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Oncostatin M and hepatocyte growth factor induce hepatic maturation via distinct signaling pathways

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Abstract Liver development is regulated by soluble factors as well as cell–cell contacts. We previously reported that oncostatin M (OSM) induced hepatic maturation in a primary culture of embryonic day 14 liver cells. While OSM expression in the liver starts in mid gestation and decreases in postnatal stages, hepatocyte growth factor (HGF) is mainly expressed in the liver in the first few days after birth. In this study, we compared the effect of OSM and HGF on the differentiation of fetal hepatic cells in vitro. Like OSM, HGF in the presence of dexamethasone induced expression of glucose-6-phosphatase, tyrosine amino transferase and carbamoyl-phosphate synthase, and accumulation of glycogen in fetal hepatic cells, although to a lesser extent than OSM. Interestingly, while both OSM and HGF up-regulated production of albumin, secretion of albumin occurred only in response to OSM. In addition, although hepatic maturation induced by OSM depends on STAT3, HGF failed to activate STAT3 and HGF-induced differentiation was independent of STAT3. These results indicate that OSM and HGF induce hepatic maturation through different signaling pathways.

Key words: Hepatocyte; Hepatic maturation; Oncostatin M; Hepatocyte growth factor

1. Introduction

Adult liver is a central organ for metabolism and maintains homeostasis, whereas fetal liver has little metabolic function [1–3]. Instead, it functions as the major hematopoietic organ in mid- to late fetus [4]. During the late-fetal stage, the hematopoietic site shifts to the spleen and bone marrow and concomitantly hepatocytes acquire various functions of the mature liver. We previously described that hematopoietic cells in mid-fetal livers produce oncostatin M (OSM), an interleukin (IL)-6 family cytokine (reviewed in [5]), and that OSM induces maturation of fetal hepatic cells derived from the embryonic day 14.5 (E14.5) liver in vitro; such as expression of glucose-6-phosphatase (G6Pase) and tyrosine amino transferase (TAT) and accumulation of glycogen [6,7]. Consistent with those in vitro results, the levels of TAT as well as glycogen accumulation were significantly reduced in the liver derived from knockout mice of gp130, the common receptor subunit of IL-6 family cytokines [6,8]. However, the level of TAT expression induced by OSM was lower than in adult liver and an adult hepatocyte marker tryptophan oxygenase was not induced in vitro by OSM, suggesting that OSM allows the E14.5 fetal hepatocytes to acquire the characteristics of the neonatal liver. The expression level of TAT in vivo is further up-regulated during postnatal development, while expression of OSM in the liver decreases during late-fetal and neonatal stages since the major hematopoietic activity shifts to the spleen and bone marrow at these stages. Therefore, another factor may be involved in the liver maturation process.

Hepatocyte growth factor (HGF) was originally identified in serum of partially hepatectomized rats and rat platelets and was shown to directly stimulate proliferation of adult hepatocytes in vitro (reviewed in [9]). In addition, HGF mRNA was remarkably increased when hepatic injury was induced by hepatotoxins (CCl_4 and D-galactosamine) in rats [10,11]. These findings indicate that HGF is an important factor for liver regeneration. It was previously described that mRNAs for HGF and c-Met (the receptor of HGF) were increased at a postnatal stage in several rat tissues such as the liver, lung and kidney [12], suggesting that HGF is involved in the development of these organs.

In this study, we investigated whether HGF is involved in the neonatal hepatic cell differentiation after expression of OSM in the liver is down-regulated. We show that HGF induces expression of liver differentiation marker genes and glycogen accumulation. Analysis of intracellular signals demonstrates that HGF does not activate signal transducer and activator of transcription 3 (STAT3) in fetal hepatic cells and the HGF-induced hepatic differentiation is not inhibited by dominant negative STAT3 (ΔSTAT3), indicating that OSM and HGF induce liver development through different signaling pathways.

