SF/HGF-c-Met autocrine and paracrine promote metastasis of hepatocellular carcinoma

Qian Xie, Kang-Da Liu, Mei-Yu Hu, Kang Zhou

Experimental Research Center of Zhongshan Hospital, Fudan University, Shanghai, 200032, China

Supported by Natural Science Foundation of China No.39970290

Correspondence to: Dr. Kang-Da Liu, Experimental Research Center, Zhongshan Hospital, Fudan University, 180 Fenlin Road, Shanghai 200032, China. kdliu@shmu.edu.cn

METHODS: SF/HGF and c-met transcription and protein expression in HCC were examined by RT-PCR and Western Blot in 4 HCC cell lines, including HepG2, Hep3B, SMMC7721 and MHCC-1, the last cell line had a higher potential of metastasis. sf/hgf cDNA was transfected by the method of Lipofectin into SMMC7721. SF/HGF and c-met antibody were used to stimulate and block SF/HGF-c-met signal transduction. Cell morphology, mobility, and proliferation were respectively compared by microscopic observation, wound healing assay and cell growth curve.

RESULTS: HCC malignancy appeared to be relative to its met-SF/HGF expression. In MHCC-1, c-met expression was much stronger than that in other cell lines with lower potential of metastasis and only SF/HGF autocrine existed in MHCC-1. After sf/hgf cDNA transfection or conditioned medium of MHCC-1 stimulation, SMMC7721 changed into elongated morphology, and the a bilities of proliferation (P<0.05) and mobility increased. Such bio-activity could be blocked by c-met antibody (P<0.05).

CONCLUSION: The system of SF/HGF-c-met autocrine and paracrine played an important role in development and metastas is potential of HCC. Inhibition of SF/HGF-c-met signal transduction system may reduce the growth and metastasis of HCC.

Subject Headings: hepatocyte growth factor/Scatter factor; c-met; hepatocellular carcinoma; metastasis

Xie Q, Liu KD, Hu MY Zhou K. SF/HGF-c-Met autocrine and paracrine promote metastasis of hepatocellular carcinoma. World J Gastroenterol, 2001;7(6):816-820

INTRODUCTION

The human proto-oncogene c-met encodes a Mr. 190, 000 heterodimeric transmembrane protein with structural features of a tyrosine kinase receptor and is expressed predominantly on epithelial cells. Its ligand HGF is a mesenchymal protein which is identical to Scatter factor (SF), a factor secreted by fibroblasts. It is well known that SF/HGF has a special binding with c-met, mainly attending mitogenic, motogenic and morphogenic effects on normal targeted cells[1-4]. Recently, its signal pathway has been explored a lot, including STAT in tubulogenesis[5], Gab-1 in cell growth[6-7], MAPK in morphogenesis[8,9], and PI-3K in cell mitogenesis[10,11]. Its relationship with ad hesive factors[12-16] and apoptotic factors[17,18] and the cross-talk between HGF/SF-c-Met and other growth factors[19] and proto-oncogenes[20] have also been discussed. However, in cancer research, controversial results have been found in various tumors. Some reports suggested that HGF/SF was potent stimulator for tumor proliferation and motility[21-28], some reports made exactly opposite conclusions[29-31]. Among all the tissues, situation in liver becomes the most complicated, since SF/HGF attends all the stages of liver growth, regeneration, cirrhosis and carcinoma. SF/HGF was first found as a serum derived factor that stimulated proliferation of primary liver cells, acted as one of the initial factors in liver regeneration[32-35] and reversed cirrhosis by suppressing the increased TGF betal, fibrogenesis and hepatocyte apoptosis[36]. It has been unexpectedly reported to inhibit the growth of hepatoma cell in many reports[37]. Little has been known about the mechanism.

We studied a HGF/SF autocrine hepatocellular carcinoma (HCC) cell line which has a high potential of metastasis and compared its characteristic with several other HCC cell lines which do not have these features. Gene transfection and other interfering methods were used to further demonstrate the SF/HGF autocrine and paracrine on malignant capability of HCC.

