Transforming Growth Factor α Levels in Liver and Blood Correlate Better than Hepatocyte Growth Factor with Hepatocyte Proliferation during Liver Regeneration

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Transforming growth factor α (TGF α) and hepatocyte growth factor (HGF) are mitogens for hepatocytes *in vitro* and *in vivo*, produced by hepatocytes or non-parenchymal cells such as stellate cells in the liver. It is still uncertain whether TGF α and HGF are essential for liver regeneration. To assess the role of these growth factors in liver regeneration, their circulating and hepatic levels were studied in various rat models of liver regeneration. Hepatic and plasma HGF levels were increased with increased number of mitotic hepatocytes in rats after partial hepatectomy or carbon tetrachloride intoxication. However, hepatic HGF levels were decreased despite an increased number of mitotic hepatocytes and increased or unchanged plasma HGF levels in rats given phenobarbital and in rats after dimethylnitrosamine intoxication, which can induce hepatic necrosis after apoptosis of hepatic stellate cells. In contrast, hepatic and serum TGF α levels were increased in all of the models. In sham-operated rats with no increased number of mitotic hepatocytes, hepatic and circulating levels of HGF were increased, whereas those levels of TGF α were unchanged. The results indicate that TGF α levels in liver and blood more closely correlate with hepatocyte mitogenesis than HGF levels. (*Am J Pathol* 1998, 153:955–961)

The liver has an ability to regenerate after loss of its volume, but its regulation mechanisms have not been known well.

Many mitogens for hepatocytes have been found since hepatocytes in primary culture were utilized to detect and characterize contributing factors.^{1–3} Transforming growth factor α (TGF α) and hepatocyte growth factor (HGF) can stimulate hepatocyte proliferation *in vivo* as well as *in vitro*.^{4–9} TGF α exerts its action by binding to the TGF α /

epidermal growth factor (EGF) receptor on the cell surface. In the liver, hepatocytes express both TGF α mRNA and TGF α /EGF receptor mRNA,^{10–16} suggesting that TGF α can stimulate hepatocyte proliferation in an autocrine manner.¹ HGF is produced by nonparenchymal cells, principally by hepatic stellate cells, in the liver, and the *c-met* proto-oncogene product identified as its receptor is present in hepatocytes.^{17–24} Thus, HGF is postulated to stimulate hepatocyte proliferation in a paracrine manner in the liver.³

TGF α and HGF have been reported to increase in the liver in several experimental models of liver regeneration,^{13,25–27} and also in the circulation of patients after partial hepatectomy and patients with acute hepatitis.^{28–31} These findings suggest that TGF α and HGF can contribute to liver regeneration.³ Recently, it was reported that there was no significant difference in hepatocyte proliferation after partial hepatectomy between TGF α knockout mice and wild-type mice.³² Also, HGF has been shown to increase resulting from its active production in inflammatory cells as well as hepatic stellate cells in rats and human.^{18,33} Systemic and hepatic inflammation can occur even in the process of liver regeneration after resection.^{30,34} In fact, serum HGF levels in patients after partial hepatectomy are more closely related to the degree of systemic and hepatic inflammation than to the degree of liver regeneration.³⁰ Liver regeneration occurs generally after partial hepatectomy, acute hepatic injury, or small-for-size liver transplantation.^{1–3,35} The levels of essential factors for liver regeneration might increase in the liver or circulation, similarly reflecting the extent of liver regeneration after these events.

The aim of this study is to determine whether TGF α and HGF levels in liver and blood correlate with hepatocyte proliferation. In different rat models of liver regeneration, we serially measured circulating levels as well as hepatic levels of both factors.

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Materials and Methods

Sources

Sep-Pak C₁₈ minicolumns were obtained from Waters Chromatography Division (Millipore Corp., Milford, MA). Twenty percent neutral buffered formalin solution was purchased from Muto Pure Chemicals Ltd. (Tokyo, Japan) and phenylmethyl-sulfonyl fluoride from Sigma Chemical Co. (St. Louis, MO). HGF enzyme-linked immunosorbent assay (ELISA) kit was a product of the Institute of Immunology (Tokyo, Japan).³⁶ Other reagents were purchased from Wako Pure Chemical Industries (Osaka, Japan).

