Hepatocyte Growth Factor (HGF), HGF Activator, and c-Met in Synovial Tissues in Rheumatoid Arthritis and Osteoarthritis

MASAKAZU NAGASHIMA, JUN HASEGAWA, KO KATO, JUNJI YAMAZAKI, KEIKO NISHIGAI, TOSHIYUKI ISHIWATA, GORO ASANO, and SHINICHI YOSHINO

ABSTRACT. ***Objective.*** Hepatocyte growth factor (HGF) is a multifunctional polypeptide that has been implicated in cancer growth, tissue development, and wound repair. Its actions are dependent on activation by HGF activator (HGFA) and its binding to a specific HGF receptor (c-Met). We examined the role of HGF, HGFA, and c-Met in synovial tissues in rheumatoid arthritis (RA) and osteoarthritis (OA), and their localization and mRNA expression.

Methods. Immunohistochemical staining, Western blotting, RT-PCR, and *in situ* hybridization (ISH) for HGF, HGFA, and c-Met were performed on synovial tissue specimens from 10 patients with RA and 4 with OA, and 2 healthy controls.

Results. Immunohistochemical staining revealed that HGFA and c-Met were strongly expressed in fibroblasts, macrophages, endothelial cells, and synovial lining cells. HGF was expressed only faintly in macrophages and fibroblasts, and not at all in the endothelial cells of RA and OA synovial tissue. HGFA was detected near 73 and 34 kDa on Western blot analysis, corresponding to inactive and active HGFA, respectively. RT-PCR showed HGF, HGFA, and c-Met mRNA in RA, OA, and control synovial tissue. ISH and immunohistochemistry revealed mRNA expression for HGF, HGFA, and c-Met in the cell types mentioned above.

Conclusion. HGFA, HGF, and c-Met mRNA are expressed in synovial tissue in RA and OA, and HGF is activated by HGFA and binds to c-Met on endothelial cells, inducing angiogenesis. (J Rheumatol 2001;28:1772–8)

Key Indexing Terms:
HEPATOCYTE GROWTH FACTOR HEPATOCYTE GROWTH FACTOR ACTIVATOR
c-MET RHEUMATOID ARTHRITIS OSTEOARTHRITIS

Angiogenesis is thought to be an essential underlying process in chronic arthritis as well as in rheumatoid arthritis (RA) and osteoarthritis (OA). Angiogenic factors as well as acidic fibroblast growth factor, basic fibroblast growth factor, platelet derived endothelial growth factor, and vascular endothelial growth factor have been isolated in several organs in RA[1-6]. The hepatocyte growth factor (HGF) was initially purified from rat platelets as a promoter of liver regeneration[7]. It is considered to be associated with the hepatotrophic factor and scatter factor secreted by mesenchymal and endothelial cells, and has mitogenic, motogenic, and morphogenic activity on epithelial and endothelial cells. This growth factor has been shown to stimulate the growth of various epithelial cells, including gastrointestinal epithelial cells[8], renal tubular cells[9], epidermal melanocytes[10] and keratinocytes[11], and further, to stimulate the proliferation of endothelial cells[12]. HGF has been shown to stimulate the organization and reconstruction of tissues, suggesting it might play an important role in tissue repair[13].

Although HGF normally exists in its inactive single-chain form, it is converted to its active heterodimeric form in response to tissue injury. Miyazawa, *et al* identified this protease as the HGF-converting enzyme (HGF activator, HGFA) and isolated and purified it from human serum[14]. HGFA is the key enzyme that regulates the activity of HGF in injured tissues[15]. On the other hand, the HGF receptor, c-Met, which has been identified as the product of the c-met protooncogene, has been reported to be a specific transmembrane receptor[16,17]. It is mainly localized in epithelial cells and endothelial cells, suggesting that the HGF/c-Met signal plays a role in mesenchymal-epithelial interaction[18]. The localization of HGF and HGF receptor in the synovial tissue in RA has been reported[19], but it has not been reported

From the Department of Joint Disease and Rheumatism, and the Department of Pathology, Nippon Medical School, Tokyo, Japan.

Supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan.

M. Nagashima, MD, Associate Professor; J. Hasegawa, MD; K. Kato, MD; J. Yamazaki, MD; S. Yoshino, MD, Professor, Department of Joint Disease and Rheumatism; K. Nishigai, Assistant; T. Ishiwata, MD, Assistant Professor; G. Asano, MD, Professor, Department of Pathology.

Address reprint requests to Dr. M. Nagashima, Department of Joint Disease and Rheumatism, Nippon Medical School, 1-1-5 Sendagi, Bunkyo-ku Tokyo, 113-8603 Japan.
E-mail: Nagashima_Masakazu/rheum@nms.ac.jp

Submitted June 15, 2000; revision accepted February 5, 2001.

whether HGFA plays a role in endothelial cell proliferation and mRNA expression of HGF, HGFA, and c-Met in synovial tissue in RA.

To examine whether HGFA, HGF, and c-Met participate in angiogenesis or synovial cell proliferation, we investigated the localization and expression of HGFA, HGF, and c-Met using immunohistochemistry, Western blot analysis, reverse transcription-polymerase chain reaction (RT-PCR) analysis, and *in situ* hybridization.

MATERIALS AND METHODS

All patients and controls gave informed consent. The study was approved by the Human Ethics Committees of the Nippon Medical School.

Synovial tissue preparation. Tissue specimens were obtained from 10 patients with RA who fulfilled the diagnostic criteria of the American College of Rheumatology with a disease duration of 10–15 years, and from 4 patients with OA. For comparative analysis, we also obtained tissues from 2 healthy subjects.

