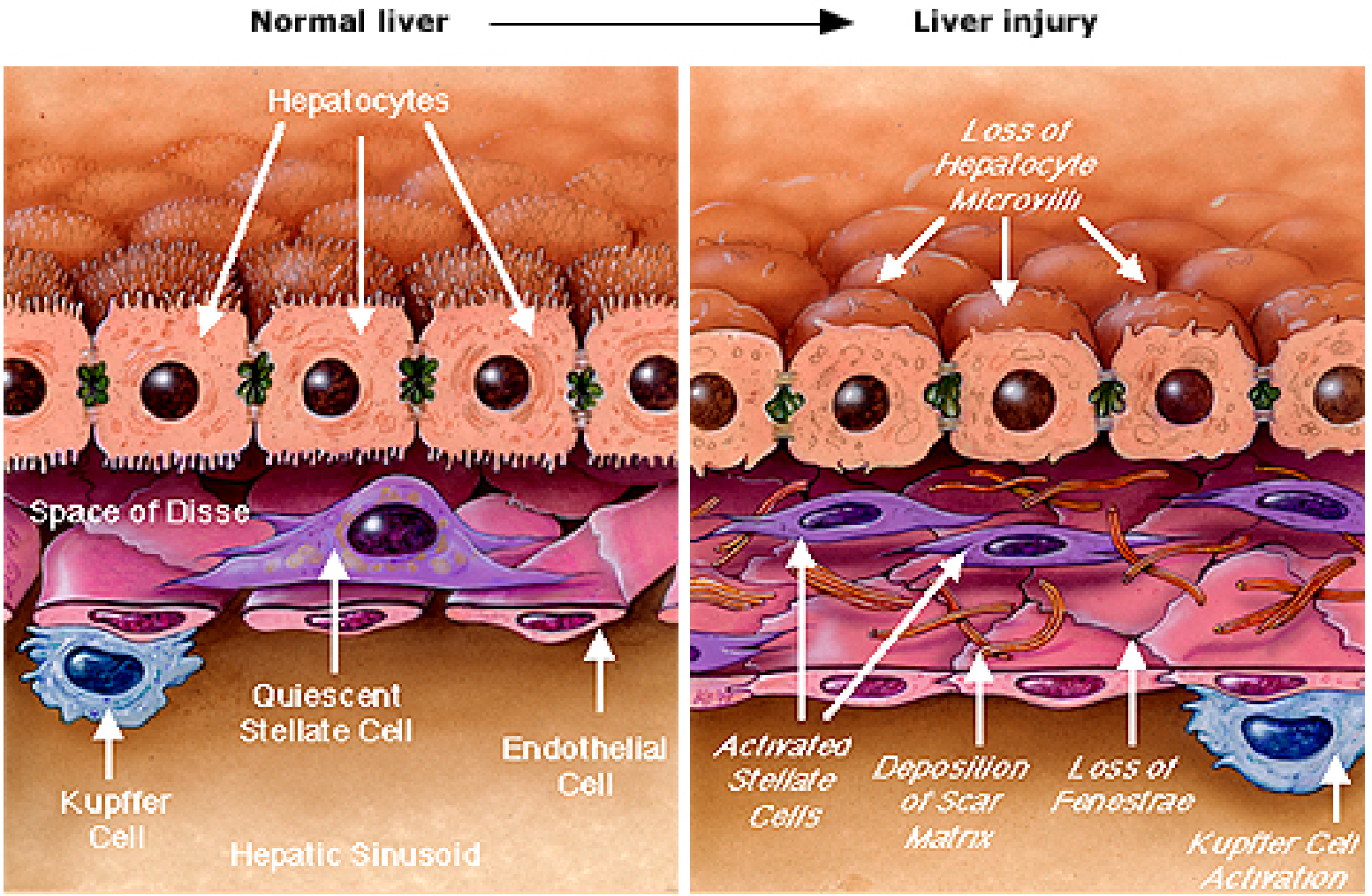
- Hepatic cell types other than stellate cells may also have fibrogenic potential.

- Myofibroblasts derived from small portal vessels proliferate around biliary tracts in cholestasis-induced liver fibrosis to initiate collagen deposition.

