The hepatocyte growth factor/scatter factor (HGF/SF) receptor, met, transduces a morphogenetic signal in renal glomerular fibromuscular mesangial cells

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SUMMARY

Previous studies have demonstrated that hepatocyte growth factor/scatter factor (HGF/SF) is secreted by mesenchymal cells and that it elicits motility, morphogenesis and proliferation of epithelia expressing the met receptor. We now report that HGF/SF may act as an autocrine factor in fibromuscular renal mesangial cells. These cells mechanically support glomerular endothelia, control the rate of plasma ultrafiltration and are implicated in the pathogenesis of a variety of chronic renal diseases. We detected met protein in the vascular stalk of metanephric glomeruli and in the mature mesangium. Mesangial lines from a mouse transgenic for a temperature-sensitive simian virus 40 T antigen expressed met mRNA and protein, and recombinant HGF/SF phosphorylated the met receptor tyrosine kinase. Cells were immortal in the permissive condition and HGF/SF enhanced proliferation in a defined medium. In the absence of the immortalising protein, division ceased and recombinant HGF/SF caused multipolar cells to become bipolar. The factor diminished stress fibres, their focal contacts and immunostaining for extracellular fibronectin, hence suggesting reduced substratum adhesion and enhanced motility. Mesangial lines also expressed HGF/SF mRNA and secreted bioactive factor; immunocytochemistry showed both ligand and receptor in individual cells. HGF/SF blocking antibody aggregated the cells, suggesting that mesangial-derived factor affects basal cell conformation in an autocrine manner. We conclude that mesangial cells express both HGF/SF and met, and the factor induces morphogenesis of cultured mesangial cells. Therefore HGF/SF may have an autocrine role in mesangial biology but further studies are now required to investigate the potential importance of the factor in vivo.

Key words: glomerulus, HGF/SF, mesangial cell, met, kidney

INTRODUCTION

Experiments based on bioassays with cultured cells (Stoker et al., 1987; Nakamura et al., 1989; Montesano et al., 1991; Berdichevsky et al., 1994) and gene expression patterns (Sonnenberg et al., 1993) led to the conclusion that hepatocyte growth factor/scatter factor (HGF/SF) is secreted by mesenchymal cells and that it elicits motility, morphogenesis and proliferation of epithelia. These paracrine effects are mediated by the met receptor (Bottaro et al., 1991; Naldini et al., 1991; Weidner et al., 1993). Met is a heterodimer composed of an α (50 kDa), and a β (140 kDa) subunit that, upon binding HGF/SF, undergoes autophosphorylation (Longati et al., 1994) and transduces growth signals into the cell (Ponzetto et al., 1994). HGF/SF null-mutant mice die in utero due to impaired trophoblast growth and perturbed liver differentiation (Schmidt et al., 1995; Uehara et al., 1995). While these experiments demonstrate the importance of HGF/SF in mesenchyme-directed epithelial growth, other studies show that non-epithelial cells may express met (Rosen et al., 1994). Fibromuscular mesangial cells support glomerular capillary loops and modulate plasma ultrafiltration, the first step in urine formation (Kriz et al., 1990; Arendshorst and Navar, 1993). Mesangial cells are also implicated in the pathogenesis of renal diseases (Floegge et al., 1993). We have derived mesangial lines from a H-2K[b]-tsA58 mouse, transgenic for an interferon-γ (IFN-γ)-inducible, temperature-sensitive simian virus 40 T antigen (SV40 T Ag) (Ikram et al., 1994). They were immortal in the permissive condition and HGF/SF enhanced proliferation in a defined medium. In the absence of the immortalising protein, division ceased and recombinant HGF/SF caused multipolar cells to become bipolar. The same cells expressed HGF/SF mRNA and protein and secreted bioactive factor, while a blocking antibody to HGF/SF aggregated monolayers of the mesangial cells. This study demonstrates an autocrine effect of HGF/SF in cultured...
mesangial cells with implications for glomerular physiology and pathology.

