Original Article

Hepatocyte growth factor and its receptor Met are induced in crescentic glomerulonephritis

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Abstract

Background. In experimental extracapillary glomerulonephritis (EG) podocytes migrate, proliferate and change phenotype, and play a pivotal role in crescent formation. Hepatocyte Growth Factor (HGF) is an injury-induced effector of tissue repair that causes cell migration, growth and transdifferentiation via its receptor Met.

Methods. In 11 patients with EG we measured serum levels of HGF and investigated whether serum induces the release of HGF by Peripheral Blood Mononuclear Cells (PBMC). In renal biopsies we studied the expression of Met. In cultured podocytes we studied Met expression, migration, growth and morphological changes induced by recombinant (r) HGF.

Results. In patients with EG average serum levels of HGF (0.73 ng/ml) were higher than in normal volunteers (N, 0.10 ng/ml, p <0.01) and in patients with non-crescentic glomerular disease (GD, 0.18 ng/ml, p <0.01). Serum of EG induced a significant HGF release by PBMC (mean 0.58 ng/ml) in comparison with serum of N and GD (0.07 and 0.06 ng/ml, respectively, both p<0.001). Met was strongly expressed in crescents. Cultured podocytes expressed Met, and rHGF induced in podocytes a time- and dose-dependent migration, growth and epithelial to mesenchymal transdifferentiation.

Conclusions. These results suggest that HGF/Met system participates in the process of crescent formation by inducing podocyte migration, growth and mesenchymal transformation.

Keywords: crescentic glomerulonephritis; hepatocyte growth factor; podocytes; scatter factor

Introduction

Extracapillary glomerulonephritis (EG) is a severe form of glomerular disease, with a rapid progression to renal failure. The histological marker of EG is the glomerular crescent that originates from proliferation of glomerular epithelial cells. Recent studies in experimental models of EG have shown that podocytes play a pivotal role in crescent formation. In fact, in EG podocytes detach from the glomerular basement membrane (GBM), assume a migratory phenotype and trigger crescent formation by establishing bridges between the tuft and Bowman's capsule [1]. Furthermore, cells derived from migrated podocytes proliferate and represent a substantial part of the crescent cell population [2]. Pathophysiological features of EG include de novo glomerular expression of Hageman's factor, production of urokinase-type plasminogen activator by proliferating glomerular epithelial cells and the transdifferentiation of crescentic cells to a myofibroblast phenotype.

Hepatocyte growth factor (HGF), also known as scatter factor, and its receptor, the product of Met oncogene, represent a system with pleiotropic activities, which include a potent mitogenic effect, detachment of cells from their substrate, induction of cell migration and transformation of cells from epithelial to fibroblast-like phenotype [3]. Experimental and human studies have shown that HGF is released in organs acutely injured by toxic, inflammatory or mechanical insults. In addition, as yet unidentified substances released in serum from the damaged tissue induce HGF production in distant intact organs [4]. HGF is produced as an inactive precursor that is enzymatically cleaved to the active form by urokinase [5] and coagulation factor XIIa [6]. The mitogenic, motogenic and angiogenic effects of HGF and its prompt local and systemic release after tissue injury HGF as an effector of tissue repair, acting both in paracrine and endocrine modes.
However, HGF has a potential fibrogenic effect that may result in deposition of collagen and sclerosis [7]. The prototypic producers of HGF are fibroblasts, but we have shown that HGF is released also by mesangial cells and by peripheral blood mononuclear cells (PBMC) [8,9]. The target cells of HGF, expressing HGF receptor Met, include cells of epithelial lineage, for example renal tubular cells, and monocytes.