Animals

Male Sprague-Dawley rats ages 5 to 6 weeks were obtained from Japan SLC (Shizuoka, Japan). They were housed in cages at 22 ± 2°C under a 12-hour light-dark cycle and fed a commercial diet and water *ad libitum*. All animal study protocols conformed to the guidelines of the Faculty of Medicine, University of Tokyo for humane care.

Animal Experiments

The rats were subjected to either of the following: an operation of two-thirds resection of the liver under diethyl ether anesthesia, a single subcutaneous injection of 3.0 ml/kg body weight of carbon tetrachloride (CCl₄) as a 20% solution in olive oil, a single intraperitoneal injection of 35 mg/kg body weight of dimethylnitrosamine (DMN) as a 3.5% solution in saline, or oral administration of phenobarbital sodium *ad libitum* at a concentration of 0.10% in the drinking water. In sham-operated rats, the abdomen was cut open under similar anesthesia, and the liver was briefly exposed outside the peritoneal cavity. Rats given water were used as controls for phenobarbital-treated rats.

The rats were serially anesthetized with diethyl ether. Blood was collected through the inferior vena cava with an empty plastic syringe and a plastic syringe containing 3.1% sodium citrate solution (1:10 v/v) to prepare serum and plasma, respectively. The liver was perfused with 25 ml of saline at 37°C through the portal vein after near-total exsanguination, and excised. A portion of the liver was immersed in 20% neutral buffered formalin solution for histological examination. The remaining liver was frozen in liquid nitrogen and stored at -80°C until use for TGF α and HGF assays.

Assay for TGF α in the Liver and Serum

Liver extracts were prepared by homogenizing 400 mg liver tissue in 1.8 ml of 0.1 mol/L phosphate-buffered saline (pH 7.2) using a Tenbrock tissue grinder as previously reported.³⁷ The supernatants were collected after centrifugation at 100,000 × *g* for 60 minutes at 4°C. One milliliter of the supernatants or sera was applied to Sep-Pak C₁₈ minicolumns.^{38,39} The columns were washed

with 20 ml distilled water, and the trapped materials were eluted with 40% acetonitrile (3 ml). The column eluates were lyophilized, redissolved in 0.2 ml of 10 mmol/L phosphate-buffered saline (pH 7.2), and used for TGF α ELISA as previously reported.^{28,29}

Assay for HGF in the Liver and Plasma

Liver extracts were prepared according to the protocol from the manufacturer of the HGF ELISA kit.³⁶ Briefly, liver tissues were homogenized in 4 vol/g of a buffer composed of 20 mmol/L Tris[hydroxymethyl]aminomethane-HCl (pH 7.5), 2 mol/L NaCl, 1 mmol/L phenylmethyl-sulfonyl fluoride, 1 mmol/L ethylenediaminetetraacetic acid, and 0.1% polyoxyethylenesorbitan mono-oleate. The homogenates were centrifuged at 20,000 × *g* for 60 minutes at 4°C, and the resultant supernatants were collected. These supernatants and the plasma were applied to HGF ELISA.³⁶

Assay for Serum Alanine Aminotransferase (ALT) Levels

Serum ALT levels were measured by modified Reitman-Framkel method using a commercial kit.⁴⁰

Histological Examination

Liver specimens fixed in formalin were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The mitotic index of hepatocytes was determined by counting more than 3000 hepatocytes in each liver specimen.⁷

Statistical Analyses

The differences between unpaired two samples were defined as significant when *P* values by both Student's *t*-test and Mann-Whitney *U* test were less than 0.05. Spearman's correlation test was used for analysis of correlations.