2. Materials and methods

2.1. Materials

C57BL/6CrSlc mice (Nihon SLC, Sizuoka, Japan) were used in all experiments in this study. Dulbecco's modified Eagle's medium (DMEM), fetal calf serum (FCS), liver perfusion medium, liver digest medium, MEM non-essential amino acid solution and insulin–trans-

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Abbreviations: OSM, oncostatin M; HGF, hepatocyte growth factor; IL-6, interleukin 6; G6Pase, glucose-6-phosphatase; TAT, tyrosine amino transferase; Dex, dexamethasone; PAS, periodic acid-Shiff; CPS, carbamoyl-phosphate synthase; STAT, signal transducer and activator of transcription

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ferrin–selenium X (ITS) were purchased from Gibco-BRL. Human HGF was from Becton Dickinson. Murine OSM was from R&D Systems. Anti-mouse albumin antibody was purchased from Nordic Immunological Laboratories. Antibodies against murine phospho-STAT3, phospho-ERK1/2 and ERK1/2 were purchased from New England Biolabs. Anti-STAT3 antibody was from Transduction Lab.

2.2. Reverse transcription (RT)-PCR analysis

Total RNA was extracted from livers derived from E14, E18, neonatal, 10-day-old and adult mice by the acid guanidinium–phenol–chloroform (AGPC) method [13]. First-strand cDNA was synthesized from 1 μg of total RNA using the First-Strand cDNA Synthesis kit (Amersham-Pharmacia) and was used as a template for PCR amplification with murine HGF primers (forward primer: 5′-AGA-CAC-CAC-ACC-GGC-ACA-AGT, reverse primer: 5′-ATA-GGG-CAA-TAA-TCC-CAA-GG) or murine OSM primers [14]. The primers were annealed at 50°C for 60 s and amplification was repeated for 30∼40 cycles. Amplified products were separated by electrophoresis on a 1.5% agarose gel and stained with ethidium bromide.

2.3. Cell culture

Fetal hepatic culture derived from E14 mouse livers was previously described [6]. Briefly, eight or nine embryonic liver tissues were minced and dissociated with liver digest medium followed by hemolysis with hypotonic buffer. Cells were cultured in DMEM supplemented with 10% FCS, 2 mM L-glutamine, 1× non-essential amino acid solution, 1× ITS, 50 μg/ml gentamicin and 10^{-7} M dexamethasone (Dex) onto 0.1% gelatin-coated tissue culture dishes. Several hours later, these cells were washed with culture media to remove contaminating hematopoietic cells and cell debris. Culture media were replaced every 2 days. Each experiment described in the paper was repeated at least twice and typical results are shown in figures.

2.4. Periodic acid-Shiff (PAS) staining

Fetal hepatic cells were cultured in the presence of 10 ng/ml OSM or 20 ng/ml HGF for 6 days. Cells were fixed with 20% formaldehyde. Intercellular glycogen was stained with the PAS staining solution (Muto Pure Chem., Japan) according to the standard protocol.

2.5. Northern blot analysis

Cellular and tissue total RNA samples were extracted by the AGPC method. 10 μg total RNA from each sample was separated by electrophoresis on a 1.5% agarose gel containing 2% formaldehyde. RNA was transferred to a positively charged Nylon membrane (Boehringer-Mannheim) and was hybridized with digoxigenin (DIG)-labeled cDNA probes generated by the rTaq DNA polymerase reaction. The membrane was then incubated with an alkaline phosphatase-labeled anti-DIG antibody (Boehringer-Mannheim) and developed with the CDP-star reagent (New England Biolabs).

2.6. Western blot analysis

Cells were washed with phosphate-buffered saline and lysed with lysis buffer (150 mM NaCl, 1.0% NP-40, 50 mM Tris–HCl (pH 8.0), 1 mM phenylmethylsulfonyl fluoride, 1 μg/ml leupeptin, 1 mM sodium vanadate and 1 mM EDTA). The protein lysates were mixed with sodium dodecyl sulfate (SDS) sample loading buffer containing β-mercaptoethanol, electrophoresed on an 8 or 10% SDS–polyacrylamide gel (SDS–PAGE) and electrotransferred onto an Immobilon-P membrane (Millipore). Membranes were blocked with Tris-buffered saline with Tween 20 (TBS-Tween; 20 mM Tris–HCl, 150 mM NaCl, 0.1% Tween 20) containing 3% bovine serum albumin and incubated with a primary antibody in TBS-Tween. Membranes were washed with TBS-Tween and incubated with a horseradish peroxidase-conjugated secondary antibody (Amersham-Pharmacia). After another wash with TBS-Tween, immunoreactive proteins were developed by the ECL reagent (Amersham-Pharmacia) and blots were exposed to a Bio-Max film (Kodak).