We reasoned that HGF/Met may be involved as culprit in crescentic glomerulonephritis because (i) activated podocytes that participate in crescent formation undergo changes (migration and growth) that are characteristically induced by HGF, (ii) resident glomerular cells (i.e. mesangial cells) and invading monocytes are potential intraglomerular producers of HGF, (iii) coagulation proteases that cleave pro-HGF to its active form are expressed in the crescent and (iv) HGF induces epithelial–mesenchymal transformation. In addition, EG is a disorder with an acute, necrotizing inflammatory presentation in which we expect that, in analogy with what occurs in other acute injuries like acute hepatitis [10], tubular necrosis [8] and peritonitis [7], substances are released in serum that induce systemic HGF production. Based on this rationale, we investigated whether HGF/Met participate in the inflammatory process associated with glomerular crescent formation.

Subjects and methods

General design of the study

In patients with biopsy-proven EG with cellular crescents we measured serum levels of HGF and tested whether the serum acquires a HGF-inducing activity, i.e. stimulates HGF release by cultured PBMC. Serum of normal volunteers (N) and of patients with glomerular disease without extracapillary proliferation (GD) was used as control. In renal biopsies we investigated by immunohistochemistry the expression of Met, CD 68 (monocyte marker) and cytokeratin (epithelial marker). Biopsies from GD and renal tissue obtained from the normal part of tumoral kidneys were used as control.

Patients and normal volunteers

The study was performed in 11 patients with EG (mean age: 49.3 ± 6.9 years; mean creatinine clearance: 15.6 ± 5.1 ml/min) whose clinicopathological features are illustrated in Table 1. Nine patients with GD (one Alport’s syndrome, two idiopathic membranous glomerulonephritis, three diabetic nephropathy, two membranoproliferative glomerulonephritis and one renal amyloidosis) matched for age (52.1 ± 4.6 years) and renal function (creatinine clearance: 18.3 ± 3.2 ml/min) and six normal volunteers (age: 44.3 ± 7.5 years; creatinine clearance: 106.3 ± 8.9 ml/min) were used as control.

HGF serum levels

Blood (5 ml) was centrifuged soon after collection and serum was stored at −80°C. HGF was measured in serum by a commercial enzyme immunoassay (EIA) (R&D Systems, Minneapolis, MN, USA) that we have already described in detail [9]. The EIA detects 0.1 ng/ml of purified HGF and displays no cross-reactivity with a wide variety of cytokines.

HGF-inducing serum activity

In serum stored at −80°C we investigated the presence of a HGF-inducing activity. We tested the activity of serum on cultured PBMC collected from normal volunteers, because PBMC express Met and release HGF and monocytes are a potential source of HGF in the crescent. PBMC were separated by Hystopaque gradient (Sigma-Aldrich Co., St Louis, MO, USA) and cultured in suspension (2 × 10^6/ml) in Iscove's (Sigma-Aldrich) containing 100 IU/ml penicillin (Sigma-Aldrich) and 100 μg/ml streptomycin (Sigma-Aldrich) to which 5% decomplemented fetal calf serum (FCS; Sigma-Aldrich) was added. Haemocytometry yielded 80–83% lymphocytes, 11–12% monocytes and 4–6% neutrophils. Cell viability was tested by trypan blue (Sigma-Aldrich) exclusion test and yielded 96–98% viable cells. We conditioned PBMC in culture with three distinct pools of sera obtained by mixing 2-ml aliquots of serum from

Table 1. Clinicopathological features of patients with crescentic glomerulonephritis

