Partial blockage of hepatocyte maturation in hepatoma-derived growth factor transgenic mice

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Abstract

AIM: To investigate the role of hepatoma-derived growth factor (HDGF) in liver development, especially in the hepaticocyte differentiation.

METHODS: We generated transgenic mice which over-expressed HDGF in hepatocytes under the transcriptional control of mouse albumin promoter/enhancer. To examine the effects of HDGF overexpression on hepatocytic differentiation, we investigated the expression patterns of the differentiation marker genes.

RESULTS: The HDGF transgenic mice developed normally and showed no apparent abnormality in the liver. However, the gene expression patterns of the liver in adult transgenic mice were similar to those of the neonatal liver in control mice.

CONCLUSION: These findings suggest that HDGF-overexpression partially suppresses hepatocyte maturation.

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Key words: Hepatoma-derived growth factor; Hepatocyte; Maturation; Transgenic mice

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INTRODUCTION

The liver is the major hematopoietic organ during the fetal period, and immature hepatocytes function as stromal cells which support hematopoiesis. During liver development, immature hepatocytes differentiate and acquire many functions in preparation for the metabolic conditions after birth.[1,2] The expression patterns of differentiation marker genes can represent the maturational stage of hepatocytes. Alpha-fetoprotein (AFP) is one of the early marker genes of the immature hepatocytes and its expression remarkably decreases after birth.[3] The expression of albumin, the most abundant protein synthesized by hepatocytes, begins during the mid-gestational stage, and this expression increases with the progression of liver development, especially after birth.[4] In the late-gestational stage hepatocytes begin to produce metabolic enzymes including tyrosine amino transferase (TAT) and glucose-6-phosphatase (G-6-Pase).[5] Subsequently, hepatocytes
gain a fully matured phenotype, characterized by the expression of tryptophan oxygenase (TO) within two weeks after birth[10]. The expression levels of TAT and G-6-Pase peak in the neonatal liver and decrease in the adult liver. In contrast, TO is barely expressed in the fetal and neonatal liver and is highly expressed in the adult liver. Although the gene expression patterns of hepatocytes continue to alter after birth, few studies have documented the growth and differentiation of post-natal hepatocytes.

Hepatoma-derived growth factor (HDGF) is a heparin-binding protein, which has been identified from the conditioned media of HuH-7 hepatoma cells[8,9]. HDGF stimulates the proliferation of fibroblasts, endothelial cells, vascular smooth muscle cells and hepatocytes[8,14]. Its primary sequence contains nuclear localization signals and the HDGF can be transported to the nucleus, thus indicating that HDGF is a unique nuclear/growth factor[13,15]. Recently, several novel genes have been identified for proteins which share a highly homologous amino terminal region consisting of about 100 amino acids; so-called HDGF-related proteins (HRPs)[14,15]. It is thought that HDGF and HRPs form a new gene family. Although HDGF was initially identified in human hepatoma-derived cells, HDGF mRNA is expressed in various normal adult tissues of mice and humans, thus suggesting that HDGF has some physiological functions in non-tumor cells[9].

Previous studies have suggested that HDGF participates in fetal organ development and adult tissue repair as an autocrine growth factor[10,11,16]. We have shown that HDGF is highly expressed in immature fetal hepatocytes and promotes their proliferation[10]. Furthermore, HDGF is induced in two animal models of liver regeneration[17], suggesting that HDGF plays an important role in the proliferation of both fetal and adult hepatocytes. Although the involvement of HDGF in cell differentiation has not been clarified, the suppressive effects of HDGF on gut cell maturation have been suggested[18]. We generated transgenic mice which overexpressed HDGF in hepatocytes under the control of the albumin promoter/ enhancer, in order to examine the functional role of HDGF in liver development. The gene expression patterns of hepatocytes in adult transgenic mice resemble those of neonatal hepatocytes in wild-type mice, thus suggesting that HDGF overexpression partially suppresses hepatocyte maturation.