MATERIALS AND METHODS

Derivation of mesangial cell lines

Unless otherwise stated, all reagents were obtained from Sigma (Poole, UK). Kidneys of 6 week old heterozygous H-2Kb-tsA58 mice were placed in Heps-buffered salts solution (pH 7.40; 118.0 mM NaCl, 4.7 mM KCl, 1.25 mM CaCl2, 1.25 mM MgCl2, 1.18 mM KH2PO4, 5 mM glucose, 5 mM glycine, 20 mM sodium cyclamate). Cortex slices were disaggregated at 37°C for 45 minutes in collagenase (Worthington type IV: 0.75 g/l) in a 1:1 (v/v) mixture of Heps solution and Ham’s F12/Dulbecco’s modified Eagle’s medium (DMEM) with 5 mg/l transferrin, 5 mg/l insulin, 50 nM hydrocortisone and 50 nM sodium selenite. Tubules were separated by centrifuging on a 50% isotonic Percoll density gradient (26,000 g for 30 minutes in a Beckman Ti60 rotor). The uppermost band of glomeruli and distal nephrons (White et al., 1992) was passed through 200-300 mesh sieves. Glomeruli were cultured in 6-well dishes in a 5% CO2/air atmosphere in DMEM with 5% (v/v) fetal calf serum (FCS), L-glutamine (4 mM), penicillin (104 i.u./l), streptomycin (104 units/l) and amphotericin (500 mg/l) in the permissive condition (33°C with 4x10^4 units/l murine IFN-γ). After 2 weeks outgrowths were dissociated with trypsin (10^6 units/l) and EDTA (0.02% in Ca2+ - and Mg2+-free DMEM). Four mycoplasma-free lines (G1-G4) were isolated by limiting dilution and investigated at passage 5-15.

Proliferation assays

Proliferation was examined at 33°C and 39°C, in the presence or absence of IFN-γ. Cells (5x10^3) were plated into 24-well plates and viable cells excluding trypan blue were counted after 4 days culture in DMEM with 5% FCS. Although low levels of HGF/SF may be present in some sera (Gherardi et al., 1989), the serum we used did not contain bioactive HGF/SF as assessed by the absence of scatter-activity and the inability to phosphorylate met in the assays described below (data not shown). In some experiments cells we used serum-free defined medium (DMEM supplemented with L-glutamine (4 mM), 5 mg/l transferrin, 5 mg/l insulin and 50 nM sodium selenite) and 20-200 pM recombinant HGF/SF purified by heparin affinity chromatography from supernatants of a mouse myeloma line (N50) transfected with mouse HGF/SF (Woolf et al., 1995).

Nitric oxide (NO) production

Cells (10^5/cm^2) were seeded into 24-well plates and incubated in DMEM with 5% FCS for 3 days at 39°C to inactivate the SV40 T Ag. They were washed with PBS and 0.5 ml of fresh medium added. NO synthase (NOS) was induced using lipopolysaccharide (LPS; 1 mg/l) and IFN-γ (10^3 units/l) while control cells received vehicle only. Conditioned medium was harvested at 0-48 hours for measurement of nitrite, the stable break-down product of NO (Marletta et al., 1988). In some experiments N^G-monomethyl-L-arginine (2 mM), a competitive inhibitor of NOS activity, or dexamethasone (300 nM), an inhibitor of NOS induction, were added. At no time was SV40 T Ag detected by immunocytochemistry (not shown). Nitrite was measured with Greiss reagent (Green et al., 1982). Absorbance was measured at 570 nm using an MR5000 microplate reader (Dynatech, Billingshurst, UK). The assay was linear over 1-100 μM using sodium nitrite as a standard.

RT-PCR for met and HGF/SF

RNA was extracted from tissues by the acid phenol-chloroform method (Chomczynski and Sacchi, 1987) and mRNA subjected to reverse transcription and PCR amplification (RT-PCR) as described (Woolf et al., 1995). Primers were: met sense primer nt 159-179 and antisense primer nt 866-886 of mouse c-met cDNA (Chan et al., 1988); HGF/SF sense primer nt 338-358 and antisense primer nt 876-896 of rat HGF/SF cDNA (Tashiro et al., 1990). Met and HGF/SF RT-PCR products were subcloned in Bluescript XL1 Blue (Stratagene) and sequencing confirmed their identities (not shown).

