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K. Yamaguchi · M. A. Nalesnik · G. K. Michalopoulos

Expression of HGF mRNA in human rejecting kidney as evidenced by in situ hybridization

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Abstract In situ hybridization was performed to demonstrate hepatocyte growth factor (HGF) mRNA in two patients with normal kidney and in 23 patients with allograft nephrectomy. In situ hybridization was combined with immunohistochemistry to identify HGF-producing cells. In the two patients with normal kidney, no HGF mRNA was obtainable. In 15 of the 23 allograft patients, signals of HGF mRNA were detectable. In six of these 15 patients, the signals were present mainly at the medullocortex junction, and in the other nine patients at the cortex and/or medulla. Strong and frequent signals were present in gland-like structures in 15 cases. Some scattered signals were also present in the fibrosed glomeruli in five cases, in the thickened intimas of large arteries in three cases, and in the arterial muscle coats in two cases. Combined immunohistochemistry and in situ hybridization showed that HGF mRNA-positive cells in gland-like arrangements were also positive for cytokeratin and negative for factor VIII. Cells with HGF mRNA signal and located in the arterial media were also positive for actin. These findings suggest that HGF mRNA is transcribed both in the urinary tubular epithelium and in the mesenchymal cells (fibroblasts, and smooth muscle cells in chronic vascular rejection and endothelial cells and/or mesangial cells in transplant glomerulopathy) in human rejecting kidney.

Key words Hepatocyte growth factor · In situ hybridization · Rejection · Kidney · Transplant

K. Yamaguchi · M. A. Nalesnik
Division of Transplant Pathology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213, USA

G. K. Michalopoulos
Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213, USA

K. Yamaguchi (✉)
Department of Surgery I, Kyushu University Faculty of Medicine, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-82, Japan

Reprints from: M. A. Nalesnik, E1549, Biomedical Science Tower, University of Pittsburgh School of Medicine, Terrace and Lothrop Streets, Pittsburgh, PA 15213, USA

Introduction

Hepatocyte growth factor (HGF), which is a potent mitogen for the mature hepatocyte, has been identified [1–3], purified [4–6], and molecularly cloned [7–9]. Recently, HGF has been thought to play an important role in regeneration of various organs and tissues, such as the liver, kidney [10, 11], lung [12], and skeletal muscle [13]. Several reports link changes in HGF levels with renal alterations, and an abnormal increase of serum HGF levels is present in patients with chronic renal failure [14]. HGF strongly stimulates DNA synthesis of rabbit renal tubular epithelium in secondary culture [15]. HGF mRNA increases markedly, reaching a maximum 6 h after unilateral nephrectomy [10], followed by an increase of HGF activity at 12 h. The number of HGF receptors decreases to 30% of the normal value 12 h after unilateral nephrectomy. In 1992, Ishibashi et al. [16] reported that c-*met* oncogene protein (HGF receptor) mRNA increases rapidly in rat 6 h after unilateral nephrectomy. Together these results suggest that HGF may be involved in kidney regeneration.

In addition, an involvement of HGF in the immune response process has been suggested [17]. HGF mRNA is present in the spleen [18], thymus [18], and human leukocytes [19], and HGF receptor is expressed in the spleen [20]. HGF has also been shown to enhance B-cell and T-cell activity [17].

These findings prompted us to study the expression of HGF in the human rejecting kidney. Our purpose was to demonstrate HGF mRNA in human allograft nephrectomy and to identify HGF-producing cells therein.