<table>
<thead>
<tr>
<th>Disease</th>
<th>Glomeruli with crescents (%)</th>
<th>Glomeruli with necrosis (%)</th>
<th>Extrarenal involvement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wegener’s granulomatosis</td>
<td>100</td>
<td>100</td>
<td>Alveolar haemorrhage</td>
</tr>
<tr>
<td>Wegener’s granulomatosis</td>
<td>25</td>
<td>12</td>
<td>None</td>
</tr>
<tr>
<td>Wegener’s granulomatosis</td>
<td>56</td>
<td>56</td>
<td>Neuritis</td>
</tr>
<tr>
<td>Wegener’s granulomatosis</td>
<td>100</td>
<td>86</td>
<td>Necrotizing pneumonitis</td>
</tr>
<tr>
<td>Wegener’s granulomatosis</td>
<td>82</td>
<td>76</td>
<td>Sinusitis</td>
</tr>
<tr>
<td>Wegener’s granulomatosis</td>
<td>100</td>
<td>89</td>
<td>Lung granuloma</td>
</tr>
<tr>
<td>Necrotizing IgA nephropathy</td>
<td>85</td>
<td>35</td>
<td>None</td>
</tr>
<tr>
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<td>46</td>
<td>12</td>
<td>None</td>
</tr>
<tr>
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<td>94</td>
<td>87</td>
<td>Coeliac disease</td>
</tr>
<tr>
<td>Necrotizing IgA nephropathy</td>
<td>64</td>
<td>34</td>
<td>None</td>
</tr>
<tr>
<td>Anti-GBM Ab glomerulonephritis</td>
<td>89</td>
<td>24</td>
<td>Alveolar haemorrhage</td>
</tr>
</tbody>
</table>

In cultured human podocytes we studied by immunocytochemistry and western blot the expression of Met. In addition, we conditioned podocytes with recombinant HGF to investigate whether HGF induces their proliferation, movement and epithelial to mesenchymal transition.
single subjects with EG, GD and N. Serum was added to culture medium at a 5% concentration. Experiments were repeated six times with each pool of serum. The supernatant of PBMC, collected after 48 h of treatment with serum, was stored at −80°C until being processed for measurement of HGF with the same EIA described above.

**Immunohistochemical studies**

Renal biopsies and samples of normal renal tissue obtained from kidneys surgically excised for tumour were fixed in 6% formalin acetate for 48 h and then embedded in paraffin wax. Three μm thick sections were mounted on poly-L-lysine-coated glass slides (Dako, Glostrup, Denmark), dewaxed, rehydrated and subsequently exposed to microwave antigen retrieval (two cycles of 5 min each in citrate buffer 0.01 mol/l, pH 6.0, at 450 watts). Endogenous peroxidase was blocked with H₂O₂ 3.7% vol/vol in ethanol 95% for 10 min at room temperature. After three washings in 150 mmol/l phosphate-buffered saline (PBS; Sigma-Aldrich), the sections were exposed in a dark humid chamber overnight at 4°C to primary antibodies (Ab): monoclonal anti-human Met Ab (clone 8F11; Novocastra Laboratories, Ltd, Newcastle, UK) and monoclonal anti-cytokeratin Ab (CAM 5.2; Becton Dickinson, San José, CA, USA) diluted 1:2 v/v in PBS/BSA 1%. Monoclonal anti-human CD68 Ab (clone PG-M1, Dickinson, San José, CA, USA) diluted 1:2 v/v in PBS/BSA 1%. Monoclonal anti-human CD68 Ab (clone PG-M1, Dako) diluted 1:100 v/v in PBS/BSA 1%. The reaction product was detected with an avidin–biotin–peroxidase complex LSAB³ Kit (Dako) and the colour reaction was developed with 3,3-diaminobenzidine (Dako) and dehydrated in increasing alcohol scale (95° to 100°, xylol) and the coverslip was mounted with synthetic non-aqueous mounting media for microscope analysis (Zeiss microscope).

**Studies in podocytes in vitro**

These studies were performed in a human podocyte line obtained by infection of pure primary cultures with a hybrid Adeno5/SV40 virus as described previously [11]. Primary cultures were established from decapsulated glomeruli isolated by differential sieving from renal cortex fragments of surgically removed kidneys [12,13]. Phenotypic characterization was performed according to cell morphology (polyhill cells with cobblestone-like appearance) and positive staining for CALLA (common acute lymphoblastic antigen). Furthermore, the cells showed positive staining for synaptopodin, podocalyxin and zona occludens-1 (ZO-1); negative staining for smooth muscle-type myosin, FVIIIr: Ag and CD45; and cytotoxicity in response to puromycin aminonucleoside [11]. The selected clones of immortalized cells were used at passages 25-40.