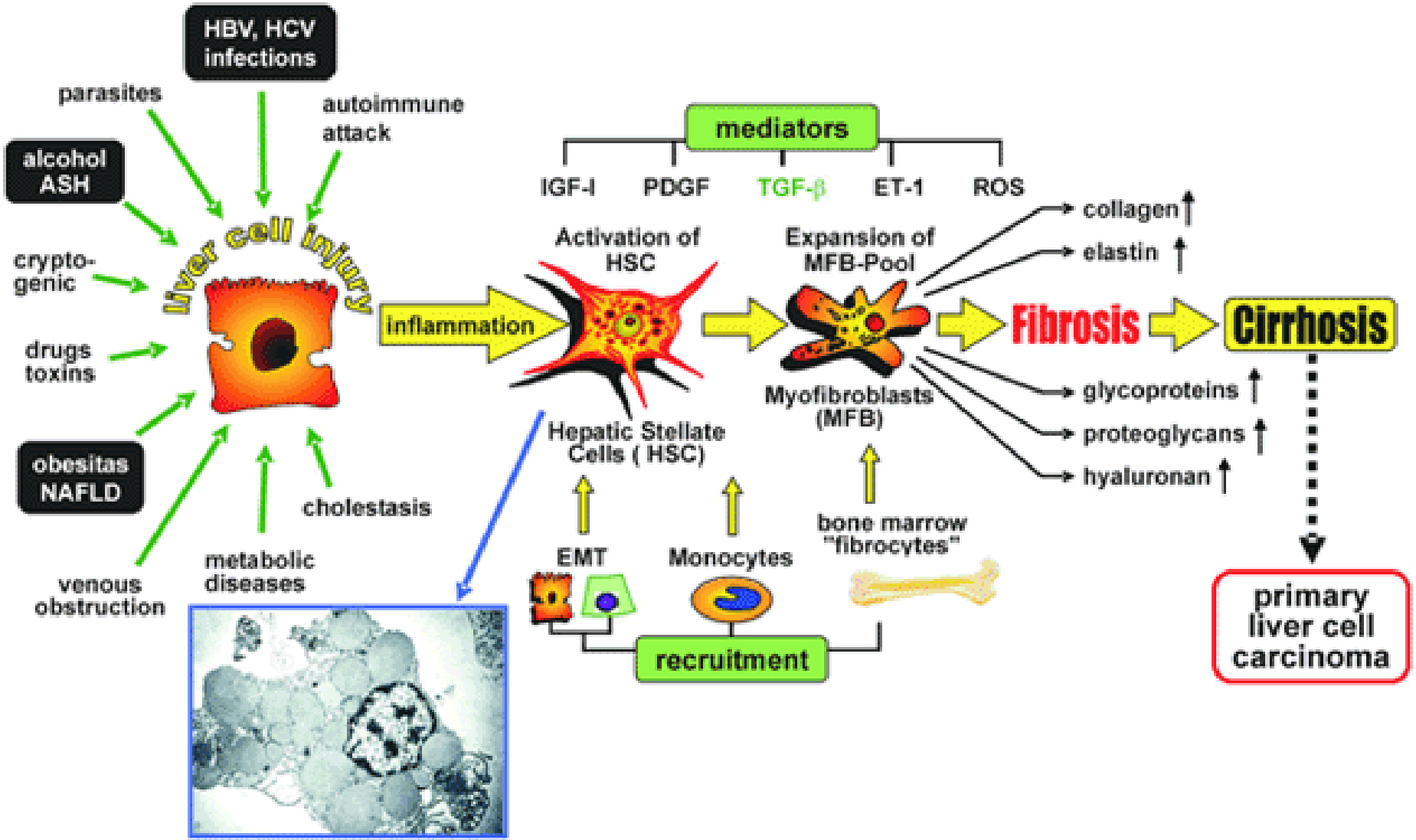
- The relative importance of each cell type in liver fibrogenesis can depend on the origin of the liver injury.

