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Hepatic fibrogenesis and carcinogenesis

Isao Sakaida

First Department of Internal Medicine, Yamaguchi University, School of Medicine, Kogushi 1144 Ube, Yamaguchi 755, Japan
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Abstract The stellate cell plays a central role in liver fibrosis in human liver diseases as well as in experimental animal models. Direct prevention of liver fibrosis by a newly synthesized prolyl 4-hydroxylase inhibitor (HOE 077) reduced the number of preneoplastic lesions in rat liver. Fibrosuppression may limit the development of hepatic neoplasms.

Key words: fibrosis, stellate cell, prolyl 4-hydroxylase inhibitor, preneoplastic lesion, glutathione S-transferase placental form, choline-deficient L-amino acid-defined diet

Introduction

Hepatic fibrosis is a common response to chronic liver injury from many causes, including alcohol, viral hepatitis, auto-immune responses, hereditary metal overload, and drugs (toxins) which can cause hepatocyte cell death (1-8), resulting in liver cirrhosis. Among the various causes of liver cirrhosis, one common feature is the increased deposition of extracellular matrix, which consists mainly of collagen, in the liver, leading to the development of portal hypertension, esophageal varices, and liver failure.

In addition, hepatocellular carcinoma (HCC) is frequently (>80%) associated with liver cirrhosis, mainly as a consequence of chronic liver injury as described above (9).

Advances in the isolation and characterization of non-parenchymal cells in the liver, referred to as sinusoidal cells, comprising stellate cells (also known as Ito cells, fat-storing cells, lipocytes, or perisinusoidal cells), Kupffer cells (10), endothelial cells, and pit cells, in conjunction with progress in matrix and cytokine biology, have led to important new insights into the mechanisms of hepatic fibrosis (11,12). In particular, the stellate cell has been clearly identified as the primary source of cellular matrix in chronic liver injury leading to liver cirrhosis (13). In this review, I will emphasize the role of the stellate cell in the development of liver fibrosis (cirrhosis) as it relates to hepatocellular carcinogenesis based on studies in which a choline-deficient L-amino acid-defined (CDAA) diet was used to induce rat liver cirrhosis associated with preneoplastic lesions. I will also discuss the rationale for possible therapies for fibrosuppression and chemoprevention of HCC.

Cellular sources of extracellular matrix in hepatic fibrosis (cirrhosis): The role of the stellate cell

The identification, isolation, and characterization of stellate cells represent a major advance in our understanding of hepatic fibrosis. These stellate cells are interposed in the subendothelial space of Disse between sinusoidal endothelial cells and hepatocytes and are distributed throughout the hepatic lobule.

Stellate cells in normal liver are distinguished by the presence of prominent intracellular droplets containing vitamin A. Based on their cytoskeletal phenotype, these cells are analogous to perivascular cells (pericytes) such as the renal mesangial cells.

Current evidence suggests that the process of stellate cell activation in the non-physiological liver includes at least two stages. Initiation is characterized by enlargement of the stellate cell, the expression of a smooth muscle actin, and the induction of cytokine receptors, e.g., transforming growth factor (TGF)-β and platelet-derived growth factor(PDGF). These initiating stimuli may include as yet uncharacterized paracrine factors from injured hepatocytes (lipid peroxide?) and hepatic macrophages (Kupffer cells). TGF-β1 from Kupffer cells may play an important role (14), as well as lymphocytes and platelets (15,16). Once stellate cells have been induced to express cytokine receptors, a number of cytokines from Kupffer cells or platelets, as paracrine sources, may stimulate and sustain proliferation and fibrogenesis. To date, TGF-β1 has been identified as the most potent fibrogenic mediator, and PDGF as the most proliferative. In addition, acetaldehyde or lipid peroxides may perpetuate stellate cell activation (17,18). Autocrine pathways for cytokines produced by the stellate cell, e.g., TGF- β1, have been suggested, and activation may continue as perpetuation. It seems likely that in chronic viral hepatitis, activation is sustained by the persistence of inflammatory cells, but this is poorly understood and further research is necessary.

