

## An Adipocentric View of Liver Fibrosis and Cirrhosis

Jiin-Haur Chuang<sup>1,3</sup>, MD; Pei-Wen Wang<sup>2</sup>, MD; Min-Hong Tai<sup>4</sup>, PhD

Liver fibrosis is the consequence of chronic or repeated liver injury caused by hepatotoxic agents like alcohol and viruses, as well as immune and congenital metabolic disorders. Nonalcoholic fatty liver disease (NAFLD), caused by obesity and abnormal lipid metabolism, may be the latest known cause of liver fibrosis and cirrhosis. Furthermore, NAFLD with obesity can provide a terrain in which alcoholic and viral liver diseases, such as chronic hepatitis C, are prone to cause liver cirrhosis. Insulin, insulin-like growth factor (IGF)-1, peroxisome proliferator-activated receptors (PPARs), leptin, adiponectin, and preadipocyte factor-1/delta-like1 (Pref-1/dlk1) are hormones, growth factors, nuclear receptors, and cytokines that are actively involved in lipid metabolism. They share common target cells important in liver fibrosis, i.e., hepatic stellate cells (HSCs). Activation of HSCs is known to initiate and perpetuate liver fibrosis. Insulin and IGF-1 stimulate HSC activation and collagen production in vitro. However, IGF-1 alleviates liver fibrosis in vivo. Ligands of PPAR $\gamma$  inhibit HSC activation and collagen synthesis in vivo and in vitro, and are helpful in decreasing liver fibrosis. But ligands of PPAR $\beta$  enhance proliferation of HSCs. Leptin is profibrogenic, and liver fibrosis is decreased in leptin- or leptin receptor-deficient mice. Adiponectin is, on the contrary, anti-fibrogenic. Extensive liver fibrosis may develop in adiponectin-knockout mice and is alleviated by administration of recombinant adiponectin. Pref-1/dlk1 is implicated in fibrogenesis of the liver through its modulation of HSCs. The use of such biologically active molecules in lipid metabolism as ligands of PPAR $\gamma$  and adiponectin might not help slim down a patient on the whole, but can potentially be used to halt the progression of liver fibrosis. Weight reduction, a strategy for controlling obesity and metabolic syndromes, may also be a tool for decreasing NAFLD and alleviating liver cirrhosis. (*Chang Gung Med J* 2004;27:855-68)

**Key words:** adipocytokine/adipokine, adiponectin, cirrhosis, hepatic stellate cells, insulin, insulin-like growth factor, leptin, liver fibrosis, nonalcoholic fatty liver disease, obesity, preadipocyte factor1/delta-like protein1.

Hepatic fibrosis is a scarring response of the liver to chronic liver injury. When fibrosis progresses to cirrhosis, morbid complications can develop.<sup>(1)</sup> Accumulation of extracellular matrix (ECM) is the paradigm, and activation of hepatic stellate cells (HSCs) has been established as the unequivocal source of the ECM.<sup>(2,3)</sup> HSCs comprise 15% of the total number of resident liver cells.<sup>(4)</sup> HSCs are also

known as lipocytes, because in a normal liver, they are not only the principal storage site for retinoids, but they also possess numerous intracellular lipid droplets that contain discernible amounts of triglyceride.<sup>(5,6)</sup> Following liver injury, HSCs undergo a process known as "activation", in which HSCs are transformed from quiescent vitamin A-rich cells into fibrogenic myofibroblasts.<sup>(7)</sup> Obesity is a risk factor

---

From the <sup>1</sup>Division of Pediatric Surgery, Department of Surgery; <sup>2</sup>Division of Endocrinology and Metabolism, Department of Internal Medicine, Chang Gung Memorial Hospital, Kaohsiung; <sup>3</sup>Graduate Institute of Clinical Medical Sciences, Chang Gung University, Taoyuan; <sup>4</sup>Department of Medical Education and Research, Kaohsiung Veterans General Hospital, Kaohsiung.

Received: Aug. 18, 2004; Accepted: Oct. 19, 2004

Address for reprints: Dr. Jiin-Haur Chuang, Department of Surgery, Chang Gung Memorial Hospital, 123, Dabi Road, Niasung Shiang, Kaohsiung, Taiwan 833, R.O.C. Tel.: 886-7-7317123 ext. 8811; Fax: 886-7-7338009; E-mail: jhchuang@adm.cgmh.org.tw

in precipitating fibrosis in a few chronic liver diseases and in the normal population with no previous liver disease.<sup>(8,9)</sup> Several cytokines that are important in lipid metabolism and adipocyte differentiation have emerged as modulators of HSC activation. In this review, the nurturing effects of obesity on liver fibrogenesis are addressed. Subsequently, we discuss how insulin, peroxisome proliferator-activated receptors (PPARs), and several adipocyte-derived biologically active molecules are involved in liver fibrosis, at least in part through their modulation of HSC activation.

#### **A. Obesity may exacerbate liver fibrogenesis**

Although ethanol has long been established as a hepatotoxic agent, only 8%~20% of chronic alcoholics develop liver cirrhosis.<sup>(10)</sup> Factors that increase the risk of cirrhosis have mostly not been firmly established. Steatosis is the earliest manifestation of alcohol-associated liver injury. In a study of 1604 alcoholic patients, the presence of excess weight for at least 10 years was a risk factor for steatosis, acute alcoholic hepatitis, and cirrhosis.<sup>(11)</sup> Nonalcoholic fatty liver disease (NAFLD) encompasses all of the features of alcohol-induced liver injury except that obesity may be the underlying factor. In a study of 351 apparently nonalcoholic patients at autopsy, severe fibrosis had developed in 13.8% of markedly obese patients and in 6.6% of lean patients.<sup>(12)</sup> Although NAFLD did not progress to steatohepatitis or cirrhosis in 1 group of patients, obesity was 1 of the significant predictors of severe liver fibrosis in another study of 144 patients with nonalcoholic steatohepatitis (NASH).<sup>(13,14)</sup> In another study of 93 patients with abnormal liver function tests but without alcoholic, viral, autoimmune, drug-induced, or genetic liver disease, septal fibrosis was 6 times more prevalent in overweight patients than in lean counterparts.<sup>(9)</sup> It is estimated that 20%~30% of adults in the US and other Western countries have excess fat accumulation in the liver, and 10% of these individuals, or fully 2%~3% of all adults are estimated to meet the current criteria for NAFLD. Older age, obesity, and diabetes are predictive factors for fibrosis.<sup>(15)</sup>

Studies of liver fibrosis in patients with chronic hepatitis C also support the important role of obesity in fibrogenesis of this viral liver disease. In a series of 148 consecutive patients with chronic hepatitis C,

Hourigan et al. found that an increasing body mass index (BMI) has a role in the pathogenesis of steatosis in chronic hepatitis C and that steatosis may contribute to fibrosis.<sup>(16)</sup> Bressler et al. further showed that a high BMI is an independent risk factor for a nonresponse to antiviral treatment in patients with chronic hepatitis C.<sup>(17)</sup> Since insulin resistance in obesity is associated with metabolic syndrome and promotes chronic inflammatory conditions such as atherosclerosis, the role of insulin resistance was examined in patients with chronic hepatitis C.<sup>(18,19)</sup> A study of 121 hepatitis C virus patients with stage 0 or 1 hepatic fibrosis and 137 healthy volunteers matched by gender, BMI, and waist-hip ratio showed that insulin resistance may contribute to fibrotic progression in chronic hepatitis C virus infection.<sup>(20)</sup> Glucose intolerance increased 2-fold in Chinese patients with chronic hepatitis C, compared to an age-adjusted control population.<sup>(21)</sup> Consequently, it was hypothesized that weight reduction in patients with chronic hepatitis C might decrease the severity of hepatic steatosis. Indeed, in a 3-month weight reduction program in 19 subjects with steatosis and chronic hepatitis C, serum alanine aminotransferase levels progressively fell in 16 cases with weight loss. Nine of 10 patients with paired liver biopsies showed a reduction in steatosis and significant decreases in the Knodell fibrosis score and the number of activated HSCs. Therefore, weight reduction may provide an important adjunct treatment for patients with chronic hepatitis C.<sup>(22)</sup>

#### **B. Molecules that bridge the troubled waters of lipid metabolism and liver fibrosis**

Adipokines and adipocytokines are series of adipocyte-derived biologically active molecules which may influence the function as well as the structural integrity of other tissues. Included are substances like leptin, acylation-stimulating protein, tumor necrosis factor- $\alpha$ , plasminogen activator inhibitor-1, interleukin-6, and adiponectin.<sup>(23)</sup> As obesity is associated with altered gene expression in adipose tissue and the biochemical phenotype of insulin resistance,<sup>(19)</sup> a review of obesity-associated liver changes therefore should begin with insulin and insulin-like growth factors (IGFs) (Table 1).