MATERIALS AND METHODS

Cells

SMMC7721, HepG2, and Hep3B human HCC cell lines were purchased from Shanghai Institute of Cell Biology, Chinese Academy of Science (Shanghai, China). MHCC-1 was a cell line with high potential of metastasis from a resected lesion of a HCC patient. Cells were cultured in DMEM supplemented with 100 mL·L-1 fetal calf serum or AB serum (MHCC-1), penicillin (100 kU·L-1), streptomycin (100 kU·L-1).

Collection of MHCC-1 conditioned medium (MHCC-1-CM)

Cells were planted in 100 ml culture bottles. When 80% cells were subconfluent, they were washed and replaced with serum free DMEM (0.1 mL·cm-2). Three to five days later, conditioned medium was collected, centrifuged under 2000 r·min-1 for 20 minutes and stored at -20°C until use.

Plasmid and DNA transfection

The plasmid pBS7.3 containing human full-length sf/hgf(kindly provided by Dr. George Vande Woode) was cloned into the BamHI-Apal site of the p cDNA3.0(+) mammalian expression vector. SMMC7721 HCC cell line was transfected overnight with the constructed plasmid, using lipofectamin (Gibco ). Cells were selected by G418. Colonies of cells were tryspinized within cloning rings, then they were transferred to 24-well dishes, and grown to confluency. After conditioned medium was changed to serum free DMEM for 3 d, both supponent and whole lysised cells were screened for SF expression (ELISA, R&D). The highest expressing...
clone (SF7721) was selected for further research.

RT-PCR
Total RNA was extracted (QIAGEN) and 1 µg was reversing transcribed in a 25 µL volume using SuperScriptII (Gibco), according to the manufacturer’s instructions. Five µL reverse transcription product was used for amplification with the following primers: SF: 5’-CAG CGT TGG ATT CTC AGT AT-3’, 5’-CTT CAG TTT GGT CTT GT T GGA-3’, c-met: 5’-ACA GTG GCA TGT CAA CAT CGC T-3’, 5’-GCT CGG TAC TC T ACA GAT TC-3’. Forty cycles were performed, each consisting of 95°C, 45 s; 60°C, 1 min. There was a time delay for 7 min at 72°C. The reaction products were visualized by 15 g·L-1 agarose gel electrophoresis.

Western blotting
Goat anti-human HGF antibody was purchased from R&D. Rabbit anti-human c-met anti body, anti-rabbit IgG-AP and anti goat IgG-AP were purchased from Santa Crus. Cells were washed with PBS, and lysed at 0°C for 30 min in lysis buffer (TrisCl 50 mmol·L-1 pH8.0, NaCl 150 mmol·L-1, NaN3 0.2 g·L-1, PMSF 100 mg·L-1, Aprotinin 2 g·L-1, TritonX-100 10 g·L-1). In c-met detection, protein content was examined using BCA Protein Assay (Pierce), and 20 µg protein per lane was electrophoresed on 80 g·L-1 SDS polyacrylamide gels after boiling for 5 min in 2X loading buffer. As to SF/HGF, conditioned medium was mixed with 2X loading buffer and added to 100 g·L-1 SDS polyacrylamide gels. Protein was blotted onto nitrocellulose membranes. After electroblotting, the membranes were blocked in PBS-50 mg·L-1 non-fat dry milk, washed with PBS-Tween buffer, and incubated with the primary antibody (1:500) diluted in blocking buffer for 2 h. Membranes were then washed, incubated with the appropriate second antibody (1:500) in blocking buffer for 2 h, and re-washed. Blotted membranes were stained with BICP-DAB (Huamei).

Cell scatter and morphology
5×103 cells of SMMC7721 and SF7721 were planted into the wells of 96-wells plates. Three replicated wells. Twenty-four hours later, wells of 96-wells plates. And 200 µL conditioned medium was replaced in the experimental group with 1 mL MHCC-1-CM or SM MC7721-CM, respectively. After 72 h incubation, 20 µL MTT (Methabenztiazauron, Serva Co, USA) was added into each well to a final concentration at 50 g·L-1. Four hours later, the medium with 100 µL DMSO was replaced, and asorbance (OD) was read under 540 nm (Bio-rad).