Synovial tissue samples were obtained from patients with RA and OA during total knee joint arthroplasty. Normal synovial tissues were obtained from autopsy cases. The tissue samples were frozen in liquid nitrogen and stored until use for protein and RNA extraction. For histological examination, the tissues were fixed in 4% paraformaldehyde and embedded in paraffin, and paraffin sections were cut at a thickness of 3–4 μm.

Synovial cell preparation. Synovial cell preparation was as reported[20]. The synovial tissue was cut into small pieces, washed 3 times in phosphate buffered saline (PBS), and treated with 1 mg/ml collagenase (Sigma Chemical Co., St. Louis, MO, USA) for 30–60 min at 37°C. The cells were suspended in Ham F-12 medium (Nikken Bio Medical Laboratories, Kyoto, Japan) containing 10% fetal calf serum (FCS; Flow Laboratories, Burlingame, CA, USA), 100 U/ml penicillin, and 100 μg/ml streptomycin. The cell suspension was plated onto 90 mm culture dishes and cultured in a humidified 5% CO_2 incubator. When cell cultures reached confluence, synovial cells were treated with trypsin and passaged further on other dishes. Cells from passages 2 to 5 were used in the present experiments.

Immunohistochemistry. Highly specific goat anti-human HGF-α (C-20) polyclonal antibody (antibody raised against a peptide corresponding to amino acids 476–495 at the carboxyl terminus of HGF-α of human origin) and goat anti-human HGFA polyclonal antibody (raised against a peptide corresponding to an amino acid sequence mapped at the amino terminus of the long chain subunit of the precursor of human HGFA), and rabbit anti-human c-Met polyclonal antibody (raised against a peptide corresponding to an amino acid sequence mapped at the carboxy terminus of c-Met p140 of human origin) and anti-CD68 monoclonal antibody against macrophages were used for immunohistochemistry.

Paraffin embedded sections were subjected to immunostaining using the streptavidin-peroxidase technique. For anti-CD68 monoclonal antibody, tissues were preincubated in 0.1% trypsin for 60 min at 37°C. Endogenous peroxidase activity was blocked by incubation for 30 min with 0.3% hydrogen peroxide in methanol. Serial sections of the tissue were incubated for 10 min at room temperature with 10% normal rabbit for HGF-α and HGFA, and goat serum against c-Met and CD68, and incubated 2 h at room temperature with HGF antibody (1:75), HGFA antibody (1:100), c-Met antibody (1:300), and CD68 antibody (1:150) in PBS containing 1% bovine serum albumin (BSA). Bound antibodies were detected with biotinylated anti-rabbit or goat or mouse IgG secondary antibodies and the streptavidin-peroxidase complex, using diaminobenzidine tetrahydrochloride (DAB) as the substrate. Sections were counterstained with Mayer's hematoxylin. To confirm the specificity of the primary antibodies, HGF, HGFA, and c-Met were preincubated with a blocking peptide for these antibodies for 1 h and then applied to the sections, which did not yield positive immunoreactivity[21].

Western blot analysis. Synovial tissues were solubilized in lysis buffer containing 75 mM potassium phosphate buffer (pH 7.4), 75 mM KCl, and 100 μM Pefabloc SC. Fifty micrograms of proteins were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to immobilon transfer membranes (Millipore). Then the membranes were incubated 3 h with the same anti-human HGFA antibody used for immunohistochemistry. Membranes were washed and incubated with secondary horseradish peroxidase conjugated donkey anti-goat IgG antibody for 45 min. After washing, antibodies were visualized by DAB.

RT-PCR analysis. Total RNA extraction, cDNA synthesis, and RT-PCR of tissues and synovial cultured cells were performed as reported[6]. The sequences of the primers used were 271–299: 5′-CGCGGATCCATCCG-GCTGAAGAAGAAAGG-3′ and 607–634: 5′-CGGAATTCATGATGC-CGTAGAGGTAAGC-3′ for human HGFA cDNA[14]; 1071–1090: 5′-GAGCATGACATGACTCCTGA-3′ and 1335–1354: 5′-GGTTCCCA-GAAGATATGACG-3′ for human HGF cDNA[22]; and 3667–3686: 5′-TCTCGCTCCTGGGAATCTGC-3′ and 4035–4054: 5′-AAGG-ACCACACATCTGACTT-3′ for human C-Met[23]. Total RNA without reverse transcription was employed as a negative control.

In situ hybridization. The RT-PCR product for HGFA was subcloned into pGEM-T vector (Promega, Madison, WI, USA). Then the authenticity was confirmed by sequencing. The probes for HGFA were labeled with digoxigenin-UTP by SP6 or T7 RNA polymerase using the DIG RNA labeling kit (Boehringer-Mannheim, Mannheim, Germany). *In situ* hybridization was performed as reported[24]. To carry out *in situ* hybridization, tissue sections (4 μm thickness) were placed on superfrosted slides with adhesion coating (Matsunami Glass Ltd., Japan), deparaffinized, and incubated at room temperature for 20 min with 0.2 N HCl and at 37°C for 15 min with 40 μg/ml proteinase K. The sections were then postfixed for 5 min in PBS containing 4% paraformaldehyde, incubated 15 min twice with PBS containing 2 mg/ml glycine and once in 50% (v/v) formamide/2 × SSC for 1 h before initiation of the hybridization reaction. Hybridization was performed with 200 ng/ml of the indicated digoxigenin labeled riboprobe in a moist chamber for 16 h at 50°C.

