Introduction

Stem cells are generally defined as cells exhibiting two properties: capacity for dividing for long periods and potency for multilineage differentiation ***(Oertel et al., 2008).***

Hepatic progenitors (hepatic stem cells) have been isolated from the following sources: 1. Extrahepatic sources: these include autologous bone marrow, umbilical cord blood, Wharton's jelly and peripheral blood monocytes. 2. Intrahepatic sources: these include cadaver livers considered of insufficient quality for organ transplantation or hepatocytes isolated from aborted human fetuses ***(Schmelzer et al., 2007).***

Another effective way to obtain hepatic differentiation is genetic modulation. This can be achieved by transfecting stem cells with recombinant DNA encoding for hepatospecific proteins ***(Tabei et al., 2003).***

Hepatic stem cells are good candidate donor cells for the treatment of liver diseases. Because they are specified to liver, functional differentiation of hepatic stem cells would be technically easier than differentiation of embryonic stem/ induced pluripotent stem cells.

A unique mechanism is recognized to be the main reason for development of liver fibrosis: sustained hepatic stellate cell activation and transformation. Therefore, the use of anti-oxidants and anti-fibrotic molecules inhibiting or

treating liver fibrosis can offer moderate hope ***(Rygiel et al., 2008).***

Autologous bone marrow cell therapies may ameliorate this condition by way of the fibrolytic actions of secreted matrix metalloproteinases that degrade the bands of collagen. ***(Higashiyama et al., 2007).***

The fact that hepatic proginator cell activation precedes the development of Hepatocellular Carcinoma (HCC) and invariably accompanies chronic liver damage, makes it almost certain that the mature hepatocyte is not the cell of origin of all HCCs; indeed, perhaps only a small minority of HCCs are derived from the mature hepatocyte ***(Tang et al., 2008).***

There is a growing realization that cancers contain a minority population of self-renewing stem cells, the cancer stem cells, which are entirely responsible for much of the cellular heterogeneity and mutations sustaining the tumour ***(Alison et al., 2008).***

Exploration of the difference between cancer stem cells and normal stem cells is crucial not only for the understanding of tumor biology but also for the development of specific therapies that effectively target these cells in patients. These ideas draw attention to the control of stem cell proliferation by the transforming growth factor beta (TGF-beta) pathway, suggesting a dual role for this pathway in tumor suppression as well as progression of differentiation from a stem or progenitor stage ***(Nguyen et al., 2007).***

Aim of Work

Liver stem cell therapy presents a new hope in the management of liver fibrosis and hepatocellular carcinoma.

Liver Fibrosis

Liver fibrosis represents the liver's wound healing response to virtually all forms of chronic liver injury. Chronic hepatic injury results in liver fibrosis with eventual progression to cirrhosis and end stage liver disease. At this point the majority of the clinical complications arise such as portal hypertension and the development of liver cancer. If the causative disease can be effectively treated the liver can regenerate and at the least partial resolution of liver fibrosis may occur. Unfortunately, unless the primary disease can be eradicated there are no specific anti-fibrotic treatments in routine clinical use. This highlights the urgent need to both increase our understanding of the mechanisms of hepatic fibrogenesis and to develop novel therapies to arrest or reverse the fibrotic process. This article initially outlines the main cellular pathway of fibrogenesis within the liver—the activation of the quiescent hepatic stellate cell into an activated myofibroblast phenotype, resulting in the production of fibrillar collagen. ***(Henderson, 2008).***

Etiology

Single or multifactorial insults to the liver ultimately lead to cirrhosis, the most common being alcohol abuse, chronic hepatitis C, and obesity with concomitant nonalcoholic fatty liver disease (Table 1) ***(Crawford, 2005).***

Etiologies of Hepatic Cirrhosis

Most common causes

Alcohol (60 to 70 percent)
Biliary obstruction (5 to 10 percent)
- Biliary atresia/neonatal hepatitis
- Congenital biliary cysts
- Cystic fibrosis

Primary or secondary biliary cirrhosis
Chronic hepatitis B or C (10 percent)
Hemochromatosis (5 to 10 percent)
NAFLD (10 percent)-most commonly resulting from obesity; also can occur after jejunoileal bypass