##### **B.1 Insulin and insulin-like growth factors**

Evidence for the involvement of insulin in liver

**Table 1.** Molecules Involved in Lipid Metabolism and Liver Fibrosis

Name	Category	Plasma levels in cirrhosis	Human diseases	In vitro or animal studies	Effects on HSC or fibrosis
Insulin/IGF	Hormone / growth factor	High or -/ low	Mixed <sup>*(26,27)†</sup>	CCl4 or BDL (rat) <sup>(28,71)</sup> and in vitro <sup>(24,25)</sup>	In vivo protective against fibrosis, inconsistent with the in vitro proliferation of HSCs; Increased CTGF and collagen
PPAR	Transcription factor	N/A	N/A	DMN, CCl4, and BDL (rat) <sup>(39)</sup> and in vitro <sup>(40)</sup>	PPAR $\gamma$ ligands inhibit HSC activation in vitro and decrease liver fibrosis in vivo; PPAR $\beta$ enhances HSC proliferation
Leptin	Hormone	High	Alcoholic; <sup>(45-47)</sup> chronic hepatitis C <sup>(48)</sup>	CCl4 (mouse); <sup>(50)</sup> TAA (ob/ob mouse); <sup>(51)</sup> TAA (fa/fa rat) <sup>(52)</sup>	Inflammation and fibrosis augmented by leptin, but decreased in leptin- or leptin receptor-deficient mice
Adiponectin	Cytokine	High or low	Mixed; <sup>(58)</sup> healthy Caucasians <sup>(59)</sup>	CCl4 (mouse); <sup>(60,61)</sup> alcohol (ob/ob mouse) <sup>(62)</sup>	Extensive fibrosis in adiponectin-knockout mice; fibrosis alleviated by recombinant adiponectin
Pref-1/dlk 1	Cytokine	N/A	Biliary atresia <sup>(68)</sup>	N/A	Downregulated in cirrhosis

**Abbreviations:** BDL: bile duct ligation; CCl4: carbon tetrachloride; CTGF: connective tissue growth factor; DMN: dimethylnitrosamine; HSCs: hepatic stellate cells; IGF: insulin-like growth factor; Pref-1/dlk1: preadipocyte factor-1/delta-like 1; PPAR: peroxisome proliferator-activated receptor; TAA: thioacetamide.

\* "Mixed" indicates that the population with liver cirrhosis included a mixture of patients with chronic hepatitis C, alcoholic liver cirrhosis, primary sclerosing cholangitis, etc.

† Numbers in parentheses indicate the references.

fibrosis comes from 2 studies which show that insulin treatment increases the proliferation of HSCs and the production of connective tissue growth factor in vitro.<sup>(24,25)</sup> IGFs are 4 to 5 times more potent than insulin for the proliferation of HSCs and promotion of type I collagen accumulation by HSCs.<sup>(24)</sup> Interestingly, cirrhotic patients have low blood IGF-I and IGF-binding protein (IGFBP)-3 levels.<sup>(26,27)</sup> Treatment of carbon tetrachloride (CCl4)-induced rat liver cirrhosis with IGF-1 reduces oxidative damage, improves liver function, and ameliorates fibrosis.<sup>(28)</sup> Impaired intestinal sugar transport in cirrhotic rats is also corrected by low doses of IGF-I.<sup>(29)</sup> These findings illustrate the controversial results from the in vitro study, which indicated a profibrogenic effect of IGFs, and those from the in vivo study, which revealed an anti-fibrogenic effect of IGFs. Currently, there are insufficient data to explain such discordant findings. Both phosphatidylinositol 3-kinase (PI3-K) and extracellular signal-regulated kinase (ERK) are involved in IGF-I-induced HSC mitogenesis, whereas insulin stimulates mitogenesis through a PI3-K-dependent and ERK-independent pathway.<sup>(24)</sup>

Insulin resistance is a pathogenic factor in non-alcoholic steatohepatitis (NASH), a progressive form

of NAFLD that can lead to liver fibrosis and cirrhosis.<sup>(30,31)</sup> A comparative study of a subset of 36 patients with less-severe NASH contrasted with 36 age- and gender-matched patients with chronic hepatitis C virus (HCV) of comparable fibrotic severity showed that insulin resistance was significantly higher in those with NASH than in comparable cases of HCV.<sup>(32)</sup> Even in non-diabetic HCV-infected patients, insulin resistance is related to the grade of liver fibrosis and occurs at an early stage of HCV infection.<sup>(33)</sup> Interestingly, in a study of 30 adults with NASH and a BMI of 25 kg/m<sup>2</sup> treated for 48 weeks with rosiglitazone, a PPAR-gamma ligand, insulin sensitivity improved along with the histological markers of NASH, including zone 3 perisinusoidal fibrosis.<sup>(34)</sup>

## B.2. Peroxisome proliferator-activated receptors

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors with pleiotropic effects on lipid metabolism, glucose homeostasis, cell proliferation, inflammation, and fibrosis. The 3 PPAR subtypes, alpha, gamma, and delta (or beta), have distinct expression patterns. PPAR $\alpha$ , the most-abundant form in the liver, potenti-

ates fatty acid catabolism and is the molecular target of the lipid-lowering fibrates (e.g., fenofibrate and gemfibrozil). PPAR $\gamma$  is essential for adipocyte differentiation and mediates the activity of the insulin-sensitizing thiazolidinediones (e.g., rosiglitazone and pioglitazone). PPAR $\beta$  ( $\delta$ ) may be important in controlling triglyceride levels by sensing very low density lipoprotein.<sup>(35)</sup> In the liver, Kupffer cells, like other macrophages, express alpha and gamma isoforms, while HSCs express the gamma isoform.<sup>(36)</sup> Ligands of PPAR $\gamma$  dose-dependently inhibit HSC proliferation and chemotaxis induced by platelet-derived growth factor. Activation of PPAR $\gamma$  also results in complete inhibition of the expression of monocyte chemoattractant protein 1 at the gene and protein levels.<sup>(37)</sup> Curcumin, an antioxidant and the yellow pigment in curry, induces the expression of PPAR $\gamma$  in activated HSCs and inhibits cell proliferation.<sup>(38)</sup> The use of pioglitazone and rosiglitazone, 2 synthetic PPAR $\gamma$  ligands, in a rat model of liver fibrosis induced by either a toxin (dimethylnitrosamine or carbon tetrachloride) or bile duct ligation significantly reduced extracellular matrix deposition and HSC activation.<sup>(39)</sup>

However, not all of the PPAR isoforms react similarly during liver fibrosis. In a recent study, cultured HSCs from rat livers constitutively expressed high levels of PPAR $\beta$  without significant expression of PPAR $\alpha$  and  $\gamma$ . Transcriptional activation of PPAR $\beta$  by L165041 enhances HSC proliferation.<sup>(40)</sup> These contradictory results reflect the fact that despite their sequence homology, PPAR $\beta$  differs from PPAR $\alpha$  and  $\gamma$  during tissue responses to injury, inflammation, and fibrosis.

### B.3. Leptin

Leptin, named from the Greek leptos, which means thin, is a 16-kd hormone. It was initially portrayed as a cure for obesity, and more than 600 publications were generated in the first 3 years after its identification in 1994.<sup>(41,42)</sup> Leptin is the product of the obese (ob) gene and is mainly produced in white adipose tissue, but can also be found in other sites such as the placenta, skeletal muscle, the gastric fundus, and culture-activated HSCs.<sup>(42-44)</sup> Serum levels of leptin are higher in females than males, and this trend remains true for female alcoholic cirrhotics compared with controls. Furthermore, women in both the cirrhotic and control groups had higher

serum leptin levels than males, denoting gender-dependent alterations in alcoholic cirrhosis.<sup>(45)</sup> In another study of alcoholic cirrhosis, the increased serum leptin levels in patients with cirrhosis were directly correlated to the ascitic-free body mass index and inversely correlated with serum creatinine. Results indicate that the elevated circulating leptin in patients with cirrhosis is likely caused by a combination of decreased renal extraction and increased release from fat tissues.<sup>(46)</sup> Elevated serum leptin correlates with energy expenditure in cirrhotics.<sup>(47)</sup> A study of 77 consecutive patients with chronic hepatitis C and 22 healthy controls further confirmed that the severity of liver fibrosis is associated with high serum leptin levels.<sup>(48)</sup> Contrasting results were found in another study on NASH, which showed that serum leptin levels in NASH patients correlated with hepatic steatosis but not fibrosis. The authors of the latter study proposed that the relationship between leptin and steatosis reflects a pathogenic role of leptin in hepatic insulin resistance and/or a failure of the anti-steatotic actions of leptin in NASH.<sup>(49)</sup>

The role of leptin in liver fibrogenesis is clearly demonstrated in the murine model of liver fibrosis. In C57B1/6 mice treated with an intraperitoneal injection of CCl<sub>4</sub>, simultaneous intraperitoneal administration of recombinant murine leptin augments the inflammatory and profibrogenic responses.<sup>(50)</sup> In another study of mice treated with thioacetamide for 4~8 weeks, prominent hepatic fibrosis was found in lean littermates, but not in ob/ob mice devoid of leptin.<sup>(51)</sup> A study using male Zucker (fa/fa) rats and their lean (+/?) littermates treated with thioacetamide also showed that in contrast to the lean littermates, fibrosis was almost completely prevented in the Zucker rats in which a mutation in the leptin receptor precludes signaling by leptin.<sup>(52)</sup> Given that there is increasing incidences of obese patients worldwide at risk for NASH, along with the possibility of leptin-mediated liver fibrogenesis, these studies create another tantalizing story in the field of liver fibrosis.<sup>(53)</sup>

### B.4. Adiponectin

Adiponectin is an adipocytokine identified by screening adipose-specific genes in the human cDNA project.<sup>(54)</sup> Adiponectin and its mouse homolog, Acrp30/AdipoQ, are abundantly synthesized by adipose tissue and secreted in plasma.