2.7. Detection of albumin secretion

Fetal hepatic cells (4×10^6) were inoculated in 60-mm dishes and incubated with either 10 ng/ml OSM or 20 ng/ml HGF for 6 days. The culture medium was changed to serum-free medium and incubated for 48 h. Culture supernatants were collected and the amount of secreted albumin was measured by the bromocresol green (BCG) method [15]. The rate of albumin secretion per cell was determined by dividing the total amount of secreted albumin by the number of cells in the culture.

2.8. Retrovirus-mediated gene transfer

Retrovirus carrying the cDNA for ΔSTAT3 [16] was produced using the packaging cell line BOSC23, and was infected to fetal hepatic cells as described previously [17]. Briefly, 3 μg of the retrovirus DNA constructs was transfected into BOSC23 cells (2×10^6 cells grown in a 60-mm tissue culture dish) using the Lipofectamine-plus reagent (Gibco-BRL) according to the manufacturer's instructions. Two days after transfection, the supernatant containing viral particles was collected. Freshly isolated fetal hepatic cells (4×10^5 cells in a 35-mm culture dish) were incubated with 1.5 ml of the supernatant solution in the presence of 1×10^{-6} M Dex and 1× ITS for 12 h. Then, the culture medium was changed to virus-free growth medium.

3. Results

3.1. Expression of HGF and OSM during liver development

We previously demonstrated that OSM induces maturation of fetal hepatic cells in vitro [6]. However, as OSM is expressed in the mid-fetal to perinatal liver and its expression level is significantly decreased after birth, another factor may be involved in hepatic maturation. It was reported that HGF and c-Met are mainly expressed in the first few days after birth in rat livers [12]. We therefore examined whether expression of HGF in mouse livers is also detected during postnatal stages. Total RNA isolated from E14, E18, neonatal, 10-day-old and adult livers was used to detect HGF and OSM mRNA by the semi-quantitative PCR method. Fig. 1 shows that OSM mRNA was expressed during mid- to late-fetal stages and decreased after birth. In contrast, the HGF level was significantly increased at the perinatal stage and reached a maximum several days after birth.

3.2. Induction of hepatic maturation by HGF and OSM

We next examined the effects of HGF on gene expression in primary culture of E14 fetal hepatic cells (Fig. 2A). Cells were cultured for 7 days in the presence or absence of 10 ng/ml OSM or 20 ng/ml HGF and mRNAs for liver differentiation

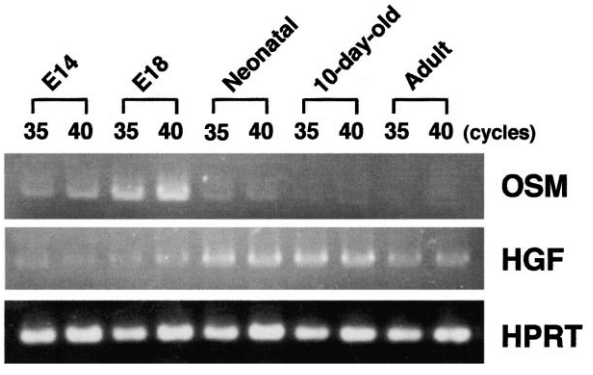

Fig. 1. Developmental changes of OSM and HGF expression. First-strand cDNAs were synthesized from murine liver tissues derived from E14, E18, neonatal, 10-day-old and adult mice. Synthesized cDNA samples were used as templates for RT-PCR analyses of OSM, HGF and hypoxanthine guanine phospho-ribosyltransferase (HPRT as an internal control) mRNAs during liver development. HGF mRNA was significantly increased for several days after birth. In contrast, expression of OSM was down-regulated in the postnatal liver.