Inhibition assay by c-met antibody
2×105 MHCC-1 or SF7721 were planted in 96-wells plates, 3 replicated wells. After 24 h incubation, cells were washed by DMEM, and incubated in 50 µL c-met polyantibody within a concentration range of 20, 10, 5, 2.5, 0 mg·L-1. Rabbit IgG was used as control. Two hours later, 150 µL control medium was added into each well and 72 h later, cells were examined by MTT. As to SMMC7721, 106 cells incubated with 2 µg c-met antibody for 2 h. Afterward, cells were washed by D-Hanks and added into wells of 96-wells plates within 100 µL conditioned medium and 100 µL MHCC-1-CM or SM MC7721-CM, respectively. Twenty-four h later, cells were observed under microcope. Rabbit IgG was used as negative control.

Statistical analysis
In SMMC7721 cell proliferation experiments, Student’s t test was used to compare the difference of each corresponding dosage groups. Values were expressed as means ± standard. The cell growth curve and c-met blocking assay were tested by two-way anova.

RESULTS

SF and c-met expression in HCC cell lines (Figure 1)
RT-PCR showed that all the cell lines had the transcription and protein expression of c-met, in which MHCC-1 most actively expressed. Only MHCC-1 had the transcription and protein expression of SF/HGF to the medium.

**SF/HGF expression in SF7721**

After sf/hgf transfection, in SF7721 cell extracts, the highest expression of SF/HGF reached 692 µg·L⁻¹, compared with 0.026 µg·L⁻¹ in control cells. However, no SF/HGF was detected in conditioned medium.

**SF/HGF-c-met autocrine stimulate HCC malignancy**

After gene transfection, SF7721 cells displayed scattered distribution and elongated morphology (Figure 2A) together with increased ability of proliferation. The cell growth curve showed that after 8 days, cell number of SF7721 reached almost double of that of SMMC7721 (Figure 2C, \( P<0.05 \)). Also, the transfected SF7721 acquired stronger mobility. In “wound healing assay”, SF7721 moved faster than SMMC7721 cells into cell free area (Figure 2B). However, the c-met expression in SF7721 and SMMC7721 did not show great difference.

![Cell morphology comparison](image)

**Figure 2A** Cell morphology comparison. ×400

**Table 1** MHCC-1-CM stimulated proliferation of SMMC7721 (contrasted by SMMC7721-CM, \( \bar{x}±S\bar{x} \))

| Group             | Conditioned medium/culture medium (volume ratio) |
|-------------------|-----------------------------------------------|
|                   | 0:16                                         |
| SMMC7721-CM       | 0.596±0.090                                  |
| MHCC-1-CM         | 0.513±0.043                                  |
|                   | 1:8                                          |
| SMMC7721-CM       | 0.535±0.315                                  |
| MHCC-1-CM         | 0.581±0.080                                  |
|                   | 1:4                                          |
| SMMC7721-CM       | 0.568±0.099                                  |
| MHCC-1-CM         | 0.570±0.061                                  |
|                   | 1:2\(^b\)                                    |
| SMMC7721-CM       | 0.524±0.053                                  |
| MHCC-1-CM         | 0.620±0.033                                  |

\( ^bP<0.01, 0.541±0.377 \text{ vs } 0.853±0.031. \)

**Assay of C-met antibody blocking**

Both cell proliferation and motility could be blocked by c-met antibody. After 3-day’s incubation with c-met antibody, either MHCC-1 or SF7721 showed inhibition of growth, and was correlative with antibody concentration (Figure 3, \( P<0.05 \)). As to SMMC7721, the effect of inhibition could be seen under microscope, their shape turned round, and became shrank.

![C-met inhibition assay](image)