Concentrations of adiponectin in human plasma range from 5 to 30  $\mu\text{g/ml}$ , which is 3 orders of magnitude higher than concentrations of most other hormones.<sup>(23,55)</sup> Concentrations are lower in non-obese diabetic subjects as well as in type 2 diabetes patients.<sup>(55,56)</sup> Adiponectin is known to increase insulin sensitivity in both muscles and the liver by increasing tissue fat oxidation and suppressing adhesion molecule expression in vascular endothelium and cytokine production by macrophages, thus inhibiting inflammatory processes such as atherosclerosis.<sup>(23,57)</sup> Strangely, despite adiponectin's function as an insulin-sensing hormone, a recent study of 20 patients with advanced cirrhosis revealed elevated circulating adiponectin levels in cirrhotics compared with controls. The levels were negatively correlated with hepatic protein synthesis and positively with increased portal pressure, but not with serum transaminases.<sup>(58)</sup> The latter finding is not consistent with the results of a study of 257 healthy Caucasian subjects, which showed that adiponectin levels were negatively correlated with transaminases. A low level of plasma adiponectin in the latter study predicted hepatocellular dysfunction.<sup>(59)</sup>

A study using the murine model of CCl<sub>4</sub>-induced liver injury also showed marked elevation of adiponectin mRNA expression within 18 h after CCl<sub>4</sub> treatment, which suggests that adiponectin may act as an anti-inflammatory protein that participates in the repair process during tissue injury.<sup>(60)</sup> In adiponectin-knockout mice, administration of 300  $\mu\text{l}$  CCl<sub>4</sub>/kg body weight for 12 weeks induced extensive liver fibrosis compared with wild-type mice. Injection of adenovirus-producing adiponectin before CCl<sub>4</sub> (1000  $\mu\text{l}$ /kg body weight) treatment prevented liver fibrosis in wild-type mice.<sup>(61)</sup> In a study of alcoholic and nonalcoholic fatty liver diseases in mice, circulating concentrations of adiponectin significantly decreased following chronic consumption of a high-fat, ethanol-containing diet. Delivery of recombinant adiponectin to nonalcoholic obese ob/ob mice dramatically alleviated the hepatomegaly, steatosis, and alanine aminotransferase abnormality.<sup>(62)</sup> Results of these studies imply that in addition to decreasing hyperglycemia and reversing insulin resistance, adiponectin may attenuate liver inflammation and fibrosis, which suggests a potential clinical application for adiponectin and its agonist in the treatment of liver diseases.

### **B.5. Preadipocyte factor-1 (Pref-1)/Delta-like 1 (Dlk1)**

Preadipocyte factor-1 (Pref-1) was first cloned and characterized by Smas and Sul as a novel regulator of adipocyte differentiation.<sup>(63)</sup> Pref-1 mRNA is abundant in 3T3-L1 preadipocytes, but its expression is completely abolished during their differentiation into adipocytes. The protein contains an epidermal growth factor-like repeat and is also known as Delta-like1 or dlk1, because it is highly homologous to invertebrate homeotic proteins, including Delta and Notch.<sup>(64)</sup> Pref-1/dlk1 is differentially expressed in small cell lung carcinomas and neuroendocrine tumor cell lines and functions as a growth inhibitor of hematopoietic progenitor proliferation.<sup>(64,65)</sup> The transformation of adipocytes from cells that store triglycerides to fatty acid-oxidizing cells is accompanied by loss of the adipocyte markers including leptin, and by the appearance of Pref-1.<sup>(66)</sup> These findings suggest the potential of Pref-1 for treating tumors and obesity.

Two recent studies used a DNA microarray to simultaneously identify Pref-1/dlk1 as a regulator of leiomyoma growth and liver fibrogenesis, respectively.<sup>(67,68)</sup> In the former study which screened genes associated with the growth regulation of uterine leiomyomata, Pref-1 was upregulated by 70-fold in leiomyomas, indicating a role for Pref-1 in the oncogenesis of smooth muscle cells.<sup>(67)</sup> In the latter study on genes participating in liver fibrosis associated with biliary atresia, Pref-1/dlk1 was downregulated by 4-fold in the late stage of biliary atresia compared with the early stage. Moreover, while Pref-1/dlk1 mRNA was present only in hepatocytes, the protein was found in alpha-smooth muscle actin-positive cells that are morphologically and immunohistochemically identical to activated HSC/myofibroblasts. The expression of Pref-1/dlk1 mRNA was correlated with the expression pattern of procollagen alpha 1(I).<sup>(68)</sup> These results imply that Pref-1/dlk1 is involved in the activation of HSC and in the production of collagen.

### **C. Pro- or anti-fibrogenic mechanisms**

#### **C.1. Insulin and insulin-like growth factors (IGFs)**

When incubated in the presence of insulin, HSCs are activated, and connective tissue growth factor mRNA is upregulated by more than 5-fold.<sup>(24,25)</sup> IGFs are more potent than insulin in stimulating the

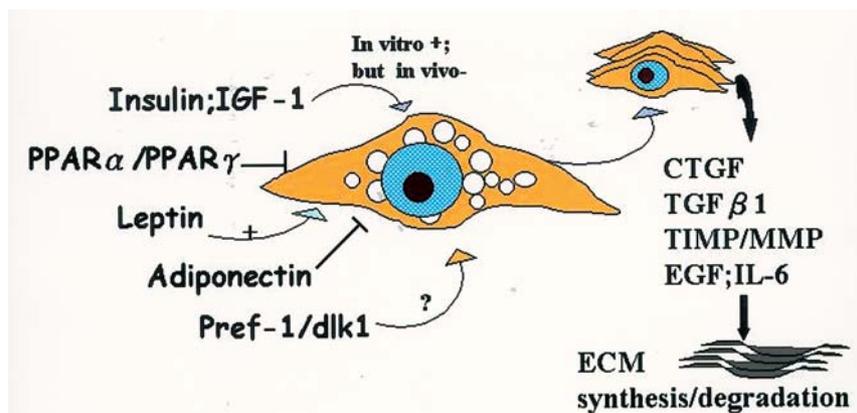
proliferation and production of type I collagen by human HSCs in vitro.<sup>(24)</sup> The above results imply a direct effect of IGFs and insulin on HSC activation. In the resolution of biliary fibrosis induced by bile duct ligation and subsequent biliodigestive anastomosis, apoptosis by HSCs plays a critical role. The addition of IGF-I to the medium that contains activated HSCs isolated from rat liver inhibits HSC apoptosis.<sup>(69)</sup> These in vitro adverse effects of IGF-I on the activation of HSCs and inhibition of apoptosis are reflected by the potential role of IGF-I in fibrotic lung disease,<sup>(70)</sup> but are inconsistent with the in vivo findings that showed a protective effect of IGF-I on experimental liver cirrhosis induced by common bile duct ligation and by CCl<sub>4</sub> intoxication (Fig. 1).<sup>(28,29,71)</sup> Controversy also exists concerning the role of IGF-I in inflammation. In clinical studies, a low plasma IGF-I level confers a high risk of disability and death in older women and is associated with an increased risk of cardiovascular disease.<sup>(72,73)</sup> But in rodents, calorie restriction decreases the levels of plasma glucose and IGF-I, which in turn postpones or attenuates immunosenescence and inflammation and extends longevity.<sup>(74)</sup> One recent study of 13 thermally injured children showed that insulin treatment increases serum IGF-I and IGFBP-3, decreases fatty acid and serum triglycerides, decreases proinflammatory cytokines and proteins, and significantly

decreases the requirement of albumin substitution to maintain normal levels.<sup>(75)</sup> Another study treating patients with NASH and obesity using the PPAR-gamma ligand, rosiglitazone, for 48 weeks found that it improves insulin sensitivity and histological markers of NASH.<sup>(34)</sup>

To date, the results of available studies indicate pleiotropic roles of insulin and IGFs in liver inflammation and fibrosis. The fibrogenetic mechanism of these molecules is still far from clear.