Immunoprecipitation and western blotting

Cells (2x10^6) were serum-starved for 12 hours and incubated with 300 pM HGF/SF for 30 seconds to 15 minutes. They were lysed in 1 ml of ice-cold RIPA buffer (PBS with 1% Nonidet P40, 0.5% sodium deoxycholate, 0.1% SDS, 1 mM phenylmethylsulphonyl fluoride, 10 mg/l aprotinin and 1 mM sodium orthovanadate), disrupted by aspiration through a 21 gauge needle and cooled on ice for 10 minutes. Debris was pelleted by centrifugation at 5,000 g at 4°C for 15 minutes and the lysate was precleared with 1.0 μg normal rabbit IgG and 20 μl of Protein A-agarose (Santa Cruz Biotechnology, Santa Cruz, CA, USA) followed by centrifugation at 2,500 g at 4°C for 5 minutes. Supernatant was mixed with 1.0 μg of anti-mouse c-met rabbit antiserum raised against the carboxy-terminal 21 amino acids of mouse met (Santa Cruz Biotechnology) and incubated for one hour at 4°C, followed by addition of 20 μl of Protein A-agarose and incubation overnight at 4°C. Immunoprecipitates were collected by centrifugation at 4,000 g at 4°C for 5 minutes. Pellets were washed with RIPA buffer, resuspended in 40 μl electrophoresis buffer (1 ml glycerol, 0.5 ml β-mercaptoethanol, 3 ml of 10% SDS, 1.25 ml 1M Tris-HCl (pH 6.7) and 1 mg bromophenol blue) and heat denatured at 100°C for 5 minutes. Proteins were separated by electrophoresis in a 6% SDS-polyacrylamide gel (Laemmli, 1970) and were transferred

Fig. 1. SV40 T Ag immunostaining in conditionally immortal mesangial cells. (A) In the permissive condition all cell nuclei stain for the SV40 T Ag. (B) After 4 days in the non-permissive condition the immortalising protein is undetectable. Bar, 40 μm
onto 0.45 μm nitrocellulose (Hybond-C Extra; Amersham, UK) (Matsudaïra, 1987). Membranes were incubated in blocking solution (5% non-fat milk in TBS) at 4°C overnight and reacted with phosphotyrosine (Upstate Biotechnology, USA) or met antibodies. Primary antibodies were detected with horseradish peroxidase-conjugated second antibodies and Enhanced Chemi-Luminescence reagent (Amersham, UK). Proteins were sized with Rainbow markers.

**Bioassays**

Cells were cultured at $10^5$ cells/cm$^2$ in DMEM with 5% FCS and conditioned medium and harvested after 2 days. Scattering bioactivity was measured using Madin-Darby canine kidney (MDCK) epithelia as target cells as described (Stoker and Perryman, 1985; Stoker et al., 1987; Woolf et al., 1995). In this assay, a titre of <2 indicates no bioactivity. Conditioned medium from the mouse D4-ras NIH3T3 line was a positive control (Gherardi et al., 1989). In some experiments the conditioned medium was incubated for one hour at room temperature with a neutralising goat antiserum against human HGF/SF (10 mg/l; R and D Systems Inc., MN, USA) before bioassay. This antibody shows not cross-reactivity with a wide ranges of

**Fig. 2.** Characterisation of mesangial cell lines. 
(A–C) Permissive condition; (D–I) non-permissive condition. 
(A and D) Staining with anti-smooth muscle actin antibody; 
(B and E) with anti-desmin antibody; and (C and F) with anti-myosin antibody. 
(G–I) Negative controls without first antibodies. Smooth muscle actin and desmin staining are more prominent in the non-permissive condition and cells are larger in the absence of the SV40 T Ag. Bars, 10 μm.
cytokines produced by mesangial cells (e.g. TGF-β, PDGF and VEGF) and the blocking ED50 is 2-4 mg/l for 1 nM recombinant human HGF/SF (R and D Systems data sheet). Our preliminary experiments (not shown) also established that this antibody blocked MDCK scattering induced by recombinant mouse HGF/SF and by D4-ras NIH3T3 conditioned medium. To study the effects of recombinant HGF/SF and its blocking antibody on mesangial shape, we plated cells at 10^4/cm^2 in DMEM and 5% FCS with recombinant HGF/SF (20-200 pM) or neutralising serum (10 mg/l), or non-immune IgG at the same concentration.