**DISCUSSION**

Metastatic dissemination of solid tumors is a complex pathophysiological process including various factors. When we started to research on a HCC cell line (M HCC-1) with high potential of metastasis, we found that it had a high expression of c-met and with SF/HGF autocrine, which did not exist in other cell lines without or with low potential of metastasis. Thus, it became a promising approach to discuss c-met-HGF/SF signal transduction and tumor metastasis. Our result showed that cell malignancy of HCC is relative to its SF/HGF-c-met expression. The c-met expression of cell line with a higher potential of metastasis is much stronger than those with lower potential of metastasis and appeared with SF/HGF autocrine. Previous studies reported that many cancer cells expressed HGF/SF and c-met in vivo, but few of carcinoma cell lines produced HGF/SF in vitro, indicating that SF/HGF is a negative regulator in tumor progression. To further elucidate the phenomena, we transfected SF/HGF cDNA into SMMC7721 cell line, trying to demonstrate that acquired SF/HGF autocrine may increase the malignancy and improve the metastatic potential in less metastatic cells. Our results showed that the proliferation, mobility and cell morphology had greatly changed in
SF7721 cells. Although the c-met expression in SF7721 cell did not increase significantly, it did improve greatly in SF7721 tumors in nude mouse assay, later in the in vivo research. (Data not shown) When SF/HGF-c-met system was blocked by c-met antibody, both MHCC-1 and SF7721 were blocked, demonstrating that SF/HGF-c-met was a positive regulator in HCC progression. Thus, we postulated that carcinoma cells may lose the ability to produce HGF/SF during in vitro passage, or the expression of HGF/SF need an activation from matrix. The high potential of metastasis of MHCC-1 may, to a great extent, contribute to its preservation of HGF/SF expression and keep the c-met activated all the way. In vivo, fibroblasts can produce HGF/SF, which may induce the expression of HGF/SF and c-met in cancer cells, thus establishing an autocrine and paracrine system and promoting cell scatter, proliferation and invasion[30-33]. We also studied the paracrine role of HGF/SF by stimulating SMMC7721 with MHCC-1-CM. Results were consistent with that from experiment of autocrine. Cell scatter, proliferation and mobility in SMMC7721 increased after they were stimulated by conditioned medium of M HCC-1. Such biological functions can be blocked by anti c-met polyclonal anti body. However, the influence of SMMC-7721 proliferation by the conditioned medium of MHCC-1 happened only when the medium was in 1:2 dilution, suggesting that the c-met receptor needs a certain amount of SF/HGF to activate. Once activated, the biological activity may depend on the quantity of c-met expression and the extent of receptor phosphorylation, thereby initiating downstream regulations. There were two reasons why we choose c-met rather than SF/HGF to be blocked. One was the conflicting reports of SF/HGF. Up to now, various of SF/HGF variants have been found, each having different structure and bioactivity in vitro and/or in vivo. This could be another reason why different results of SF/HGF are reported[40-47]. Compared with HGF/SF, c-met introduces many biological functions, but all the signal transductions start from a same ‘multifunctional docking site’[45-48]. The different biological functions come from different signal messages, thus making it a better choice compared with HGF/SF and other members of tyrosine kinase family. Recently, c-met inhibition has become a hot spot in anticancer research[1,2,4,10]. In our research, the tumor cells blocked by c-met antibody shrank in morphology and decreased in cell proliferation. Results suggested that the inhibition of met-SF/HGF could become one of the potential approaches to reduce tumor growth and metastasis. In conclusion, our experiment showed that the system of SF/HGF-c-met autocrine and paracrine play an important role in invasion and metastasis of hepatocellular carcinoma. Inhibition of c-met-H GF/SF system may reduce the proliferation and metastasis of hepatocellular carcinoma by lowering the expression of c-met or its downstream signal transduction.

ACKNOWLEDGMENTS Dr. George Vande Woude providing us PBS7.3 and Dr. Bo-Heng Zhang performing statistical analysis.