Materials and methods

Patients

Twenty-three patients in this study were referred to the University of Pittsburgh Medical Center for treatment of their rejecting kidney. The two remaining patients were referred for treatment of renal cell carcinoma. Allograft nephrectomy was done in the 23 patients and radical nephrectomy was performed in the 2 patients. The 23 allograft nephrectomies were performed 2 days after transplant of the kidney in 3 cases, 1 month in 2, 3 months in 2, and 1–10 years in the remaining 16. The present allograft nephrectomy was the first allograft nephrectomy in 12 patients, the second in 7, the third in 3, and the fourth in 1. Histopathologic diagnoses were hyperacute rejection in three patients, acute rejection in four, chronic rejection with superimposed acute rejection in eight, and without in the remaining eight. The basic renal disease was glomerulonephritis in seven, diabetes mellitus in four, polycystic disease in four, hypertension in two, vesicoureter reflex in two, lupus nephritis in one, Albert's disease in one, dysplastic kidney in one, and undetermined in the remaining one. The state and type of immunosuppressants were available in 21 patients.

Preparation of tissue

Tissue sections were cut from fresh material immediately after the organ was resected in the operating room, mounted in optimum cutting temperature (OCT) compound (Miles Inc. Elkhart, IN, USA), fixed, and stored at −70°C for times ranging from 1 month to 2 years. Five-micrometer-thick sections were cut from the OCT compound blocks.

In situ hybridization

RNA probe

A 2.4-kb cDNA for human HGF was inserted into the plasmid vector PSport (Gibco BRL, Life Technologies, Gaithersburg, MD, USA) and flanked by SP6 and T7 promoters. Plasmid DNA was isolated and linearized with *Sph*1 and *Sst*1. The anti-sense and sense single-strand radiolabeled RNA probes complementary to the coding sequence of human HGF were transcribed from the linearized plasmid in the presence of ^{35}S-UTP with T7 (anti-sense) and SP6 (sense) RNA polymerases using the Riboprobe Gemini Transcription system (Promega Corporation, Madison, WI, USA). The newly synthesized RNA probes were separated from unincorporated nucleotides by Sephadex G50 column (Boehringer Mannheim, Biochemical Products, Indianapolis, IN, USA). The RNA probes were treated with alkaline hydrolysis (25 min, 60 °C).

Pretreatment

The tissue sections were pretreated by sequential incubations in: 5 mM $MgCl_2$/PBS (10 min, at room temperature), 0.1 M glycine/0.2 M TRIS-HCl (10 min at room temperature), 4% paraformaldehyde (20 min at room temperature), 0.2 N HCl (15 min at room temperature), with or without proteinase K (0.1, 0.5, or 1.0 μg/ml proteinase K); 500 mM TRIS-HCl (pH 8) and 5 mM ethylenediamine tetraacetic acid (EDTA) solution (15, 30, or 45 min at room temperature), after immersion in 4% paraformaldehyde (5 min at room temperature) to stop the previous reaction.

Prehybridization and hybridization

Prehybridization was carried out in a solution containing 50% formamide, 10 mM DTT, 300 mM NaCl, 10 mM TRIS-HCl, 10 mM $NaPO_4$, 1 mM EDTA, 10% dextran, 1X Denhart's solution, 1 mg/ml yeast tRNA, and 0.5 mg/ml salmon sperm DNA for 1–2 h at 50 °C. Hybridization was carried out with probe (20 000 000 –100 000 000 cpm in 1 ml hybridization solution) diluted in this cocktail overnight in a moist chamber at 50 °C.

RNase treatment and washing

After hybridization, the sections were rinsed in 0.1x standard saline citrate (SSC) for 90 or 120 min at 60 °C and 0.1x SSC for 90 or 120 min at room temperature with constant stirring. The total washing time was 180 min or 240 min. Nonspecifically bound single-strand probe was removed by treatment with a solution containing 25 μg/ml RNase A, 10 mM TRIS-HCl (pH 8), and 1 mM EDTA for 30 min at 37 °C.

Autoradiography and visualization

After dipping in Kodak NTB 2 emulsion, the slides were exposed in a light-tight black box for 7–10 days at 4 °C. The slides were developed in Kodak D-19 developer, and fixed in Kodak fixer. The slides were counterstained with hematoxylin. Sense probe slides were used as a negative control.