MATERIALS AND METHODS

Mice
The DNA fragment covering the entire coding region of mouse HDGF was cloned into the Eco RI site of an expression vector which contains the promoter and enhancer of the mouse albumin gene[19]. A schematic representation of the constructed transgene (Alb-HDGF) is illustrated in Figure 1. Transgenic founders were generated by pronuclear injection according to standard techniques. Using a 32P-labeled fragment of HDGF-specific cDNA as a probe, transgene integration and expression were identified by Southern and Northern blot analyses, respectively, C57BL/6CrSlc mice (Nihon SLIC, Shizuoka, Japan) or non-transgenic mice were used as controls. All animal experiments were performed according to the guidelines of Osaka University Medical School.

Hybridization probes
The probes used for the Northern blot analysis were as follows: a 0.4 kb fragment of mouse HDGF cDNA[20], a 0.5 kb fragment of mouse G-6-Pase cDNA[20], and TO cDNA[24].

Southern blot and Northern blot analyses
Genomic DNA was isolated from individual mouse tails, and then was blotted onto nylon membranes according to standard protocols. Total RNA was extracted from liver tissues using ISOGEN (Nippon Gene, Tokyo, Japan), denatured with formamide and blotted onto nylon membranes. The mouse cDNAs described above were labeled with (α-32P) dCTP using a Megaprime DNA labeling kit (Amersham Life Science, Tokyo, Japan) and then were used for hybridization[16,17].

RESULTS

An expression unit was constructed that contained the entire HDGF cDNA under the control of the mouse albumin promoter/enhancer (Alb-HDGF, Figure 1). Purified fragments were used for pronuclear injection and potential founders were analyzed for the genomic integration(s) of the transgene. Three founders containing the Alb-HDGF sequence were identified by Southern blot, and the transgenes were successfully transmitted in two lines (Figure 2A: Tg-48, and Tg-21). Northern blot analysis revealed that HDGF was highly expressed in the adult liver of Tg-48 mice, whereas HDGF expression in the liver of Tg-21 mice was almost equal to the wild-type mice (Figure 2B). We therefore used the Tg-48 mice to analyze the effects of HDGF overexpression in hepatocytes.

HDGF overexpressing mice (Tg-48) developed normally and did not show any abnormality in appearance. In addition, no obvious histological abnormality associated with the expression of HDGF was detected in these mice up to 12 mo of age (data not shown). The expression patterns of genes related to hepatocyte differentiation were investigated by Northern blotting to examine the effects of HDGF overexpression on liver development in detail. In normal mice, consistent with the previous studies, G-6-
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figure 2 genomie integration and mrna expression of the hdgf transgene. a: southern blot analysis with hdgf cDNA probe. Genomic DNA was isolated from individual mouse tails and southern blot analysis was performed according to standard methods. The bands representing endogenous HDGF and transgenes are shown. Genomic DNA of a transgenic mouse (line number 21: Tg-21) contains high copy numbers of the transgene. Mammalian DNA of the other transgenic mice (line number 48: Tg-48) contains low copy numbers of the transgene. B: Northern blot analysis with HDGF cDNA probe. Total RNA was isolated from liver tissues of both transgenic (Tg) and wild-type (WT) mice. Twenty micrograms of total RNA extracted was loaded and hybridized with mouse cDNA of the HDGF-specific sequence. HDGF expression was high in the liver of the Tg-48 mouse. The expression level of HDGF in the liver of Tg-21 was almost equal to the level expressed in the control liver. Ribosomal RNA of 18S is shown in the lower panel.

discussion

A number of studies have suggested that HDGF is involved in the development of various organs[10,11,16,18,21]. We have demonstrated that HDGF is a unique growth factor, which is highly expressed in fetal liver and promotes fetal hepatocyte proliferation[16]. However, familial genes often compensate for the functions of other family members, and HDGF-null mice have been reported to show no obvious phenotype, perhaps as a result of the redundant functions of HDGF related genes[22]. We therefore generated the HDGF transgenic mice and examined the functional role of HDGF in vivo according to the gain-of-function method.