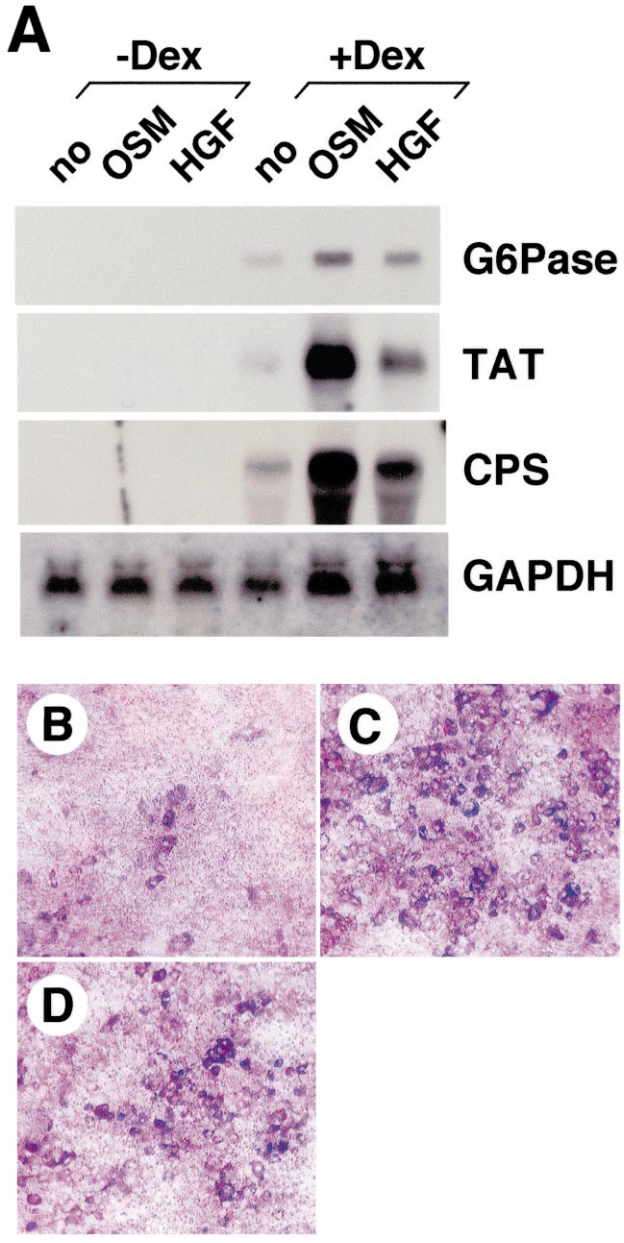

Fig. 2. Induction of hepatic maturation by HGF. (A) Induction of G6Pase, TAT and CPS requires OSM or HGF in combination with Dex. Fetal hepatic cells were cultured for 7 days, and expression of hepatic genes and GAPDH (internal control) was analyzed using DIG-labeled cDNA probes. (B–D) Glyconeogenesis in cultured hepatocytes induced by OSM and HGF. Fetal hepatic cells derived from E14 embryonic livers were cultured for 6 days in the absence (B) or presence of OSM (C) or HGF (D) in combination with 10^{-7} M Dex. Fetal hepatocytes cultured with Dex alone accumulated a limited amount of glycogen. In contrast, a number of PAS-positive cells appeared when cultured with either OSM or HGF in the presence of Dex for 6 days.

marker genes were detected by Northern blotting. Expression of G6Pase, TAT and carbamoyl-phosphate synthase (CPS) was significantly induced by HGF in combination with 1×10^{-7} M Dex, suggesting that HGF is capable of inducing liver maturation in vitro. Dex was strictly required for the induction of hepatic differentiation markers by HGF since no mRNAs of these genes were detected in the absence of Dex.

In our previous work, we showed that OSM is able to induce glycogen accumulation, one feature of adult liver [18], in fetal hepatic cells. We investigated glyconeogenesis in culture stimulated by HGF. Fetal hepatic cells were cultured for 6 days either with OSM or with HGF and the intracellular glycogen accumulation was analyzed by staining the cells with the PAS reagent. A slight accumulation of glycogen was detected in the presence of Dex alone (Fig. 2B) and this was significantly increased by OSM and HGF (Fig. 2C,D). Since no accumulation of glycogen was induced by HGF alone in the absence of Dex, induction of glycogenesis strictly requires glucocorticoid (data not shown).