Combined immunohistochemistry and in situ hybridization

Sections were first applied to immunohistochemistry. The tissues were treated with methanol containing 0.3% hydrogen peroxide for 10 min, washed in phosphate-buffered solution (PBS) 5 min 2 times, and mounted with primary antibodies. The primary antibodies used were factor-VIII-related antigen (rabbit polyclonal) (×400) and muscle actin (mouse monoclonal) (×40) purchased from Dako Corporation. Carpinteria, CA, USA, and AE1/AE3 (cytokeratin) (mouse monoclonal) (×500) from Boehringer Mannheim, Biotechnological Products, Indianapolis, IN, USA. The avidin-biotin complex method was performed. The immunoreaction was developed by DAB (3,3′-diaminobenzidine). Solutions of every staining step were supplemented with 0.04% diethylpyrocarbonate (DEPC). Thereafter, the sections were processed for in situ hybridization in the same manner as mentioned above.

Results

In situ hybridization

In the two patients with normal kidney, no signals of HGF mRNA were observed. In 15 of the 23 patients with allograft nephrectomy, signals of HGF mRNA were seen (Table 1). In six cases, the great majority of signals were present in the medullocortex interjunction. In the other nine cases, signals were present in the cortex and/or medulla. In all 15 cases, strong signals were seen along gland-like structures (Table 2, Figs. 1, 2). Scattered signals were also present within the fibrosed glomeruli in five cases (Fig. 3), in the thickened intimas of the large arterial branches in three

Table 1 HGF mRNA and histopathology of allograft nephrectomy

	Hyperactue rejection	Acute rejection	Chronic rejection
HGF mRNA	2/3	2/4	11/16

cases, in the muscle coats of a large artery in one, and in a medium-sized artery in another.

There was no relationship between the histopathology of allograft nephrectomy and HGF mRNA signals. Nine of the 14 patients with HGFmRNA signals had no immunosuppressants around the allograft nephrectomy, while 5 of the 7 patients with no HGFmRNA signals received immunosuppressants. HGFmRNA signals seemed to be related to the state of immunosuppressants. However, the distribution of the patients was not statistically significant. There was no relationship between HGFmRNA signals and type of immunosuppressant. There were no or few inflammatory infiltrates around HGF mRNA-positive cells. No or few signals were seen with a sense probe.

Combined immunohistochemistry and in situ hybridization

HGF mRNA-positive cells forming grandular structures were also positive for cytokeratin and negative for factor VIII (Fig. 4), suggesting urinary tubular epithelium as the cells of origin. Cells having HGF mRNA signals in the smooth muscle coat of the interlobular and arcuate artery were also positive for smooth muscle actin.

Discussion

HGF was first detected in the serum of the partially hepatectomized rat in 1984 [2] and was purified to homogeneity from rat platelets in 1986 [4]. In 1989, HGF was cloned and the primary structure was identified [7]. HGF gene is located in 7q21.1 [21]. HGF is identical to scatter factor [22]. Its receptor is c-*met* proto-oncogene product [23]. HGF is a pleiotropic factor which regulates construction of normal tissue architecture, acting as mitogen (induction of mitogenesis), motogen (induction of cell movement), and morphogen (induction of epithelial tissue-like structures) [24]. HGF and regulators for HGF expression seem to be a unique self-defense system which supports organogenesis, organ regeneration, and tissue homeostasis. It also works as a tumor supressor on hepatoma, melanoma, and squamous cell carcinoma cell lines, suggesting a bidirectional growth factor.

