Role of Leptin in Pathogenesis of NASH

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Summary. Increasing lines of evidence indicate that obesity is an important risk factor for the exacerbation of alcoholic liver disease (ALD) and nonalcoholic steatohepatitis (NASH). Leptin, an obese gene product, is a cytokine-type hormone mainly produced from adipose tissue. Recently, it has been demonstrated that serum leptin levels are increased in patients with alcoholic cirrhosis. In this study, therefore, we investigated the role of leptin in hepatic fibrogenesis. Activated hepatic stellate cells (HSCs) produced leptin during hepatic fibrogenesis. Xenobiotic-induced hepatic fibrogenesis was almost completely abolished in ob/ob mice and Zucker (fa/fa) rats, which are inborn leptin- and leptin receptor (Ob-R)-deficient animals, respectively. Further, leptin increased transforming growth factor (TGF)-β mRNA in isolated sinusoidal endothelial cells and Kupffer cells. Moreover, leptin augmented platelet-derived growth factor (PDGF)-dependent proliferation of HSCs. Taken together, these findings lead to the postulation that leptin acts as a profibrogenic cytokine in the sinusoidal microenvironment. In conclusion, leptin most likely plays a pivotal role in the progression of hepatic fibrosis in a variety of chronic liver diseases, including NASH.

Key words. NASH, Hepatic fibrogenesis, Leptin, Sinusoidal cells, TGF-β

Introduction

Accumulating lines of evidence suggest that alcoholic liver disease (ALD) and nonalcoholic steatohepatitis (NASH) share a common pathophysiological basis, in terms of inflammation and fibrogenesis. Because NASH is often associated with metabolic syndrome, comprising obesity, type-2 diabetes, and hypertension, it is hypothesized that adipocytokines, insulin resistance, and autonomic nervous regulation play causative roles in the disease progression of NASH. Leptin, an obese gene product mainly produced from adipocytes, is a cytokine-type hormone that regulates food intake and fat metabolism through actions on the central nervous system [1]. Recently, McCullough et al. [2] reported that serum leptin levels were increased in patients with alcoholic cirrhosis. Further, hepatic stellate cells (HSCs) have been shown to produce leptin when they become activated [3]. Moreover, the coadministration of recombinant

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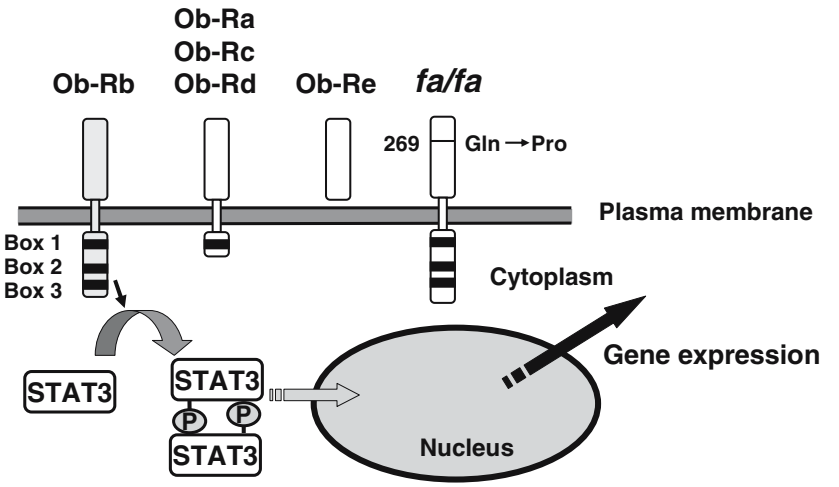

Fig. 1. Isoforms of leptin receptors (Ob-R). *Ob-Ra*, short-form Ob-R; *Ob-Rb*, long-form Ob-R.

leptin augments the inflammation and fibrogenesis in the liver caused by hepatotoxic xenobiotics [4]. These findings lead to the hypothesis that leptin plays a pivotal role in profibrogenic responses in the liver.