The sections were then washed sequentially with 50% formamide/2 × SSC for 30 min at 55°C, 2 × SSC for 20 min at 55°C, and 0.2 × SSC for 20 min at 55°C. For immunological detection, the DIG nucleic acid detection kit was used. After the color reaction was stopped with TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0), the sections were washed briefly with water and fixed with 4% paraformaldehyde in PBS for 5 min. The sections were then counterstained with nuclear fast red and mounted in Malinol mounting medium (Mutoh Chemical Co., Tokyo, Japan).

RESULTS

Immunohistochemical findings. To confirm the localization of HGF, HGFA, and c-Met, immunohistochemistry was performed. In RA, HGFA and c-Met were localized in stromal cells and synovial lining cells (Figures 1A, 1B), whereas HGF was only weakly stained in these stromal cells (Figure 1D). Next, to confirm the histological type of the HGFA and c-Met positive cells, we investigated the localization of these polypeptides and CD68 (macrophage surface marker) in serial sections (Figure 1C). Some cells were positive for HGFA, c-Met, and CD68 (Figures 1A, 1B, 1C). On the other hand, in serial sections of the OA synovial tissue, whereas both HGF activator and c-Met were clearly localized in the fibroblasts, macrophages, synovial lining cells, vascular smooth muscle cells, and endothelial cells (Figures 2A, 2B), HGF was localized only in a production of synovial lining cells and stromal cells and not at all in most of the endothelial cells and vascular smooth muscle cells (Figure 2C).

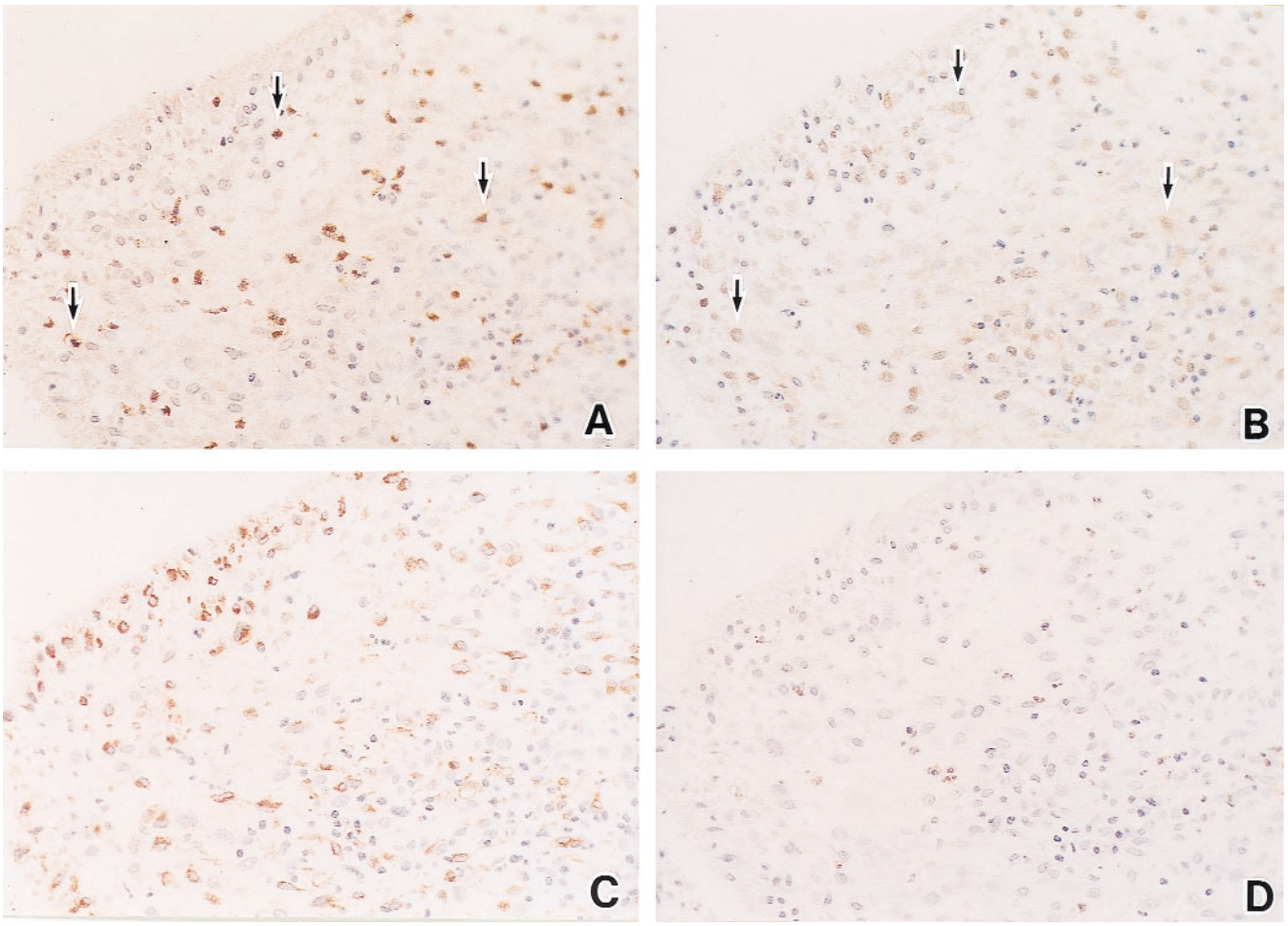

Figure 1. Immunohistochemical staining for HGFA, c-Met, HGF, and CD68 on serial sections of RA synovial tissue specimens. HGFA (A) and c-Met (B) were abundant on fibroblasts, macrophages, endothelial cells, and synovial lining cells. Arrows indicate cells positively stained for both HGFA and c-Met on serial sections. However, HGF was weakly stained (D) in stromal cells including fibroblasts and macrophages. Positive CD68 staining (C) was noted on macrophages. A: HGFA (×200), B: c-Met (×200), C: CD68 (×200), D: HGF (×200).