Less common causes

Autoimmune chronic hepatitis types 1, 2, and 3
Drugs and toxins
- Alpha-methyldopa (Aldomet)
- Amiodarone (Cordarone)
- Isoniazid (INH)
- Methotrexate
- Oxyphenisatin (Prulet)
- Perhexiline
- Troglitazone (Rezulin)
- Vitamin A

Genetic metabolic disease
- a1-Antitrypsin deficiency
- Amino acid disorders (e.g., tyrosinemia)
- Bile acid disorders
- Carbohydrate disorders (e.g., fructose intolerance, galactosemia, glycogen storage diseases)
- Lipid disorders (e.g., abetalipoproteinemia)
- Porphyria
- Urea cycle defects (e.g., ornithine carbamoyltransferase deficiency)
- Wilson's disease

Idiopathic/miscellaneous
- Granulomatous liver disease (e.g., sarcoidosis)
- Idiopathic portal fibrosis
- Indian childhood cirrhosis
- Polycystic liver disease

Infection
- Brucellosis
- Congenital or tertiary syphilis
- Echinococcosis
- Schistosomiasis

Vascular abnormalities
- Chronic, passive hepatic congestion caused by right-sided

	heart failure, pericarditis
	Hereditary hemorrhagic telangiectasia (Osler-Weber-Rendu disease)
Veno-occlusive disease	

NAFLD = nonalcoholic fatty liver disease.

Table 1. Etiologies of Hepatic Cirrhosis ***(Friedman, 2004)***

Pathophysiology

Regardless of the cause, the primary event that leads to cirrhosis is injury to hepatocellular elements, which leads to architectural destruction if the damage exceeds the regenerative capacity of the organ. Both acute and chronic injury initiate an inflammatory response with associated cytokine release; elaboration of toxic substances; destruction of hepatocytes, bile duct cells, and vascular endothelial cells; repair through cellular proliferation and regeneration; and formation of fibrous scar (Fig.1) ***(Mulholland, 2006).***

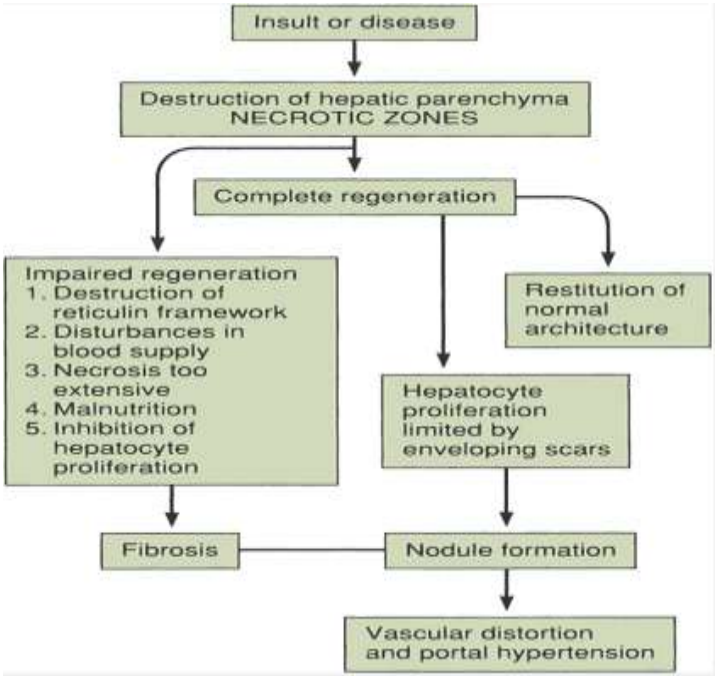

Fig. (1): Evolution of cirrhosis. Fibrosis develops in nonregenerative necrotic areas, producing scars. The pattern of nodularity and scars reflects the type of response to injury (e.g., uniform vs. nonuniform necrosis) and the extent of injury ***(Mulholland, 2006).***

Hepatic Stellate Cells:

The primary cell implicated in the formation of fibrosis is the hepatic stellate cell (Ito cell, lipocyte, perisinusoidal cell), located in the perisinusoidal space of Disse (Fig. 2) ***(Friedman et al., 1992).***

As a result of the activation of stellate cells and a subsequent enhancement in collagen and extracellular matrix synthesis, the space of Disse becomes thickened, so that capillarization develops and the normal fenestrated architecture of the sinusoidal endothelium is lost ***(Schaffner, 1963).***