3.3. Production and secretion of albumin by HGF and OSM

Serum albumin is the most abundant protein synthesized by

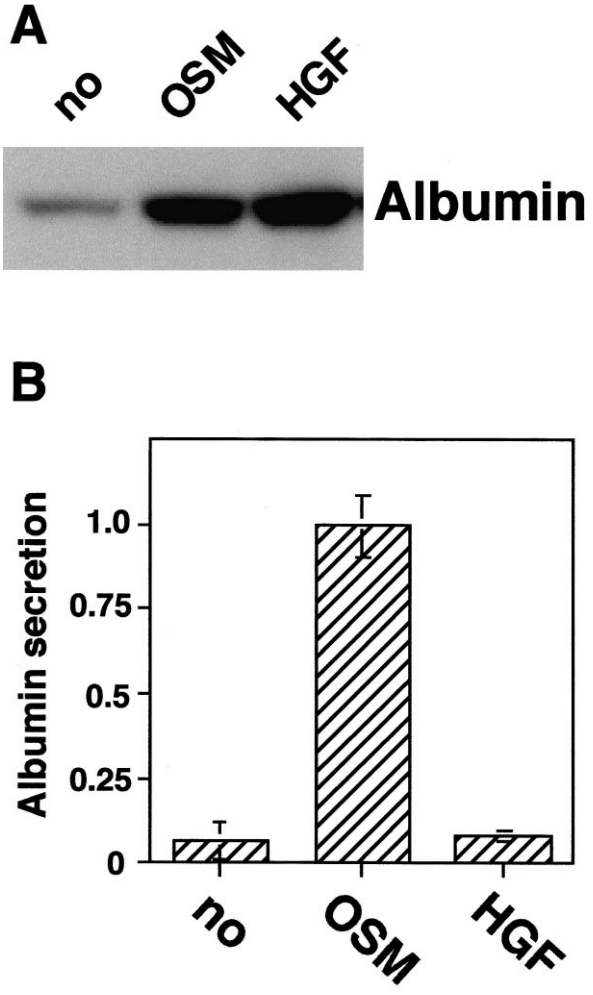

Fig. 3. Differential regulation of albumin production and secretion by OSM and HGF. (A) E14 hepatic cells were cultured for 7 days in the presence of OSM and HGF in combination with Dex. Cells were lysed in NP-40 lysis buffer and albumin was detected by Western blotting. OSM and HGF significantly induced albumin production. (B) Hepatocytes were cultured for 6 days in the presence of OSM and HGF, and culture media were changed to serum-free media. After 48 h, secreted albumin in media was detected by the BCG method. Albumin secretion was induced by OSM but not HGF. Columns, amount of secreted albumin in media relative to control. The amount of albumin secreted in the presence of OSM sets to 1.0. Values and vertical bars are each indicated as the mean and S.D. of triplicate experiments, respectively.

hepatocytes and its production starts in the early stage of liver development and reaches the maximum level in the adult liver [19]. We measured the intracellular content of albumin to examine the effect of HGF on albumin production in fetal hepatic culture. After 7 days of incubation with or without HGF, equal amounts of total cellular proteins were used for Western blotting. The albumin levels were increased when fetal hepatic cells were cultured with either OSM or HGF (Fig. 3A). These results were consistent with a previous report that HGF in combination with Dex induced albumin production in rat fetal and adult hepatocytes [20]. Interestingly, however, secretion of albumin into the culture medium was induced only by OSM and not by HGF (Fig. 3B). These results indicate that OSM and HGF activate distinct sets of signaling molecules and that secretion of albumin depends on the pathway specifically stimulated by OSM.

3.4. STAT3 is activated by OSM but not by HGF in fetal hepatic cells

Previously, we described that gp130 is essential for OSM-induced hepatic differentiation [6]. STAT3 is a latent transcription factor that is activated by IL-6 family cytokines through gp130 [21,22]. Recently, we found that STAT3 plays a major role in the OSM-induced differentiation of fetal hepatic cells [23]. We investigated whether or not STAT3 is involved in the induction by HGF of the hepatic differentiation markers in our culture system. Fetal hepatocytes were cultured for 5 days in the presence or absence of either 10 ng/ml OSM or 20 ng/ml HGF in combination with Dex (Fig. 4A). Phosphorylation of STAT3 was detected in the cells cultured with OSM but not HGF. Furthermore, phosphorylation of STAT3 was rapidly induced by OSM in 5 or 15 min, whereas HGF failed to induce it. While it was previously

