Vol. 177, No. 1, 1991 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 31, 1991 Pages 330-335

Possible Endocrine Control by Hepatocyte Growth Factor of Liver Regeneration after Partial Hepatectomy

Taisei Kinoshita, Shigeaki Hirao, Kunio Matsumoto,

and Toshikazu Nakamura

Department of Biology, Faculty of Science, Kyushu University,

Fukuoka 812, Japan

Received April 20, 1991

SUMMARY: Hepatocyte Growth Factor (HGF), which is a most potent growth factor for primary cultured hepatocytes, may act as a trigger for liver regeneration. After 70% of the rat liver was removed, HGF activity in the remnant liver began to increase within 24 h. In parallel with the activity, the HGF mRNA level in the remnant liver increased at 12 h after the operation and reached a maximum at 24 h. Increases in HGF activity and in the mRNA level were much lower and later than those in the liver of rats with hepatitis induced with CCl_4. However, the first increase in HGF activity in the plasma of hepatectomized rats was noted 3 h after the resection, that is much earlier than the initial DNA synthesis in the remnant liver. Thus, while HGF production was induced in the remnant liver during regeneration after partial hepatectomy, the initial trigger may not be the liver-derived HGF, rather, it may be HGF derived from extrahepatic organs, via blood circulation.

Hepatocyte Growth Factor (HGF) was originally detected in the sera of partially hepatectomized rats (1). We purified HGF to apparent homogeneity from rat platelets (2,3). HGF is a heterodimer composed of an α-subunit with 69kD and a β-subunit with 34kD (3). Thereafter, HGF was purified from human plasma (4, 5). The cloning and sequencing of cDNAs from humans (6, 7) and rats (8) revealed that HGF is synthesized in a single polypeptide chain of 728 amino acid residues and is then proteolitically processed to form a mature heterodimeric HGF. We also cloned and characterized the human HGF gene (9).

HGF may be a hepatotropic factor acting as a trigger for liver regeneration. An elevated level of HGF was found in the plasma of a patient with fulminant hepatitis (4), and that of a patient after partial hepatectomy (10), and in the ascites and the plasma of a patient with cirrhosis (11). We showed that HGF and HGF mRNA were remarkably increased in the liver of

0006-291X/91 $1.50

rats when hepatitis was experimentally induced by the administration of hepatotoxins, such as carbon-tetrachloride (CCl_4) and D-galactosamine (12, 13). HGF mRNA was detected and increased only in non-parenchymal cells, but not in parenchymal hepatocytes following CCl_4-administration (13). Using *in situ* hybridization, we noted that HGF producing tissues in liver are Kupffer cells and sinusoidal endothelial cells (14).

To determine whether liver regeneration after partial hepatectomy is driven by HGF in a paracrine manner, as is hepatitis, we examined changes in HGF mRNA in the liver and HGF activity in the remnant liver and the plasma. Evidence suggests that liver regeneration after partial hepatectomy seems to mainly depend on HGF supplied in an endocrine manner rather than a paracrine manner.

MATERIALS AND METHODS

***Animal*:** Adult male Wistar rats (130-150 g) were used in the following experiments.

Partial purification of HGF from the liver: The liver was homogenized in 4 volumes of the buffer composed of 0.05 M Tris-HCl, 0.15 M NaCl and 0.01 M HEPES (pH 8.5) containing protease inhibitors (1 mM PMSF, 1 mM monoiodoacetate and 1 mM EDTA). After centrifugation at 10,5000 x *g* for 60 min, the supernatant was applied to a S-Sepharose column (1 x 10 cm) equilibrated with the above buffer, and washed in 2 volumes of the same buffer. The column was eluted with 1 M NaCl in the buffer and the eluate was passed through a 0.22 μm pore sized filter (GMV, Millipore) before the assay of HGF activity.

Preparation of Rat Plasma: Rat peripheral blood samples were obtained from the abdominal aorta and EDTA was used as the anti-coagulant. Blood samples were centrifuged at 1,400 x *g* for 10 min, and the plasma was clotted by an excess volume of calcium chloride, dialyzed against 100 times volumes of culture medium and sterilized by filtration.

Assay for HGF activity: HGF activity was determined by measuring the stimulatory effect on DNA synthesis of rat parenchymal hepatocyte in primary culture. Adult rat hepatocytes were isolated by the *in situ* collagenase perfusion method (15). The isolated cells were plated at 2.5 x 10^5 cells/cm^2 on 24-well plastic dish (Corning) coated with Type I collagen and cultured in Williams' E medium containing, 10^{-9} M of insulin and 10^{-9} M dexamethasone (16). After 24 h, the samples were added to the culture and further cultured for 22 h. [^{125}I]-deoxyuridine was then for a 6 h pulse-labeling. Incorporation of [^{125}I]-deoxyuridine into the nuclei was determined as described elsewhere (17). One unit represents a half maximal value of the stimulatory effect by EGF (10 ng/ml) on DNA synthesis in hepatocytes.