Western blot analysis. Western blot analysis was performed to detect HGFA polypeptide in the RA synovial tissue. As shown in Figure 3, inactive HGFA and active HGFA bands were detected near 73 kDa and 34 kDa in RA samples (lanes 2–6), respectively, while the sample from human placental tissue (lane 7) showed only one band corresponding to inactive HGFA.

RT-PCR analysis. Appropriate RT-PCR were performed to estimate the expression of HGFA, HGF, and c-Met mRNA. The PCR products of HGFA, HGF, and c-Met were recognized as being 337 bp, 284 bp, and 388 bp, respectively. HGFA and c-Met mRNA were detected in the RA and OA synovial tissue specimens and those from controls, but HGF mRNA was only faintly detected in all of these cases (Figure 4). On the other hand, HGFA, HGF, and c-Met mRNA were detected by RT-PCR in cultured RA synoviocytes as well as in synovial tissue as being 337 bp, 284 bp, and 388 bp, respectively (Figure 5).

In situ hybridization. *In situ* hybridization (ISH) was performed using serial sections to identify cells that overexpressed HGFA, HGF, and c-Met mRNA. HGFA mRNA was overexpressed in fibroblasts, endothelial cells, and macrophages of the RA synovial tissue specimens (Figures 6A, 6B). In the OA synovial tissues, HGFA mRNA was overexpressed in synovial lining cells and endothelial cells, consistent with the immunohistochemical findings (Figures 6C, 6D). ISH using a sense probe showed no positive signals (data not shown).

DISCUSSION

HGF is a kringle-containing polypeptide growth factor that possesses structural homology to plasminogen[25]. It has many roles in wound healing in various tissues and in cellular proliferation, cell motility, tissue organization, formation of duct-like structures, and neovascularization[26,27]. HGF is synthesized as pro-HGF and is then digested by a proteolytic enzyme named HGFA[14]. Mature HGF is a heterodimeric protein consisting of an α subunit