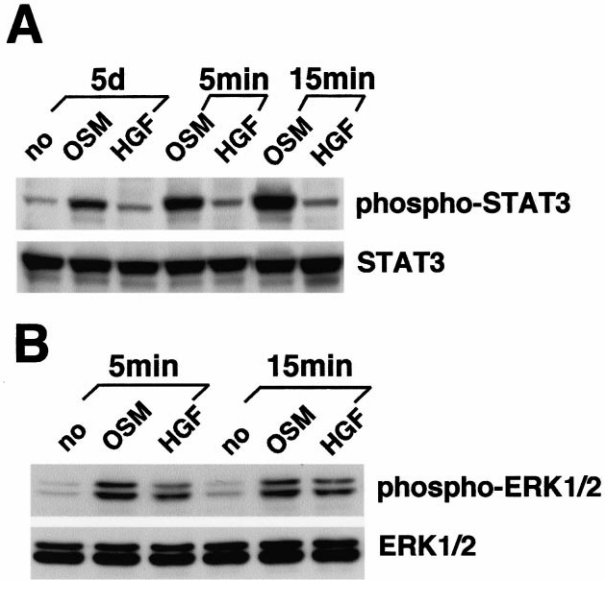

Fig. 4. Activation of STAT3 and ERK1/2 induced by OSM and HGF. (A) Fetal hepatocytes were cultured for 5 days in the absence or presence of OSM or HGF in combination with Dex. Phosphorylation of STAT3 was detected only in the culture with OSM. Furthermore, fetal hepatocytes were cultured for 5 days with Dex alone and were then stimulated by either OSM or HGF for 5 or 15 min. (B) Consistent with previous results, OSM and HGF induced phosphorylation of ERK1/2 equally, while phosphorylation of STAT3 was only detectable in the OSM-stimulated cells.

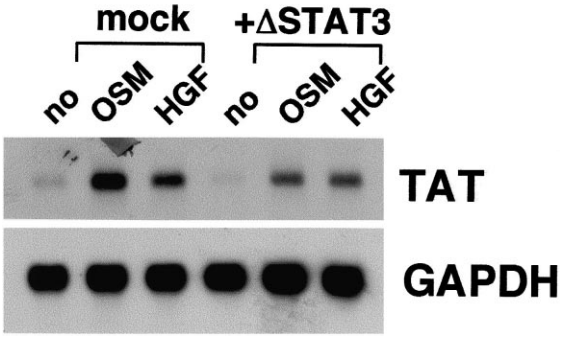

Fig. 5. Hepatic maturation induced by HGF is independent of STAT3. E14 fetal hepatic cells were infected with the retrovirus vectors, pMX (mock) and pMX-ΔSTAT3 (dominant negative STAT3). The cells were cultured for 7 days in the presence of OSM or HGF, and TAT mRNA levels were determined by Northern blotting. Expression of TAT induced by OSM was significantly reduced by the infection with pMX-ΔSTAT3 while HGF-induced TAT mRNA expression was unaffected.

reported that the STAT3 activation by HGF was observed after a long latency [24], no phosphorylation of STAT3 was detected even after 7 h of HGF stimulation in our culture (data not shown). The inability of HGF to phosphorylate STAT3 was not due to the lack of receptor activation, since ERK, another signaling molecule activated by these factors, was significantly phosphorylated under the same conditions (Fig. 4B).

3.5. HGF induced hepatic maturation through STAT3-independent signaling pathways

To further examine the role of STAT3 in HGF-induced differentiation, ΔSTAT3, a dominant negative form of STAT3, was expressed in primary fetal hepatic culture using the retroviral gene delivery system [23]. As a control, we also infected the cells with the vector pMX-IRES-GFP [25]. Fetal hepatic cells were cultured in the presence of OSM or HGF for 6 days and expression of TAT was analyzed by Northern blotting (Fig. 5). Expression of ΔSTAT3 resulted in inhibition of OSM-induced expression of TAT. In contrast, it failed to inhibit HGF-induced expression of TAT. These results indicate that activation of STAT3 is responsible for the hepatic differentiation regulated by OSM, while HGF-induced maturation is mediated by STAT3-independent pathways.

4. Discussion

In this report, we showed that HGF, in addition to OSM, has the ability to induce hepatic maturation as evidenced by expression of hepatic differentiation marker genes and glycogen accumulation. Interestingly, HGF was not able to induce the phosphorylation of STAT3 that is required for OSM-induced hepatic maturation and ΔSTAT3 blocked hepatic differentiation induced by OSM but not HGF, indicating that OSM and HGF induce hepatic maturation via distinct signal molecules. As OSM induces higher levels of expression of liver differentiation marker genes and of glycogen accumulation than HGF, it may play a more active role in the maturation of hepatic cells at these stages.