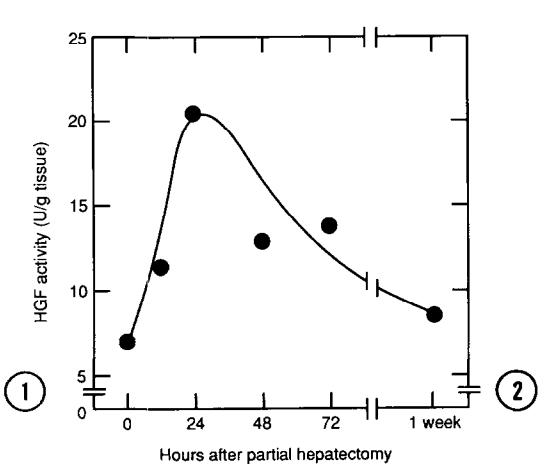
***Northern-blot analysis*:** Total RNA was isolated from rat livers by the AGPC method (18). RNA (10 μg of total RNA) was separated by 1.0% agarose/ formaldehyde gel electrophoresis and transferred to a Hibond-N nylon membrane filter. EcoRI fragment (1.4 kb) of rat HGF cDNA (RBC-1 clone) was labeled using the multiprime DNA labeling system (Amersham) according to the manufacturers' instruction. Hybridization were performed at 42°C for 24 h in solution composed of 50% (w/v) formamide, 5 x SSC, 10 mM sodium phosphate (pH 6.8), 0.5% (w/v) SDS, 5 x Denhardt's, 0.05% (w/v) BSA and 200 μg/ml salmon sperm DNA. The filter was washed twice

with 0.2 x SSC-0.1% SDS for 30 min at 65°C, then was dried and autoradiographed on Fuji X-ray film.

RESULTS

First we examined the change in HGF activity in the remnant liver of rats after 70% partial hepatectomy. Fig. 1 shows that the HGF activity increased from 12 h after the operation, reached a maximal level at around 24 h. This maximal activity was three times higher than that in the normal liver, then, it decreased to the initial level after 7 days, a time when liver regeneration was almost complete. The maximum HGF activity after partial hepatectomy was only about one-tenth that after CCl_4-treatment.

To determine whether the increase of HGF activity in the remnant liver was due to an increased net synthesis of HGF, the HGF mRNA level was examined by Northern hybridization. As shown in Fig. 2, the expression of HGF mRNA after partial hepatectomy showed a time-dependent increase similar to the HGF activity. Increase in the HGF mRNA was seen at 12 h and a maximal value was noted at 24 h. In sham operated rats, there was no detectable induction of HGF mRNA. Although HGF mRNA increased in the remnant liver after partial hepatectomy, the maximal level was much lower than that seen with CCl_4-treatment.



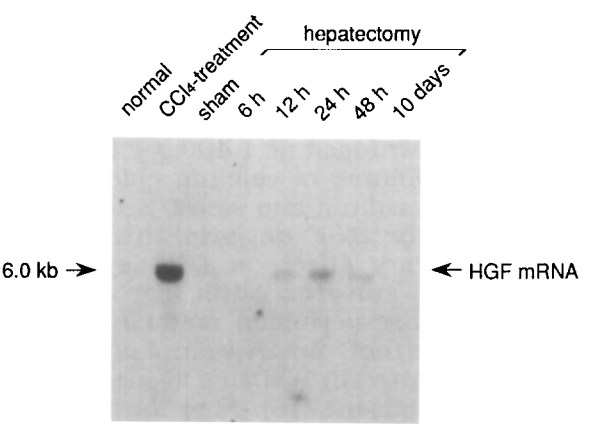


Fig. 1. Increase in HGF activity in the remnant rat liver. HGF activity was assayed by measuring the effect of partially purified HGF on DNA synthesis in rat parenchymal hepatocytes in primary culture, as described in materials and methods. Each point represents the mean value of triplicate measurements. In the case of CCl_4-treatment, HGF concentration in the liver was increased to 220 U/g wet weight 36 h later (Data not shown).