### C.2. Peroxisome proliferator-activated receptors (PPARs)

Peroxisomes are organelles in vertebrate animal cells, especially liver and kidney cells, which are rich in the enzymes peroxidase, catalase, and D-amino acid oxidase and are involved in fatty acid oxidation, purine metabolism, and gluconeogenesis. In 1990, PPARs were cloned. These receptors are members of the large steroid/retinoid nuclear receptor family.<sup>(36)</sup> Fatty acids and eicosanoids are natural PPAR ligands. Endogenous regulators such as glucocorticoids may play critical roles in the tissue expression of PPARs, notably PPAR $\alpha$ .<sup>(76)</sup> PPAR $\alpha$  deficiency results in reduced  $\beta$ -oxidative degradation of these inflammatory fatty acid derivatives and a prolonged response to inflammatory stimuli. On the other hand, PPAR activators may inhibit the activation of inflam-



**Fig. 1** Diagram showing the effect of insulin and other molecules on hepatic stellate cell (HSC) proliferation, and the production of fibrogenic or anti-fibrogenic factors such as connective tissue growth factor (CTGF), transforming growth factor (TGF)  $\beta$ 1, matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs), epidermal growth factor (EGF), and interleukin-6 (IL-6), which may subsequently cause synthesis or degradation of the extracellular matrix (ECM). Adiponectin and ligands of PPAR $\alpha$  and PPAR $\gamma$  are inhibitory to HSC activation, while leptin and in vitro effects of insulin and IGF-1 are stimulatory to HSC proliferation. However, the in vivo effects of insulin and IGF-1 are anti-fibrogenic. The effect of Pref-1/dlk1 on HSC activation is currently undetermined.

matory response genes, such as IL-2, IL-6, IL-8, TNF- $\alpha$ , and metalloproteases, by negatively interfering with the NF- $\kappa$ B, STAT, and AP-1 signaling pathways.<sup>(77)</sup> A study of murine ischemia-reperfusion injury indicated that endogenous PPAR $\gamma$  mediates anti-inflammatory activity.<sup>(78)</sup> However, the results of another study using embryonic stem cells in which both alleles of the gene expressing PPAR $\gamma$  were deleted showed that the anti-inflammatory effects of PPAR $\gamma$  agonists are independent of PPAR $\gamma$ .<sup>(79)</sup>

In the liver, in addition to decreasing HSC proliferation through their direct effects, PPAR $\gamma$  ligands may attenuate alcohol-induced fatty liver through upregulation of c-Met.<sup>(80)</sup> As c-Met is the receptor of the hepatocyte growth factor (HGF) and HGF gene therapy may prevent liver cirrhosis or cause its regression, it is reasonable to assume that PPAR $\gamma$  ligands may prevent liver fibrosis through the c-Met/HGF receptor.<sup>(81,82)</sup> The mechanism of attenuation of ethanol-induced liver dysfunction or nutritional fibrosis and steatohepatitis in mice by the PPAR $\alpha$  agonist, Wy-14,643, is less well known, but may involve lipid peroxidation.<sup>(83,84)</sup>

Taken together, the anti-inflammatory effects of PPARs are less well established. PPAR $\alpha$  and PPAR $\gamma$  ligands or agonists are potential antifibrotic agents through their direct inactivation of HSC activity or their indirect upregulation of the c-Met/HGF receptor and interference with oxidative stress.

### C.3. Leptin

Among liver cells, HSCs are known to regulate hepatic fibrosis. The profibrogenic role of leptin is manifested by induction of alpha2 (I) collagen gene production in cultured rat HSCs.<sup>(85)</sup> However, leptin may also act on other liver cells, such as Kupffer cells. Isolated Kupffer cells from leptin receptor-deficient Zucker (fa/fa) rats showed significantly reduced lipopolysaccharide (LPS) uptake and TNF-alpha production compared with control rats. Kupffer cell dysfunction may explain why leptin receptor-deficient Zucker (fa/fa) rats exhibit retarded development of pig serum-induced liver fibrosis.<sup>(86)</sup>

The secondary structure of leptin is similar to that of interleukin-6 (IL-6), and the leptin receptor is homologous to the gp130 signal-transducing subunit of IL-6-type cytokine receptors.<sup>(87)</sup> The full-leptin receptor, not a major naturally occurring truncated or mutant form, has signaling capabilities of IL-6-type

cytokine receptors.<sup>(88)</sup> However, in 1 study, IL-6/gp130 pathways were protective during fibrosis progression in CCl4-induced chronic liver diseases in mice.<sup>(89)</sup> IL-6-deficient mice are therefore prone to develop CCl4-induced or bile duct ligation-induced liver fibrosis.<sup>(90,91)</sup> If leptin and the leptin receptor function in the same way as IL-6, their roles in liver fibrosis should be similar to the results of a study which showed attenuated liver fibrosis in CCl4-treated IL-6-deficient mice.<sup>(92)</sup>

In summary, leptin and the leptin receptor are profibrogenic by their activation of HSCs, modulation of Kupffer cell function, or signaling through the IL-6/gp130 pathway.

### C.4. Adiponectin

In cultured HSCs, adiponectin suppresses platelet-derived growth factor (PDGF)-induced proliferation and migration of HSCs and attenuates expressions of transforming growth factor (TGF) beta 1 and connective tissue growth factor gene.<sup>(61)</sup> In cultured human aortic smooth muscle cells (HASMCs), adiponectin strongly suppresses HASMC proliferation and migration through directly binding with PDGF-BB and generally inhibits growth factor-induced ERK signals in HASMCs.<sup>(93)</sup> As PDGF is the most-potent mitogen for HSCs,<sup>(94)</sup> it is likely that adiponectin may inhibit HSC proliferation and migration by directly binding with PDGF. In 3T3-L1 adipocytes, adiponectin gene expression and secretion are inhibited by IL-6.<sup>(95)</sup> The reciprocal effect of these 2 adipokines on adipocytes may imply that the same effect also holds true for HSCs. Adiponectin may regulate liver fibrosis through its modulation of IL-6 expression.

In addition to HSCs, adiponectin may exert its effect on Kupffer cells and thus affect liver fibrosis. A study on human monocyte-derived macrophages incubated with physiological concentrations of human recombinant adiponectin showed that adiponectin treatment dose-dependently increases TIMP-1 mRNA levels.<sup>(96)</sup> The expression of adiponectin receptors in human macrophages is induced by agonists of PPAR $\alpha$  and PPAR $\gamma$ . The latter implies crosstalk between adiponectin and the nuclear receptors PPAR $\alpha$  and PPAR $\gamma$ .<sup>(97)</sup> The fact that leptin treatment markedly increases plasma adiponectin in ob/ob mice indicates direct stimulation of adiponectin gene expression by leptin.<sup>(98)</sup> The

interaction of adiponectin with PPARs and leptin in cells or organ systems other than the liver can also be inferred in the liver.

Together, the above data indicate a diverse mechanism in which adiponectin may exert its effect on attenuating liver fibrosis.

### **C.5. Preadipocyte factor-1 (Pref-1)/Delta-like 1 (Dlk1)**

Pref-1/dlk1 is a member of the epidermal growth factor (EGF)-like homeotic family. EGF alone may stimulate the proliferation and migration of HSCs, and the effect is markedly enhanced when co-stimulated with TGF-beta1.<sup>(94,99,100)</sup> The presence of EGF in a cirrhotic liver may also indicate EGF-mediated hepatocyte proliferation within the regenerative nodule.<sup>(101)</sup> The same was true for Pref-1/dlk1 in liver regeneration after a partial hepatectomy in the rat.<sup>(102)</sup> A study of ear healing in mice showed high expression of Pref-1/dlk1 in the wound, which may have contributed to the regenerative capacity of the ear wound.<sup>(103)</sup> Our study of patients with biliary atresia denoted a possibility that Pref-1/dlk1 activates HSCs, which may in turn increase the transcription of procollagen mRNA and the synthesis of collagen.<sup>(68)</sup> However, the exact role Pref-1/dlk1 plays in liver fibrosis is still unresolved (Fig. 1).