Liver development is known to proceed through several distinct steps and it seems that different key soluble factors are involved in each process. In mice, the initial event in liver

development occurs at E9. The foregut endoderm commits to become the liver through interaction with the cardiogenic mesoderm [26,27]. Recent study shows that fibroblast growth factors 1 and 2 produced by mesodermal cells are involved in hepatic differentiation in this step [28]. In contrast, we previously reported that OSM is a paracrine factor produced by hematopoietic cells and plays an important role in hepatic maturation during the mid- to late-fetal stages [6]. These results suggest that liver development is controlled by several distinct paracrine factors. Expression of OSM gradually decreases during late-fetal and neonatal stages (Fig. 1). In contrast, expression of TAT is increased after birth, therefore, another factor might be involved in postnatal liver development. HGF is significantly expressed after birth in the liver and induces maturation of fetal hepatic cells (Figs. 1 and 2). During late-fetal to neonatal development, hematopoietic cells relocate to the spleen and bone marrow, and non-parenchymal cells (e.g. sinusoidal endothelial cells and stellate cells) increase in the liver. As HGF is produced by non-parenchymal cells during liver regeneration [29,30], the increase of HGF expression in the postnatal liver is correlated with the proliferation of non-parenchymal cells. Thus, HGF seems to be a paracrine factor that is involved in the postnatal hepatic maturation.

Although both OSM and HGF induced hepatic differentiation in the presence of Dex, their signaling mechanisms are quite different. A notable difference between OSM and HGF was their capability to activate STAT3. Hepatic maturation induced by OSM is mediated through STAT3 since expression of hepatic differentiation markers was efficiently inhibited by expression of ΔSTAT3 in fetal hepatic culture [23]. In sharp contrast, HGF did not activate STAT3 in fetal hepatic cells and expression of ΔSTAT3 failed to inhibit expression of the liver differentiation marker gene induced by HGF (Figs. 4A and 5). It is currently unknown what signaling molecules are responsible for HGF-mediated hepatic differentiation. However, gp130 knockout mice exhibit severe defects in hepatic maturation but the expression of TAT and accumulation of glycogen are not totally abrogated in these mice, suggesting that OSM- and STAT3-independent signaling pathways are involved in the in vivo liver development. It is, therefore, possible that the signal molecules activated by HGF partly substitute for the functions of OSM in liver development of gp130 knockout mice. In addition, we recently found that fetal hepatic cells differentiate in the absence of OSM in a high cell density culture and differentiation under these conditions is STAT3-independent [15]. Taken together, there appears to be an alternative signaling pathway for hepatic maturation and it would be interesting to identify the signaling molecules involved in hepatic maturation induced by HGF and/or high cell density culture.