**Expression of Met.** The expression of Met was investigated by western blot analysis and immunocytochemistry. For western blot, 2.4 × 10⁶ cells were lysed in lysis buffer containing 20% glycerol (Sigma-Aldrich), 30% sodium dodecyl sulphate (SDS) 10× (Sigma-Aldrich), 12.5% upper Tris, 0.25 TlU/ml aprotinin (Sigma-Aldrich) and 10 mg/ml leupeptin (Sigma-Aldrich). Aliquots of lystate (30 μl) were sized-fractionated by SDS gel electrophoresis (10% gradient gel) and the proteins were transferred to polyvinylidene difluoride membranes (Bio-Rad, Hercules, CA, USA). The membranes were blocked with 5% skim milk (Sigma-Aldrich) and incubated overnight at 4°C with mouse anti-human c-Met monoclonal Ab (Novoceastra) diluted 1:20 in PBS/BSA 1%. After three washings with 150 mM PBS, the membranes were incubated at room temperature with the secondary Ab and the complex streptavidin–biotin–peroxidase according to the manufacturers of the LSAB³ Kit. Visualization was in 3,3-diaminobenzidine. For immunocytochemistry, podocytes (1 × 10⁶/well) were seeded on chamber slides (Nunc, Roskilde, Denmark) and grown to confluence. Subsequently, cells were fixed in 6% formalin–acetate for 10 min, then washed in PBS and incubated in a dark humid chamber at room temperature for 60 min with mouse anti-human Met monoclonal Ab. The secondary Ab and the complex alkaline phosphatase–anti-alkaline phosphatase mouse steps were performed according to the manufacturers of the APAP Kit (Dako). The immunoreaction resulted in an intense red precipitate. Harris haematoxylin was used to counterstain the nuclei lightly. The coverslip was mounted with synthetic aqueous mounting media for microscope analysis (Zeiss microscope).

**Growth.** Podocytes were seeded at the concentration of 2 × 10⁶ cells/cm². After resting for 5 h in Dulbecco’s modified Eagle’s medium (DMEM; Sigma-Aldrich)–2% FCS, cells were stimulated with different doses of recombinant HGF (rHGF, 0.2–125 ng/ml; R&D Systems) in medium containing 0.1% BSA, 200 μg/ml Tetrazolium salt XTT sodium salt (Sigma-Aldrich) and 0.2% phenazine methosulphate (Sigma-Aldrich). Cell proliferation was assessed at 12 h intervals up to 48 h. The assay is based on bioreduction of Tetrazolium salt XTT by metabolically active cells into a coloured product that is soluble in culture medium. Colour intensity recorded at 450 nm is proportional to the number of viable cells. The results were confirmed by viable cell counting with the trypan blue exclusion test. To avoid colour interference, DMEM used in these experiments did not contain phenol red. Neutralization experiments were performed by pre-incubating HGF (5 ng/ml) with or without HGF-neutralizing Ab (1 μg/ml; R&D System) for 1 h at 37°C in DMEM–1% BSA and then challenging podocyte cultures as described above.

**Scatter.** Podocytes (2 × 10⁶ cells/flask) were treated with rHGF (100 ng/ml). Cell migration was evaluated over the first 4 h period of treatment under a Nikon Diaphot inverted microscope with a 5× phase-contrast objective in an attached, hermetically sealed Plexiglas Nikon NP-2 incubator at 37°C. Images of cell migration were recorded by a JVC ICCD video camera and stored at 20-min intervals. Image analysis was performed with a MicroImage analysis system (Casti Imaging, Venice, Italy) and an IBM-compatible system equipped with a video card (Targa 2000; Truevision, Santa Clara, CA, USA). Migration tracks were generated by marking the position of the nucleus of individual cells on each image. The net migratory speed (straight-line velocity) was calculated by the MicroImage software on the basis of the straight-line distance between the starting and end points divided by the time of observation. Migration of at least 30 cells was analysed for each experimental condition.