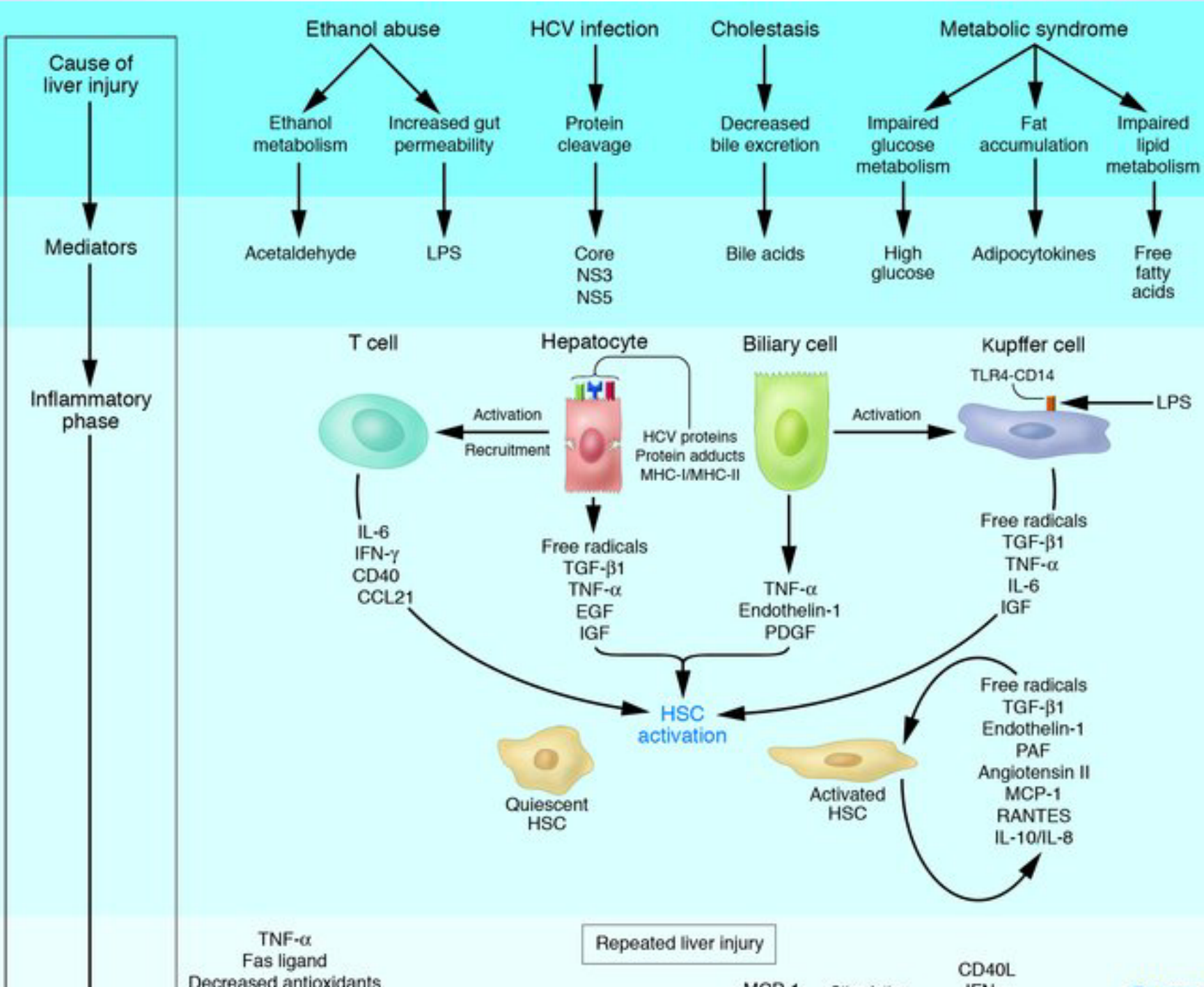
- Stellate cells are the main fibrogenic cell type in pericentral areas, but portal myofibroblasts may predominate when liver injury occurs around portal tracts.