Results

TGF α and HGF Levels after Partial Hepatectomy and Sham Operation in Rats

Mean (±SEM) hepatic and circulating levels of TGF α in five normal rats were 9.8 ± 1.2 ng/g liver and 5.3 ± 0.85 ng/ml, respectively, and those of HGF were 168 ± 17.2 ng/g liver and 0.14 ± 0.013 ng/ml, respectively. As shown in Figure 1, the mitotic index of hepatocytes was unchanged until 18 hours after partial hepatectomy, but increased to the maximal level at 24 hours. It decreased to the preoperative level at 120 hours, with a smaller peak at 72 hours. Hepatic and serum TGF α levels were significantly increased after 12 hours compared with the preoperative levels, reached the maximum at 24 hours, and

Figure 1. Changes in mitotic index of hepatocytes, serum and hepatic TGF α levels, and plasma and hepatic HGF levels after partial hepatectomy in rats. Data are means $\pm$ SEM of five rats. **Top:** Circles indicate mitotic index of hepatocytes. **Middle and bottom:** Circles indicate serum or plasma levels, and columns indicate hepatic levels. * $P < 0.05$ and ** $P < 0.01$ compared with the levels before operation.

thereafter changed similarly to the mitotic index of hepatocytes. Hepatic and plasma HGF levels were significantly increased at 6 hours compared with the preoperative levels, reached the maximum around 18 hours, and returned to the preoperative level at 48 hours.

After sham operation, the mitotic index of hepatocytes and hepatic and serum TGF α levels showed no significant changes. However, hepatic and plasma HGF levels were significantly increased between 6 and 18 hours compared with the preoperative levels (Figure 2).

TGF α and HGF Levels after Intoxication with CCl₄ or DMN in Rats

As shown in Figure 3, serum ALT levels were increased with a peak on day 1 after intoxication with CCl₄, and the mitotic index of hepatocytes and hepatic and serum TGF α levels peaked on day 3. Hepatic and plasma HGF

Figure 2. Changes in mitotic index of hepatocytes, serum and hepatic TGF α levels, and plasma and hepatic HGF levels after sham operation in rats. Data are means $\pm$ SEM of five rats. **Top:** Circles indicate mitotic index of hepatocytes. **Middle and bottom:** Circles indicate serum or plasma levels, and columns indicate hepatic levels. * $P < 0.05$ and ** $P < 0.01$ compared with the levels before operation.

levels changed similarly to serum ALT levels. In the rats on days 0.5, 1, and 2, plasma HGF levels were correlated with serum ALT levels as shown in Figure 4 ($r = 0.81$; $P < 0.01$).

Figure 5 demonstrates the results in rats given DMN. Serum ALT levels peaked on day 2 after intoxication, but the mitotic index of hepatocytes and hepatic and serum TGF α levels peaked on day 3. In contrast, hepatic HGF levels were significantly decreased compared with the basal level, but plasma HGF levels were unchanged.

TGF α and HGF Levels during Phenobarbital Administration in Rats

As shown in Figure 6, the mitotic index of hepatocytes was gradually increased and peaked on day 7, with liver weights increasing significantly after 12 hours compared with the controls. The mitotic index decreased thereafter and returned to the basal level on day 14. Hepatic and serum TGF α levels changed similarly to the mitotic index. Plasma HGF levels were increased, reached the maximum on day 1, and returned to the basal level on day 5. In contrast, hepatic HGF levels were significantly de-

Figure 3. Changes in serum ALT levels, mitotic index of hepatocytes, serum and hepatic TGFα levels, and plasma and hepatic HGF levels after carbon tetrachloride injection in rats. Data are means $\pm$ SEM of five rats. **Top:** Open circles indicate serum ALT levels, and closed circles indicate mitotic index of hepatocytes. **Middle and bottom:** Circles indicate serum or plasma levels, and columns indicate hepatic levels. * $P < 0.05$ and ** $P < 0.01$ compared with the levels before CCl₄ injection.

creased from day 1 until day 14 compared with the basal level.

Discussion

Our results demonstrate that hepatic and circulating levels of TGFα were closely associated with liver regeneration. However, those levels of HGF were not necessarily related to it.