**Immunochemistry**

Cells were fixed for 2 minutes in 2% paraformaldehyde or acid-ethanol (95% (v/v) ethanol and 5% glacial acetic acid) or methanol. Primary antibodies were applied for 18 hours at 4°C: anti-desmin (Sigma, D8281 and D1033), anti-endothelial cell antigen (Serotec, MCA 762), anti-fibronectin (Sigma F-3648), anti-HGF/SF (raised in goat; R and D Systems Inc., MN, USA; raised in sheep, a gift from E. Gherardi, ICRF, Cambridge, UK), anti-mouse c-met (Santa Cruz Biotechnology), anti-myosin (Sigma, M7648 and M7786), anti-pancytokeratin (Sigma, C1801), anti-PECAM/CD31 (DAKO MR23), anti-smooth muscle actin (Sigma A2547 and DAKO, M851), anti-SV40 T Ag (Harlow et al., 1981) and anti-von Willebrand factor (Sigma). They were detected by FITC- or TRITC-conjugated second antibodies. For double staining of HGF/SF and met, paraformaldehyde-fixed mesangial cells were incubated with rabbit anti-met plus sheep anti-HGF/SF at 4°C for 18 hours. Secondary antibodies were applied sequentially (Texas Red-conjugated goat anti-rabbit IgG followed by FITC-conjugated donkey anti-sheep IgG) and examined at appropriate wavelengths with a laser scanning confocal microscope (Leica Lasertechnik GmbH, Heidelberg, Germany). Preliminary experiments showed that no significant signal was obtained upon omitting the primary antibodies and the met or HGF/SF primary antibodies are no longer immunoreactive after respective preincubation with met peptide (Santa Cruz Biotechnology) or recombinant HGF/SF. Paraformaldehyde-fixed 10 µm cryosections of non-transgenic embryonic and adult kidneys were incubated with the anti-met or anti-HGF/SF antisera as for immunocytochemistry. F-actin filaments were stained with FITC-conjugated phalloidin (Molecular Bioprobes, Oregon, USA).

**RESULTS**

**Isolation of conditionally immortal mesangial cells**

We isolated 4 lines (G1-4) that maintained similar phenotypes. Cells were maintained in the non-permissive condition to inactivate SV40 T Ag and were exposed to LPS to induce NOS. The graph shows nitrite production over the course of 48 hours (●); stimulation with LPS and addition of N^G^-monomethyl-L-arginine (▲); stimulation with LPS and addition of dexamethasone (■). Vehicle-treated cells (▼).

**Electron microscopy (EM)**

Cells were fixed in 3% glutaraldehyde in 0.1M sodium cacodylate and 5 mM NaCl (pH 7.4). Ultrathin sections were cut on an RTC MT6000 ultramicrotome using a Diatome diamond knife (Agar Scientific Ltd., Stansted, Essex, UK). Sections were stained with 25% uranyl acetate in 50% methyl alcohol and Reynold’s lead citrate, each for 20 minutes and grids examined with a JEOL 1200EX electron microscope.
to passage 15. All cell lines stained for SV40 T Ag in the permissive condition but this temperature-sensitive protein was absent in the non-permissive condition (Fig. 1). All lines expressed mesangial markers including smooth muscle actin, desmin, myosin (Fig. 2) and vimentin (not shown). Nitrite production was undetectable in unstimulated cells but levels rose significantly after stimulation with LPS and IFN-γ; an effect abolished by addition of Nω-monomethyl-L-arginine or dexamethasone (Fig. 3); these are physiological characteristics of mesangial cells (Shultz et al., 1994). Individual cells were multipolar and at confluence (Fig. 2) they lacked the cobblestone appearance of epithelial and endothelial cells. They were negative for cytokeratin, an epithelial marker, and for endothelial markers (von Willebrand factor, MCA 762 and anti-CD1; data not shown). Proliferation was dependant on the presence of the SV40 T Ag (Fig. 4), and cells appeared larger after withdrawal of the immortalising protein (Fig. 2). In the permissive condition, cells continued to proliferate without serum (Fig. 4). When the transgene was inactivated 5% FCS maintained viability but cells did not proliferate; in the non-permissive condition cells died in defined medium (Fig. 4). Thus we had isolated conditionally immortal mesangial cells.