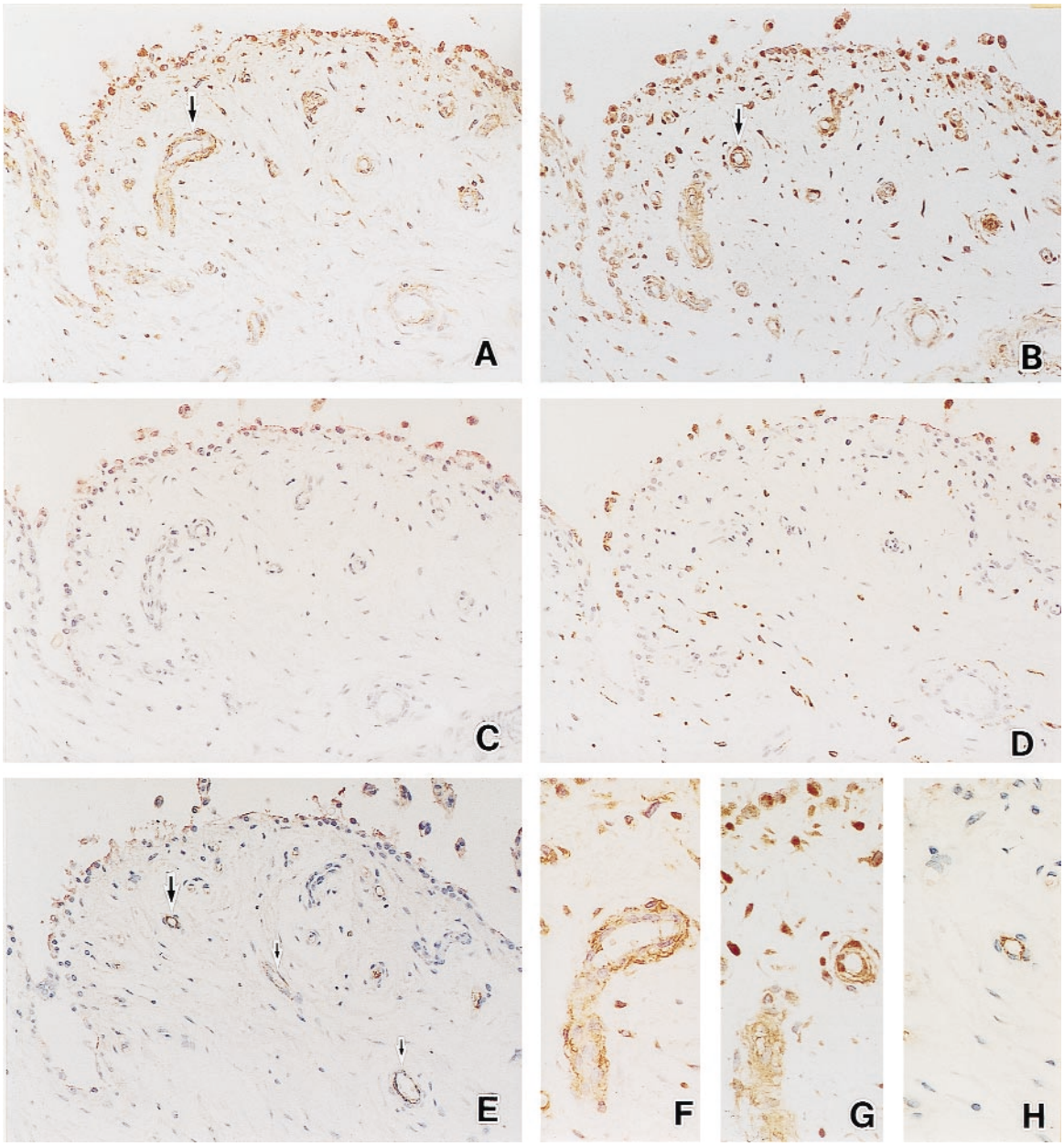

Figure 2. Immunohistochemical staining for HGFA, c-Met, HGF, CD68 and Factor VIII on serial sections of OA synovial tissue. Positive staining for HGFA, c-Met, and HGF was seen on synovial lining cells, macrophages, fibroblasts, vascular endothelial cells, and vascular smooth muscle cells (A, B), but no positive staining for HGF was seen on vascular endothelial cells (C). Arrows show the same vessels on serial sections; panels F, G, H show higher magnifications of those vessels. Arrows in E show vascular endothelial cells positively stained for Factor VIII. A and F: HGFA (×100, ×200), B and G: c-Met (×100, ×200), C: HGF (×100), D: CD68 (×100), E and H: Factor VIII (×100, ×200).

with M_r of 69 kDa and ß subunit of 34 kDa M_r joined by a disulfide bond, and possessing hydrophobic and signal peptides[28]. Only mature HGF has biological activity and its effects are dependent on its binding with the highly specific HGF receptor (c-Met) on the target cell membrane[16,17].

Yukioka, *et al* reported that HGF levels in synovial fluid specimens obtained from patients with RA were significantly higher than those in OA specimens, but they were unable to detect the HGF peptide in cultured synovial cells using ELISA[29]. This could be because lesser amounts of HGF are synthesized in synovial tissues compared to HGFA and c-Met. HGF could be synthesized in other organs or tissues, to be activated in the synovial tissue: positive staining for HGFA and c-Met was noted in synovial lining cells, endothelial cells, fibroblasts, and macrophages, but positive staining for HGF was noted in a limited number of fibroblasts and macrophages.

On the other hand, HGF stimulates proliferation of chon-

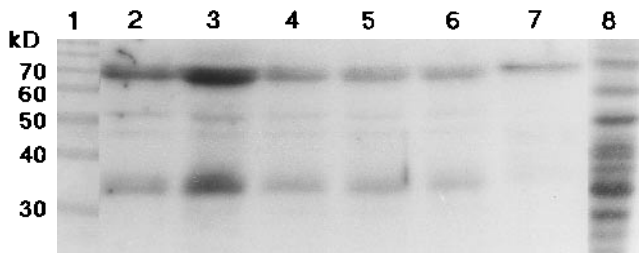

Figure 3. Western blot for HGFA polypeptides on RA synovial tissues. In all RA samples (lanes 2–6), 2 bands were detected near 70 kDa and 30 kDa, corresponding to inactive (73 kDa) and active states (34 kDa) of HGFA. Human placental sample (lane 7) showed only 73 kDa inactive band. Lane 1: size marker; lane 8: human monocytic leukemia cell lysate. HGFA protein from human monocytic leukemia cell lysate may be glycosylated and several bands were detected with no inactive or active forms.

drocytes and also has the ability to enhance proteoglycan synthesis in the chondrocytes of embryonic and 4-week-old rabbits[30]; it also stimulates the proliferation of blast cells synergistically with granulocyte-macrophage colony stimulating factor, yielding increased numbers of osteoclast precursors in a stromal-cell-free blast cell culture[31]. Grano, *et al* reported that osteoclasts synthesize and secrete biologically active HGF, and suggested the possibility of autocrine regulation of the osteoclasts by HGF and paracrine regulation of osteoblasts by the HGF produced by the osteoclasts[32].

HGFA is a serine protease that converts single-chain HGF to the active 2-chain form; it is present in the plasma as an inactive zymogen that is activated by the cleavage of the bond between Arg407 and Ile408 by thrombin[33]. HGFA is synthesized as an inactive 655 amino acid precursor that is activated to generate a 34 kDa heterodimer consisting of

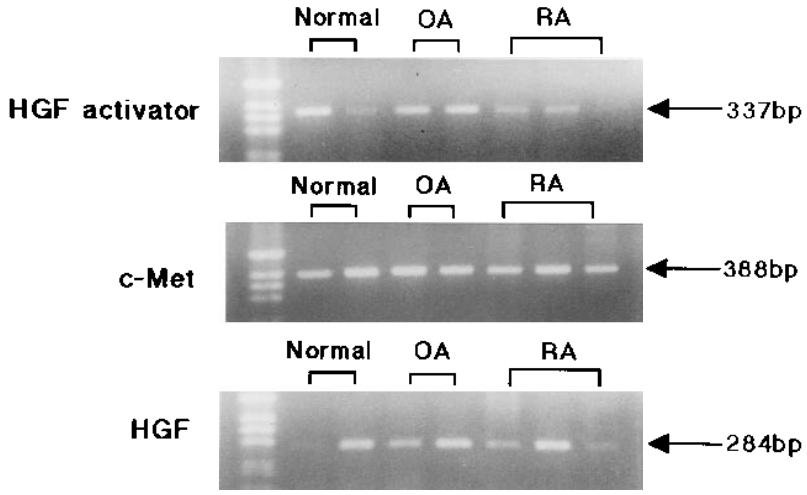

Figure 4. RT-PCR results for HGFA, HGF, and c-Met mRNA in synovial tissue specimens from patients with RA and OA and controls. PCR products for HGFA, HGF, and c-Met mRNA were electrophoretically separated by 2% agarose gel. Bands of HGFA mRNA, HGF mRNA, and c-Met mRNA of 337 bp, 284 bp, and 388 bp, respectively, are visible.