We used four different rat models of liver regeneration. Partial hepatectomy induces compensatory hyperplasia of the remaining liver with minimal necrosis.¹ Intoxication with CCl₄ also induces compensatory hepatic hyperplasia after liver necrosis due to direct damage to hepatocytes.⁴¹ Similar hepatic hyperplasia after liver necrosis is also produced by intoxication with DMN, but the mechanism of development of liver necrosis is much different from that by CCl₄ intoxication. In this model, liver necrosis occurs mainly as a result of microcirculatory disturbance

Figure 4. Serum ALT levels and plasma HGF levels after CCl₄ injection in rats. Open circles, closed circles, and triangles indicate values 0.5, 1, and 2 days after CCl₄ injection, respectively.

due to sinusoidal fibrin deposition caused by endothelial cell destruction after apoptosis of hepatic stellate cells, which are the major HGF-producing cells in the liver.^{41,42} Phenobarbital administration can induce augmentative hyperplasia of the liver without any damage to parenchymal and nonparenchymal cells in the liver.⁴³

Both hepatic and serum TGFα levels were increased in close association with the numbers of mitotic hepatocytes irrespective of the type of liver regeneration (Figures 1, 3, 5, and 6). Sham operation caused no changes in hepatic and serum TGFα levels (Figure 2). These results were in line with our previous observations in patients^{28,39} and reports in rats showing increased hepatic TGFα mRNA levels after partial hepatectomy and CCl₄ intoxication.^{1,14,25,44} TGFα produced by hepatocytes in the liver may be an essential factor for liver regeneration.

Recently, Russell et al found no difference in hepatocyte proliferation after partial hepatectomy between TGFα knockout mice and wild-type mice.³² There are many ligands for the TGFα/EGF receptor, including EGF which is abundantly produced by salivary glands in mice.^{1,15} Loss of TGFα might be masked as a result of opportunistic compensation by one such ligand in TGFα knockout mice, as was discussed in the report³². Indeed, targeting of a gene can increase the expression of other functionally related proteins.⁴⁵ Investigation with TGFα/EGF receptor knockout mice would be a way to clarify this controversy.

Hepatic and plasma HGF levels were increased in rats after partial hepatectomy and CCl₄ intoxication (Figures 1 and 3). In both models, the increase of hepatic HGF mRNA levels has also been reported.^{3,17,26,27,46,47} However, such significant increases of hepatic and plasma HGF levels were also found after sham operation, with no increase in number of mitotic hepatocytes (Figure 2). Recently, it was reported that hepatic HGF mRNA levels were increased after sham operation, as well as after partial hepatectomy in rats.^{47,48} In addition, plasma HGF levels were positively correlated with serum ALT levels in rats treated with CCl₄ (Figure 4). These results are com-

Figure 5. Changes in serum ALT levels, mitotic index of hepatocytes, serum and hepatic TGF α levels, and plasma and hepatic HGF levels after DMN injection in rats. Data are means $\pm$ SEM of five rats. **Top:** Open circles indicate serum ALT levels, and closed circles indicate mitotic index of hepatocytes. **Middle and bottom:** Circles indicate serum or plasma levels, and columns indicate hepatic levels. * P < 0.05 and ** P < 0.01 compared with the levels before DMN injection.

parable with our previous finding in patients that serum HGF levels were increased in association with hepatic necrosis and systemic inflammation.^{30,31} It can be assumed that HGF production is stimulated in response to an inflammatory reaction after a surgical procedure or hepatic injury. This assumption may be supported by the recent report that proinflammatory cytokines such as interleukin-1 can regulate HGF production.⁴⁹ Furthermore, the number of mitotic hepatocytes was increased despite decreased hepatic HGF levels after intoxication with DMN and during phenobarbital administration in rats (Figures 5 and 6). Considering that stellate cells are major HGF-producing cells in the liver,²³ such a decrease with unchanged plasma HGF levels in DMN-intoxicated rats is likely to result from apoptosis of stellate cells caused by DMN.^{41,42} The decrease in hepatic HGF levels during phenobarbital administration was accompanied by transiently increased plasma HGF levels (Figure 6). These data are comparable with reported obser-