**Mesangial cells express met**

Met transcripts were detected by RT-PCR in normal embryonic day 16 (E16) metanephros, in neonatal and in adult organs (Fig. 5). Western blot of kidney homogenates showed met protein at these times (data not shown). At E16, immunohistochemistry detected met in the vascular stalk within the crevices of primitive glomeruli (Fig. 6), an area that contains endothelial and putative mesangial cells (Saxen, 1987; Bernstein et al.,
The mature glomerulus is composed of mesangial, endothelial and epithelial cells. Met immunoreactivity was detected in mesangial cells that are multipolar and located in the centre of the glomerulus; they are surrounded by capillary loops (Fig. 6). A weaker signal was detected in other glomerular cells. As assessed by RT-PCR, cultured mesangial cells expressed met mRNA (Fig. 5). Met protein was detected in these cells by immunocytochemistry (Fig. 7), the 140 kDa met β-subunit was identified on a western blot and recombinant HGF/SF caused tyrosine phosphorylation (Fig. 8). In the presence of the SV40 T Ag, 20-200 pM HGF/SF significantly enhanced proliferation of mesangial cells in defined medium but the factor did not additionally increase cell number in the presence of serum. In the non-permissive condition HGF/SF was unable to prevent cell death in the serum-free medium (Fig. 4).

**HGF/SF changes mesangial cell shape**

Further experiments were performed in the absence on the immortalising SV40 T Ag when cells were not proliferating. Multipolar mesangial cells contained prominent F-actin stress fibres terminating in focal adhesions (Figs 9-11). Recombinant HGF/SF produced striking shape changes in all 4 lines. Cells in confluent monolayers separated (not shown) while cells at lower density became thin and bipolar, possessing lamellipodia and long processes at opposite poles (Figs 9 and 10). After 12-24 hours exposure to HGF/SF, F-actin filaments were maintained along the perimeter of cells, in the long processes and in the borders of the lamellipodia but after 48 hours F-actin staining decreased (Fig. 10). In the presence of recombinant HGF/SF the plasma membrane appeared irregular or ruffled and the cytoplasm contained prominent vacuoles (Fig. 11). No changes in immunostaining for desmin, myosin or smooth muscle actin were noted after HGF/SF was added (data not shown) but extracellular fibronectin was markedly reduced (Fig. 12). HGF/SF produced qualitatively similar effects in the permissive condition (data not shown).

**Cultured renal mesangial cells express HGF/SF**

HGF/SF mRNA was expressed by embryonic and adult kidney, and in the mesangial lines (Fig. 5), and a diffuse immunostaining was noted in adult glomeruli (not shown). HGF/SF was detected in cultured mesangial cells by immuno-
Fig. 9. Exogenous HGF/SF induces mesangial shape changes. Mesangial cells cultured in serum in the non-permissive condition. (A-D) Mesangial cell lines (G1-G4) at low density. Note the multipolar shape of the majority of cells. (E-H) The same cell lines, 24 hours after addition of 200 pM recombinant HGF/SF. The majority of cells are bipolar. Bar, 20 μm.

Fig. 10. Effects of HGF/SF on mesangial F-actin. (A-C) Phase-contrast photomicrographs; (D-F) corresponding FITC-phalloidin staining. (A and D) Mesangial cells in the non-permissive condition have prominent stress fibres. (B and E) After 12 hours exposure to 200 pM HGF/SF, cells elongated; F-actin was still present in the cell body and in long processes (large arrows). (C and F) After exposure for 24 hours, stress fibres were less prominent and cells became attenuated, often with a lamellipodium (small arrows) and long process at opposite poles. Bar, 15 μm.
cytochemistry and double-labelling demonstrated that the same cells expressed met (Fig. 7). Conditioned medium collected from confluent mesangial cells in the non-permissive condition scattered MDCK cells at a titre of 16-32 (Stoker et al., 1987), a bioactivity abolished by pre-incubation with 10 mg/l of HGF/SF neutralising antibody (data not shown). The D4-ras NIH3T3 line, which is known to secrete high levels of HGF/SF, produced a titre of 128-256. The addition of the above HGF/SF antiserum (10 mg/l) to low-density mesangial cells was associated with the formation of areas of aggregated cells after 24 hours, suggesting that HGF/SF of mesangial origin may contribute to basal cell conformation (Fig. 13). Unspecific pre-immune goat IgG had no effect (not shown).

