Role of haematopoietic stem cells in treatment of advanced liver cirrhosis

Assay

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CONTENTS

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Mohamed Owid

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LIST OF ABBREVIATIONS

AAF	Acetylaminofluorene
AFP	Alpha feto protein
ANA	Anti nuclear antibody
Anti-LKM1	Anti liver kidney microsomal antibody 1
Anti-SMA	Anti smooth muscle antibody
BMT	Bone marrow transplantation
CCI 4	Carbon tetrachloride
CD	Cluster of differentiation
CK	Cytokeratin
DPPIV+	Dipeptidyl peptidase IV-positive
EGCS	Embryonic germ cells
EGE	The European Group on Ethics
ESCS	Embryonic stem cells
EU	European Union
FACS	Fluorescence-activated cell sorting
FAH	Fumarylacetoacetate hydrolase
FGF-4	Fibroblast growth factor 4
FISH	Fluorescent in situ hybridization
G-CSF	Granulocyte-colony stimulating factor
GFP	Green fluorescent protein
HBsAg	Hepatitis B surface antigen
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HGF	Hepatocyte growth factor
HIV	Human immunodeficiency virus
HSCs	Hematopoietic stem cells
IL-3	Interleukin-3
INR	International Normalized Ratio
IVF	In vitro fertilization
LDLTX	Living-donor liver transplantation
Lin	Lineage marker
MAPCs	Multipotent adult progenitor cells
MELD	Model for End Stage Liver Disease
MHC	Major histocompatability antigens
MSCs	Mesenchymal stem cell.
NASH	Non alcoholic Steatohepatitis
NSAIDs	Non steroidal anti inflammatory drugs
NTBC	(2-Nitro-4-trifluoro-methylbenzyol)-1,3-cyclohexanedione
OLTXs	Orthotopic liver transplantation
PBC	Primary biliary cirrhosis
PCR	Polymerase chain reaction

Sca-1	Stem cell antigen -1
SCF	Stem cell factor
SP	Side population
Stro-1	Stromal cell marker
TGF-β_1	Transforming growth factor- beta1
TIMP 1 and 2	Tissue inhibitor of metalloproteinase1 and 2
TIPS	Transjugular intrahepatic portosystemic shunt
WBC	White blood cell
WLTx	Whole liver transplantation

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Cirrhosis represents a late stage of progressive hepatic fibrosis. It is generally considered to be irreversible in its advanced stages at which point the only option may be liver transplantation. Patients with cirrhosis are susceptible to a variety of complications and their life expectancy is markedly reduced**.**

Stem cells are cells that have clonogenic and self renewing capabilities and that differentiate into multiple cell lineages **(Weissman IL.; 2000)** The classic paradigm of stem cell differentiation restricted to its organ specific lineage is being challenged by the suggestion that adult stem cells including haematopoietic Stem cells retain a previously unrecognized degree of developmental plasticity that allow them to differentiate across boundaries of lineage tissue **(Quesenberry PJ, 2002)**

Liver was one of the earliest tissues recognized as having potential contribution to differentiated cells by bone marrow stem cells. Bone marrow stem cells have been induced to form hepatocytes in culture **(Miyazaki M. et al., 2002).**

In vivo bone marrow stem cells were able to incorporate into liver as hepatocytes and rescue mice from a liver enzyme deficiency, and restore normal liver function **(Lagasse E et al.; 2000)** Studies showed that similar repopulation of liver from bone marrow stem cells could take place in humans **(Theise ND. Et al.; 2000)**

AIM OF WORK

The aim of this work is to show that haematopoietic Stem cells are indeed transformed into hepatocytes and the transformed cells acquire the function of the original hepatocytes by expressing organ specific proteins and by showing specific organ function.

Liver cirrhosis

Definition

Cirrhosis represents the final common histological pathway for a wide variety of chronic liver diseases. The term cirrhosis was first introduced by Laennec in 1826. It is derived from the Greek term scirrhus and is used to describe the orange or tawny surface of the liver seen at autopsy.

Many forms of liver injury are marked by fibrosis. Fibrosis is defined as an excess deposition of the components of extra cellular matrix (i.e., collagens, glycoproteins, and proteoglycans) within the liver. This response to liver injury potentially is reversible. In contrast, in most patients, cirrhosis is not a reversible process.