**Mesenchymal transition.** To document the loss of podocyte-specific phenotype and mesenchymal transition we studied the effects of treatment with rHGF (100 ng/ml) for 24 h on cell shape, expression of CALLA, E-cadherin, α-smooth
muscle actin (α-SMA) and collagen production. The changes in cell shape were studied in viable cells (2 × 10^5 cells/flask) using an inverted microscope and in fixed cells using scanning electron microscopy. The latter procedure was performed after removing culture medium and washing to take away non-adherent cells. Cells were fixed in 3.5% paraformaldehyde + 3.5% sucrose (Fisher Scientific, Loughborough, UK) + 2.5% glutaraldehyde for 10 min at room temperature. Then cells were washed twice with PBS, dehydrated in ethanol solutions at progressively increasing room temperature. Then cells were washed twice with PBS, dehydrated in ethanol solutions at progressively increasing concentration, dried in an oven at 37°C and gold-sputtered by an automatic sputter coater (Baltec SCD 050; Balzer, Germany). The expression of CALLA, E-cadherin, α-SMA, collagen I and collagen IV was studied by immunocytochemistry. For immunocytochemistry, podocytes (1 × 10^5 /well) were seeded on chamber slides (Nunc) and grown to subconfluence, then stimulated with rHGF 100 ng/ml for 24 h. Subsequently, cells were fixed in 6% formalin-acetate for 10 min. Endogenous peroxidase was blocked with H2O2 3.7% vol/vol in H2O for 10 min at room temperature. After three washings in 150 mM PBS and microwave treatment the cells were exposed to primary Ab: anti-human CALLA monoclonal Ab (1:10; Dako), anti-human E-cadherin monoclonal Ab (1:200; Zymed Laboratories, Inc., San Francisco, CA, USA), anti-human α-SMA monoclonal Ab (1:50, Dako), rabbit anti-human collagen I (1:300) and collagen IV (1:300) polyclonal Abs (Santa Cruz Biotechnology, Santa Cruz, CA, USA) in a dark humid chamber at room temperature for 60 min. The reaction product was detected with an avidin-biotin-peroxidase complex LSAB+ Kit and the colour reaction was developed with 3,3-diaminobenzidine. The cells were counterstained with Harris haematoxylin or green light and dehydrated with 3,3-diaminobenzidine. The cells were counterstained with Harris haematoxylin or green light and dehydrated with increasing alcohol scale (95% to 100%, xylol) and the coverslip was mounted with synthetic non-aqueous mounting medium for analysis with a Zeiss microscope. Collagen IV production was measured in supernatant of podocytes by enzyme-linked immunosorbent assay. Standards (human collagen IV, diluted 0.25–8 ng/μl in 0.02 N acetic acid; Sigma-Aldrich) and samples of supernatants (100 μl) were incubated for 24 h at 4°C in 96-well plates (Corning Costar Corp., Cambridge, MA, USA). The plates were then washed three times with 0.05% PBS before being incubated overnight at 4°C with 1% PBS in BSA. After three washings with 0.05% PBS, wells were loaded with primary Ab (mouse monoclonal anti-human collagen IV Ab; Chemicon, Temecula, CA, USA) diluted 1:1000 in 0.2% PBS/BSA and incubated on a stirrer at room temperature for 90 min. After four washings with 0.05% PBS, secondary Ab (anti-mouse polyclonal Ab; Chemicon) diluted 1:5000 was added and incubated at room temperature for 60 min. After four washings with 0.05% PBS, the streptavidin (Dako)/pNTPP (Sigma-Aldrich) reaction was developed as described previously [14]. Readings were at 405 nm.

Statistical analysis

Analysis of variance and the Tukey-Kramer test were used for comparison of the means.