Obliteration of sinusoidal fenestrations may be the essential component of fibrosis-induced hepatocellular dysfunction in cirrhosis, preventing the normal flow of

nutrients to hepatocytes and increasing vascular resistance ***(Friedman, 1999).***

In addition, production of endothelin-1, a potent vasoconstrictor, by endothelial or stellate cells can cause contraction of the myofilaments within the stellate cell, influencing blood flow to injured areas and contributing to portal hypertension. Initially, fibrosis may be reversible if the inciting agents are removed. With sustained injury, the process of fibrosis becomes irreversible and leads to cirrhosis ***(Mulholland, 2006).***

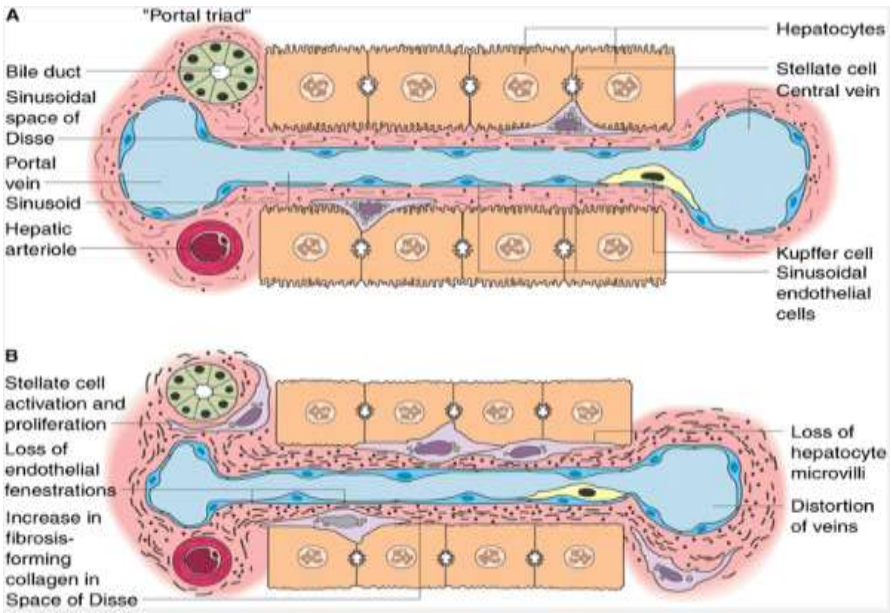

Fig. (2): Matrix and cellular alterations in hepatic fibrosis. (A) In normal liver, a modest amount of low-density matrix is present in the subendothelial space of Disse. (B) In the fibrotic liver, the accumulation of fibril-forming matrix in this region leads to capillarization of the sinusoid and functional changes in all neighboring cell types ***(Friedman et al., 1992).***

Non-hepatic stellate cells, origins of myofibroblasts in liver fibrogenesis

The anatomical location, source and lineage of hepatic myofibroblasts have recently generated a great deal of interest. As well as hepatic stellate cells there are now a substantial number of different postulated subclasses of hepatic scar forming cells. Fig.3 summarises our current understanding of the potential origins of the hepatic myofibroblast pool, and also highlights the functionally distinct subpopulations of macrophages which exist within the liver during hepatic injury and repair ***(Henderson, 2008).***