**DISCUSSION**

**Fibromuscular mesangial cells express met**

We isolated 4 conditionally immortal mesangial lines from glomeruli of H-2Kb-tsA58 mice, which are transgenic for an inducible and temperature-sensitive SV40 T Ag (Ikram et al., 1994). The cells stained for desmin, myosin, smooth muscle actin and vimentin, and they made a fibronectin matrix. Vimentin and fibronectin are also expressed by renal fibroblasts but desmin and myosin are specific for mesangial cells in this context (Ishino et al., 1991). In addition, smooth muscle actin expression is characteristic of cultured mesangial cells (Elger et al., 1993). Finally, the lines exhibited an important physiological characteristic of mesangial cells (Shultz et al., 1994): they released NO when stimulated with LPS, and NO production was inhibited by dexamethasone or N\(^\circ\)-monomethyl-L-arginine.

Mesangial cells bind various growth factors that transduce signals via receptor tyrosine kinases. These include epidermal growth factor (EGF; Maxwell et al., 1993), fibroblast growth factor (Floge et al., 1993), insulin-like growth factor (Ohashi et al., 1993), nerve growth factor (Alpers et al., 1993), and platelet-derived growth factor (PDGF; Alpers et al., 1992; Floge et al., 1993). Our study demonstrates that mouse mesangial cells express met in vivo and in vitro. Although a
brief report suggested that cultured rat mesangial cells do not express *met* mRNA (Ishibashi et al., 1992), we suggest that in that study *met* may have been down-regulated after prolonged passaging. Alternatively, there might be a difference in expression between mouse and rat cells. Our study broadens the known range of non-epithelial targets for HGF/SF, which include nephrogenic mesenchymal cells (Woolf et al., 1995), haemopoietic progenitors (Galimi et al., 1994), skeletal muscle precursors (Bladt et al., 1995), endothelial cells (Grant et al., 1993) as well as neurons, chondrocytes and Kaposi’s Sarcoma cells (Rosen et al., 1994).

**Met transduces a morphogenetic signal in mesangial cells**

The effects of HGF/SF on epithelial cells in monolayer culture are well documented. The factor induces cell spreading followed by a loss of cell-cell contact and scattering (Stoker et al., 1987). Early effects include membrane ruffling and micropinocytosis in actin-rich lamellipodia (Dowrick et al., 1993), followed by a reduction in stress fibres (Dowrick et al., 1991; Ridley et al., 1995). Ras, and the related GTP-binding protein, Rac, mediate HGF/SF-induced spreading of MDCK cells, while activated Rho, which enhances stress fibres, prevents HGF/SF-induced scattering and motility in this model (Ridley et al., 1995). Microtubules have also been implicated in these effects (Prescott et al., 1992; Dugina et al., 1995). In the current study we found that addition of recombinant HGF/SF caused multipolar mesangial cells to become bipolar. This was accompanied by a decrease in stress fibres and focal contacts, changes consistent with the acquisition of motility (Stossel et al., 1992; Dugina et al., 1995). In the current study we found that addition of recombinant HGF/SF caused multipolar mesangial cells to become bipolar. This was accompanied by a decrease in stress fibres and focal contacts, changes consistent with the acquisition of motility (Stossel, 1993). HGF/SF also reduced immunostaining for extracellular fibronectin, which could result from decreased synthesis of this matrix molecule. Another explanation would be increased degradation of fibronectin resulting from HGF/SF-induced upregulation of proteases (Pepper et al., 1992). Fibronectin is a major component of the mesangial matrix and its loss could contribute to reduced adhesion to the substratum. Elevations of cAMP induced by isoproterenol also cause dissolution of mesangial stress fibres and extension of processes (Kreisberg et al., 1985); although these effects resemble those induced by HGF/SF they occur in minutes while the HGF/SF-induced changes take 12 hours.

**Mesangial cells express HGF/SF**

We found that cultured mesangial cells expressed HGF/SF

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**Fig. 12.** HGF/SF reduces mesangial fibronectin. (A) Mesangial cells show immunostaining for extracellular fibronectin. (B) After 2 days exposure to HGF/SF, staining is reduced. (C) No significant staining after omission of first antibody. Bar, 50 μm.