### **D. Will treatment of obesity affect the progression of liver fibrosis?**

The epidemic of obesity is steadily increasing around the world, and has caused enormous public impacts with at least 300,000 premature deaths and at least US\$90 billion in direct health care costs annually in the US alone.<sup>(104)</sup> In Taiwan, the prevalence of obesity in children and adolescents also follows the trend in Western countries.<sup>(105,106)</sup> Surgery for severe obesity, a bariatric operation, has enormously increased from about 16,000 in the early 1990s to about 103,000 in 2003 in the US.<sup>(107)</sup> One interesting finding following this trendy operation is a change in the liver's status. In addition to a high incidence of steatosis (86%) in a group of 551 severely obese patients undergoing anti-obesity surgery, fibrosis was found in 74% and cryptogenic cirrhosis in 2%.<sup>(108)</sup> Furthermore, among the 104 patients who underwent a reoperation in a total of 689 patients receiving bariatric surgery, severe fibrosis (grades 3~5) decreased in 28 while mild fibrosis (grades 1~2)

appeared in 42. Most impressively, there was a decrease in fibrosis from a mean grade of 5 to 3, as well as reduced inflammation, in 11 patients with cirrhosis.<sup>(109)</sup> The results of the latter study are meaningful, as obesity appeared to be a risk factor for cirrhosis-related death or hospitalization among persons who consumed little or no alcohol in a population-based cohort study.<sup>(110)</sup> The results are also in agreement with those of a previous study, which showed a reduction in steatosis and a significant decrease in Knodell fibrosis scores after a 3-month weight reduction program in obese patients with chronic hepatitis C.<sup>(22)</sup>

How does weight reduction affect the liver status of patients with morbid obesity? Based on 2 studies involving Asian populations, weight reduction significantly elevated plasma adiponectin levels in diabetic as well as nondiabetic subjects.<sup>(111,112)</sup> In a study of 60 Western women randomly assigned to an intervention group receiving a change of lifestyle to reduce weight by 10% or more and the control group without weight reduction over a period of 2 years, a decrease in the body mass index in the intervention group was associated with significant decreases in serum IL-6, IL-18, and CRP and a significant increase in adiponectin.<sup>(113)</sup> The decrease in inflammatory markers, including IL-6, IL-18, TNF-alpha, tissue plasminogen activator, and von Willebrand factor, is also significant in women who reduce weight using liposuction or by using long-acting pegylated recombinant leptin.<sup>(114,115)</sup> Therefore, at least the decrease in IL-6 and the increase in adiponectin are implicated in the change of liver status with weight reduction, as these 2 cytokines are involved in liver fibrogenesis. Since the liver is not the sole organ for the production of IL-6 and adiponectin, the exact mechanism underlying the alleviation of liver cirrhosis by weight reduction remains to be elucidated.

### **E. Perspectives in tackling liver fibrosis exacerbated by deranged lipid metabolism**

The liver is central for the metabolism of nutrients, hormones, growth factors, and cytokines. The recent epidemic explosion in the conditions associated with NAFLD lies in public awareness of type 2 DM, obesity, and dyslipidemia. The fact that steatosis contributes to the progression of fibrosis in HCV-related disease in a pattern similar to that observed in

NAFLD may lead to multimodal therapy, including weight loss, antioxidants, and exploiting the interactions of HCV with host insulin and lipid metabolism.<sup>(116)</sup> Since certain HCV proteins, such as core and NS5A, induce derangement of lipid metabolism and alter signal transduction of infected hepatocytes leading to the production of oxygen radicals and profibrogenic mediators, a combination of cytokine strategies with other potential antifibrotic agents appears promising.<sup>(117)</sup> The same is true for alcohol liver disease and NAFLD, as there is multi-dimensional regulation of gene expression by fatty acids<sup>(118,119)</sup> and adipocytokines.

Although there are no data to support the association of obesity with liver fibrosis in chronic hepatitis B, 1 recent publication indicated that DM may play a role in the progression of liver cirrhosis in chronic HBsAg carriers in Taiwan.<sup>(120)</sup> Further study is required to implicate aberrant lipid metabolism in liver cirrhosis associated with chronic hepatitis B and to develop a strategy to halt this untoward process.

## REFERENCES

1. Albanis E, Safadi R, Friedman SL. Treatment of hepatic fibrosis: almost there. *Curr Gastroenterol Rep* 2003;5:48-56.
2. Eng FJ, Friedman SL. New insights into hepatic stellate cell activation: the simple becomes complex. *Am J Physiol Gastrointest Liver Physiol* 2000;279:G7-11.
3. Cassiman D, Roskams T. Beauty is in the eye of the beholder: emerging concepts and pitfalls in hepatic stellate cell research. *J Hepatol* 2002;37:527-35.
4. Friedman SL. Molecular regulation of hepatic fibrosis, and integrated cellular response to tissue injury. *J Biol Chem* 2000;275:2247-50.
5. Yamada M, Blaner WS, Soprano DR, Dixon JL, Kjeldbye HM, Goodman DS. Biochemical characteristics of isolated rat liver stellate cells. *Hepatology* 1987;7:1224-9.
6. Moriwaki H, Blaner WS, Piantedosi R, Goodman DS. Effects of dietary retinoid and triglyceride on the lipid composition of rat liver stellate cells and stellate cell lipid droplets. *J Lipid Res* 1988;29:1523-34.
7. Reeves HL, Friedman SL. Activation of hepatic stellate cells--a key issue in liver fibrosis. *Front Biosci* 2002;7:d808-26.
8. McCullough AJ. Obesity and its nurturing effect on hepatitis C. *Hepatology* 2003;38:557-9.
9. Ratziu V, Giral P, Charlotte F, Bruckert E, Thibault V, Theodorou I, Khalil L, Turpin G, Opolon P, Poynard T. Liver fibrosis in overweight patients. *Gastroenterology* 2000;118:1117-23.
10. Lelbach WK. Epidemiology of alcoholic liver disease. In: Popper H, Schaffner F, eds. *Progress in Liver Diseases*. Philadelphia: Grune and Stratton, 1976:434-515.
11. Naveau S, Giraud V, Borotto E, Aubert A, Capron F, Chaput J-C. Excess weight risk factor for alcoholic liver disease. *Hepatology* 1997;25:108-11.
12. Wanless IR, Lentz JS. Fatty liver hepatitis (steatohepatitis) and obesity: an autopsy study with analysis of risk factors. *Hepatology* 1990;12:1106-10.
13. Teli MR, James OF, Burt AD, Bennett MK, Day CP. The natural history of nonalcoholic fatty liver: a follow-up study. *Hepatology* 1995;22:1714-9.
14. Angulo P, Keach JC, Batts KP, Lindor KD. Independent predictors of liver fibrosis in patients with nonalcoholic steatohepatitis. *Hepatology* 1999;30:1356-62.
15. Neuschwander-Tetri BA, Caldwell SH. Nonalcoholic steatohepatitis: summary of an AASLD Single Topic Conference. *Hepatology* 2003;37:1202-19.
16. Hourigan LF, Macdonald GA, Purdie D, Whitehall VH, Shorthouse C, Clouston A, Powell EE. Fibrosis in chronic hepatitis C correlates significantly with body mass index and steatosis. *Hepatology* 1999;29:1215-9.
17. Bressler BL, Guindi M, Tomlinson G, Heathcote J. High body mass index is an independent risk factor for nonresponse to antiviral treatment in chronic hepatitis C. *Hepatology* 2003;38:639-44.
18. Haffner SM, Stern MP, Hazuda HP, Mitchell BD, Patterson JK. Cardiovascular risk factors in confirmed prediabetic individuals. Does the clock for coronary heart disease start ticking before the onset of clinical diabetes? *JAMA* 1990;263:2893-8.
19. Sanyal AJ. Insulin resistance and tissue repair: a "fateological" phenomenon. *Gastroenterology* 2003;125:1886-9.
20. Hui JM, Sud A, Farrell GC, Bandara P, Byth K, Kench JG, McCaughan GM, George J. Insulin resistance is associated with chronic hepatitis C and virus infection fibrosis progression. *Gastroenterology* 2003;125:1695-704.
21. Chen LK, Hwang SJ, Tsai ST, Luo JC, Lee SD, Chang FY. Glucose intolerance in Chinese patients with chronic hepatitis C. *World J Gastroenterol* 2003;9:505-8.
22. Hickman IJ, Clouston AD, Macdonald GA, Purdie DM, Prins JB, Ash S, Jonsson JR, Powell EE. Effect of weight reduction on liver histology and biochemistry in patients with chronic hepatitis C. *Gut* 2002;51:89-94.
23. Diez JD, Iglesias P. The role of the novel adipocyte-derived hormone adiponectin in human disease. *Europ J Endocrin* 2003;148:293-300.
24. Svegliati-Baroni G, Ridolfi F, Di Sario A, Cassini A, Marucci L, Gaggiotti G, Orlandoni P, Macarri G, Perego L, Benedetti A, Folli F. Insulin and insulin-like growth factor-1 stimulate proliferation and type I collagen accumulation by human hepatic stellate cells: differential effects on signal transduction pathways. *Hepatology* 1999;29:1743-51.
25. Paradis V, Perlemuter G, Bonvoust F, Dargere D, Parfait