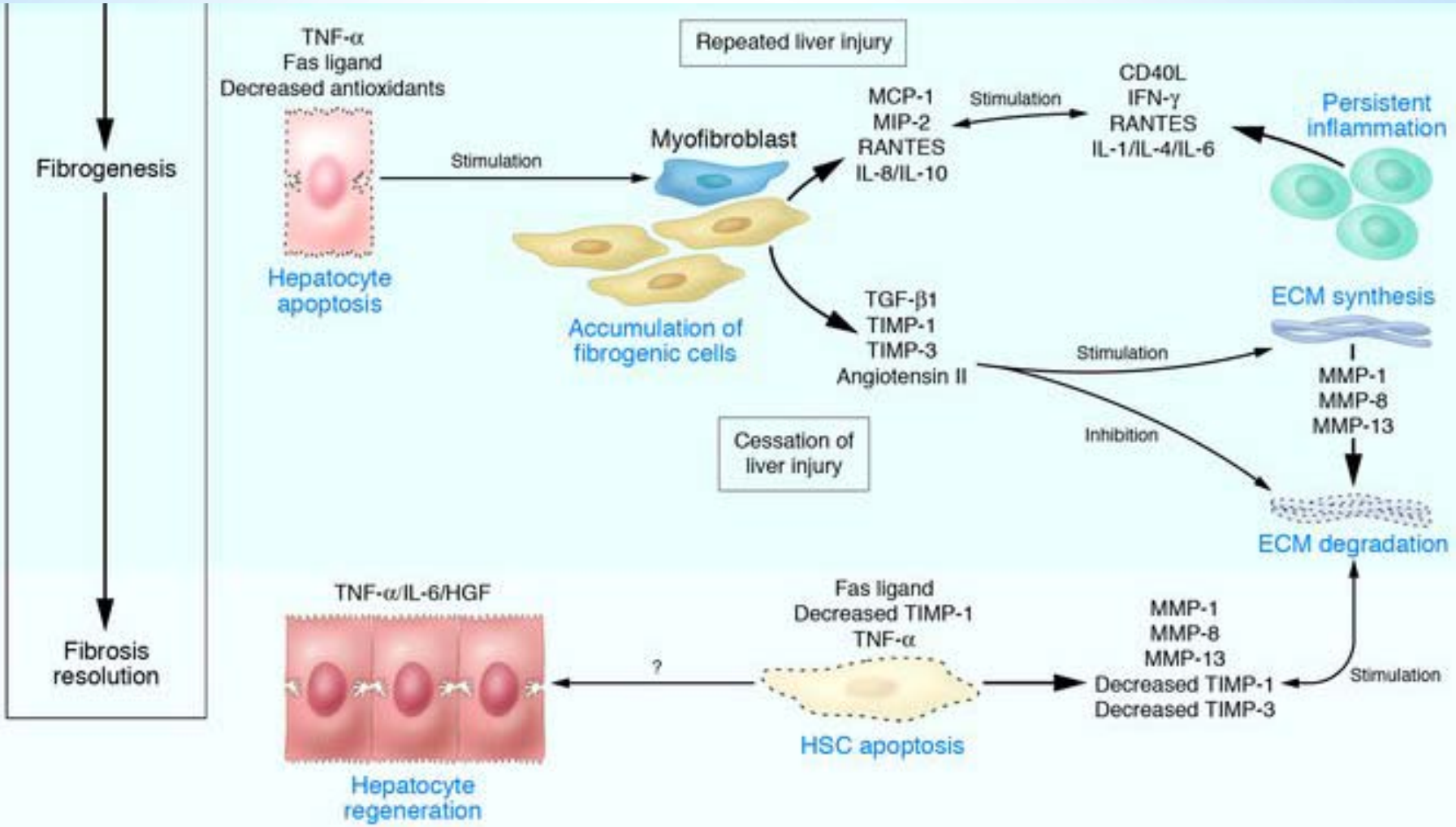

A
Normal liver
Chronic liver injury
B
Liver with advanced fibrosis
Hepatocyte
Hepatic stellate cell
Sinusoidal endothelial cell
Kupffer cell
Sinusoid lumen with normal resistance to blood flow
Infiltrating lymphocyte
Extracellular matrix proteins
Apoptotic hepatocyte
Activated Kupffer cell
Sinusoid lumen with increased resistance to blood flow



Changes in the subendothelial space of Disse and sinusoid as fibrosis develops in response to liver injury include alterations in both cellular responses and extracellular matrix composition. Stellate cell activation leads to accumulation of scar (fibril-forming) matrix. This in turn contributes to the loss of hepatocyte microvilli and sinusoidal endothelial fenestrae, which result in deterioration of hepatic function. Kupffer cell (macrophage) activation accompanies liver injury and contributes to paracrine activation of stellate cells.