Leptin receptors (Ob-R) were originally shown in hypothalamic neurons, through which leptin regulates food intake and body weight [5]. In fact, homozygous mutations of the leptin receptor gene have been identified in rodents (i.e., db/db mice and Zucker rats), which are also associated with obesity [6, 7]. There are several isoforms of Ob-R, which are splice variants with the same extracellular domain. The most ubiquitous form of Ob-R is a short-form receptor (Ob-Ra); however, the function of this receptor isoform remains unclear. In contrast, a long-form leptin receptor (Ob-Rb), which contains a longer intracellular domain, is known to activate the Janus kinase (JAK)-STAT-3 pathway, leading to the transcriptional regulation of target genes (Fig. 1). In the present study, we investigated the expression and functions of Ob-R in hepatic sinusoidal cells in order to elucidate the mechanisms underlying the profibrogenic action of leptin in the liver.

Poor Hepatic Fibrogenesis in Leptin- and Ob-R-Deficient Animals

In a previous study, we [4], demonstrated that administration of recombinant leptin augmented profibrogenic responses in the liver caused by xenobiotics (i.e., carbon tetrachloride and thioacetamide [TAA]) in mice. These observations led us to investigate whether endogenous leptin promoted hepatic fibrogenesis. To answer this question, we utilized ob/ob mice, which lack leptin due to naturally occurring disruption of the leptin gene. Interestingly, ob/ob mice demonstrate extremely poor profibrogenic responses to xenobiotic treatment [8], suggesting that leptin is one of the key regulators of hepatic fibrogenesis.

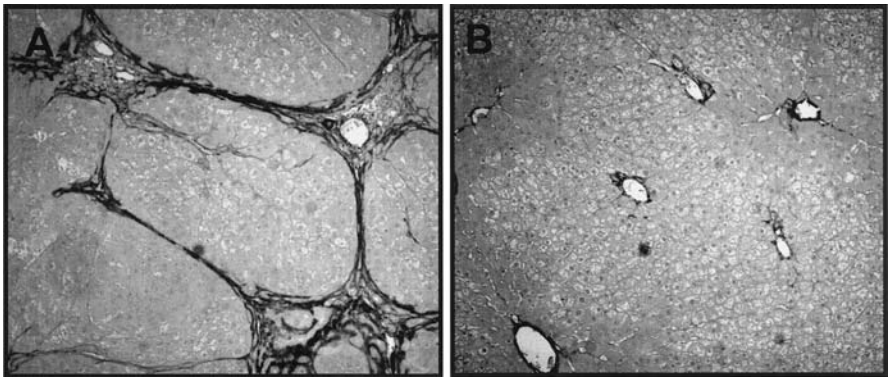

Fig. 2A,B. Thioacetamide (TAA)-induced hepatic fibrosis in Zucker (fa/fa) rats. Male Zucker (fa/fa) rats and their lean littermates (+/?) were given repeated intraperitoneal injections of TAA, (200 mg/kg body weight [BW], three times/week) for 8 weeks, and liver histology was assessed by picro-sirius red staining. **A** Control rats (+/?) treated with TAA. **B** Zucker (fa/fa) rats given TAA. ×40.

Next, we evaluated the role of Ob-R in hepatic fibrogenesis, using Zucker (fa/fa) rats, which lack functional Ob-R due to a missense mutation in the common, extracellular domain [9]. Zucker rats presented extremely poor profibrogenic responses in the liver caused by chronic TAA treatment as compared to their lean littermates (Fig. 2), indicating that Ob-R is involved in the profibrogenic response in the liver. Overt expression of α smooth muscle actin (SMA) in the liver was observed in lean littermates given TAA, whereas αSMA staining was almost negative in Zucker rats even when they were treated with the equivalent amount of TAA. In clear contrast, HSCs isolated from Zucker rats transactivated in vitro were almost the same as the cells isolated from their lean littermates, in terms of the induction of αSMA and steady-state mRNA levels of α1(I)procollagen. This discrepancy in HSC transactivation in vivo and in vitro led us to investigate the expression of Ob-R isoforms in sinusoidal cells.