- B, Vidaud M, Conti M, Huet S, Ba N, Buffet C, Bedossa P. High glucose and hyperinsulinemia stimulate connective tissue growth factor expression: a potential mechanism involved in progression to fibrosis in nonalcoholic steatohepatitis. *Hepatology* 2001;34:738-44.
26. Shmueli E, Miell JP, Stewart M, Alberti KG, Record CO. High insulin-like growth factor binding protein 1 levels in cirrhosis: link with insulin resistance. *Hepatology* 1996;24:127-33.
27. Donaghy AJ, Delhanty PJ, Ho KK, Williams R, Baxter RC. Regulation of the growth hormone receptor/binding protein, insulin-like growth factor ternary complex system in human cirrhosis. *J Hepatol* 2002;36:751-8.
28. Castilla-Cortazar I, Garcia M, Muguerza B, Quiroga J, Perez R, Santidrian S, Prieto J. Hepatoprotective effects of insulin-like growth factor I in rats with carbon tetrachloride-induced cirrhosis. *Gastroenterology* 1997;113:1682-91.
29. Castilla-Cortazar I, Prieto J, Urdaneta E, Pascual M, Nunez M, Zudaire E, Garcia M, Quiroga J, Santidrian S. Impaired intestinal sugar transport in cirrhotic rats: correction by low doses of insulin-like growth factor I. *Gastroenterology* 1997;113:1180-7.
30. Chitturi S, Farrell GC. Etiopathogenesis of nonalcoholic steatohepatitis. *Semin Liver Dis* 2001;21:27-41.
31. Dixon JB, Bhathal PS, O'Brien PE. Nonalcoholic fatty liver disease: predictors of nonalcoholic steatohepatitis and liver fibrosis in the severely obese. *Gastroenterology* 2001;121:91-100.
32. Chitturi S, Abeygunasekera S, Farrell GC, Holmes-Walker J, Hui JM, Fung C, Karim R, Lin R, Samarasinghe D, Liddle C, Weltman M, George J. NASH and insulin resistance: Insulin hypersecretion and specific association with the insulin resistance syndrome. *Hepatology* 2002;35:373-9.
33. Petit JM, Bour JB, Galland-Jos C, Minello A, Verges B, Guiguet M, Brun JM, Hillon P. Risk factors for diabetes mellitus and early insulin resistance in chronic hepatitis C. *J Hepatol* 2001;35:279-83.
34. Neuschwander-Tetri BA, Brunt EM, Wehmeier KR, Oliver D, Bacon BR. Improved nonalcoholic steatohepatitis after 48 weeks of treatment with the PPAR-gamma ligand rosiglitazone. *Hepatology* 2003;38:1008-17.
35. Lee CH, Olson P, Evans RM. Minireview: lipid metabolism, metabolic diseases, and peroxisome proliferator-activated receptors. *Endocrinology* 2003;144:2201-7.
36. Everett L, Galli A, Crabb D. The role of hepatic peroxisome proliferator-activated receptors (PPARs) in health and disease. *Liver* 2000;20:191-9.
37. Marra F, Efsen E, Romanelli RG, Caligiuri A, Pastacaldi S, Batignani G, Bonacchi A, Caporale R, Laffi G, Pinzani M, Gentilini P. Ligands of peroxisome proliferator-activated receptor gamma modulate profibrogenic and proinflammatory actions in hepatic stellate cells. *Gastroenterology* 2000;119:466-78.
38. Xu J, Fu Y, Chen A. Activation of peroxisome proliferator-activated receptor-gamma contributes to the inhibitory effects of curcumin on rat hepatic stellate cell growth. *Am J Physiol Gastrointest Liver Physiol* 2003;285:G20-30.
39. Galli A, Crabb DW, Ceni E, Salzano R, Mello T, Svegliati-Baroni G, Ridolfi F, Trozzi L, Surrenti C, Casini A. Antidiabetic thiazolidinediones inhibit collagen synthesis and hepatic stellate cell activation in vivo and in vitro. *Gastroenterology* 2002;122:1924-40.
40. Hellemans K, Michalik L, Dittie A, Knorr A, Rombouts K, De Jong J, Heirman C, Quartier E, Schuit F, Wahli W, Geerts A. Peroxisome proliferator-activated receptor-beta signaling contributes to enhanced proliferation of hepatic stellate cells. *Gastroenterology* 2003;124:184-201.
41. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. *Nature* 1994;372:425-32.
42. Auwerx J, Staels B. Leptin. *Lancet* 1998;351:737-42.
43. Masuzaki H, Ogawa Y, Sagawa N, Hosoda K, Matsumoto T, Mise H, Nishimura H, Yoshimasa Y, Tanaka I, Mori T, Nakao K. Nonadipose tissue production of leptin: leptin as a novel placenta-derived hormone in humans. *Nat Med* 1997;3:1029-33.
44. Potter JJ, Womack L, Mezey E, Anania FA. Transdifferentiation of rat hepatic stellate cells results in leptin expression. *Biochem Biophys Res Commun* 1998;244:178-82.
45. McCullough AJ, Bugianesi E, Marchesini G, Kalhan SC. Gender-dependent alterations in serum leptin in alcoholic cirrhosis. *Gastroenterology* 1998;115:947-53.
46. Henriksen JH, Holst JJ, Moller S, Brinch K, Bendtsen F. Increased circulating leptin in alcoholic cirrhosis: relation to release and disposal. *Hepatology* 1999;29:1818-24.
47. Ockenga J, Bischoff SC, Tillmann HL, Rifai K, Widjaja A, Boker KH, Manns MP, Brabant G. Elevated bound leptin correlates with energy expenditure in cirrhotics. *Gastroenterology* 2000;119:1656-62.
48. Piche T, Vandenbos F, Abakar-Mahamat A, Vanbiervliet G, Barjoan EM, Calle G, Giudicelli J, Ferrua B, Laffont C, Benzaken S, Tran A. The severity of liver fibrosis is associated with high leptin levels in chronic hepatitis C. *J Viral Hepat* 2004;11:91-6.
49. Chitturi S, Farrell G, Frost L, Kriketos A, Lin R, Fung C, Liddle C, Samarasinghe D, George J. Serum leptin in NASH correlates with hepatic steatosis but not fibrosis: a manifestation of lipotoxicity? *Hepatology* 2002;36:403-9.
50. Ikejima K, Honda H, Yoshikawa M, Hirose M, Kitamura T, Takei Y, Sato N. Leptin augments inflammatory and profibrogenic responses in the murine liver induced by hepatotoxic chemicals. *Hepatology* 2001;34:288-97.
51. Honda H, Ikejima K, Hirose M, Yoshikawa M, Lang T, Enomoto N, Kitamura T, Takei Y, Sato N. Leptin is required for fibrogenic responses induced by thioacetamide in the murine liver. *Hepatology* 2002;36:12-21.
52. Ikejima K, Takei Y, Honda H, Hirose M, Yoshikawa M, Zhang YJ, Lang T, Fukuda T, Yamashina S, Kitamura T, Sato N. Leptin receptor-mediated signaling regulates