Results

HGF serum levels

Serum levels of HGF in patients with EG, GD and N are shown in Figure 1. In patients with EG, HGF averaged 0.73 ± 0.06 ng/ml, a level that was significantly higher than in GD (0.18 ± 0.02 ng/ml; P < 0.01) and N (0.10 ± 0.01 ng/ml; P < 0.01).

Serum HGF-inducing effect

Release of HGF by PBMC stimulated with EG serum averaged 0.58 ± 0.09 ng/ml and was much higher than release induced by GD serum (0.06 ± 0.02 ng/ml; P < 0.001) and N serum (0.07 ± 0.01 ng/ml; P < 0.001) (Figure 2).

Expression of Met in renal tissue

The expression of Met in renal tissue is shown in Figure 3. Tubular cells diffusely expressed Met in all renal samples. In normal glomeruli Met was occasionally detected in parietal and visceral epithelial cells (A); no upregulation of Met was observed in glomerular

Fig. 1. Serum levels of HGF in patients with extracapillary crescentic glomerulonephritis (EG), patients with non-crescentic glomerular disease (GD) and normal volunteers (N). Columns are means of serum concentrations in single patients and controls and bars represent SDs. *P < 0.01 vs GD and N.

Fig. 2. Release of HGF in supernatant of cultured PBMC. PBMC from normal volunteers were treated with pooled sera of patients with extracapillary crescentic glomerulonephritis (EG), patients with non-crescentic glomerular disease (GD) and normal volunteers (N). Columns are means of six experiments performed with each pooled sera and bars represent SDs. *P < 0.001 vs GD and N.
diseases without extracapillary proliferation (panel B shows as example one case of membranous nephropathy). In contrast, in biopsies from patients with EG, Met was strongly expressed in cellular crescents (C).

Expression of cytokeratin and CD 68 in crescents

In EG, immunostaining with anti-human cytokeratin Ab showed a positive reaction in cellular crescents and in tubular cells (data not shown). Immunostaining with anti-human CD 68 Ab showed clusters of positive cells in cellular crescents; some positive cells were present in the glomerular tuft and the periglomerular infiltrate (Figure 4).

Expression of Met in podocytes

Cultured podocytes expressed Met, as shown by the intense fuchsia red immunostaining and by the precipitation of a 190 kDa band corresponding to Met protein in the western blot (Figure 5).

Effects of HGF on podocytes

Growth. HGF caused a dose- and time-dependent proliferation of podocytes. HGF-induced growth was prevented by neutralizing anti-HGF Ab (Figure 6).

Scatter. The scatter effect of HGF on podocytes is shown in Figure 7. Representative migration tracks (A and B) clearly demonstrate the increase in migratory activity caused by HGF (B). In fact, net migratory speed (graph on the right) was significantly higher in cells treated with HGF.

Mesenchymal transition. HGF changed the morphology of podocytes from their original cobblestone shape into a stellate, elongated one similar to that of fibroblasts (Figure 8). In cultured podocytes not treated with HGF, CALLA and E-cadherin were intensely expressed and a faint staining for α-SMA was observed, as reported previously [15]. HGF reduced expression of CALLA and E-cadherin and upregulated expression of α-SMA (Figure 9). HGF caused de novo expression of collagen I and increased expression of collagen IV (Figure 10). In addition, HGF significantly increased collagen IV release in supernatant (Figure 11).