Effect of fibrosuppression on preneoplastic lesions in rat liver cirrhosis induced by a CDAA diet

We examined the relationship between fibrosis and carcinogenesis using a choline-deficient L-amino acid-defined (CDAA) diet model. In this model, stellate cells play a key role in hepatic fibrosis using an in situ hybridization method (Fig. 1).

In this model, liver cirrhosis developed in 14 weeks, with severe necrosis and regeneration. Preneoplastic lesions were identified as glutathione S-transferase placental form (GST-P)-positive lesions surrounded by fibrous septa (Fig. 2). After 1 year, such

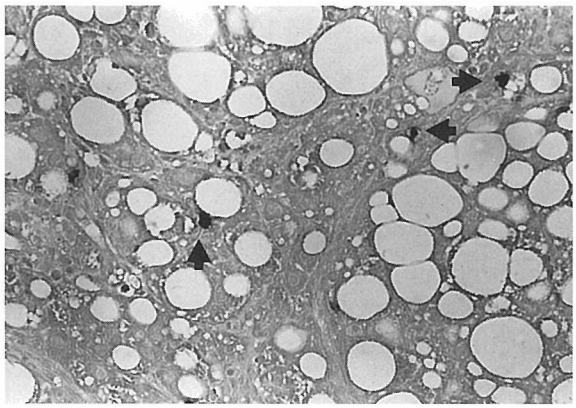

Fig. 1. Messenger expression of procollagen type III was strongly detected by in situ hybridization in the liver of rats fed a CDAA diet for 14 weeks (arrows). Magnification X 200.

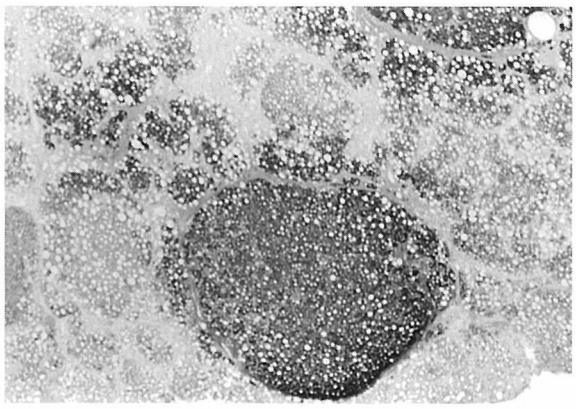

Fig. 2. Preneoplastic lesions were identified by anti-glutathione S-transferase placental form (GST-P) antibody in the liver of rats fed a CDAA diet for 14 weeks. GST-P-positive lesions were surrounded by fibrous septa. Magnification X 100.

preneoplastic lesions developed into hepatocellular carcinoma.

A newly synthesized prolyl 4-hydroxylase inhibitor (HOE 077) directly reduced the hydroxyproline content of the liver, which reflects collagen content, in parallel with the reduction in GST-P-positive lesions (Table 1) without preventing hepatocyte cell death (11, 12). These results indicate that the prevention of fibrosis may limit the development of hepatic neoplasms.

Conclusion

Stellate cells play a key role in hepatic fibrosis, and fibrosuppression may prevent carcinogenesis. As stellate cells also produce growth factor(s) such as hepatocyte growth factor (HGF), the direct effects of stellate cells on carcinogenesis should be further examined.

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Table 1 Male Wistar rats were fed a choline-deficient L-amino acid-defined (CDAA) diet with 0, 100, or 200 ppm of prolyl 4-hydroxylase inhibitor (HOE 077) for 14 weeks. Glutathione S-transferase placental form (GST-P)-positive lesions were measured by an image analysis system and expressed as a percentage of the total area of the specimen.

HOE 077 (ppm)	0	100	200
Hydroxyproline (μg/g wet wt)	752±102	661±164	[a]564±121
GST-P-positive lesions (%)	5.60±3.53	4.55±2.92	[b]2.78±1.34

a : $P<0.01$ versus HOE 077 0 ppm
b : $P<0.05$ versus HOE 077 0 ppm
Each value represents mean ± SD for 15 rats

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