REFERENCES

1. Cao B, Su Y, Oskarsson M, Zhao P, Kort EJ, Fisher RJ, Wan LM, Vande Woude GF. Neutralizing monoclonal antibodies to hepatocyte growth factor/scatter factor (HGF/SF) display antitumor activity in animal models. Proc Natl Acad Sci USA, 2001; 98:7443-7448
2. Atabay N, Gao Y, Yao ZJ, Breckenridge D, Soon L, Soriano JV, Burke TR, Bottaro DP. Potent blockade of hepatocyte growth factor-stimulated cell motility, matrix invasion and branching morphogenesis by antagonists of Grb2 Src homology 2 domain interaction. J Biol Chem, 2001;276:14308-14314
3. Furge KA, Zhang YW, Vande Woude GF. Met receptor tyrosine kinase: enhanced signaling to the adapter proteins. Oncogene, 2000; 19:5568-5574
4. Shaharabany M, Abramovitch R, Kushner T, Tsarfaty G,avid-Megido M, Horev J, Ron J, Itzhak Y and Tsarfaty I. In vivo molecular imaging of met tyrosine kinase growth factor receptor activity in normal organs and breast tumors. Cancer Res, 2001; 61:4873-4878
5. Boccaccio C, Ando M, Tamagnone L, Bardelli L, Micheli P, Battistini C, and Comoglio PM. Induction of epithelial tubules by growth factor HGF depends on the STAT pathway. Nature, 1998;391:285-288
6. Gual P, Giordano S, Anguissola S, Parker PJ, Comoglio PM. Gab1 phosphorylation: a novel mechanism for negative regulation of HGF receptor signaling. Oncogene, 2001;20:156-166
7. Sachs M, Brohmann H, Zechner D, Muller T, Hulsken J, Walther I, Schaeper U, Birchmeier C, Birchmeier W. Essential Role of Gab1 for signaling by the c-Met in vivo. J Cell Biol, 2000;150:1375-1384
8. Monachini A, Hirono S, Tsukita S, Nakama T, Uto H, Hori T, Hayashi K, Tsuchiya H. Additive and inhibitory effects of simultaneous treatment with growth factors on DNA synthesis through MAPK and G1 cyclins in rat hepatocytes. Biochem Biophys Res Commun, 2001;280:368-373
9. Karhaloja A, O'Rourke DA, Nickel C, Spokes K, Cantley LG. Differential MAPK pathways utilized for HGF- and EGF-dependent renal epithelial morphogenesis. J Biol Chem, 2001;276:9166-9173
10. Day RM, Cioce V, Breckenridge D, Castagnino P, Bottaro DP. Differential signaling by alternative HGF isoforms through c-Met: activation of both MAP and PI 3-kinase pathways is insufficient for mitogenesis. Oncogene, 1999;18:3399-3406
11. Dong G, Chen Z, Li ZY, Yeh NT, Bancroft CC, Van Waes C. Hepatocyte growth factor/scatter factor-induced activation of MEK and PI3K signal pathways contributes to expression of proangiogenic cytokines interleukin-8 and vascular endothelial growth factor in head and neck squamous cell carcinoma. Cancer Res, 2001;61:5911-5918
12. Wielenga VJM, vaert Voort R, Taher TEI, Smit L, Beuling EA, van Krimpen C, Spaargaren M, Pals ST. Expression of c-met and heparan-sulfate proteoglycan forms of CD44 in colorectal cancer. Am J Pathol, 2000;157:1563-1573
13. Trusolini L, Serini G, Cecchinelli G, Besai C, Ambesi-Impic F, Marchisio PC, De Filippi R. Growth factor-dependent activation of av3 integrin in normal epithelial cells: implications for tumor invasion. J Cell Biol, 1999;142:1145-1156
14. House S, Jiang WC. Association of the HGF/SF receptor, c-met, with the cell-surface adhesion molecule, E-cadherin, and catenins in human tumor cells. Biochem Biophys Res Commun, 1999;261:406-411
15. Ried S, Jager C, Jeffers M, Vande Woude GF, Graef H, Schmitt M, Lavelle E. Activational mechanisms of the urokinase-type plasminogen activator promoter by hepatocyte growth factor/scatter factor. J Biol Chem, 1999;274:16377-16386
16. van der Voort R, Taher TEI, Wielenga VJM, Spaargaren M, Pervoo R, Smit L, David G, Hartmann G, Gherardi E, Pals ST. Heparan sulfate-modified CD44 promotes HGF/c-met-scatter factor-induced signal transduction through the c-Met receptor tyrosine kinase c-Met. J Biol Chem, 1999;274:6499-6506
17. Kitamura S, Kondo S, Shionomura Y, Kanayama S, Miyazaki Y, Kiyohara T, Hiraoka S, Matsuzawa Y. Met/HGF receptor modulates bel-w expression and inhibits apoptosis in human colorectal cancers. Br J Cancer, 2000;83:668-673
18. Xiao GH, Jeffers M, Bellaccosa A. Anti-apoptotic signaling by hepatocyte growth factor/Met via the phosphatidylinositol 3-kinase/Akt and mitogen-activated protein kinase pathways. Proc Natl Acad Sci USA, 1999;98:247-252
19. Jo M, Stolz DB, Esplin JE, Dorko K, Michalopoulous GK, Strom SC. Cross-talk between epithidal growth factor receptor c-Met signal transduction in transformed cells. J Biol Chem, 2000;275:8860-8861
20. Follenzi A, Bakovic S, Gual P, Stella MC, Longati P, Comoglio PM. Cross-talk between the p53proto-oncogenes Met and Ron. Oncogene, 2000; 19:3041-3049
21. Wong AST, Pelech SL, Woo MMM, Yim G, Rosen B, Ehlen T, Leung PCK, Auersperg N. Coexpression of hepsin and HGF as growth factor in ovarian carcinoma? An early step in ovarian carcinogenesis? Oncogene, 2001;20:1338-1348
22. Sower HM, Corps AN, Smith SK. Hepatocyte growth factor (HGF) in ovarian epithelial tumor fluids stimulates the migration of ovarian carcinoma cells. Int J Cancer, 1999;83:476-480
23. Teofili L, Di Febo AL, Periconi F, Maggiano N, Bendandi M, Rutella S, Cingolani A, Peroni GP, Reni M, Pileri S, Leone G, Larocca LM. Expression of the c-met proto-oncogene and its ligand, hepatocyte growth factor, in Hodgkin disease. Blood, 2001;97:1063-1069
24. Kataoka H, Hamasu R, Itoh H, Kitamura N, Koomo M. Activation of hepatocyte growth factor a/cotater/scatter factor in colorectal carcinoma. Cancer Res, 2000;60:6148-6159
25. Bredin CG, Liu Z, Hauzenberger D, Klominek J. Growth-factor-dependent migration of human lung-cancer cells. Int J Cancer, 1999;82:338-345
26. Camp BL, Rimm EB, Rimm DL. Met expression is associated with poor outcome in patients with an early lymph node negative breast carcinoma. Cancer, 1999;86:2259-2265
27. Presles I SC, Hooth MJ, Borchert KM, Coleman WB, Grisham JW,
Smith GJ. Establishment of a Functional HGF/C-MET Autocrine Loop in Spontaneous Transformants of WB-F344 Rat Liver Stem-Like Cells. *Hepatology*, 1998;28:1253-1259