HGF is a mitogen of urinary tubular epithelium, [25–27], (a) motogen of the MDCK cell line from dog urinary epithelium [28], and a morphogen of urinary epithelium [29]. HGF is involved in the development of the kidney [30, 31]. A possible role of HGF as a renotropic factor in renal regeneration has been reported [10, 11]. Increased HGF mRNA [10, 11, 32], HGF activity [10, 11], HGF receptor mRNA [10, 16], and DNA synthesis [11], with downregulation of HGF receptor [10] have been noted in rat kidney after unilateral nephrectomy [10, 16, 32], ischemia [11, 16], and treatment with CCl_4 [10], $HgCl_2$ [11], or folic acid [16]. HGF activity (peak 12 h after renal injury) increases after elevation of HGF mRNA (peak 6–12 h), and this in turn is followed by downregulation of the HGF receptor (peak 48 h). Thereafter, DNA synthesis of kidney tubular epithelium peaks. These phenomena are similar to those in regenerating liver after chemical or mechanical injury. The presence of HGF mRNA in rejecting kidneys in this series suggests that HGF is involved in the repair of the kidney following repeated rejectin-associated damage.

In 1990, Zarnegar and coworkers [33] noted that rabbit kidney was negative for HGF by immunohistochemistry. In 1991, Wolf et al. [34] reported that a strong immunoreactivity for HGF was present in the distal tubules and collecting ducts of human and rat kidney using a chicken polyclonal antibody for rabbit HGF. The proximal tubules and thin segments of the loop of Henle showed a weak staining, and glomeruli were negative for HGF. In 1993, Yoshinaga and colleagues [35] developed a rabbit polyclonal antibody against recombinant human HGF and demonstrated that normal kidney at autopsy showed immunoreactivity in the basement membrane of the urinary tubule and Bowman's capsule. Localization of HGF mRNA in

Table 2 Localization of HGF mRNA in human allograft nephrectomy

	Localization of HGF mRNA			
	Gland-like structure	Transplant glomerulopathy	Large artery	
			Intimal thickening	Smooth muscle coat
No. of positive cases/ Total no. of cases	15/23	5/23	3/23	2/23