Cirrhosis is defined histologically as a diffuse hepatic process characterized by fibrosis and the conversion of normal liver architecture into structurally abnormal nodules. The progression of liver injury to cirrhosis may occur over weeks to years. Indeed, patients with hepatitis C may have chronic hepatitis for as long as 40 years before progressing to cirrhosis. Often a poor correlation exists between histological findings and the clinical picture. Some patients with cirrhosis are completely asymptomatic and have a reasonably normal life expectancy. Other individuals have a multitude of the most severe symptoms of end-stage liver disease and have a limited chance for survival

. **(DAVID C WOLF, 2005)**

AETIOLOGY

1. Viral hepatitis B with or without delta virus, and hepatitis C.
2. Alcohol.

3. Metabolic e.g. haemochromatosis, Wilson's disease, α1-antitrypsin deficiency, type IV glycogenesis, galactosaemia, congenital tyrosinosis, non alcoholic steatohepatitis, intestinal bypass.
4. Prolonged cholestasis, intra-and extra-hepatic.
5. Hepatic venous outflow obstruction, e.g. veno-occlusive disease, Budd-Chiari syndrome, constrictive pericarditis.
6. Disturbed immunity (autoimmune hepatitis).
7. Toxins and therapeutic agents, e.g., methotrexate, amiodarone.
8. Indian childhood hepatitis.
9. Cryptogenic cirrhosis.

(Sherlock, 2002)

RISK FACTORS

-Risk Factors in People with Alcoholism;

- 10% of heavy drinkers develop advanced liver disease.
- Obesity is a major factor for all stages of liver disease.
- Women develop liver disease at lower quantities of alcohol intake than men. The reason for this may be due to women's inability to metabolize alcohol as quickly as men, so it stays in the bloodstream longer.
- Genetic factors that regulate the immune responses in the intestine also play role in increasing the risk for liver injury from alcoholism.

-Risk Factors in People with Chronic Hepatitis;

A- Risk Factors for Developing Cirrhosis from Hepatitis C. Between 20% and 30% of people with hepatitis C develop cirrhosis after twenty years. The following conditions put people with hepatitis C at higher risk for liver damage:

- Having a specific genetic type of hepatitis C (genotype 1). This is the strongest risk factor for severity and risk for cirrhosis.
- Being male may pose a higher risk for severity than being female.
- Developing hepatitis C at an older age.
- Heavy alcohol use.
- Co-infection with HIV or hepatitis B.
- A history of transfusions.
- Being overweight, particularly if fat is distributed in the abdomen (an apple-shape). This condition may pose a higher risk for a fatty liver, which in turn is more able to become scarred and cirrhotic.
- Having large iron stores in the liver.

B-Risk Factors for Developing Cirrhosis from Hepatitis B.

The great majority of people with chronic persistent hepatitis B have a good long-term outlook, but between 5% and 10% become carriers of the virus and 5% to 10% of these individuals eventually develop cirrhosis. The addition of hepatitis D is a particular danger and increases the risk for cirrhosis.

-Risk Factors for Cirrhosis in Autoimmune Liver Diseases;

Primary biliary cirrhosis accounts for only 0.6% to 2% of deaths from cirrhosis. And in patients with chronic persistent autoimmune hepatitis, the outlook is very favorable and survival rates are equal to the general population. If it becomes active, it must be treated, since if untreated, the five-year survival rates are 50%.

-Risk Factors for Cirrhosis in Non alcoholic Steatohepatitis (NASH);

Evidence now suggests that NASH is an under-recognized cause of cirrhosis, and, in fact, nearly 25% of the adult American population have evidence of it. Severe obesity and diabetes are major risk factors in these patients. Men are at higher risk

than women and African Americans have a higher risk than Caucasians. Patients with NASH-associated cirrhosis generally do better than patients with alcoholic cirrhosis. **(Harvey S. et al.; 2002)**

PATHOGENESIS

Cirrhosis can be divided into two steps, these steps may overlap. Firstly, the aetiological agent causing hepatocyte damage and death, which then stimulates a repair process including inflammation and fibrosis.

If the cell death and repair process is of sufficient severity and duration, and is more or less continuous it results in diffuse fibrosis, hepatocyte nodule formation and distortion of liver architecture. Then the second stage of the process starts in which; fibrosis, nodule formation, distorted liver architecture produce, by themselves, further hepatocyte damage and death.