Figure 6. Changes in mitotic index of hepatocytes, liver weight/body weight, serum and hepatic TGF α levels, and plasma and hepatic HGF levels during phenobarbital administration in rats. Data are means $\pm$ SEM of five rats. **Top:** Circles indicate mitotic index of hepatocytes, and columns indicate liver weight/body weight. **Middle and bottom:** Circles indicate serum and plasma levels, and columns indicate hepatic levels. * P < 0.05 and ** P < 0.01 compared with the levels of controls.

vations that plasma HGF levels were increased, even with no increase of hepatic HGF mRNA levels in the model of augmentative hepatic hyperplasia.^{43,50,51} The reason for such an increase should be investigated, especially in relation to reduced uptake of HGF.

There were some differences in the duration and the timing of the increase in TGF α and HGF levels in rats after partial hepatectomy between our results and reported observations.^{52,53} The differences might arise from assay systems used. Russell et al reported that the increase of hepatic TGF α levels after partial hepatectomy in rats continued longer than in rats in the present study.⁵² The TGF α ELISA we used can measure exclusively mature TGF α ,^{28,29,38,39} whereas their radioimmunoassay seems to detect both the mature TGF α and its precursors. They found that mature TGF α was increased in regenerating liver but not in the liver of sham-operated rats by gel chromatography, suggesting that the increase of mature TGF α is related to liver regeneration. Lindroos et al showed two peaks of plasma HGF levels as early as 2 and 6 hours after operation, and this elevation continued for 72 hours,⁵³ whereas the levels in the present study

reached the maximum around 18 hours and returned to the preoperative levels at 48 hours (Figure 1). In the determination of HGF, we used sandwich ELISA composed of a polyclonal antibody and a monoclonal antibody specific for the heavy chain of HGF.³⁶ In contrast, only one polyclonal antibody was utilized in their assay system. There are many forms of HGF.^{12,39,54} Furthermore, HGF-like protein, a novel protein synthesized by hepatocytes, contains the same structural domains as HGF.⁵⁵ The ELISA we have used might detect a smaller number of HGF forms and HGF-like protein compared with other assays.

From our study, both TGF α and HGF seemed not to initiate liver regeneration, because the timing of their increases in the liver and circulation occurred too late to act as initiation factors. Recently, tumor necrosis factor and interleukin-6 have been reported to initiate liver regeneration.^{56,57} The relation of TGF α and HGF to tumor necrosis factor and interleukin-6 should be investigated in the future.