Sinusoidal Endothelial Cells and Kupffer Cells Express a Functional Ob-R

To determine whether sinusoidal cells express Ob-R isoforms, mRNA for Ob-Ra and Ob-Rb were detected by reverse transcription-polymerase chain reaction (RT-PCR). As expected, Ob-Ra mRNA was detected ubiquitously in HSCs and sinusoidal endothelial cells (SECs). In contrast, Ob-Rb was detected clearly only in SECs and Kupffer cells, but not in 7-day cultured HSCs. Next, to confirm the expression of functional receptors, phosphorylation of STAT-3 was measured by Western blotting. Tyrosine phosphorylation of STAT-3 was clearly detected in SECs when they were treated with leptin (100 nM) for 1 h. In sharp contrast, STAT-3 phosphorylation was barely detectable in rat HSCs even they were incubated with the same dose of leptin. These findings indicated that primary cultured rat SECs express both Ob-Ra and functional

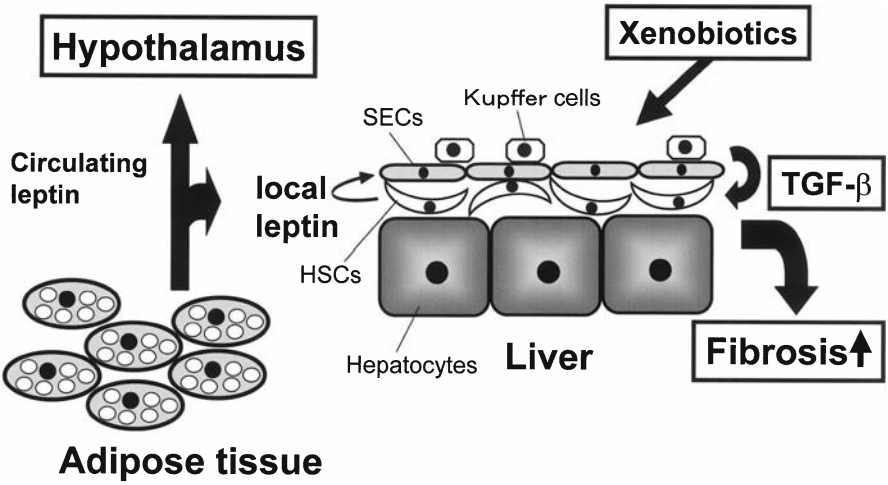

Fig. 3. Leptin is involved in hepatic fibrogenesis: working hypothesis. *SECs*, sinusoidal endothelial cells; *HSCs*, hepatic stellate cells; *TGF-β*, transforming growth factor-β.

Ob-Rb constitutively. Kupffer cells are also positive for the constitutive expression of Ob-Rb. Isolated HSCs, however, appear to express only Ob-Ra, but not Ob-Rb.

Next, we examined whether leptin activated the activator protein (AP)-1 DNA binding in isolated Kupffer cells. The addition of leptin to the medium for 1 h increased AP-1 DNA binding activity in a dose-dependent manner. Further, the effect of leptin on steady-state levels of mRNA for transforming growth factor (TGF)-β1 mRNA in Kupffer cells was measured by ribonuclease protection assay. TGF-β1 mRNA levels increased nearly twofold over controls 3–6 h after leptin treatment (100 nM). Similar, increases in AP-1 DNA binding activity and steady-state mRNA levels of TGF-β1 were observed in LSE cells, a human sinusoidal endothelial cell-derived cell line [9]. In contrast, leptin did not increase steady-state mRNA levels of TGF-β1 in isolated HSCs [8]. Collectively, these observations indicate that leptin most likely upregulates the transcriptional activities of the TGF-β1 gene in Kupffer cells and SECs through the activation of AP-1. It is hypothesized that Ob-Rb-mediated signaling, which is predominant in Kupffer cells and SECs, upregulates the production/activation of TGF-β, thereby facilitating tissue repair and profibrogenic responses in the sinusoidal microenvironment (Fig. 3).

Leptin Facilitates the Proliferation of Hepatic Stellate Cells

As stated above, we have observed that HSCs isolated from Ob-R-deficient Zucker (fa/fa) rats undergo an almost normal transactivation process in vitro [9], suggesting that Ob-R in HSCs is not essential for their activation. On the other hand, emerging lines of evidence suggest the possibility that leptin affects collagen synthesis in isolated HSCs [10]. In line with these observations, we investigated the effect of leptin on the proliferation of HSCs in vitro. The proliferation of 3-day cultured rat HSCs was