This in turn, stimulates further fibrosis and inflammation, exacerbating the cirrhotic process which in turn causes further hepatocyte death and so on. Thus once cirrhosis is established, it is self perpetuating, irreversible and ultimately fatal. **(Flemming and Mc Gee, 1984)**

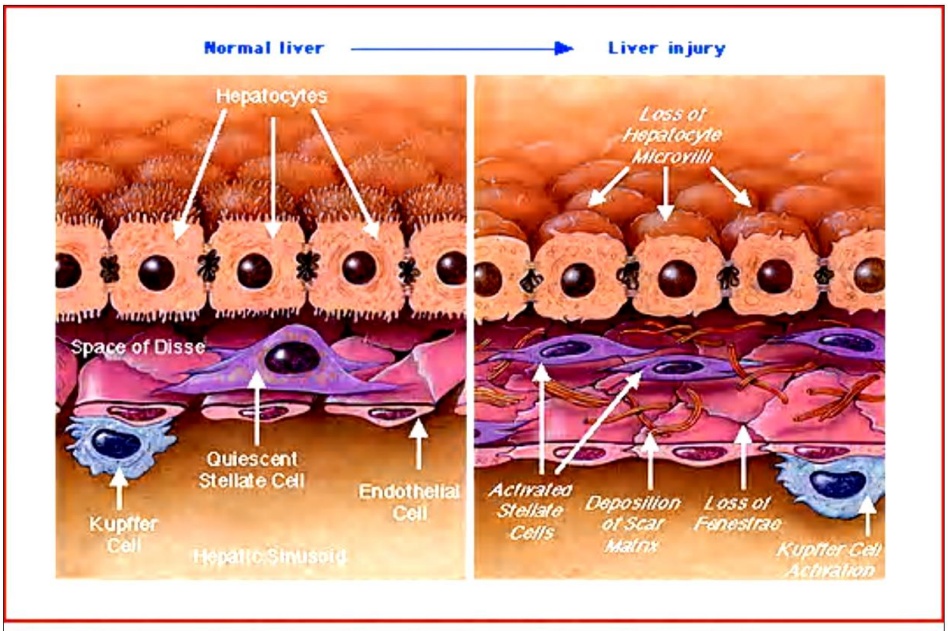
Normal liver has a connective tissue matrix which includes type Ⅳ (non fibrillary) collagen, glycoproteins (including fibronectin and laminin) and proteoglycans including (heparan sulfate). These comprise the low density basement membrane in the space of disse. Following hepatic injury there is three- to eight- fold increase in the extra-cellar matrix which is of a high density interstitial type, containing fibril- forming collagens (types Ⅰ and Ⅲ) as well as cellular fibronectin, hyaluronic acid and other matrix proteoglycans and glycoconjugates. There is loss of endothelial cell fenestrations and hepatocyte microvilli, and capillarization of sinusoids, which impedes the metabolic exchange between blood and liver cells.

(Sheila Sherlock & James Dooley; 2002)

Stellate cell activation, the central event in hepatic fibrosis

Hepatic stellate cells, located in subendothelial space of Disse between hepatocytes and sinusoidal endothelial cells, represent one-third of the non parenchymal population or about 15 percent of the total number of resident cells in normal liver. In normal liver they are the principal storage site for retinoids (vitamin A metabolites).

(Friedman SL.2000, Greets. 2001)