Although several other groups have also reported the involvement of HDGF in the development of various organs through its growth stimulating activity, little is known about the role of HDGF in cellular maturation. As for hepatocyte differentiation, Kamiya et al[23] established a primary culture system of murine fetal hepatocytes to investigate the mechanism that controls late fetal liver
development. In the culture system, the administration of Oncostain M and dexamethasone can induce hepatocyte differentiation and recapitulate the maturational process of hepatocytes ranging from mid-gestation to new-born stage. This culture system was used to clarify the involvement of HDGF in hepatocyte differentiation although down-regulation of HDGF could not induce the cellular differentiation process of the late gestation stage.

In the present study, the overexpression of HDGF under the control of the albumin promoter did not cause any apparent morphological abnormalities in the liver. However, the gene expression patterns showed the possibility that the maturational process of hepatocytes during the post-natal stage was disturbed. This result is consistent with the report by Lepourculet et al., which documented that overexpression of HDGF in the mouse fetal gut explants retards epithelial differentiation, suggesting a suppressive role of HDGF in epithelial differentiation.

Several proteins strongly expressed in both tumors and fetal organs, such as carcinoembryonic antigen and AFP, are known as oncofetal proteins. HDGF is expressed exclusively in both fetal and cancer tissues, indicating that HDGF can also be regarded as an oncofetal protein. Although several oncofetal proteins are clinically used as tumor markers, there are few proteins whose functional roles in cancer cells have so far been demonstrated. HDGF expression is strongly associated with the prognosis of many malignant diseases including pancreatic cancer, esophageal cancer, colorectal cancer, gastrointestinal stromal tumor, gastric cancer and hepatocellular carcinoma (HCC). Recently, Lee et al. showed that individuals with HCC who shared a gene expression pattern with fetal hepatoblasts had a poor prognosis. The gene expression pattern that distinguished this subtype from other types of HCC contained the markers of oval cells (hepato-cholangio progenitor cells), thus suggesting that the HCC of this subtype may be derived from hepatic progenitor/stem cells. Two groups have shown that high expression of HDGF is closely related to the poor prognosis of HCC and HDGF stimulates the growth of immature fetal hepatocytes. Recently, we have found that HDGF is highly expressed in oval cells and promotes their proliferation (Iwamoto et al. in preparation), thereby suggesting the involvement of HDGF in the proliferation of immature hepatic cells. Therefore, HDGF may stimulate the proliferation of HCC cells derived from hepatic progenitor/stem cells and thereby cause the poor prognosis. HDGF expression may maintain the characteristics of immature cells and be associated with high growth activity of malignant cells. HDGF not only promotes hepatocyte proliferation but also inhibits their differentiation, indicating that HDGF is an oncofetal protein which participates both in the cellular growth and differentiation. Clarifying the functional role of HDGF would give us new insights into molecular mechanisms common to normal and malignant hepatic cell growth.

Since HDGF-null mice did not show any remarkable abnormalities, perhaps as a result of the compensation by HDGF-related genes, the down-regulation of HDGF should inhibit the growth of cancer cells without any serious side effects on normal organs. Therefore, HDGF is considered to be a candidate therapeutic target. Although little is known about the regulation of HDGF expression, we recently have found that Vitamin K2 negatively controls the transcription of HDGF in hepatoma cells. However, the suppressive effects of the Vitamin K2 are limited and it is necessary to elucidate the whole regulation of the HDGF expression in hepatic cells, especially in hepatoma cells.

In conclusion, HDGF overexpressing transgenic mice showed the possible inhibitory role of HDGF on hepatocyte differentiation. The identification of both the regulation and signal transduction of HDGF makes it possible to obtain a better understanding of liver development, regeneration, and carcinogenesis.

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REFERENCES
1. Panduro A, Shalaby F, Shafritz DA. Changing patterns of transcriptional and post-transcriptional control of liver-specific gene expression during rat development. Genes Dev 1987; 1: 1172-1182
2. Derman E, Krauter K, Walling L, Weinberger C, Ray M, Enomoto H et al. Transgenic expression of HDGF in liver
Darnell JE Jr. Transcriptional control in the production of liver-specific mRNAs. Cell 1981; 2: 731-739

3 Shiojiri N, Lemire JM, Fausto N. Cell lineages and oval cell progenitors in rat liver development. Cancer Res 1991; 51: 2611-2620