**Fig. 13.** Anti-HGF/SF antibody alters shape of mesangial cells. (A) Cells in the non-permissive condition. (B) Blocking antibody to HGF/SF (10 mg/l) causes aggregation of cells after 24 hours. Bar, 20 μm.
mRNA and secreted bioactive factor; the titre was, however, an order of magnitude below that produced by D4-ras NIH3T3 fibroblasts (Gherardi et al., 1989). Our observation accords with a report in which human mesangial cells released immunoreactive HGF/SF (Couper et al., 1994); bioactivity was not measured in that study. Mesangial cells mechanically support glomerular endothelial cells, a role analogous to that of vascular smooth muscle cells (Kriz et al., 1990). It is therefore of note that both smooth muscle cells (Rosen et al., 1989) and mesangial cells secrete HGF/SF. Mesangial cells grown in tissue culture do not form tight colonies characteristic of epithelia and endothelia. We found that a neutralising antibody to HGF/SF enhanced cell aggregation, hence suggesting that endogenously produced factor may alter cell shape by an autocrine mechanism. The effect of mesangial-derived factor, however, cannot be maximal because the addition of 200 pM HGF/SF produced changes of shape in all 4 cell lines. This concentration was used because it produces a maximal effect in various bioassays: for comparison, cultured fibroblasts secrete 10-100 pM (Stoker et al., 1987). The ability of one type of cell to express both met and HGF/SF is unusual, but occurs in renal mesenchymal cells (Woolf et al., 1995) and the ndk epithelial strain (Adams et al., 1991). When Tsarfaty and colleagues (1994) genetically engineered fibroblasts to coexpress HGF/SF and met, the cells acquired some markers of epithelia. Our current study, however, shows that the expression of both genes in a cell does not inevitably lead to an epithelial phenotype.

Possible roles of the mesangial HGF/SF-met axis in health and disease

HGF/SF produced by renal mesenchyme supports cell survival and epithelial differentiation in serum-free metanephric organ culture (Woolf et al., 1995) but early nephrogenesis appears to be normal in mice with homozygous null mutations of HGF/SF (Schmidt et al., 1995; Uehara et al., 1995) or met (Bladt et al., 1995). A caveat here is that the renal phenotype of mice with null mutations of another receptor tyrosine kinase, the EGF receptor, is highly dependent on the genetic background. The ndk epithelial strain (Adams et al., 1991). When Tsarfaty and colleagues (1994) genetically engineered fibroblasts to coexpress HGF/SF and met, the cells acquired some markers of epithelia. Our current study, however, shows that the expression of both genes in a cell does not inevitably lead to an epithelial phenotype.

production of HGF/SF by mesangial cells represents another renal source of the factor. Mesangial cells mechanically support glomerular endothelial cells (Kriz et al., 1990) and in vitro they contract in response to a wide variety of bioactive agents such as angiotensin. It has been argued that if similar shape changes were to occur in vivo, then these cells could control the rate of glomerular ultrafiltration (Arendshorst and Navar, 1993). Since HGF/SF alters the shape of cultured mesangial cells, future experiments should investigate whether this factor is capable of modulating the glomerular ultrafiltration coefficient in whole animals.

In a variety of human and animal glomerular diseases such as diabetic nephropathy and immune-mediated disorders (the glomerulonephritides) growth factors cause excessive mesangial proliferation and matrix production (Floge et al., 1993; Border et al., 1990). It is therefore of note that human mesangial cells release increased immunoreactive HGF/SF when cultured in high glucose medium, a model that mimics diabetes mellitus (Couper et al., 1994). Furthermore, in immune-mediated mesangiocapillary glomerulonephritis mesangial cells extend fine processes around the perimeter of capillary loops, hence reducing the rate of production of glomerular filtrate (Nakamoto et al., 1992); from our in vitro observations, HGF/SF should be regarded as a potential candidate for producing this effect. Rat platelets are rich in HGF/SF and they could be another important local source of the factor because they infiltrate the glomerulus in many inflammatory disorders; human platelets, however, make less, if any, HGF/SF and are therefore unlikely to play a similar role in patients with glomerulonephritis (Kamura et al., 1989). Finally, it is possible that HGF/SF from mesangial cells could have paracrine effects on the growth of glomerular epithelial cells, which are known to express met (Ishibashi et al., 1992). It would now seem appropriate to study the in vivo expression of HGF/SF in inflammatory and metabolic glomerular diseases. If HGF/SF could be implicated in these disorders it might be possible to modulate the course of such diseases by, for example, the administration of neutralising antibodies (Border et al., 1990).

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