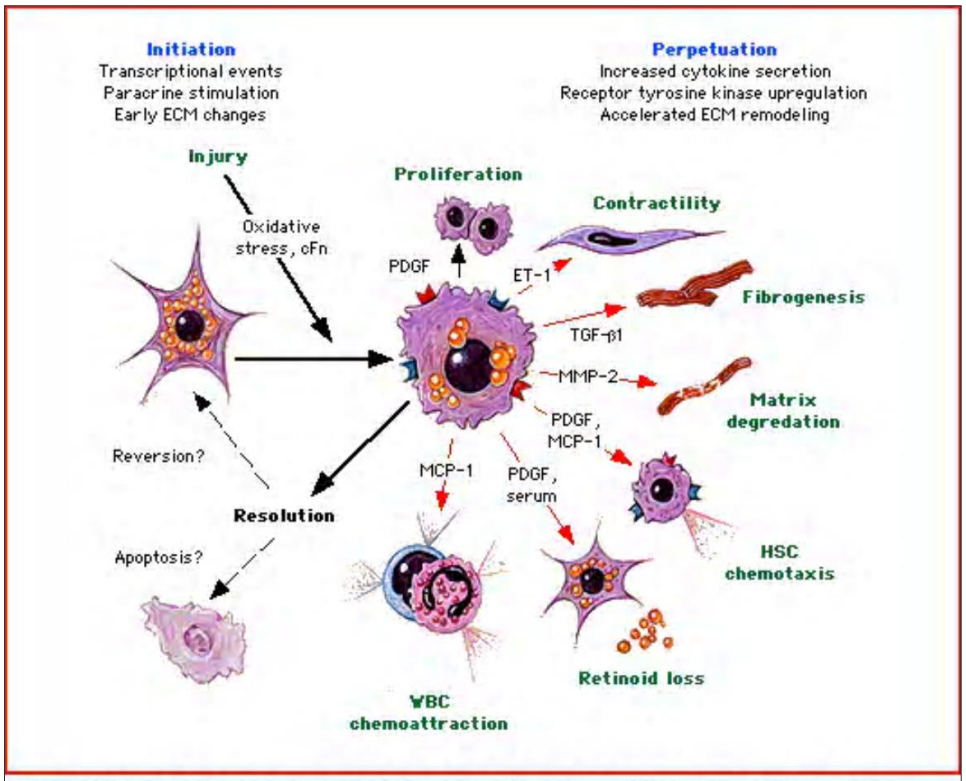


Sinusoidal events during fibrosing liver injury Changes in the subendothelial space of Disse and sinusoid as fibrosis develops in response to liver injury include alterations in both cellular responses and extracellular matrix composition. Stellate cell activation leads to accumulation of scar (fibril-forming) 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. Courtesy of Scott L Friedman, MD.

(Figure: 1)

Damage to the hepatic parenchyma leads to activation of the stellate cell, which becomes contractile and obstructs blood flow in the circulation. In addition, it secretes TGF-β_1, which leads to a fibrotic response and proliferation of connective tissue. Furthermore, it disturbs the balance between matrix metalloproteinases and the naturally occurring inhibitors (TIMP 1 and 2), leading to matrix breakdown and replacement by connective tissue-secreted matrix. (Figure: 1)

(John Iredal; 2003)



Phenotypic features of hepatic stellate cell activation during liver injury and resolution Following liver injury, hepatic stellate cells undergo "activation," which connotes a transition from quiescent vitamin A-rich cells into proliferative, fibrogenic and contractile myofibroblasts. The major phenotypic changes after activation include proliferation, contractility, fibrogenesis, matrix degradation, chemotaxis, retinoid loss, and white blood cell chemoattraction. Key mediators underlying these effects are shown. The fate of activated stellate cells during resolution of liver injury is uncertain but may include reversion to a quiescent phenotype and/or selective clearance by apoptosis. Courtesy of Scott L Friedman, MD.

(Figure: 2)

Activation consists of two major phases:

- Initiation: which 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. **(Friedman SL.2000)**
- Perpetuation: Perpetuation of stellate cell activation involves at least seven discrete changes in cell behavior: proliferation, chemotaxis,

fibrogenesis, contractility, matrix degradation, retinoid loss, and WBC chemoattractant and cytokine release. The net effect of these changes is to increase accumulation of extracellular matrix. (Figure: 2)

(Li and Fridman.1999)

PATHOLOGY

Macroscopically, the liver is initially enlarged, but with progression of the disease, it becomes smaller. Its surface is irregular, the consistency is firm and the color is often yellow (if associates steatosis) Depending on the size of the nodules there are three macroscopic types: micronodular, macronodular and mixed cirrhosis. In micronodular form (Laennec cirrhosis or portal cirrhosis) regenerating nodules are less than 3 mm. In macronodular cirrhosis (post-necrotic cirrhosis), the nodules are larger than 3 mm. The mixed cirrhosis consists in a variety of nodules with different sizes. Microscopically; cirrhosis is characterized by regeneration nodules surrounded by fibrous septa.

In these nodules the regenerating hepatocytes are disorderly disposed, biliary tracts, central veins and the raider pattern of hepatocytes are absent. Fibrous septae are important and may present inflammatory infiltrate (lymphocytes, macrophages).

(NDDIC: 2003)