Discussion

In this study, we show that serum levels of HGF are elevated in patients with crescentic glomerulonephritis. In addition, we demonstrate that serum of these patients contains HGF-inducer(s), which stimulate the production of HGF by circulating mononuclear cells. These phenomena do not depend simply on loss of renal function, because they do not occur in patients with non-crescentic glomerular disease matched for glomerular filtration rate. Therefore, crescentic glomerulonephritis is a type of injury that raises HGF production. Other conditions in which HGF
production is stimulated are acute severe hepatic insults [10], tubular necrosis [8] and peritonitis [7] and in addition a massive release of HGF occurs during extracorporeal circulation in haemodialysis [9]. In these conditions, as in crescentic glomerulonephritis, the release of HGF is sustained by circulating factor(s) that induce HGF production in distant intact organs [4], in mesangial cells [8] and in PBMC [9]. The nature of these circulating factors is still quite obscure. A glycoprotein with a molecular weight of ~12000 Da that induced HGF mRNA expression was isolated in the serum of rats subjected to hepatectomy or nephrectomy and was named ‘injurin’ [4]. Another factor distinct from injurin was identified in the conditioned media of tumour cell lines. Studies in vitro and in human disease suggest that inflammatory cytokines work as HGF-inducers [16].

HGF acts through its receptor Met, a transmembrane tyrosine kinase that is expressed in various types of normal and tumoral cells and is inducible by inflammatory cytokines in vitro [16] and by ischaemic or toxic injury in vivo. In this study we show that in normal glomeruli Met is scarcely expressed in occasional podocytes and parietal cells. In addition, we show that in glomerular diseases in which extracapillary proliferation is absent Met is not upregulated. In contrast, Met is diffusely and strongly expressed in cellular crescents. Crescents originate from proliferation of cells of epithelial lineage, as indicated by their positivity for cytokeratin. Traditionally, it has been assumed that crescents derive from parietal cells, because in the crescent no podocyte marker is identifiable. However, this assumption is not based on sound evidence, i.e. the demonstration of markers specific for parietal cells. Furthermore, recent studies in mice in which podocytes have been genetically tagged, so that cells derived from podocytes can be identified independently of their phenotype, have shown that up to 50% of crescentic cells originate from podocytes and the rest from parietal cells. Interestingly, these studies show that podocytes, upon transforming to crescentic cells, lose any known podocyte-specific antigens so that their origin is no longer identifiable. These observations and the current lack of specific markers for parietal cells frustrate any attempt to precisely define the origin of cells expressing Met in the crescent. However, the uniform expression of Met in crescents and the experimental evidence that about half of crescentic cells originate from either cell type [2] suggest that Met is expressed in cells derived both from podocytes and from parietal cells. Cytokines released locally, such as tumour necrosis factor and interleukin-1 or ischaemia

Fig. 5. Expression of Met in cultured podocytes, shown by immunocytochemistry in the left panel (purple colour) and by western blot in the right panel. MW, molecular weight markers.

Fig. 6. HGF-induced proliferation of podocytes. Growth is measured as percentage increase in cell number vs untreated cells. Upper panel shows dose-dependence (columns are means, bars are SDs). Lower panel shows time-dependence (points are means, bars are SDs). *P<0.01 vs untreated podocytes (w/o HGF) and podocytes treated with HGF and neutralizing anti-HGF Ab (Ab anti-HGF).
secondary to glomerular capillary necrosis may induce new expression of Met, as observed in other types of epithelial and non-epithelial cells.

The expression of Met makes crescentic cells a potential target for the high levels of HGF induced by extracapillary glomerulonephritis. Assuming that podocytes are part of the cells that express Met in the crescent, we investigated in vitro whether HGF has biological effects on podocytes. We studied only podocytes, not parietal cells, for two reasons: the first and main reason is the experimental evidence that podocytes play a pivotal role in initiating and sustaining crescent formation, while proliferation of parietal cells is secondary to activation of podocytes [1]. The second reason is that as yet no cell line or primary culture has been raised that originates definitely from parietal epithelium. In contrast, we have raised a glomerular cell line that presents a full array of podocyte-specific phenotypes. In vitro, podocytes expressed Met, as demonstrated by western blot and immuno-cytochemistry, and responded to HGF with pleiotropic reactions. In fact, HGF caused a dose- and time-dependent proliferation of podocytes and induced in podocytes detachment from substrate and a spectacular migratory activity. In addition, HGF transformed their cobblestone, typically epithelial form to an elongated,
stellate shape looking like that of fibroblasts, caused the loss of CALLA antigen and of E-cadherin, induced overexpression of α-SMA and increased the expression of collagens I and IV and the release of collagen IV in supernatant. These effects induced by HGF in podocytes in vitro mimic perfectly the transformation occurring in podocytes in crescentic glomerulonephritis in vivo. In fact, in crescentic glomerulonephritis podocytes detach from the GBM and elongate, forming bridges between the tuft and Bowman’s capsule, then lose their contact with the tuft and move beneath the parietal epithelium. This migratory behaviour is associated with proliferation, loss of podocyte-specific phenotypes and secretion of extracellular matrix material [1].