Otsuka T, Takayama A, Sharp R, Celi G, LaRochele WJ, Bottaro DP, Ellmore B, Vieira W, Owens J W, Aner M, and Merlino G. c-Met autocrine activation induces development of malignant melanoma and acquisition of the metastatic phenotype. *Cancer Research*, 1998;58:5157-5167

Nakashiro K, Okamoto M, Hayashi Y, Oyasu R. Hepatocyte growth factor secretion by prostate-derived stromal cells stimulates growth of an androgen-independent human prostatic carcinoma cell line. *Am J Pathology*, 2000;157:7-803

Ronan D, Altstock RT, Firon M, Mittelman L, Sober T, Resau JH, Vande Woude GF, Tsarlaty I. Met-HGF/SF mediates growth arrest and differentiation in T47D breast cancer cells. *Cell Growth & Differentiation*, 1999; 10: 131-140

Von Schweinitz D, Faundez A, Teichmann B, Birnbaum T, Koch A, Hecker H, Gluer S, Fuchs J, Pietsch T. Hepatocyte growth factor/sca factor can stimulate post-operative tumor-cell proliferation in childhood hepatoblastoma. *Int J Cancer*, 2000; 85: 151-159

Matteucci E, Castoldi R, Desiderio MA. Hepatocyte growth factor induces pro-apoptotic genes in HepG2 hepatoma but not in B16-F1 melanoma cells. *J Cell Physiol*, 2001;186:387-396

Qadan LR, Perez-stable CM, Schwall RH, Burnstein KL, Liftsen RC, Howard GA, Roos BA. Hepatocyte growth factor and Vitamin D cooperatively inhibit androgen-unresponsive prostate cancer. *Endocrinology*, 2000;141:2567-2573

Shimizu M, Hara A, Okuno M, Matsuno H, Okada K, Ueshima S, Matsuo O, Niwa M, Akita K, Yamada Y, Yoshimi N, Uematsu T, Kojim S, Friedman SL, Dvorak H, Moni H. Mechanism of retarded liver regeneration in plasminogen activator-deficient mice: impaired activation of hepatocyte growth factor after Fas-mediated massive hepatic apoptosis. *Hepatology*, 2001;33:569-576

Stolz DB, Max DM, Petersen BE, Kim TH, Michalopoulos GK. Growth factor signal transduction immediately after two-thirds partial hepatectomy in the rat. *Cancer Res*, 1999;59:3954-3960