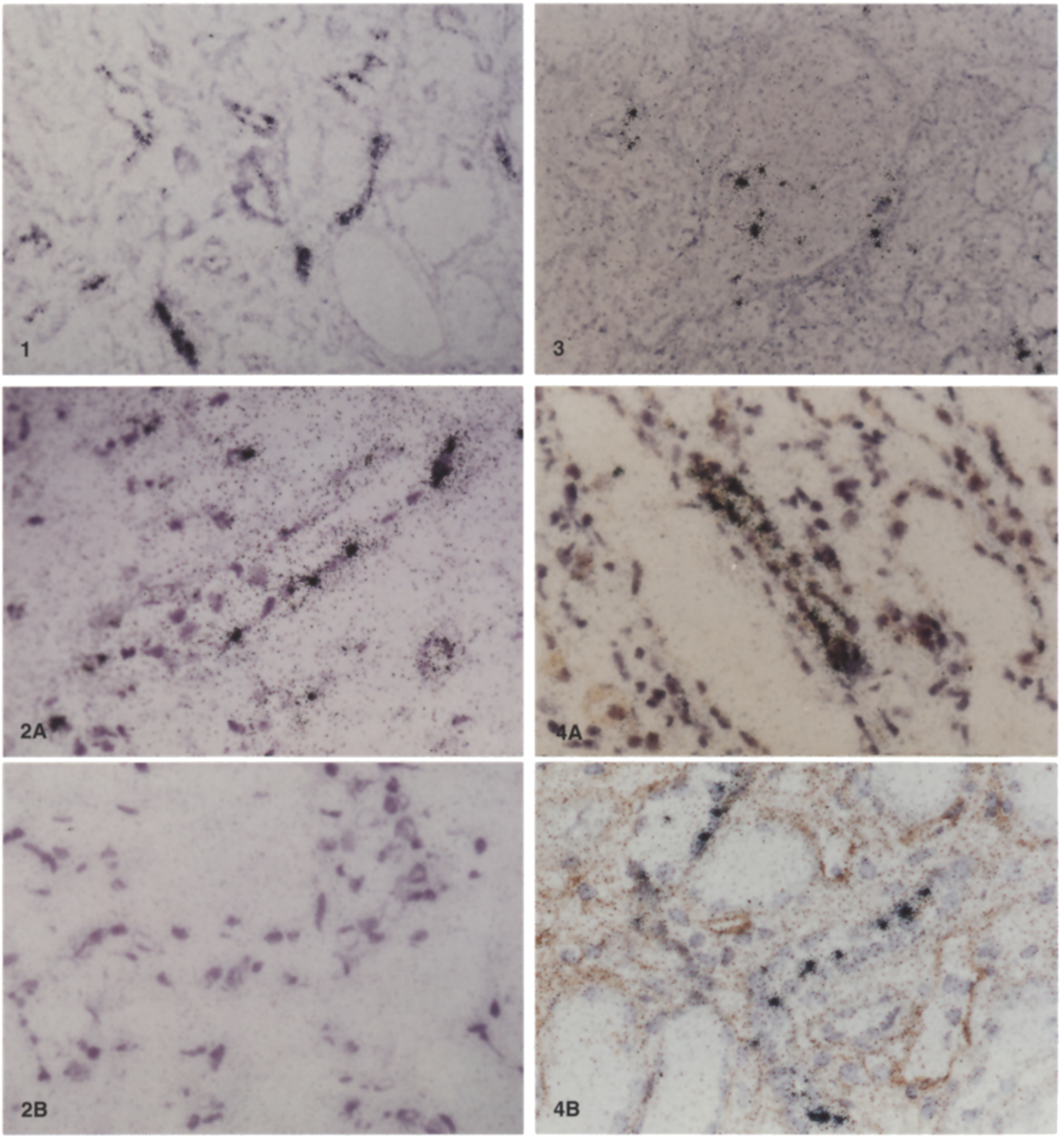

Fig. 1 HGF mRNA is seen in glandular structures in an area of chronic interstitial fibrous and tubular atrophy, × 32

Fig. 2A, B HGF mRNA is present in glandular structures (**A** × 320) and no HGF mRNA is seen in glandular structures with a sense probe (**B** × 320)

Fig. 3 HGF mRNA is present in transplant glomerulopathy, × 170

Fig. 4 HGF mRNA is present in cytokeratin-positive cells (**A** × 350, combined cytokeratin and HGF mRNA) and in factor-VIII-negative cells (**B** × 350, combined factor VIII and HGF mRNA)

human rejecting kidney in this study appears to be similar to that of HGF reported by Wolf [34].

In 1991, Nagaike et al. [10] reported that HGF mRNA was seen in the endothelial cells but not in the tubular epithelium of rat kidneys, both untreated and treated with CCl_4. Their speculation was based on morphological findings; the HGF mRNA-positive cells were located between the renal tubules, were small and flat in shape, and their nuclei stained well with hematoxylin. In 1992, Ishibashi et al. [36] mentioned that rat mesangial cells in primary culture exhibit HGF mRNA by Northern blotting, while cultured renal epithelial cell lines (OK, LLCPK1, and MDCK cells) show c-*met* mRNA. In 1993, Igawa et al. [11], using immunohistochemistry and in situ hybridization, reported that both HGF mRNA and HGF were expressed in the renal interstitial cells, presumably endothelial cells and macrophages, after $HgCl_2$ administration. In the present study, combined immunohistochemistry and in situ hybridization was performed. HGF mRNA-positive cells forming gland-like structures were also positive for cytokeratin (a marker of epithelium) and negative for factor VIII (a marker of endothelial cells). Cells having HGF mRNA in the muscle coat of large arteries were positive for smooth muscle actin. In addition, scattered signals were present in the fibrosed glomeruli and thickened intimas of large arteries. The present results suggest that HGF gene transcription is present mainly in the urinary tubular epithelium, with lesser expression in the mesenchymal cells (the fibroblasts, endothelial cells, or mesangeal cells) in human rejecting kidney.