References

1. Fausto N: Hepatic regeneration. *Hepatology*. Edited by D Zakim, TD Boyer. Philadelphia, WB Saunders, 1996, pp 32–57
2. Michalopoulos G: Liver regeneration: molecular mechanisms of growth control. *FASEB J* 1990, 4:176–187
3. Zarnegar R, DeFrances MC, Michalopoulos GK: Hepatocyte growth factor: its role in hepatic growth and pathology. *Liver: Biology and Pathology*. Edited by IM Arias, JL Boyer, N Fausto, WB Jakoby, DA Schachter, DA Shafritz. New York, Raven Press, 1994, pp 1047–1057
4. Jhappan C, Stahle C, Harkins RN, Fausto N, Smith GH, Merlino GT: TGF α overexpression in transgenic mice induces liver neoplasia and abnormal development of the mammary gland and pancreas. *Cell* 1990, 61:1137–1146
5. Shiota G, Wang TC, Nakamura T, Schmidt EV: Hepatocyte growth factor in transgenic mice: effects on hepatocyte growth, liver regeneration and gene expression. *Hepatology* 1994, 19:962–972
6. Ishiki Y, Ohnishi H, Muto Y, Matsumoto K, Nakamura T: Direct evidence that hepatocyte growth factor is a hepatotropic factor for liver regeneration and has a potent antihepatitis effect in vivo. *Hepatology* 1992, 16:1227–1235
7. Fujiwara K, Nagoshi S, Ohno A, Hirata K, Ohta Y, Mochida S, Tomiya T, Higashio K, Kurokawa K: Stimulation of liver growth by exogenous human hepatocyte growth factor in normal and partially hepatectomized rats. *Hepatology* 1993, 18:1443–1449
8. Webber EM, Godowski PJ, Fausto N: In vivo response of hepatocytes to growth factors requires an initial priming stimulus. *Hepatology* 1994, 19:489–497
9. Liu ML, Mars WM, Zarnegar R, Michalopoulos GK: Collagenase pretreatment and mitogenic effects of hepatocyte growth factor and transforming growth factor- α in adult rat liver. *Hepatology* 1994, 19:1521–1527
10. Todaro GJ, Fryling C, deLarco JE: Transforming growth factors produced by certain human tumor cells: polypeptides that interact with epidermal growth factor receptors. *Proc Natl Acad Sci USA* 1980, 77:5258–5262
11. Massague J: Epidermal growth factor-like transforming growth factor. II. Interaction with epidermal growth factor receptors on human placenta membrane and A431 cells. *J Biol Chem* 1983, 258:13614–13620
12. Derynck R: Transforming growth factor α . *Cell* 1988, 54:593–595
13. Johnson AC, Garfield SH, Merlino GT, Pastan I: Expression of epidermal growth factor receptor proto-oncogene mRNA in regenerating rat liver. *Biochem Biophys Res Commun* 1988, 150:412–418
14. Mead JE, Fausto N: Transforming growth factor α may be a physiological regulator of liver regeneration by means of an autocrine mechanism. *Proc Natl Acad Sci USA* 1989, 86:1558–1562
15. Fisher DA, Lakshmanan J: Metabolism and effects of epidermal growth factor and related growth factors in mammals. *Endocr Rev* 1990, 11:418–442
16. Fausto N, Webber EM: Liver regeneration. *The Liver: Biology and Pathology*. Edited by IM Arias, JL Boyer, N Fausto, WB Jakoby, DA Schachter, DA Shafritz. New York, Raven Press, 1994, pp 1059–1084
17. Kinoshita T, Tashiro K, Nakamura T: Marked increase of HGF mRNA in non-parenchymal liver cells of rats treated with hepatotoxins. *Biochem Biophys Res Commun* 1989, 165:1229–1234
18. Noji S, Tashiro K, Koyama E, Nohno T, Ohyama K, Taniguchi S, Nakamura T: Expression of hepatocyte growth factor gene in endothelial and Kupffer cells of damaged rat livers, as revealed by in situ hybridization. *Biochem Biophys Res Commun* 1990, 173:42–47
19. Zarnegar R, DeFrances MC, Oliver L, Michalopoulos G: Identification and partial characterization of receptor binding sites for HGF on rat hepatocytes. *Biochem Biophys Res Commun* 1990, 173:1179–1185