- hepatic fibrogenesis and remodeling of extracellular matrix in the rat. *Gastroenterology* 2002;122:1399-410.
53. Anania FA. Leptin, liver and obese mice - fibrosis in the fat lane. *Hepatology* 2002;36:246-8.
54. Maeda K, Okubo K, Shimomura I, Funahashi T, Matsuzawa Y, Matsubara K. cDNA cloning and expression of a novel adipose specific collagen-like factor, apM1 (AdiPose Most abundant Gene transcript 1). *Biochem Biophys Res Commun* 1996;221:286-9.
55. Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, Hotta K, Shimomura I, Nakamura T, Miyaoka K, Kuriyama H, Nishida M, Yamashita S, Okubo K, Matsubara K, Muraguchi M, Ohmoto Y, Funahashi T, Matsuzawa Y. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem Biophys Res Commun* 1999;257:79-83.
56. Hotta K, Funahashi T, Arita Y, Takahashi M, Matsuda M, Okamoto Y, Iwahashi H, Kuriyama H, Ouchi N, Maeda K, Nishida M, Kihara S, Sakai N, Nakajima T, Hasegawa K, Muraguchi M, Ohmoto Y, Nakamura T, Yamashita S, Hanafusa T, Matsuzawa Y. Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. *Arterioscler Thromb Vasc Biol* 2000;20:1595-9.
57. Kadowaki T, Hara K, Yamauchi T, Terauchi Y, Tobe K, Nagai R. Molecular mechanism of insulin resistance and obesity. *Exp Biol Med* 2003;228:1111-7.
58. Tietge UJ, Boker KH, Manns MP, Bahr MJ. Elevated circulating adiponectin levels in liver cirrhosis are associated with reduced liver function and altered hepatic hemodynamics. *Am J Physiol Endocrinol Metab* 2004;287:E82-9.
59. Lopez-Bermejo A, Botas P, Funahashi T, Delgado E, Kihara S, Ricart W, Fernandez-Real JM. Adiponectin, hepatocellular dysfunction and insulin sensitivity. *Clin Endocrinol (Oxf)* 2004;60:256-63.
60. Yoda-Murakami M, Taniguchi M, Takahashi K, Kawamata S, Saito K, Choi-Miura NH, Tomita M. Change in expression of GBP28/adiponectin in carbon tetrachloride-administrated mouse liver. *Biochem Biophys Res Commun* 2001;285:372-7.
61. Kamada Y, Tamura S, Kiso S, Matsumoto H, Saji Y, Yoshida Y, Fukui K, Maeda N, Nishizawa H, Nagaretani H, Okamoto Y, Kihara S, Miyagawa JI, Shinomura Y, Funahashi T, Matsuzawa Y. Enhanced carbon tetrachloride-induced liver fibrosis in mice lacking adiponectin. *Gastroenterology* 2003;125:1796-807.
62. Xu A, Wang Y, Keshaw H, Xu LY, Lam KS, Cooper GJ. The fat-derived hormone adiponectin alleviates alcoholic and nonalcoholic fatty liver diseases in mice. *J Clin Invest* 2003;112:91-100.
63. Smas CM, Sul HS. Pref-1, a protein containing EGF-like repeats, inhibits adipocyte differentiation. *Cell* 1993; 73:725-34.
64. Laborda J, Sausville EA, Hoffman T, Notario V. dlk, a putative mammalian homeotic gene differentially expressed in small cell lung carcinoma and neuroendocrine tumor cell line. *J Biol Chem* 1993;268:3817-20.
65. Han W, Ye Q, Moore MA. A soluble form of human Delta-like-1 inhibits differentiation of hematopoietic progenitor cells. *Blood* 2000;95:1616-25.
66. Zhou YT, Wang ZW, Higa M, Newgard CB, Unger RH. Reversing adipocyte differentiation: implications for treatment of obesity. *Proc Natl Acad Sci U S A* 1999;96: 2391-5.
67. Tsibris JC, Segars J, Coppola D, Mane S, Wilbanks GD, O'Brien WF, Spellacy WN. Insights from gene arrays on the development and growth regulation of uterine leiomyomata. *Fertil Steril* 2002;78:114-21.
68. Huang CC, Chuang JH, Huang LL, Chou MH, Wu CL, Chen CM, Hsieh CS, Lee SY, Chen CL. The human Delta-like 1 homologue is implicated in the progression of liver fibrosis in biliary atresia. *J Pathol* 2004;202:172-9.
69. 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.
70. Krein PM, Winston BW. Roles for insulin-like growth factor I and transforming growth factor-beta in fibrotic lung disease. *Chest* 2002;122:289S-93S.
71. Canturk NZ, Canturk Z, Ozden M, Dalcik H, Yardimoglu M, Tulubas F. Protective effect of IGF-1 on experimental liver cirrhosis-induced common bile duct ligation. *Hepatogastroenterology* 2003;50:2061-6.
72. Cappola AR, Xue QL, Ferrucci L, Guralnik JM, Volpato S, Fried LP. Insulin-like growth factor I and interleukin-6 contribute synergistically to disability and mortality in older women. *J Clin Endocrinol Metab* 2003;88:2019-25.
73. Heald AH, Anderson SG, Ivison F, Laing I, Gibson JM, Cruickshank K. C-reactive protein and the insulin-like growth factor (IGF)-system in relation to risk of cardiovascular disease in different ethnic groups. *Atherosclerosis* 2003;170:79-86.
74. Longo VD, Finch CE. Evolutionary medicine: from dwarf model systems to healthy centenarians? *Science* 2003; 299:1342-6.
75. Jeschke MG, Klein D, Herndon DN. Insulin treatment improves the systemic inflammatory reaction to severe trauma. *Ann Surg* 2004;239:553-60.
76. Sterchele PF, Sun H, Peterson RE, Vanden Heuvel JP. Regulation of peroxisome proliferator-activated receptor-alpha mRNA in rat liver. *Arch Biochem Biophys* 1996; 326:281-9.
77. Chinetti G, Fruchart JC, Staels B. Peroxisome proliferator-activated receptors (PPARs): nuclear receptors at the crossroads between lipid metabolism and inflammation. *Inflamm Res* 2000;49:497-505.
78. Nakajima A, Wada K, Miki H, Kubota N, Nakajima N, Terauchi Y, Ohnishi S, Saubermann LJ, Kadowaki T, Blumberg RS, Nagai R, Matsushita N. Endogenous PPAR gamma mediates anti-inflammatory activity in murine ischemia-reperfusion injury. *Gastroenterology*

- 2001;120:460-9.
79. Chawla A, Barak Y, Nagy L, Liao D, Tontonoz P, Evans RM. PPAR-gamma dependent and independent effects on macrophage-gene expression in lipid metabolism and inflammation. *Nat Med* 2001;7:48-52.
  80. Tomita K, Azuma T, Kitamura N, Nishida J, Tamiya G, Oka A, Inokuchi S, Nishimura T, Suematsu M, Ishii H. Pioglitazone prevents alcohol-induced fatty liver in rats through up-regulation of c-Met. *Gastroenterology* 2004; 126:873-85.
  81. Ueki T, Kaneda Y, Tsutsui H, Nakanishi K, Sawa Y, Morishita R, Matsumoto K, Nakamura T, Takahashi H, Okamoto E, Fujimoto J. Hepatocyte growth factor gene therapy of liver cirrhosis in rats. *Nat Med* 1999;5:226-30.
  82. Matsuno Y, Iwata H, Umeda Y, Takagi H, Mori Y, Kosugi A, Matsumoto K, Nakamura T, Hirose H. Hepatocyte growth factor gene transfer into the liver via the portal vein using electroporation attenuates rat liver cirrhosis. *Gene Ther* 2003;10:1559-66.
  83. Fischer M, You M, Matsumoto M, Crabb DW. Peroxisome proliferator-activated receptor alpha (PPARalpha) agonist treatment reverses PPARalpha dysfunction and abnormalities in hepatic lipid metabolism in ethanol-fed mice. *J Biol Chem* 2003;278:27997-8004.
  84. Ip E, Farrell G, Hall P, Robertson G, Leclercq I. Administration of the potent PPARalpha agonist, Wy-14,643, reverses nutritional fibrosis and steatohepatitis in mice. *Hepatology* 2004;39:1286-96.
  85. Saxena NK, Saliba G, Floyd JJ, Anania FA. Leptin induces increased alpha2(I) collagen gene expression in cultured rat hepatic stellate cells. *J Cell Biochem* 2003; 89:311-20.
  86. Sakaida I, Jinhua S, Uchida K, Terai S, Okita K. Leptin receptor-deficient Zucker (fa/fa) rat retards the development of pig serum-induced liver fibrosis with Kupffer cell dysfunction. *Life Sci* 2003;73:2491-501.
  87. Faggioni R, Feingold KR, Grunfeld C. Leptin regulation of the immune response and the immunodeficiency of malnutrition. *FASEB J* 2001;15:2565-71.
  88. Baumann H, Morella KK, White DW, Dembski M, Bailon PS, Kim H, Lai CF, Tartaglia LA. The full-length leptin receptor has signaling capabilities of interleukin 6-type cytokine receptors. *Proc Natl Acad Sci U S A* 1996;93: 8374-8.
  89. Streetz KL, Tacke F, Leifeld L, Wustefeld T, Graw A, Klein C, Kamino K, Spengler U, Kreipe H, Kubicka S, Muller W, Manns MP, Trautwein C. Interleukin 6/gp130-dependent pathways are protective during chronic liver diseases. *Hepatology* 2003;38:218-29.
  90. Kovalovich K, DeAngelis RA, Li W, Furth EE, Ciliberto G, Taub R. Increased toxin-induced liver injury and fibrosis in interleukin-6-deficient mice. *Hepatology* 2000;31: 149-59.
  91. Ezure T, Sakamoto T, Tsuji H, Lunz JG 3rd, Murase N, Fung JJ, Demetris AJ. The development and compensation of biliary cirrhosis in interleukin-6-deficient mice. *Am J Pathol* 2000;156:1627-39.
  92. Natsume M, Tsuji H, Harada A, Akiyama M, Yano T, Ishikura H, Nakanishi I, Matsushima K, Kaneko S, Mukaida N. Attenuated liver fibrosis and depressed serum albumin levels in carbon tetrachloride-treated IL-6-deficient mice. *J Leukoc Biol* 1999;66:601-8.
  93. Arita Y, Kihara S, Ouchi N, Maeda K, Kuriyama H, Okamoto Y, Kumada M, Hotta K, Nishida M, Takahashi M, Nakamura T, Shimomura I, Muraguchi M, Ohmoto Y, Funahashi T, Matsuzawa Y. Adipocyte-derived plasma protein adiponectin acts as a platelet-derived growth factor-BB-binding protein and regulates growth factor-induced common postreceptor signal in vascular smooth muscle cell. *Circulation* 2002;105:2893-8.
  94. Pinzani M, Gesualdo L, Sabbah GM, Abboud HE. Effects of platelet-derived growth factor and other polypeptide mitogens on DNA synthesis and growth of cultured rat liver fat-storing cells. *J Clin Invest* 1989;84:1786-93.
  95. Fasshauer M, Kralisch S, Klier M, Lossner U, Bluher M, Klein J, Paschke R. Adiponectin gene expression and secretion is inhibited by interleukin-6 in 3T3-L1 adipocytes. *Biochem Biophys Res Commun* 2003; 301: 1045-50.
  96. Kumada M, Kihara S, Ouchi N, Kobayashi H, Okamoto Y, Ohashi K, Maeda K, Nagaretani H, Kishida K, Maeda N, Nagasawa A, Funahashi T, Matsuzawa Y. Adiponectin specifically increased tissue inhibitor of metalloproteinase-1 through interleukin-10 expression in human macrophages. *Circulation* 2004;109:2046-9.
  97. Chinetti G, Zawadzki C, Fruchart JC, Staels B. Expression of adiponectin receptors in human macrophages and regulation by agonists of the nuclear receptors PPARalpha, PPARgamma, and LXR. *Biochem Biophys Res Commun* 2004;314:151-8.
  98. Delporte ML, Ait El Mkaem S, Quisquater M, Brichard SM. Leptin treatment markedly increased plasma adiponectin, but barely decreased plasma resistin of ob/ob mice. *Am J Physiol Endocrinol Metab* 2004;287:E446-53.
  99. Gressner AM. Cytokines and cellular crosstalk involved in the activation of fat-storing cells. *J Hepatol* 1995; 22:28-36.
  100. 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.
  101. Komuves LG, Feren A, Jones AL, Fodor E. Expression of epidermal growth factor and its receptor in cirrhotic liver disease. *J Histochem Cytochem* 2000;48:821-30.
  102. Jensen CH, Jauho EI, Santoni-Rugiu E, Holmskov U, Teisner B, Tygstrup N, Bisgaard HC. Transit-amplifying ductular (oval) cells and their hepatocytic progeny are characterized by a novel and distinctive expression of delta-like protein/preadipocyte factor 1/fetal antigen 1. *Am J Pathol* 2004;164:1347-59.
  103. Samulewicz SJ, Seitz A, Clark L, Heber-Katz E.