After hepatic injury, HGF is produced rapidly not only in the injured liver but also in the lung and spleen [32]. Most authors report that HGF is produced in the mesenchymal cells and acts on epithelial cells in an endocrine and/or paracrine mechanism. However, some authors have shown that epithelial cells or epithelial neoplastic cells produce HGF and have proposed that HGF acts on epithelial cells in an autocrine mechanism. For example, Adams and colleagues [37] demonstrated that a human keratinocyte strain secretes HGF and proposed the autocrine stimulation. In 1992, Yoshinaga et al. [38] first reported that human lung cancer cell lines produce HGF. In 1993 Tsao and coworkers [39] noted that HGF/SF is an autocrine factor for normal and neoplastic human bronchial epithelial cells in culture. In 1993, Rygaard et al. [40] reported that 1 of 25 small cell lung carcinoma cell lines and nude mouse xenografts exhibited HGF mRNA and c-*met* oncogene product expression. Localization of HGF mRNA in the urinary tubular epithelium may support an autocrine mechanism of HGF on the urinary tubular epithelium, although further studies are mandatory.

Recently, an involvement of HGF in the immune response process has been suggested, HGF enhances B-cell and T-cell activity [17] and it is also produced in immunologic tissues, including the spleen [18, 32] after partial hepatectomy or chemical injury [32], thymus [18], and human leukocytes [19]. A small number of HGF receptors exist on spleen cells [20]. Of interest is that B-cell numbers increase in the spleen after partial hepatectomy in mice [41]. The present demonstration of HGF mRNA in allograft nephrectomy specimens may suggest an involvement of HGF in the immunorejection of the allograft kidney, although there seemed to be no topological relationship between HGF mRNA-positive cells and mononuclear cell infiltrates.

References

1. Michalopoulos G, Houck KA, Dolan ML, Luetteke NC (1984) Control of hepatocyte replication by two serum factors. Cancer Res 44:4414
2. Nakamura T, Nawa K, Ichihara A (1984) Partial purification and characterization of hepatocyte growth factor from serum of hepatectomized rats. Biochem Biophys Res Commun 122:1450
3. Russell WE, McGowan JA, Bucher NLR (1984) Partial characterization of a hepatocyte growth factor from rat platelets. J Cell Physiol 119:183
4. Nakamura T, Teramoto H, Ichihara A (1986) Purification and characterization of a growth factor from rat platelets for mature parenchymal hepatocytes in primary cultures. Proc Natl Acad Sci USA 83:6489
5. Ghoda E, Tsubouchi H, Nakayama H, Hirono S, Sakiyama O, Takahashi K, Miyazaki H, Hashimoto S, Daikuhara Y (1988) Purification and partial characterization of hepatocyte growth factor from plasma of a patient with fulminant hepatic failure. J Clin Invest 81:414
6. Zarnegar R, Michalopoulos G (1989) Purification and biological characterization of human hepatopoietin A, a polypeptide growth factor for hepatocytes. Cancer Res 49:3314
7. Miyazawa K, Tsubouchi H, Naka D, Takahashi K, Okigaki M, Arakaki N, Nakayama H, Hirono S, Sakiyama O, Takahashi K, Ghoda E, Daikuhara Y, Kimura N (1989) Molecular cloning and sequence analysis of cDNA for human hepatocyte growth factor. Biochem Biophys Res Commun 163:967
8. Zarnegar R, Muga S, Enghild J, Michalopoulous G (1989) NH_2-terminal amino acid sequence of rabbit hepatopoietin A, a heparin-binding polypeptide growth factor for hepatocytes. Biochem Biophys Res Commun 163:1370
9. Nakamura T, Nishizawa T, Hagiya M, Seki T, Shimonishi M, Sugimura A, Tashiro K, Shimizu S (1989) Molecular cloning and expression of human hepatocyte growth factor. Nature 342:440
10. Nagaike M, Hirao S, Tajima H, Noji S, Taniguchi S, Matsumoto K, Nakamura T (1991) Renotropic functions of hepatocyte growth factor in renal regeneration after unilateral nephrectomy. J Biol Chem 266:22781
11. Igawa T, Matsumoto K, Kanda S, Saito Y, Nakamura T (1993) Hepatocyte growth factor may function as a renotropic factor for regeneration in rats with acute renal injury. Am J Physiol 265:F61
12. Yanagita K, Matsumoto K, Sekiguchi K, Ishibashi H, Niho Y, Nakamura T (1993) Hepatocyte growth factor may act as a pulmotrophic factor on lung regeneration after acute lung injury. J Biol Chem 268:21212
13. Jennische E, Ekberg S, Matejka GL (1993) Expression of hepatocyte growth factor in growing and regenerating rat skeletal muscle. Am J Physiol 265:C122