20. Naldini L, Vigna E, Narsimhan RP, Gaudino G, Zarnegar R, Michalopoulos GK, Comoglio PM: Hepatocyte growth factor (HGF) stimulates the tyrosine kinase activity of the receptor encoded by the proto-oncogene *c-MET*. *Oncogene* 1991, 6:501–504
21. Bottaro DP, Rubin JS, Faletto DL, Chan AM-L, Kmieciak TE, Vande Woude GF, Aaronson SA: Identification of the hepatocyte growth factor receptor as the *c-met* proto-oncogene product. *Science* 1991, 251:802–804
22. Higuchi O, Nakamura T: Identification and change in the receptor for hepatocyte growth factor in rat liver after partial hepatectomy or induced hepatitis. *Biochem Biophys Res Commun* 1991, 176:599–607
23. Schirmacher P, Geerts A, Pietrangelo A, Dienes HP, Rogler CE: Hepatocyte growth factor/hepatopoietin A is expressed in fat-storing cells from rat liver but not myofibroblast-like cells derived from fat-storing cells. *Hepatology* 1992, 15:5–11
24. Hu Z, Everts RP, Fujio K, Marsden ER, Thorgeirsson SS: Expression of hepatocyte growth factor and *c-met* genes during hepatic differentiation and liver development in the rat. *Am J Pathol* 1993, 142:1823–1830
25. Webber EM, FitzGerald MJ, Brown PI, Bartlett MH, Fausto N: Transforming growth factor- α expression during liver regeneration after partial hepatectomy and toxic injury, and potential interactions between transforming growth factor- α and hepatocyte growth factor. *Hepatology* 1993, 18:1422–1431
26. Kinoshita T, Hirao S, Matsumoto K, Nakamura T: Possible endocrine control of liver regeneration after partial hepatectomy. *Biochem Biophys Res Commun* 1991, 177:330–335
27. Zarnegar R, DeFrances MC, Kost DP, Lindroos P, Michalopoulos G: Expression of hepatocyte growth factor mRNA in regenerating rat liver after partial hepatectomy. *Biochem Biophys Res Commun* 1991, 177:559–565
28. Tomiya T, Fujiwara K: Serum levels of transforming growth factor- α in patients after partial hepatectomy as determined with an enzyme-linked immunosorbent assay. *Hepatology* 1993, 18:304–308
29. Tomiya T, Fujiwara K: Liver Regeneration in fulminant hepatitis as evaluated by serum transforming growth factor α levels. *Hepatology* 1996, 23:253–257
30. Tomiya T, Tani M, Yamada S, Hayashi S, Umeda N, Fujiwara K: Serum hepatocyte growth factor levels in hepatectomized and nonhepatectomized surgical patients. *Gastroenterology* 1992, 103:1621–1624
31. Tomiya T, Nagoshi S, Fujiwara K: Significance of serum human hepatocyte growth factor levels in patients with hepatic failure. *Hepatology* 1992, 15:1–4
32. Russell WE, Kaufmann WK, Sitaric S, Luetteke NC, Lee DC: Liver regeneration and hepatocarcinogenesis in transforming growth factor- α -targeted mice. *Mol Carcinog* 1996, 15:183–189
33. Sakaguchi H, Seki S, Tsubouchi H, Daikuhara Y, Nittani T, Kobayashi K: Ultrastructural localization of human hepatocyte growth factor in human. *Hepatology* 1994, 19:1157–1163
34. Mochida S, Ogata I, Hirata K, Ohta Y, Yamada S, Fujiwara K: Provocation of massive hepatic necrosis by endotoxin after partial hepatectomy in rats. *Gastroenterology* 1990, 99:771–777
35. Kawasaki S, Makuuchi M, Ishizone S, Matsunami H, Terada M, Kawarazaki H: Liver regeneration in recipients and donors after transplantation. *Lancet* 1992, 339:580–581
36. Yamada A, Matsumoto K, Iwanari H, Sekiguchi K, Kawata S, Matsuzawa Y, Nakamura T: Rapid and sensitive enzyme-linked immunosorbent assay for measurement of HGF in rat and human tissues. *Biomed Res* 1995, 16:105–114