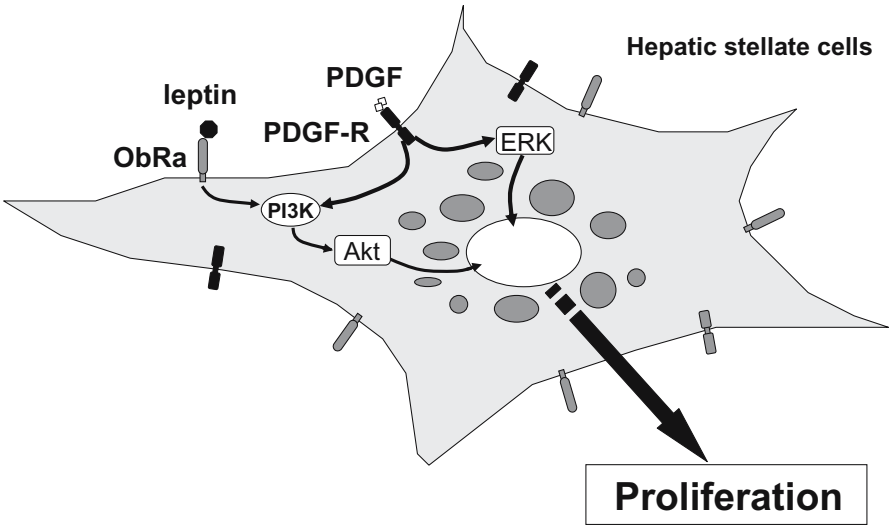

Fig. 4. Leptin enhances platelet-derived growth factor (*PDGF*)-dependent proliferative responses in hepatic stellate cells.

assessed by the incorporation of 5-bromo-2′-deoxyuridine (BrdU) into the nuclei. The percentages of BrdU-positive cells were increased in the presence of platelet-derived growth factor (PDGF)-BB (5 ng/ml) for 8 h, as expected. Co-incubation with leptin (10–100 nM) potentiated this PDGF-dependent increase in BrdU-positive cells in a dose-dependent manner. Messenger RNA for PDGF receptor α and β subunits was increased almost two- to threefold by incubation with leptin for 6 h. Further, preincubation with leptin for 6 h enhanced PDGF-induced increases in phospho-p44/42 mitogen activated protein (MAP) kinase and phospho-Akt levels in a dose-dependent manner. In the same condition, however, leptin per se did not increase phospho-STAT 3 and phospho-p44/42 MAP kinase levels. Instead, leptin increased phospho-Akt levels in HSCs within 30 min, suggesting that the phosphatidylinositol 3 kinase (PI3K)/Akt pathway is involved in the mechanism by which leptin accelerates the proliferation of HSCs (Fig. 4). Taken together, these findings clearly indicate that leptin potentiates the PDGF-dependent proliferative responses of HSCs in vitro.

Conclusion

Leptin and its functional receptors play a crucial role in hepatic fibrogenesis, most likely through the upregulation of TGF-β expression in the liver. Further, leptin augments PDGF-dependent proliferative responses in HSCs, most likely through actions involving the PI3K/Akt pathway. On the other hand, it is likely that leptin ameliorates hepatic steatosis and inflammatory responses. Taken together, these findings suggest that leptin can be characterized as a tissue-repairing cytokine (Fig. 5). In conclusion, it is postulated that leptin, produced systemically from adipose tissue and locally from

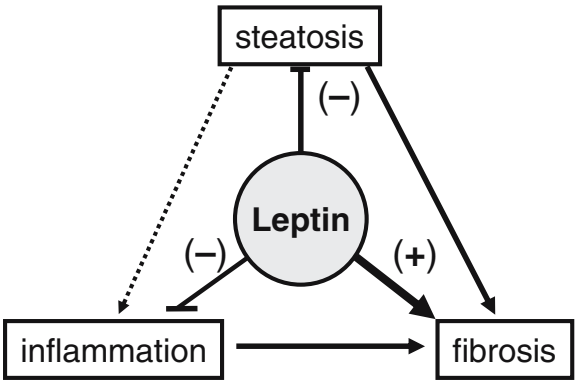

Fig. 5. Role of leptin in progression of steatohepatitis.

HSCs, contributes to the progression of hepatic fibrosis in a variety of obesity-related chronic liver diseases, including NASH.

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