14. Tomiya T, Nagoshi S, Fujiwara K (1992) Significance of serum human hepatocyte growth factor levels in patients with hepatic failure. Hepatology 15:1
15. Igawa T, Kanda S, Kanetake H, Saitoh Y, Ichihara A, Tomita Y, Nakamura T (1991) Hepatocyte growth factor is a potent mitogen for cultured rabbit renal tubular epithelial cells. Biochem Biophys Res Commun 174:831
16. Ishibashi K, Sasaki S, Sakamoto H, Hoshino Y, Nakamura T, Marumo F (1992) Expression of receptor gene for hepatocyte growth factor in kidney after unilateral nephrectomy and renal injury. Biochem Biophys Res Commun 187:1454
17. Delaney B, Koh WS, Yang KH, Strom SC, Kaminski NE (1993) Hepatocyte growth factor enhances B-cell activity. Life Science 53:89
18. Tashiro K, Hagiya M, Nishizawa T, Seki T, Shimonishi M, Shimizu S, Nakamura T (1990) Deduced primary structure of rat hepatocyte growth factor and expression of the mRNA in rat tissues. Proc Natl Acad Sci USA 87:3200
19. Seki T, Ihara I, Sugimura A, Shimonishi M, Nishizawa T, Asami O, Hagiya M, Nakamura T, Shimizu S (1990) Isolation and expression of cDNA for different forms of hepatocyte growth factor from human leukocyte. Biochem Biophys Res Commun 172:321
20. Tajima H, Higuchi O, Mizuno K, Nakamura T (1992) Tissue distribution of hepatocyte growth factor receptor and its exclusive down-regulation in a regenerating organ after injury. J Biochem 111:401
21. Fukuyama R, Ichijoh Y, Minoshima S, Kitamura N, Shimizu N (1991) Regional localization of the hepatocyte growth factor (HGF) gene to human chromosome-7 band q21.1. Genomics 11:410
22. Weidner KM, Arakaki N, Hartmann G, Vandekerckhove J, Weingart S, Rieder H, Fonatsch C, Tsubouchi H, Hishida T, Daikuhara Y, Birchmeier W (1991) Evidence for the identity of human scatter factor and human hepatocyte growth factor. Proc Natl Acad Sci USA 88:7001
23. Bottaro DP, Rubin JS, Faletto DL, Chan AML, Kmiecik TE, Vande Woude GF, Aaronson SA (1991) Identification of the hepatocyte growth factor receptor as the c-*met* protooncogene product. Science 251:802
24. Vande Woude GF (1992) Hepatocyte growth factor-mitogen, motogen and morphogen. Jpn J Cancer Res 83:1388
25. Igawa T, Kanda S, Kanetake H, Saitoh Y, Ichihara A, Tomita Y, Nakamura T (1991) Hepatocyte growth factor is a potent mitogen for culture rabbit renal tubular epithelial cells. Biochem Biophys Res Commun 174:831
26. Kan M, Zhang G, Zarnegar R, Michalopoulos G, Myoken Y, McKeehan WL, Stevens JI (1991) Hepatocyte growth factor/hepatopoietin A stimulates the growth of rat kidney proximal tubular epithelial cells (RPTE), rat nonparenchymal liver cells, human melanoma cells, mouse keratinocytes and stimulates anchorage-independent growth of SV-40 transformed RPTE. Biochem Biophys Res Commun 174:331