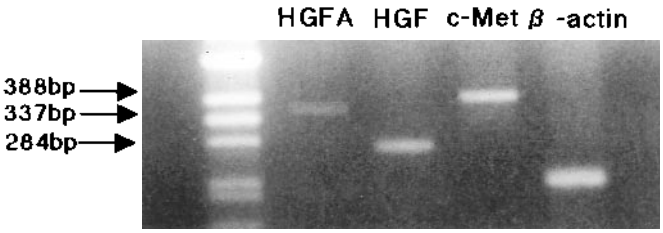

Figure 5. RT-PCR results for HGFA, c-Met, and HGF mRNA in cultures of RA synovial cells. PCR products for HGFA, HGF, and c-Met mRNA were electrophoretically separated by 2% agarose gel. All lanes show the size marker, HGFA mRNA, HGF mRNA, c-Met mRNA, and ß-actin as a positive control. Bands show HGFA mRNA 337 bp, HGF mRNA 284 bp, and c-Met mRNA 388 bp.

a 35 amino acid short chain and a 248 amino acid long chain joined by a disulfide bond[14,33]. We could reasonably suggest that the aforementioned phenomenon takes place in the synovial tissue, because thrombin-converting enzyme was detected in RA and OA synovial fluid specimens, and at a markedly higher level in RA than in OA specimens[34]. Thrombin receptors were also expressed in macrophage-like cells and fibroblasts in RA and OA synovial tissue[35]. Shimomura, *et al* reported that HGFA is homologous to blood coagulation factor XIIa and has the ability to activate single-chain HGF, although the specific activity of factor XIIa was slightly lower than that of HGFA[36].

Thrombin directly stimulates proliferation of fibroblasts and is mitogenic for fibroblasts, lymphocytes, endothelial cells, vascular smooth muscle cells, and monocytes, and also has an indirect effect on cellular growth by mediating the release of platelet derived growth factor[37]. It acts