Fig. 2. Northern blot analysis of HGF mRNA in rat liver after partial hepatectomy and CCl_4-treatment. Ten μg of total RNAs were separated by 1.0% agarose/formaldehyde gel electrophoresis and transferred to Hibond-N nylon membrane filter. The membrane was then hybridized with a ^{32}P-labelled rat HGF cDNA probe.

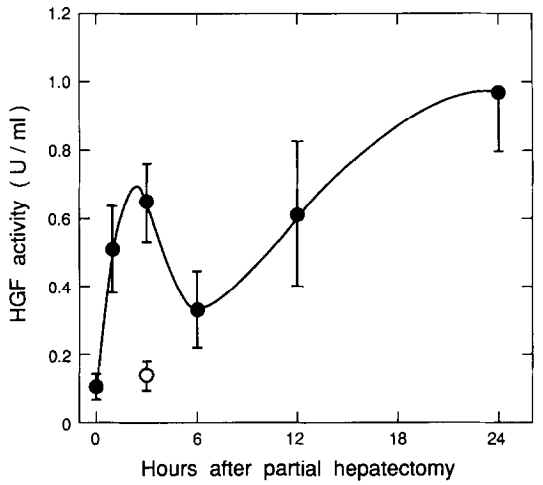


Fig. 3. HGF activity in the plasma of partially hepatectomized rats at the early stage of liver regeneration. ● ; Partial hepatectomy. O ; sham operation. HGF activity was assayed by the method described in "Materials and Methods". Values are expressed as mean ± S. E. of three rats.

Since the initial prominent DNA synthesis in the remnant liver occurs about 24 h after hepatectomy (19), HGF synthesized in the remnant liver seems to be insufficient to compensate for regenerative responses of the liver after partial hepatectomy. Therefore, we measured HGF activity in the plasma of hepatectomized rats to assess the extrahepatic supply of HGF, at the early stage of liver regeneration. More than a 5-fold increase in HGF activity was noted in the blood plasma as early as 3 h after hepatectomy (Fig. 3). HGF activity in the plasma decreased transiently at 6 h and increased again at 12 h, reaching the second maximum (9-fold of normal level) at 24 h after the operation. Thus, the change in HGF activity in the plasma after partial hepatectomy showed two distinct peaks.

DISCUSSION

We reported that HGF mRNA was detected only in non-parenchymal cells of the liver and that it markedly increased within 5 h after administration of CCl_4 to rats (13). The marked increase occurred mainly in Kupffer cells as demonstrated by *in situ* hybridization (14). More recently, we confirmed this finding by Northern blot analysis after isolating Kupffer cells, using centrifugal elutriation (our unpublished observation). From these findings, we suggested that the growth of hepatocytes during liver regeneration may be controlled by HGF synthesized by Kupffer cells in a paracrine manner, after hepatitis induced by CCl_4-treatment.

As described in the present report, HGF mRNA was also significantly increased in the remnant liver of partially hepatectomized rats, whereas the increase was much less and later than that after CCl_4-treatment. Jansson et

al. of the University of Göteborg also found that the HGF mRNA level in the remnant liver was increased following partial hepatectomy and that growth hormone treatment promotes the increase in HGF mRNA at an earlier time; 10 h after the operation (personal communication). However, the initial mitogenic signal should act within 10 h because the initial peak of DNA synthesis in the remnant liver was seen to appear about 24 h after hepatectomy (19). Therefore, HGF newly synthesized in the remnant liver is unlikely to trigger an initial prominent DNA synthesis in hepatocytes even when rats are given a growth hormone. HGF synthesized in the liver may function to sustain the following hepatocyte proliferation and functional maturation.

On the other hand, HGF activity in the plasma increased at an early stage of regeneration, hence, the initiation of the liver regeneration after hepatectomy probably does not depend on HGF newly produced in the liver, but rather HGF derived from extrahepatic organs via the blood circulation. We recently found supportive evidence for the importance of blood-borne HGF for the initiation of the liver regeneration following partial hepatectomy. The number of HGF receptors on plasma membranes of the remnant liver decreased to a negligible level at 6 h after the operation (20). The rapid decrease in HGF binding sites may be the result of the occupation and/or internalization of receptors by HGF supplied from extrahepatic organs or cells within 6 h. As we reported elsewhere, significant amounts of HGF mRNA were detected in the kidney, lung and spleen (8), and rat platelets contain relatively large amounts of HGF (3). Some of these organs or cells are candidates for storage sites of HGF. Thus, the growth of hepatocytes during liver regeneration after partial hepatectomy might be mainly supported by HGF from extrahepatic sources rather than from Kupffer cells.