The findings of this study, i.e. (a) that in crescentic glomerulonephritis HGF production is induced and crescentic cells express de novo Met, becoming a target for HGF, and (b) that podocytes stimulated in vitro with HGF assume the same functional and phenotypic features that characterize their behaviour in vivo in crescentic glomerulonephritis, suggest that HGF plays a pathogenic role in the disease, by stimulating podocyte migration, growth and mesenchymal transformation. The participation of HGF in the disease is circumstantiated also by some characteristics of the glomerular environment, such as the presence of mesangial cells in the tuft and of CD 68-positive cells, urokinase and factor XIIa in the crescent. In fact, mesangial cells and macrophages are a potential source of paracrine HGF [8] while the coagulation enzymes that convert HGF to its dimeric active form [6] make the local inflammatory environment suited to activate HGF.

While we provide a sound demonstration that the HGF/Met system is induced in crescentic glomerulonephritis, we give only indirect evidence for a link between HGF/Met and crescent formation, so that the conclusion that HGF is pathogenic remains hypothetical. Actually, this limitation is imposed by studying the disease in humans, in which no manipulation of the HGF/Met system is possible. However, our
observations in patients supply good grounds for experiments in animals in which HGF and/or Met can be neutralized. Such experiments are beyond the objective of the present investigation and their burden deserves an independent study. A possible criticism of our interpretation of HGF as culprit in crescentic glomerulonephritis issues from the protective effect of HGF in some experimental models of renal disease. These were models of toxic–ischaemic tubular necrosis and of chronic renal disease in which HGF sustained tubular cell growth and antagonized tubulointerstitial fibrosis by contrasting transforming growth factor (TGF)-β. In fact, distinctive traits of HGF, such as its mitogenic and motogenic effects, candidate HGF as an effector of tissue regeneration and repair and HGF may be induced in crescentic glomerulonephritis as part of a healing response. However, the eventual effects produced by HGF in an injured tissue depend on the type of cells and their structural arrangement. In fact, while in tubular necrosis tubular cell growth and spreading along the denuded tubular basement membrane will prompt the replacement of damaged cells and tubular recovery, in crescentic glomerulonephritis invasive growth of glomerular epithelial cells in Bowman’s space and podocyte adhesion to the capsule will lead to glomerular obliteration. Similarly equivocal are the effects of HGF on cell phenotype and collagen deposition. In fact, it has been shown that HGF blocks the transformation of tubular epithelial cells to myofibroblasts triggered by TGF-β [17], reduces extracellular matrix accumulation by proximal tubular cells and attenuates glomerulosclerosis and interstitial fibrosis in a murine model of diabetes [18]. In contrast, HGF is per se a well-known inducer of epithelial to mesenchymal transformation, increases collagen production in mesothelial cells [7], upregulates fibronectin and connective tissue growth factor production in tubular cells [19] and downregulates extracellular matrix turnover in endothelial cells; in addition, transgenic mice overexpressing HGF develop a severe renal disease characterized by glomerulosclerosis and, most interestingly, by crescent formation [20]. It seems, therefore, that HGF may be either antifibrogenic or fibrogenic, depending on cell type and experimental conditions.

Conflict of interest statement. None declared.

References


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