4 Tilghman SM, Belayew A. Transcriptional control of the murine albumin/alpha-fetoprotein locus during development. Proc Natl Acad Sci USA 1982; 79: 5254-5257

5 Greengard O. The developmental formation of enzymes in rat liver. In: Litwack G, editor. Biochemical Actions of Hormones. New York: Academic Press Inc, 1970: 53-87

6 Haber BA, Chin S, Chuang E, Baikhuwisen W, Naji A, Taub R. High levels of glucose-6-phosphatase gene and protein expression reflect an adaptive response in proliferating liver and diabetes. J Clin Invest 1995; 95: 832-841

7 Nagao M, Nakamura T, Ichihara A. Developmental control of gene expression of trypsinogen T3-dioxigenase in neonatal rat liver. Biochin Biophys Acta 1986; 867: 179-186

8 Nakamura H, Kambe H, Egawa T, Kimura Y, Ito H, Hayashi E, Yamamoto H, Saito J, Kishimoto S. Partial purification and characterization of human hepatoma-derived growth factor. Clin Chim Acta 1989; 183: 273-284

9 Nakamura H, Izumo Y, Kambe H, Kuroda T, Mori T, Kawamura K, Yamamoto H, Kishimoto T. Molecular cloning of complementary DNA for a novel human hepatoma-derived growth factor. Its homology with high mobility group-1 protein. J Biol Chem 1994; 269: 25143-25149

10 Oliver JA, Al-Awqati Q. An endothelial growth factor involved in rat renal development. J Clin Invest 1998; 102: 1208-1219

11 Everett AD, Lobe DR, Matsumura ME, Nakamura H, McNamara CA. Hepatoma-derived growth factor stimulates smooth muscle cell growth and is expressed in vascular development. J Clin Invest 2000; 105: 567-575

12 Kishima Y, Yamamoto H, Izumo Y, Yoshida K, Enomoto H, Yamamoto M, Kuroda T, Ito H, Yoshizaki K, Nakamura H. Hepatoma-derived growth factor stimulates cell growth after translocation to the nucleus by nuclear localization signals. J Biol Chem 2002; 277: 10315-10322

13 Everett AD, Stoops T, McNamara CA. Nuclear targeting is required for hepatoma-derived growth factor-stimulated mitogenesis in vascular smooth muscle cells. J Biol Chem 2001; 276: 37564-37568

14 Izumo Y, Kuroda T, Harada H, Kishimoto T, Nakamura H. Hepatoma-derived growth factor belongs to a gene family in mice showing significant homology in the amino terminus. Biochem Biophys Res Commun 1997; 238: 26-32

15 Ikegame K, Yamamoto M, Kishima Y, Enomoto H, Yoshida K, Suemura M, Kishimoto T, Nakamura H. A new member of a hepatoma-derived growth factor gene family can translocate to the nucleus. Biochem Biophys Res Commun 1999; 266: 81-87

16 Enomoto H, Yoshida K, Kishima Y, Kinoshita T, Yamamoto M, Everett AD, Miyajima A, Nakamura H. Hepatoma-derived growth factor is highly expressed in developing liver and promotes fetal hepatocyte proliferation. Hepatology 2002; 36: 1519-1527

17 Enomoto H, Nakamura H, Liu W, Yoshida K, Okuda Y, Imanishi H, Saito M, Shimomura S, Hada T, Nishiguchi S. Hepatoma-derived growth factor is induced in liver regeneration. Hepatol Res 2009; 39: 988-997

18 Lepoutrelet M, Tou L, Cai L, Sawada J, Lazar AJ, Glickman JN, Williamson JA, Everett AD, Redston M, Fox EA, Nakatani Y, Shivasra NA. Insights into developmental mechanisms and cancers in the mammalian intestine derived from serial analysis of gene expression and study of the hepatoma-derived growth factor (HDGF). Development 2005; 132: 415-427

19 Pinkert CA, Ornitz DM, Brinster RL, Palmiter RD. An albumin enhancer located 10 kb upstream functions along with its promoter to direct efficient, liver-specific expression in transgenic mice. Genes Dev 1987; 1: 268-276