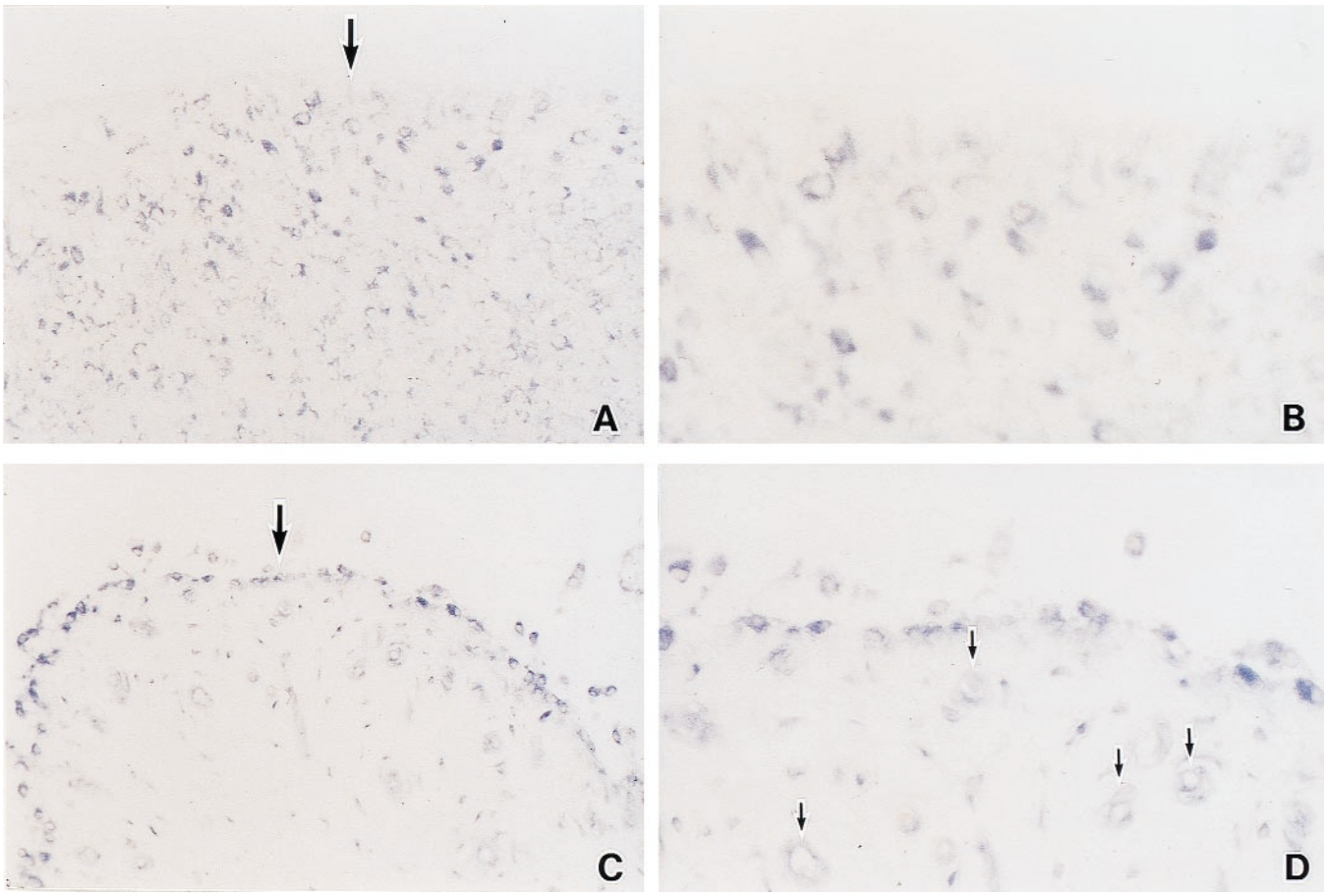

Figure 6. In situ hybridization for HGFA mRNA in RA and OA synovial tissue specimens. Positive signals are observed mainly in stromal cells (subsynovial lining cells) and partially in synovial lining cells in RA (A, B); in OA, positive signals are observed mainly in synovial lining cells, but not in subsynovial lining cells (C, D). Panels B and D show higher magnifications of areas indicated with arrows in A and C. Arrows in D show vessels (A: ×200, B: ×400, C: ×200, D: ×400).

together with basic fibroblast growth factor to stimulate the proliferation of vascular smooth muscle cells[38].

Immunohistochemical analyses have clarified that distributions of HGFA and c-Met are localized in the same cells, namely synovial lining cells, endothelial cells, and stromal cells, whereas HGF, while localized in fibroblasts and macrophages, was not detected in endothelial cells. On the other hand, according to RT-PCR and *in situ* hybridization, HGFA, HGF, and c-Met were expressed in both synovial tissues and cultured synoviocytes.

It is noted that synovial tissues from patients with RA recruit a greater number of macrophages and fibroblasts than those of patients with OA and healthy subjects, resulting in increased levels of HGFA, HGF, and c-Met in the synovial lining and subsynovial lining layers. However, it must be noted that synovial tissues were obtained from total knee arthroplasties in patients with RA, and may not represent the early stage of RA. Nonetheless, these results show that macrophages and fibroblasts in synovial and subsynovial lining layers may be the main sources of angiogenic growth factors. Our findings indicate that immature HGF partly synthesized in inflammatory synovial tissue was activated to mature HGF by HGFA, and binds to c-Met in the synovial lining cells and endothelial cells of synovial tissue, to act in a paracrine or autocrine manner.

REFERENCES

1. Esch F, Baird A, Ling N, et al. Primary structure of bovine pituitary basic fibroblast growth factor and comparison with the amino-terminal sequence of bovine brain acidic FGF. Proc Natl Acad Sci USA 1985;82:6507-11.
2. Thomas KA, Rios-Candelore M, Gimenez-Gallego G, et al. Pure brain-derived acidic fibroblast growth factor is a potent angiogenic vascular endothelial cell mitogen with sequence homology to interleukin 1. Proc Natl Acad Sci USA 1985;82:6409-13.
3. Ishikawa F, Miyazono K, Hellman U, et al. Identification of angiogenic activity and cloning expression of platelet-derived endothelial growth factor. Nature 1989;338:557-62.
4. Ferrara N, Henzel WJ. Pituitary follicular cells secrete a novel heparin-binding growth factor specific for vascular endothelial cells. Biochem Biophys Res Commun 1989;161:851-8.
5. Gospodalowicz D, Abraham JA, Schilling J. Isolation and characterization of a vascular endothelial mitogen produced by pituitary derived folliculostellate cells. Proc Natl Acad Sci USA 1989;86:7311-5.