Ueki T, Kaneda Y, Tsutsui H, Nakanishi K, Saw a Y, Morishita R, Matsumoto K, Nakamura T, Takahashi H, Okamoto E, Fujimoto J. Hepatocyte growth factor gene therapy of liver cirrhosis in rats. *Am J Pathol*, 1999;5:226-230

Tokunou M, Niki T, Eguchi K, Iba S, Tsuda H, Yamada T, Matsuno Y, Kond o H, Saitoh Y, Hirohashi S. c-met expression in ovarian surface epithelial cell mitosis or apoptosis depending on presence or absence of an extracellular matrix. *Endocrinology*, 1999;140:290-2916

Blanquart F, Delany AM, Canalis E. Fibroblast growth factor-2 induces hepatocyte growth factor/scatter factor expression in osteoblasts.

Jakuubczak J, LaRochele WJ, Merlino G. NK1, a natural splice variant of hepatocyte growth factor/Scatter factor, is a partial agonist in vivo. *Mol Cell Biol*, 1998;18:1275-1283

Otsuka T, Jakuubczak J, Vieira O, Bottaro DP, Breckenridge D, Larochelle WJ, and Michalementia of M eutopey growth factor on c-Met bio-

Jiang WJ, Hsocus SE, Parr C, Martin TA, Matsumoto K, Nakamura T and Mansell RE. Antagonistic effect of NK4a onl hepatocyte growth factor variant, on in vitro angiogenesis of human vascular endothelial cells. *Clin Cancer Res*, 1999;5:3695-3703

Maehara N, Matsumoto K, Kuba K, Mizumoto K, Tanaka M, Nakamura T. NK4, a four-kringle antagonist of HGF, inhibits s preading and invasion of human pancreatic cancer cells. *Br J Cancer*, 2001;84:864-873

Kuba K, Matsumoto K, Ohashi K, Shiratsuchi T, Tanaka M, Nakamura T. Kringle 4-4of hepatocyte growth factor inhibits proliferation and migration of human microvascular endothelial cells. *Biochem Biophys Res Commun*, 2000; 279: 46-852

Bell A, Chen QY, DeFrances MC, Michalopoulos GK, Zarnegar R. The five amino acid-deleted isoform of hepatocyte growth factor promotes carcinogenesis in transgenic mice. *Oncogene*, 1999;18:887-895

Kuba K, Matsumoto K, Date K, Shimura H, Tanaka M, Nakamura T. HGF/NIK, a four-kringle antagonist of hepatocyte growth factor, is an angiogenesis inhibitor that suppress tumor growth and metastasis in mice. *Cancer Res*, 2000;60:6737-6743

Matsumoto K, Kataoka H, Date K, Nakamura T. Cooperative interaction between α- and β-chains on hepatocyte growth factor enhance receptor confers induced tyrosine phosphorylation and multiple biological responses. *J Biol Chem*, 1998;273:22913-22920

Tulasne D, Paumelle R, Weidner KM, Vandenbunder B, Fafure V. The multifunctional docking site of the Met receptor is dispensable for Met-mediated Ras signaling and cell scattering. *Mol Cell Biol*, 1999; 10: 551-565

Abounader R, Ranganathan S, Lal B, Fielding K, Book A, Dietz H, Burger P, Laterra J. Reversion of human glioblastoma malignancy by U small nuclear RNA/Ribozyme targeting of scatter factor/h epotyope growth factor and c-met expression. *Nat Cell Biol*, 1999; 91:1548-1556

Webb CP, Hose CD, Koochekpour S, Jeffers M, Oskarsson M, Sausville E, Monks A and Vande Woude GF. The Geldanamycins Are Potent Inhibitors of the Hepatocyte Growth Factor/Scatter Factor-Met-Oriki- nase Plasminogen Activator-Pl asmin Proteolytic Network. *Cancer Research*, 2000;60:342-349

Michieli P, Basilico C, Pennacchietti S, Maffe A, Tamagnone L, Giordano S, Bardelli A, Comoglio PM. Mutant Met-mediated trans- formation is ligand-dependent that can be inhibited by HGF antagonists. *Oncogene*, 1999;18:5221-5231

Edited by Xu XQ, Wang JH and Ma JY