Acidic fibroblast growth factor (aFGF) and transforming growth factor-α (TGF-α) are also known to be mitogens for hepatocytes and transient increases of mRNAs for aFGF and TGF-α were demonstrated (21, 22). However, expression of the mRNAs in the liver was not evident until after 24 h posthepatectomy and their activities to stimulate the growth of hepatocytes were much less than those of HGF. These growth factors seem to be insufficient for the initiation of liver regeneration.

We postulate that there are two mechanisms involved in liver regeneration after insult: One is a paracrine mechanism for liver repair after hepatitis. In such cases, hepatocyte growth is mainly sustained by HGF synthesized and secreted from neighbouring non-parenchymal liver cells; the other is an endocrine mechanism for liver regeneration after partial hepatectomy in which the initial hepatocyte growth depends on HGF from other organs or cells, via the blood circulation.

Acknowledgments. We thank Dr. M. Ohara for helpful comments. This work was supported by a Research Grant for Studies on Science and Cancer from the Ministry of Education, Science and Culture of Japan.

REFERENCES

1. Nakamura, T., Nawa, K. and Ichihara, A. (1984) Biochem. Biophys. Res. Commun. **122,** 1450-1459
2. Nakamura, T., Teramoto, H. and Ichihara, A. (1986) Proc. Natl. Acad. Sci. USA **83,** 6489-6493
3. Nakamura, T., Nawa, K., Ichihara, A., Kaise, A. and Nishino T. (1987) *FEBS* Lett, **224,** 311-316
4. Gohda, E., Tsubouchi, H., Nakayama, H., Hirono, S., Sakiyama, O., Takahashi, K., Miyazaki, H., Hashimoto, S. and Daikuhara, Y. (1988) J. Clin. Invest. **81,** 414-419
5. Zarneger, R. and Michalopoulos, G. (1989) Cancer Res. **49,** 3314-3320
6. Nakamura, T., Nishizawa, T., Hagiya, M., Seki, T., Shimonishi, M., Sugimura, A., Tashiro, K. and Shimizu, S. (1989) Nature **342,** 440-443
7. Miyazawa, K., Tsubouchi, H., Naka, D., Takahashi, K., Okigaki, M., Arakaki, N., Nakayama, H., Hirono, S., Sakiyama, O., Gohda, E., Daikuhara, Y. and Kitamura, N. (1989) Biochem. Biophys. Res. Comun. **163,** 967-973
8. Tashiro, K., Hagiya, M., Nishizawa, T., Seki, T., Shimonishi, M., Shimizu, S. and Nakamura, T. (1990) Proc. Natl. Acad. Sci. USA **87,** 3200-3204
9. Seki, T., Hagiya, M., Shimonishi, M., Nakamura, T. and Shimizu, S. (1991) Gene, in press.
10. Selden, C., Johnstone, R., Darby, H., Gupta, C. and Hodgeson, H. J. F. (1986) Biochem. Biophys. Res. Commun. **139,** 361-366
11. Shimizu, I., Ichihara, A. and Nakamura, T. (1991) J. Biochem. **109,** 14-18
12. Asami, O., Ihara, I., Shimidzu, N., Shimizu, S., Tomita, Y., Ichihara, A. and Nakamura, T. (1991) J. Biochem. **109,** 8-13
13. Kinoshita, T., Tashiro, K. and Nakamura, T. (1989) Biochem. Biophys. Res. Commun. **165,** 1229-1234
14. Noji, S., Tashiro, K., Koyama, E., Nohno, T., Ohyama, K., Taniguchi, S. and Nakamura, T. (1990) Biochem. Biophys. Res. Commun. **173,** 42-47
15. Seglen, P. O. (1976) Methods Cell Biol. **13,** 29-83
16. Nakamura, T., Arakaki, R. and Ichihara, A. (1988) Exp. Cell Res. **179,** 488-497
17. Raff, M. C., Abney, E., Brockes J. P. and Hornby-Smith, A. (1978) Cell **15,** 813-822
18. Chomczynski, P. and Sacchi, N. (1987) Anal. Biochem. **162,** 156-159
19. Lewan, L., Yngner, T. and Engelbrecht, C. (1977) J. Biochem. **8,** 477-487
20. Higuchi, O. and Nakamura, T. (1991) Biochem. Biophys. Res. Commun., in press.
21. Kan, M., Huang, J., Mansson, D-E., Yasumitsu, H., Carr, B. and McKeehan, W. L. (1989) Proc. Natl. Acad. Sci. USA **86,** 7432-7436
22. Mead, J. E. and Fausto, N. (1989) Proc. Natl. Acad. Sci. USA **86,** 1558-1562