6. Nagashima M, Yoshino S, Ishiwata T, Asano G. Role of vascular endothelial growth factor in angiogenesis of rheumatoid arthritis. J Rheumatol 1995;22:1624-30.
7. Nakamura T, Nawa K, Ichihara A. Partial purification and characterization of hepatocyte growth factor from serum of hepatectomized rat. Biochem Biophys Res Commun 1984;122:1450-9.
8. Takahashi M, Ota S, Shimada T, et al. Hepatocyte growth factor is the most potent endogenous stimulation of rabbit gastric epithelial cell proliferation and mitogen in primary culture. J Clin Invest 1995;95:1994-2003.
9. Igawa T, Kanda S, Kanetaka H, et al. Hepatocyte growth factor is a potent mitogen for cultured rabbit renal tubular epithelial cells. Biochem Biophys Res Commun 1991;174:831-8.
10. Matsumoto K, Tajima H, Nakamura T. Hepatocyte growth factor is a potent stimulator of human melanocyte DNA synthesis and growth. Biochem Biophys Res Commun 1991;176:45-51.
11. Matsumoto K, Hashimoto K, Yoshikawa K, Nakamura T. Marked stimulation of growth and motility of human keratinocyte by hepatocyte growth factor. Exp Cell Res 1991;196:114-20.
12. Bussolino F, Di Renzo MF, Ziche M, et al. Hepatocyte growth factor is a potent angiogenic factor which stimulates endothelial cell motility and growth. J Cell Biol 1992;119:629-41.
13. Gherardi E, Gray J, Stoker M. Purification of scatter factor, a fibroblast-derived basic protein that modulates epithelial interaction and movement. Proc Natl Acad Sci USA 1989;86:5844-8.
14. Miyazawa K, Shimomura T, Kitamura A, Kondo J, Morimoto Y, Kitamura N. Molecular cloning and sequence analysis of the cDNA for a human serine protease responsible for activation of hepatocyte growth factor. Structural similarity of the protease precursor to blood coagulation factor XII. J Biol Chem 1993;268:10024-8.
15. Miyazawa K, Shimomura T, Kitamura A. Activation of hepatocyte growth factor in the injured tissues is mediated by hepatocyte growth factor activator. J Biol Chem 1996;271:3615-8.
16. Gonzatti HM, Seth A, Patk M, Copeland T, Oroszlan S, Vande WGF. Characterization of the TPR-MET oncogene p65 and the MET protooncogene p140 protein tyrosine kinases. Proc Natl Acad Sci USA 1988;85:21-5.
17. Giordano S, Ponzetto C, Di Renzo MF, Cooper CS, Comoglio PM. Tyrosine kinase receptor indistinguishable from the c-Met protein. Nature 1989;339:155-6.
18. Weidner KM, Sachs M, Birchmeier W. The Met receptor tyrosine kinase transduces motility, proliferation, and morphologic signals of scatter factor/hepatocyte growth factor in epithelial cells. J Cell Biol 1993;121:145-54.
19. Koch AE, Halloran MM, Hosaka S, et al. Hepatocyte growth factor: A cytokine mediating endothelial migration in inflammatory arthritis. Arthritis Rheum 1996;39:1566-75.
20. Nagashima M, Yoshino Y, Aono H, Takai M, Sasano M. Inhibitory effects of antirheumatic drugs on vascular endothelial growth factor in cultured rheumatoid synovial cells. Clin Exp Immunol 1999;116:360-5.
21. Kleeff J, Ishiwata T, Friess H, Buchler MW, Israel MA, Korc M. The helix-loop-helix protein Id 2 is overexpressed in human pancreatic cancer. Cancer Res 1998;58:3769-72.
22. Weidner KM, Arakaki N, Hartmann G, et al. Evidence for the identity of human scatter factor and human hepatocyte growth factor. Proc Natl Acad Sci USA 1991;88:7001-5.
23. Park M, Dean M, Kaul K, et al. Sequence of MET protooncogene cDNA has features characteristic of the tyrosine kinase family of growth-factor receptors. Proc Natl Acad Sci USA 1987;84:6379-83.
24. Ishiwata T, Friess H, Buchler MW, Lopez ME, Korc M. Characterization of keratinocyte growth factor and receptor expression in human pancreatic cancer. Am J Pathol 1998; 153:213-22.
25. Nakamura T, Nishizawa T, Hagiya M, et al. Molecular cloning and expression of human hepatocyte growth factor. Nature 1989;342:440-3.
26. Matsumoto K, Nakamura T. Roles of HGF as a pleiotropic factor in organ regeneration. In: Gildberg ID, Rosen ER, editors. Hepatocyte growth factor-scatter factor (HGF/SF) and c-met receptor. Basel: Birhauser Verlag; 1993:225-50.
27. Matsumoto K, Nakamura T. Hepatocyte growth factor as a tissue organizer for organogenesis and regeneration. Biochem Biophys Res Commun 1997;239:639-44.
28. Gohda E, Tsubouchi H, Nakayama H, et al. Purification and partial characterization of hepatocyte growth factor from plasma of a patient with fulminant hepatic failure. J Clin Invest 1988;81:414-9.
29. Yukioka K, Inaba M, Furumitsu Y, et al. Levels of hepatocyte growth factor in synovial fluid and serum of patients with rheumatoid arthritis and release of hepatocyte growth factor by rheumatoid synovial fluid cells. J Rheumatol 1994;21:2184-9.
30. Takebayashi T, Iwamoto M, Jikko A, et al. Hepatocyte growth factor/scatter factor modulates cell motility, proliferation, and proteoglycan synthesis of chondrocytes. J Cell Biol 1995; 129:1411-9.
31. Sato T, Hakeda Y, Yamaguchi Y, et al. Hepatocyte growth factor is involved in formation of osteoclast-like cells mediated by clonal stromal cells (MC3T3-G2/PA6). J Cell Physiol 1995;164:197-204.
32. Grano M, Galimi F, Zambonin G, et al. Hepatocyte growth factor is a coupling factor for osteoclasts and osteoblasts in vitro. Proc Natl Acad Sci USA 1996;93:7644-8.
33. Shimomura T, Kondo J, Ochiai M, et al. Activation of the zymogen of hepatocyte growth factor activator by thrombin. J Biol Chem 1993;268:22927-32.
34. Nakano S, Ikata T, Kinoshita I, Kanematsu J, Yasuoka S. Characteristics of the protease activity in synovial fluid from patients with rheumatoid arthritis and osteoarthritis. Clin Exp Rheum 1999;17:161-70.
35. Morris R, Winyard PG, Brass LF, Blake DR, Morris CJ. Thrombin receptor expression in rheumatoid and osteoarthritic synovial tissue. Ann Rheum Dis 1996;55:841-3.
36. Shimomura T, Miyazawa K, Komiyama Y, et al. Activation of hepatocyte growth factor by two homologous proteases, blood-coagulation factor XIIa and hepatocyte growth factor activator. Eur J Biochem 1995;229:257-61.
37. Daniel TO, Gibbs VC, Milfay DF, Garavoy MR, Williams LT. Thrombin stimulates c-sis gene expression in microvascular cells. J Biol Chem 1986;261:9579-82.
38. Weiss R, Maduri M. The mitogenic effect of thrombin in vascular smooth muscle cells is largely due to basic fibroblast growth factor. J Biol Chem 1993;268:5724-7.