27. Harris RC, Burns KD, Alattar M, Homma T, Nakamura T (1993) Hepatocyte growth factor stimulates phosphoinositide hydrolysis and mitogenesis in cultured renal epithelial cells. Life Sci 52:1091
28. Gherardi E, Stoker M (1991) Hepatocyte growth factor-scatter factor; mitogen, motogen, and met. Cancer Cells 3:227
29. Montesano R, Matsumoto K, Nakamura T, Orci L (1991) Identification of a fibroblast-derived epithelial morphogen as hepatocyte growth factor. Cell 67:901
30. Kagoshima M, Kinoshita T, Matsumoto K, Nakamura T (1992) Developmental changes in hepatocyte growth factor mRNA and its receptor in rat liver, kidney and lung. Eur J Biochem 210:375
31. Defrances MC, Wolf HK, Michalopoulous GK, Zarnegar R (1992) The presence of hepatocyte growth factor in the developing rat. Development 116:387
32. Kono S, Nagaike M, Matsumoto K, Nakamura T (1992) Marked induction of hepatocyte growth factor mRNA in intact kidney and spleen in response to injury of distant organs. Biochem Biophys Res Commun 186:991
33. Zarnegar R, Muga S, Rahija R, Michalopoulos GK (1990) Tissue distribution of hepatopoietin-A: a heparin-binding polypeptide growth factor for hepatocytes. Proc Natl Acad Sci USA 87:1252
34. Wolf HK, Zarnegar R, Michalopoulos GK (1991) Localization of hepatocyte growth factor in human and rat tissues: an immunohistochemical study. Hepatology 14:488
35. Yoshinaga Y, Matsuno Y, Fujita S, Nakamura T, Kikuchi M, Shimosato Y, Hirohashi S (1993) Immunohistochemical detection of hepatocyte growth factor/scatter factor in human cancerous and inflammatory lesions of various organs. Jpn J Cancer Res 84:1150
36. Ishibashi K, Sasaki S, Sakamoto H, Nakamura Y, Hata T, Nakamura T, Marumo F (1992) Hepatocyte growth factor is a paracrin factor for renal epithelial cells: stimulation of DNA synthesis and NA, K-ATPase activity. Biochem Biophys Res Commun 182:960
37. Adams JC, Furlong RA, Watt FM (1991) Production of scatter factor by ndk, a strain of epithelial cells, and inhibition of scatter factor activity by suramin, J Cell Sci 98:385
38. Yoshinaga Y, Fujita S, Gotoh M, Nakamura T, Kikuchi M, Hirohashi S (1992) Human lung cancer cell line producing hepatocyte growth factor/scatter factor. Jpn J Cancer Res 83:1257
39. Tsao M, Zhu H, Giaid A, Viallet J, Nakamura T, Park M (1993) Hepatocyte growth factor/scatter factor is an autocrine factor for human normal bronchial epithelium and lung carcinoma cells. Cell Growth Differ 4:571
40. Rygaard K, Nakamura T, Spang-Thomsen M (1993) Expression of the proto-oncogene c-*met* and c-*kit* and their ligands, hepatocyte growth factor/scatter factor and stem cell factor, in SCLC cell lines and xenografts. Br J Cancer 67:37
41. Noda H (1986) Liver regeneration and the immune system III. Suppressor activity of splenic adherent cells activated in vivo by liver regeneration (in Japanese). J Jpn Surg Assoc 87:636