- Expression of preadipocyte factor-1(Pref-1), a delta-like protein, in healing mouse ears. *Wound Repair Regen* 2002;10:215-21.
104. Manson JE, Skerrett PJ, Greenland P, VanItallie TB. The escalating pandemics of obesity and sedentary lifestyle. A call to action for clinicians. *Arch Intern Med* 2004; 164:249-58.
  105. Huang YC, Wu JY, Yang MJ. Weight-for-height reference and the prevalence of obesity for school children and adolescents in Taiwan and Fuchien Areas. *J Chin Med Assoc* 2003;66:599-606.
  106. Dietz WH. Overweight in childhood and adolescence. *N Engl J Med* 2004;350:855-7.
  107. Steinbrook R. Surgery for severe obesity. *N Engl J Med* 2004;350:1075-9.
  108. Marceau P, Biron S, Hould FS, Marceau S, Simard S, Thung SN, Kral JG. Liver pathology and the metabolic syndrome X in severe obesity. *J Clin Endocrinol Metab* 1999;84:1513-7.
  109. Kral JG, Thung SN, Biron S, Hould FS, Lebel S, Marceau S, Simard S, Marceau P. Effects of surgical treatment of the metabolic syndrome on liver fibrosis and cirrhosis. *Surgery* 2004;135:48-58.
  110. Ioannou GN, Weiss NS, Kowdley KV, Dornitz JA. Is obesity a risk factor for cirrhosis-related death or hospitalization? A population-based cohort study. *Gastroenterology* 2003;125:1053-9.
  111. Hotta K, Funahashi T, Arita Y, Takahashi M, Matsuda M, Okamoto Y, Iwahashi H, Kuriyama H, Ouchi N, Maeda K, Nishida M, Kihara S, Sakai N, Nakajima T, Hasegawa K, Muraguchi M, Ohmoto Y, Nakamura T, Yamashita S, Hanafusa T, Matsuzawa Y. Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. *Arterioscler Thromb Vasc Biol* 2000;20:1595-9.
  112. Yang WS, Lee WJ, Funahashi T, Tanaka S, Matsuzawa Y, Chao CL, Chen CL, Tai TY, Chuang LM. Weight reduction increases plasma levels of an adipose-derived anti-inflammatory protein, adiponectin. *J Clin Endocrinol Metab* 2001;86:3815-9.
  113. Esposito K, Pontillo A, Di Palo C, Giugliano G, Masella M, Marfella R, Giugliano D. Effect of weight loss and lifestyle changes on vascular inflammatory markers in obese women: a randomized trial. *JAMA* 2003;289: 1799-804.
  114. Giugliano G, Nicoletti G, Grella E, Giugliano F, Esposito K, Scuderi N, D'Andrea F. Effect of liposuction on insulin resistance and vascular inflammatory markers in obese women. *Br J Plast Surg* 2004;57:190-4.
  115. Hukshorn CJ, Lindeman JH, Toet KH, Saris WH, Eilers PH, Westerterp-Plantenga MS, Kooistra T. Leptin and the proinflammatory state associated with human obesity. *J Clin Endocrinol Metab* 2004;89:1773-8.
  116. Lonardo A, Adinolfi LE, Loria P, Carulli N, Ruggiero G, Day CP. Steatosis and hepatitis C virus: mechanisms and significance for hepatic and extrahepatic disease. *Gastroenterology* 2004;126:586-97.
  117. Schuppan D, Krebs A, Bauer M, Hahn EG. Hepatitis C and liver fibrosis. *Cell Death Differ* 2003;1:S59-67.
  118. Clarke SD. The multi-dimensional regulation of gene expression by fatty acids: polyunsaturated fats as nutrient sensors. *Curr Opin Lipidol* 2004;15:13-8.
  119. Browning JD, Horton JD. Molecular mediators of hepatic steatosis and liver injury. *J Clin Invest* 2004;114:147-52.
  120. Huo TI, Wu JC, Lee PC, Tsay SH, Chang FY, Lee SD. Diabetes mellitus as a risk factor of liver cirrhosis in patients with chronic hepatitis B virus infection. *J Clin Gastroenterol* 2000;30:250-4.

## 從脂肪代謝觀點談肝臟纖維化及硬化

莊錦豪<sup>1,3</sup> 王佩文<sup>2</sup> 戴明泓<sup>4</sup>

肝臟因慢性或反覆性傷害，如酒精中毒，肝炎病毒，免疫或先天代謝異常，而致纖維化或硬化。因肥胖導致之非酒精性脂肪肝病，可能是最近才發現可導致肝硬化的病因。肥胖也提供沃土，使本來因酒精或病毒引起之肝病，更容易走向硬化。胰島素，IGF-1，PPAR，leptin，adiponectin 及 Pref-1/dlk1 是脂肪代謝相關的荷爾蒙，生長因子，細胞核受體及細胞素。但它們在肝纖維化的病程中也插一腳，主要藉由調控共同的目標—即肝臟星狀細胞HSC。HSC的活化增生，導致肝臟纖維化相關蛋白質如膠原的生成，催化肝纖維化的進行。胰島素及IGF-1在體外可活化HSC並生成膠原。但在體內，IGF-1的作用卻在減輕肝纖維化。PPAR $\gamma$ 之配體(ligand)在體外可抑制HSC活化及膠原的生成，與其體內抑制肝纖維化吻合，但與PPAR $\beta$ 之作用相反。瘦體素leptin是促纖維化的因子，因此缺leptin或其受體的小白鼠，反而較不容易因毒性肝損傷導致肝臟的纖維化。Adiponectin是抑制肝纖維化的細胞素，因此剔除adiponectin基因的小白鼠，容易產生厲害的肝纖維化，而補充重組adiponectin後，情形即好轉。Pref-1/dlk1可在人體內調控HSC而影響肝纖維化的進展。應用活躍於脂肪代謝的分子，如PPAR $\gamma$ 之配體或adiponectin，雖不一定能達到減肥的目的，但在減輕代謝徵候群或肝硬化上，或有不容忽視的潛力。有利於減輕代謝徵候群的減重，也同樣可能減少肥胖相關之肝硬化。(長庚醫誌2004;27:855-68)

**關鍵字：**肝臟，纖維化，肝硬化，肥胖，非酒精性脂肪肝病，細胞素，瘦體素。

---

長庚紀念醫院 高雄院區 <sup>1</sup>兒童外科，<sup>2</sup>新陳代謝科，<sup>3</sup>長庚大學臨研所；<sup>4</sup>高雄榮民總醫院 醫學教育研究部

受文日期：民國93年8月18日；接受刊載：民國93年10月19日。

索取抽印本處：莊錦豪醫師，長庚紀念醫院 兒童外科。高雄縣833鳥松鄉大埤路123號。Tel.: (07)7317123轉8811; Fax: (07)7338009; E-mail: jhchuang@adm.cgmh.org.tw