37. Brown PI, Lam R, Lakshmanan J, Fischer DA: Transforming growth factor alpha in developing rats. *Am J Physiol* 1990, 259:E256-E260
38. Inagaki H, Katoh M, Kurokawa-Ohsawa K, Tanaka S: A new sandwich enzyme-linked immunosorbent assay (ELISA) for transforming growth factor α (TGF α) based upon conformational modification by antibody binding. *J Immunol Methods* 1990, 128:27-37
39. Katoh M, Inagaki H, Kurosawa-Ohsawa K, Katsuura M, Tanaka S: Detection of transforming growth factor alpha in human urine and plasma. *Biochem Biophys Res Commun* 1990, 167:1065-1072
40. Reitman S, Frankel S: A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *Am J Clin Pathol* 1957, 28:56-63
41. Fujiwara K, Ogata I, Ohta Y, Hirata K, Oka Y, Yamada S, Sato Y, Masaki N, Oka H: Intravascular coagulation in acute liver failure in rats and its treatment with antithrombin III. *Gut* 1988, 29:1103-1108
42. Hirata K, Ogata I, Ohta Y, Fujiwara K: Hepatic sinusoidal cell destruction in the development of intravascular coagulation in acute liver failure of rats. *J Pathol* 1989, 158:157-165
43. Lindroos P, Tsai WH, Zarnegar R, Michalopoulos GK: Plasma levels of HGF in rats treated with tumor promoters. *Carcinogenesis* 1992, 13:139-141
44. Everts RP, Nakatsukasa H, Marsden ER, Hu Z, Thorgeirsson SS: Expression of transforming growth factor- α in regenerating liver and during hepatic differentiation. *Mol Carcinog* 1992, 5:25-31
45. Zhang W, Behringer RR, Olson EN: Inactivation of the myogenic bHLH gene MRF4 results in up-regulation of myogenin and rib anomalies. *Genes Dev* 1995, 9:1388-1399
46. Tashiro K, Hagiya M, Nishizawa T, Seki T, Shimonishi M, Shimizu S, Nakamura T: Deduced primary structure of rat hepatocyte growth factor and expression of the mRNA in rat tissues. *Proc Natl Acad Sci USA* 1990, 87:3200-3204
47. Okajima A, Miyazawa K, Kitamura N: Primary structure of rat hepatocyte growth factor and induction of its mRNA during liver regeneration following hepatic injury. *Eur J Biochem* 1990, 193:375-381
48. Aldana PR, Goerke ME, Carr SC, Tracy TF: The expression of regenerative growth factors in chronic liver injury and repair. *J Surg Res* 1994, 57:711-717
49. Matsumoto K, Okazaki H, Nakamura T: Up-regulation of hepatocyte growth factor gene expression by interleukin-1 in human skin fibroblasts. *Biochem Biophys Res Commun* 1992, 188:235-243
50. Shinozuka H, Kubo Y, Katyal SL, Coni P, Ledda-Columbano GM, Columbano A, Nakamura T: Roles of growth factors and tumor necrosis factor- α on liver cell proliferation induced in rats by lead nitrate. *Lab Invest* 1994, 71:35-41
51. Columbano A, Shinozuka H: Liver regeneration versus direct hyperplasia. *FASEB J* 1993, 10:118-128
52. Russell WE, Dempsey PJ, Sitaric S, Peck AJ, Coffey RJ Jr: Transforming growth factor- α (TGF α) concentrations increase in regenerating rat liver: evidence for a delayed accumulation of murine TGF α . *Endocrinology* 1993, 133:1731-1738
53. Lindroos P, Zarnegar R, Michalopoulos GK: Hepatocyte growth factor (hepatopoietin A) rapidly increases in plasma before DNA synthesis and liver regeneration stimulated by partial hepatectomy and carbon tetrachloride administration. *Hepatology* 1991, 13:743-749
54. Tang W, Miyazawa K, Kitamura N: Hepatocyte growth factor remains as an inactive single chain after partial hepatectomy or unilateral nephrectomy. *FEBS Lett* 1995, 362:220-224
55. Bezerra JA, Witte DP, Aronow BJ, Degen SJ: Hepatocyte-specific expression of the mouse hepatocyte growth factor-like protein. *Hepatology* 1993, 18:394-398
56. Yamada Y, Kirillova I, Peschon JJ, Fausto N: Initiation of liver growth by tumor necrosis factor: deficient liver regeneration in mice lacking type I tumor necrosis factor receptor. *Proc Natl Acad Sci USA* 1997, 94:1441-1446
57. Cressman DE, Greenbaum LE, DeAngelis RA, Ciliberto G, Furth EE, Poli V, Taub R: Liver failure and defective hepatocyte regeneration in interleukin-6 deficient mice. *Science* 1996, 274:1379-1383