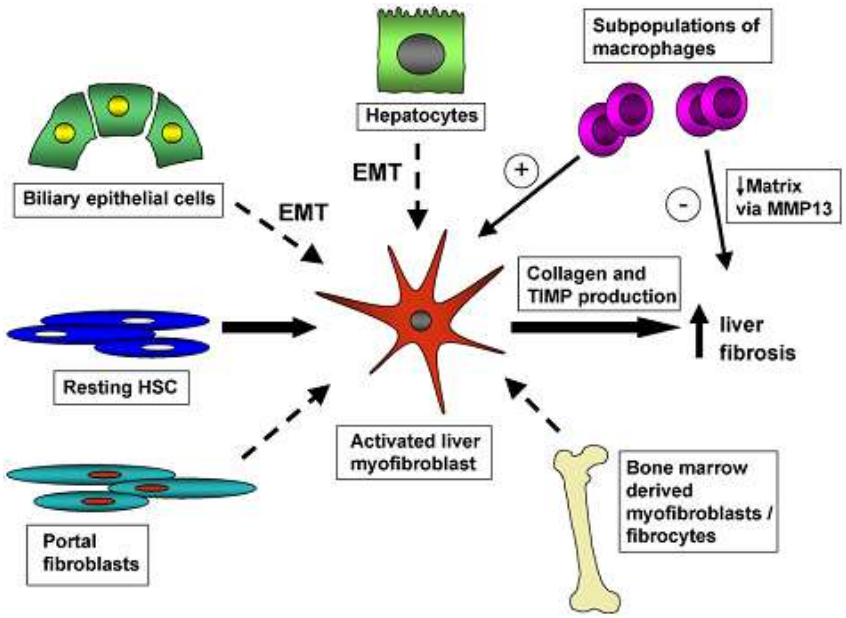

Fig. (3): Potential origins of the liver myofibroblast pool and functionally distinct subpopulations of macrophages during hepatic injury and repair. Figure modified with permission from Clinical Science ***(Henderson, 2007).***

EMT (epithelial–mesenchymal transition)

EMT is a highly orchestrated sequence of events in which cell–cell and cell–extracellular matrix interactions are modulated to release epithelial cells from the basement membrane and surrounding tissue. The resulting cytoskeletal reorganisation confers an ability to migrate through the extracellular matrix and the transcriptional programme of the cell is altered to maintain a mesenchymal phenotype ***(Radisky, 2005).*** EMT has recently come to the fore with regard to its role in the formation of fibroblasts from epithelia during organ fibrogenesis in the wound healing response in adult tissues.

Biliary EMT

Analysis of liver tissue from a single patient with posttransplantation recurrence of primary biliary cirrhosis suggested biliary EMT may be an important pathogenic mechanism in this process. Biliary epithelial cell expression of S100A4, vimentin and p-Smad2/3 were identified immunohistochemically in most biliary epithelial cells in liver tissue from this patient at the point of diagnosis of recurrent disease. The authors concluded that expression of these markers in early recurrent PBC after liver transplantation suggests that biliary epithelial cell EMT is occurring (potentially explaining biliary epithelial cell loss) and that this process is driven by TGF-β ***(Robertson et al., 2007).***

A further study has examined a larger number of paediatric and adult liver disease cases looking for evidence of biliary EMT. This study observed significant co-localisation between cytokeratin-19 and various markers of the epithelial to mesenchymal transition in biliary atresia as well as other liver diseases associated with significant bile ductular proliferation, including primary biliary cirrhosis. The authors presented histological evidence suggesting that EMT occurs in human liver fibrosis, particularly in diseases such as biliary atresia and primary biliary cirrhosis with prominent bile ductular proliferation ***(Díaz et al., 2008).***

Hepatocyte EMT

Recent data has proposed that hepatocytes may contribute to the hepatic fibrogenic process by EMT in the adult liver wound healing response to chronic injury.

Zeisberg and colleagues have examined hepatocyte EMT and liver fibrosis. TGF-β1 induced adult mouse hepatocytes to undergo EMT and demonstrating that approximately half of these fibroblasts were hepatocyte-derived. ***(Zeisberg et al., 2007).***

Zeisberg et al. also examined the effect of bone morphogenic protein-7 (BMP7) in the progression of liver fibrosis. BMP7 (an antagonist of TGF-1) administration inhibited liver fibrogenesis induced by chronic CCl4 treatment. Furthermore BMP7 administration resulted in a significant inhibition of hepatocyte EMT in vivo suggesting that the therapeutic effect of BMP7 in liver fibrosis is at least

partially secondary to inhibition of hepatocyte EMT. ***(Zeisberg et al., 2003).***

These results suggest a more intimate relationship between the mesenchymal cells and the epithelial cells in the liver than previously suspected and if confirmed may go to explain the finding that epithelial cells can switch (or now possibly revert) into a mesenchymal phenotype under conditions of injury.