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References

[1] Oliver, I.T., Martin, R.L., Fisher, C.J. and Yeoh, G.C. (1983) Differentiation 24, 234–238.
[2] Perry, S.T., Rothrock, R., Isham, K.R., Lee, K.L. and Kenney, F.T. (1983) J. Cell Biochem. 21, 47–61.
[3] Shelly, L.L., Tynan, W., Schmid, W., Schutz, G. and Yeoh, G.C. (1989) J. Cell Biol. 109, 3403–3410.
[4] Orkin, S.H. (1996) Curr. Opin. Genet. Dev. 6, 597–602.
[5] Taga, T. and Kishimoto, T. (1997) Annu. Rev. Immunol. 15, 797–819.
[6] Kamiya, A., Kinoshita, T., Ito, Y., Matsui, T., Morikawa, Y., Senba, E., Nakashima, K., Taga, T., Yoshida, K., Kishimoto, T. and Miyajima, A. (1999) EMBO J. 18, 2127–2136.
[7] Kinoshita, T., Sekiguchi, T., Xu, M.J., Ito, Y., Kamiya, A., Tsuji, K., Nakahata, T. and Miyajima, A. (1999) Proc. Natl. Acad. Sci. USA 96, 7265–7270.
[8] Yoshida, K., Taga, T., Saito, M., Suematsu, S., Kumanogoh, A., Tanaka, T., Fujiwara, H., Hirata, M., Yamagami, T., Nakahata, T., Hirabayashi, T., Yoneda, Y., Tanaka, K., Wang, W.Z., Mori, C., Shiota, K., Yoshida, N. and Kishimoto, T. (1996) Proc. Natl. Acad. Sci. USA 93, 407–411.
[9] Matsumoto, K. and Nakamura, T. (1997) Biochem. Biophys. Res. Commun. 239, 639–644.
[10] Hamanoue, M., Kawaida, K., Takao, S., Shimazu, H., Noji, S., Matsumoto, K. and Nakamura, T. (1992) Hepatology 16, 1485–1492.
[11] Kinoshita, T., Tashiro, K. and Nakamura, T. (1989) Biochem. Biophys. Res. Commun. 165, 1229–1234.
[12] Kagoshima, M., Kinoshita, T., Matsumoto, K. and Nakamura, T. (1992) Eur. J. Biochem. 210, 375–380.
[13] Chomczynski, P. and Sacchi, N. (1987) Anal. Biochem. 162, 156–159.
[14] Mukouyama, Y., Hara, T., Xu, M., Tamura, K., Donovan, P.J., Kim, H., Kogo, H., Tsuji, K., Nakahata, T. and Miyajima, A. (1998) Immunity 8, 105–114.
[15] Kojima, N., Kinoshita, T., Kamiya, A., Nakamura, K., Nakashima, K., Taga, T. and Miyajima, A. (2000) Biochem. Biophys. Res. Commun. 277, 152–158.
[16] Chida, D., Miura, O., Yoshimura, A. and Miyajima, A. (1999) Blood 93, 1567–1578.
[17] Kitamura, T., Onishi, M., Kinoshita, S., Shibuya, A., Miyajima, A. and Nolan, G.P. (1995) Proc. Natl. Acad. Sci. USA 92, 9146–9150.
[18] Nemeth, A.M., Insull Jr., W. and Flexner, L.B. (1953) J. Biol. Chem. 52, 63–77.
[19] Tilghman, S.M. and Belayew, A. (1982) Proc. Natl. Acad. Sci. USA 79, 5254–5257.
[20] Sanchez, A., Alvarez, A.M., Benito, M. and Fabregat, I. (1995) J. Cell. Physiol. 165, 398–405.
[21] Akira, S., Nishio, Y., Inoue, M., Wang, X.J., Wei, S., Matsusaka, T., Yoshida, K., Sudo, T., Naruto, M. and Kishimoto, T. (1994) Cell 77, 63–71.
[22] Lutticken, C., Wegenka, U.M., Yuan, J., Buschmann, J., Schindler, C., Ziemiecki, A., Harpur, A.G., Wilks, A.F., Yasukawa, K. and Taga, T. et al. (1994) Science 263, 89–92.
[23] Ito, Y., Matsui, T., Kamiya, A., Kinoshita, T. and Miyajima, A. (2000) Hepatology 32, 1370–1376.
[24] Schaper, F., Siewert, E., Gomez-Lechon, M.J., Gatsios, P., Sachs, M., Birchmeier, W., Heinrich, P.C. and Castell, J. (1997) FEBS Lett. 405, 99–103.
[25] Onishi, M., Kinoshita, S., Morikawa, Y., Shibuya, A., Phillips, J., Lanier, L.L., Gorman, D.M., Nolan, G.P., Miyajima, A. and Kitamura, T. (1996) Exp. Hematol. 24, 324–329.
[26] Douarin, N.M. (1975) Med. Biol. 53, 427–455.
[27] Houssaint, E. (1980) Cell Differ. 9, 269–279.
[28] Jung, J., Zheng, M., Goldfarb, M. and Zaret, K.S. (1999) Science 284, 1998–2003.
[29] Noji, S., Tashiro, K., Koyama, E., Nohno, T., Ohyama, K., Taniguchi, S. and Nakamura, T. (1990) Biochem. Biophys. Res. Commun. 173, 42–47.
[30] Ramadori, G., Neubauer, K., Odenthal, M., Nakamura, T., Knittel, T., Schwogler, S. and Meyer zum Buschenfelde, K.H. (1992) Biochem. Biophys. Res. Commun. 183, 739–742.