Cause of liver injury
Mediators
Inflammatory phase
Ethanol abuse
HCV infection
Cholestasis
Metabolic syndrome
Ethanol metabolism
Increased gut permeability
Protein cleavage
Decreased bile excretion
Impaired glucose metabolism
Fat accumulation
Impaired lipid metabolism
Acetaldehyde
LPS
Core
NS3
NS5
Bile acids
High glucose
Adipocytokines
Free fatty acids
T cell
Hepatocyte
Biliary cell
Kupffer cell
TLR4-CD14
LPS
Activation
Recruitment
HCV proteins
Protein adducts
MHC-I/MHC-II
Activation
IL-6
IFN-γ
CD40
CCL21
Free radicals
TGF-β1
TNF-α
EGF
IGF
TNF-α
Endothelin-1
PDGF
Free radicals
TGF-β1
TNF-α
IL-6
IGF
HSC activation
Quiescent HSC
Activated HSC
Free radicals
TGF-β1
Endothelin-1
PAF
Angiotensin II
MCP-1
RANTES
IL-10/IL-8
TNF-α
Fas ligand
Decreased antioxidants
Repeated liver injury
CD40L


Fibrogenesis
Fibrosis resolution
TNF-α
Fas ligand
Decreased antioxidants
Hepatocyte apoptosis
Repeated liver injury
Stimulation
Myofibroblast
Accumulation of fibrogenic cells
MCP-1
MIP-2
RANTES
IL-8/IL-10
Stimulation
CD40L
IFN-γ
RANTES
IL-1/IL-4/IL-6
Persistent inflammation
TGF-β1
TIMP-1
TIMP-3
Angiotensin II
Stimulation
ECM synthesis
MMP-1
MMP-8
MMP-13
Inhibition
Cessation of liver injury
ECM degradation
TNF-α/IL-6/HGF
Hepatocyte regeneration
?
Fas ligand
Decreased TIMP-1
TNF-α
HSC apoptosis
MMP-1
MMP-8
MMP-13
Decreased TIMP-1
Decreased TIMP-3
Stimulation

- The gold standard for diagnosis of hepatic fibrosis is liver biopsy.
- Other noninvasive tests have been developed for hepatic fibrosis
- FibroSURE is a noninvasive blood test that combines the quantitative results of six serum biochemical markers: α2-macroglobulin, haptoglobin, apolipoprotein A1, bilirubin, GGT, and ALT with a patient's age and gender in a patented artificial intelligence algorithm to generate a measure of fibrosis and necroinflammatory activity in the liver.
- It provides a numerical quantitative estimate of liver fibrosis ranging from 0.00 to 1.00 corresponding to the well-established Metavir scoring system of stages F0 to F4. (F0 = no fibrosis, F1 = portal fibrosis, F2 = bridging fibrosis with few septa, F3 = bridging fibrosis with many septa, F4 = cirrhosis).
- In addition, the test provides a numerical quantitative estimate of necroinflammatory activity ranging from 0.00 to 1.00 corresponding to the Metavir scoring system of grades A0 to A3. (A0 = no activity, A1 = minimal activity, A2 = moderate activity, A3 = severe activity).
- It can differentiate mild and severe fibrosis but is not as accurate for intermediate fibrosis

Strategies	Approach
Remove injurious stimuli	Abstinence from alcohol for alcoholic liver diseases
	Antiviral therapy for viral hepatitis
	Antihelminthic therapy for schistosomiasis
	Copper chelation for Wilson's disease
	Phlebotomy for hemochromatosis
	Discontinue hepatotoxins (eg, methotrexate) in drug-induced liver injury
Suppress hepatic inflammation	Corticosteroids in autoimmune liver diseases
	Neutralizing inflammatory cytokines using specific receptor antagonists: IL-1 receptor antagonists, soluble TNF-alpha receptor
	Ursodeoxycholic acid (UDCA)
	Others: prostaglandin E, colchicine and colchiceine, milotilate, translast, IL-10

Downregulate stellate cell activation	**Gamma Interferon**
	Antioxidants: alpha-tocopherol, resveratrol, quercetin, N-acetylcysteine, silymarin, OC-15161
	Cytokine-directed therapy:
	TGF-beta antagonists
	Endothelin receptor antagonists
	Hepatic growth factor
	Disrupt ECM-HSC interactions: Arg-Gly-Asp (RGD) analogue
	Collagen synthesis inhibitors:
	TGF-beta antagonists, relaxin, halofuginone
	Others:
	Dilinoleylphosphotidylcholine (DLPC), HMG CoA reductase,
	Pentoxyphylline, HOE077, Safironil
	Retinoids?
	Herbal medicine: Sho-saiko-to (Xiao-Chaihu-Tang), Salvia miltiorrhiza (Dan-Shen)
Promote matrix degradation	Target degradation of interstitial-type matrix, rather than basement membrane-type matrix
	TGF-beta antagonists, relaxin
Promote specific apoptosis of hepatic stellate cells	Not yet available