Bone marrow-derived myofibroblasts

In order to investigate the recruitment of BM-derived ells to the liver various groups studied mice that had received transplants with traceable BM. These studies revealed that a proportion of the scar forming cells (stellate cells, myofibroblasts and fibrocytes) could be of BM origin ***(Baba et al., 2004; Russo et al., 2006; Kisseleva et al., 2006).*** This will be discussed later in the coming chapters…

In conclusion, hepatic stellate cell activation remains the major paradigm pathway of fibrogenesis within the liver. However recent studies have highlighted the emerging role of other potentially important cellular pathways in the pathogenesis of hepatic scarring. Fibrogenic cells recruited both locally and from sites outwith the liver may play complementary roles in the dynamic process of hepatic wound healing. Increasing our understanding of the key cellular effectors of scar deposition in the liver and how these processes interplay with the liver's inflammatory

response should provide the basis for rational design of effective anti-fibrotic therapies ***(Henderson, 2008).***

Clinical Presentation

History

Cirrhosis often is a silent disease, with most patients remaining asymptomatic until decompensation occurs ***(Friedman, 2004).***

Early and well-compensated cirrhosis can manifest as anorexia and weight loss, weakness, fatigue, and even osteoporosis as a result of vitamin D malabsorption and subsequent calcium deficiency. Decompensated disease can result in complications such as ascites, spontaneous bacterial peritonitis, hepatic encephalopathy, and variceal bleeding from portal hypertension. Clinical symptoms at presentation may include jaundice of the eyes or skin, pruritus, gastrointestinal bleeding, coagulopathy, increasing abdominal girth, and mental status changes. Each of these clinical findings is the result of impaired hepatocellular function with or without physical obstruction secondary to cirrhosis. Because hepatic enzyme synthesis is required for drug metabolism, heightened sensitivity and medication toxicity may occur in patients with impaired hepatic enzyme synthesis ***(Friedman, 2004).***

Physical examination

Physical examination of patients with cirrhosis may reveal a variety of findings that should lead to a targeted hepatic- or gastrointestinal-based work-up (Table 2). Many

patients will already have had serologic or radiographic tests or an unrelated surgical procedure that incidentally uncovered signs of cirrhosis ***(Yee, 2002).***

Common Physical Examination Findings in Patients with Cirrhosis

Abdominal wall vascular collaterals (caput medusa)
Ascites
Asterixis
Clubbing and hypertrophic osteoarthropathy
Constitutional symptoms, including anorexia, fatigue, weakness, and weight loss
Cruveilhier-Baumgarten murmur-a venous hum in patients with portal hypertension
Dupuytren's contracture
Fetor hepaticus-a sweet, pungent breath odor
Gynecomastia
Hepatomegaly
Jaundice
Kayser-Fleischer ring-brown-green ring of copper deposit around the cornea, pathognomonic for Wilson's disease
Nail changes:
- Muehrcke's nails-paired horizontal white bands separated by normal color
- Terry's nails-proximal two thirds of nail plate appears white, whereas the distal one third is red

Palmar erythema
Scleral icterus
Vascular spiders (spider telangiectasis, spider angiomata)
Splenomegaly
Testicular atrophy

Table 2. Common Physical Examination Findings in Patients with Cirrhosis ***(Yee, 2002).***

Laboratory Evaluation

No serologic test can diagnose cirrhosis accurately ***(Friedman, 2004).*** The term liver function tests is a misnomer because the assays in most standard liver panels do not reflect the function of the liver correctly ***(Yee, 2002).*** Although liver function tests may not correlate exactly with

hepatic function, interpreting abnormal biochemical patterns in conjunction with the clinical picture may suggest certain liver diseases. When a liver abnormality is suspected or identified, a liver panel, a complete blood count (CBC) with platelets, and a prothrombin time test should be performed ***(Dufour DR, ..I, 2000).***

Common tests in standard liver panels include the serum enzymes aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase, and g-glutamyltransferase; total, direct, and indirect serum bilirubin; and serum albumin. The ALT is thought to be the most cost-effective screening test for identifying metabolic or drug-induced hepatic injury, but like other liver function tests, it is of limited use in predicting degree of inflammation and of no use in estimating severity of fibrosis. ***(Dufour DR, ..II, 2000).***

If clinical suspicion for liver disease is high, then further serologic work-up is warranted within six months. If a patient has a persistently increased ALT level, viral hepatitis serologies should be assayed. If these are negative, the remaining serologic work-up should include an antinuclear antibodies test or anti-smooth muscle antibody test, or both, to evaluate for autoimmune hepatitis; and a fasting transferrin saturation level or unsaturated iron-binding capacity and ferritin level to evaluate for hereditary hemochromatosis ***(Dufour DR, ..II, 2000).***