20 Kamiya A, Kinoshita T, Ito Y, Matsui T, Morikawa Y, Senba E, Nakashima K, Taga T, Yoshida K, Kishimoto T, Miyajima A. Fetal liver development requires a paracrine action of oncostatin M through the gp130 signal transducer. EMBO J 1999; 18: 2127-2136

21 Cilley RE, Zglenzowski SE, Chinary MR. Fetal lung development: airway pressure enhances the expression of developmental genes. J Pediatr Surg 2000; 35: 113-118; discussion 119

22 Gallitzenberder R, Abouzied MM, Hartmann D, Dobrowski R, Gieselmann V, Franken S. Hepatoma-derived growth factor (HDGF) is dispensable for normal mouse development. Dev Dyn 2008; 237: 1875-1885

23 Orell SR, Dowling KD. Oncofetal antigens as tumor markers in the cytologic diagnosis of effusions. Acta Cytol 1983; 27: 625-629

24 Garrett PE, Kurtz SR. Clinical utility of oncofetal proteins and hormones as tumor markers. Med Clin North Am 1986; 70: 1295-1306

25 Uyama H, Tomita Y, Nakamura H, Nakamori S, Zhang B, Hoshida Y, Enomoto H, Okuda Y, Sakon M, Aozasa K, Kawase I, Hayashi N, Monden M. Hepatoma-derived growth factor is a novel prognostic factor for patients with pancreatic cancer. Clin Cancer Res 2006; 12: 6043-6048

26 Yamamoto S, Tomita Y, Hoshida Y, Mori E, Yasuda T, Doki Y, Aozasa K, Uyama H, Nakamura H, Monden M. Expression level of hepatoma-derived growth factor correlates with tumor recurrence of esophageal carcinoma. Ann Surg Oncol 2007; 14: 2141-2149

27 Hu TH, Lin JW, Chen HH, Liu LF, Chua SH, Tai MH. The expression and prognostic role of hepatoma-derived growth factor in colorectal stromal tumors. Dis Colon Rectum 2009; 52: 319-326

28 Chang KC, Tai MH, Lin JW, Wang CC, Huang CC, Hung CH, Chen CH, Lu SN, Lee CM, Changchien CS, Hu TH. Hepatoma-derived growth factor is a novel prognostic factor for gastrointestinal stromal tumors. Int J Cancer 2007; 121: 1059-1065

29 Yamamoto S, Tomita Y, Hoshida Y, Takiguchi S, Fujiiwa Y, Yasuda T, Doki Y, Yoshida K, Aozasa K, Nakamura H, Monden M. Expression of hepatoma-derived growth factor is correlated with lymph node metastasis and prognosis of gastric carcinoma. Clin Cancer Res 2006; 12: 117-122

30 Yoshida K, Tomita Y, Okuda Y, Yamamoto S, Enomoto H, Uyama H, Ito H, Hoshida Y, Aozasa K, Nagano H, Sakon M, Kawase I, Monden M, Nakamura H. Hepatoma-derived growth factor is a novel prognostic factor for hepatocellular carcinoma. Ann Surg Oncol 2006; 13: 159-167

31 Hu TH, Huang CC, Liu LF, Lin PR, Liu SY, Chang HW, Changchien CS, Lee CM, Chuang JH, Tai MH. Expression of hepatoma-derived growth factor in hepatocellular carcinoma. Cancer 2003; 98: 1444-1456

32 Lee JS, Heo J, Libbrecht L, Chu IS, Kaposi-Novak P, Calvisi DF, Mikaelyan A, Robert LR, Demetris AJ, Sun Z, Nevens F, Rosskams T, Thorgerinson SS. A novel prognostic subtype of human hepatocellular carcinoma derived from hepatic progenitor cells. Nat Med 2006; 12: 410-416

33 Yamamoto T, Nakamura H, Liu W, Cao K, Yoshikawa S, Enomoto H, Iwata Y, Koh N, Saito M, Imanishi H, Shimomura S, Iijima H, Hada T, Nishiguchi S. Involvement of hepatoma-derived growth factor in the growth inhibition of hepatocellular carcinoma cells by vitamin K2. J Gastroenterol 2009; 44: 228-235

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