Immunohistochemical Detection of C-MET/HGF Receptor in Glomerulonephritis

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Introduction

In a variety of human and animal glomerular diseases, growth factors cause excessive mesangial proliferation and matrix production (1). The proto-oncogene c-met encodes a transmembrane tyrosine kinase receptor for Hepatocyte Growth Factor (HGF) (2,3); in detail, c-met is a heterodimer composed of an α (50 KDa) and a β (140 kDa) subunit that, upon binding HGF, undergoes autophosphorylation and transduces growth signals into the cell (1). HGF is a multifunctional cytokine and its interaction with c-met is a prototype of mesenchymal to epithelial signaling, which has been implicated in morphogenesis of the kidney (4), acceleration of glomerular repair (5) and negative regulation of activated mesangial cell proliferation (6). Dysfunction of cell-cell regulation in the kidney due to decreased local HGF production may be an initial trigger for the development of glomerulonephritis (GN) (7). C-met expression in mesangial cells is considered a functional receptor for at least HGF secretion in experimental models (7). The purpose of this study was to investigate the tissue expression of c-met protein in a well documented series of renal biopsies from patients with various types of GN and search for statistical correlation between the patients' groups.

Material and Methods

Percutaneous renal biopsies were obtained from 100 patients with various types of GN. Patients' clinical data (i.e. hematuria, proteinuria, hypertension and creatinine serum levels) had been collected. The examined biopsies were divided in two main groups (primary-secondary GNs and these groups were further divided in proliferative and non-proliferative GNs). Twenty normal controls were also examined.

Tissue samples were uniformly fixed in buffered formadlehyde solution. The streptABC immunostaining method was carried out by utilizing the Dako strept ABC-HRP kit (Dako, Denmark). The paraffin, 4 μm-thick sections were deparaffinized with xylene and alcohol and then immersed in a phosphate-buffered saline solution and in methanol with 0.5% hydrogen peroxide to block endogenous peroxidase activity. Pepsin digestion prior to incubation with antibodies was performed. Affinity purified rabbit IgG antibody against the synthetic peptide corresponding to the last 12 amino acids at the carboxy terminal of human c-met protein (C-12) (Santa Cruz Biotechnology Inc, California, USA) was used at a dilution of 1:75 with an overnight incubation at 4°C in a humidity chamber. The subsequent reactions were made by using the Dako streptABC-HRP method. A freshly prepared diaminobenzidine solution was used as a chromogen. Substitution of the primary antibody by phosphate-buffered saline was used as a substitute control. As a negative control, a paraffin section of a normal spleen, which is reported to express neither c-met protein nor mRNA, was also used.

Statistical analysis was performed by chi-square statistics. Statistical significance was set at 5%.

Results

The patient group of primary GN was more frequently characterized by macroscopic hematuria and increased blood pressure by comparison to the group of secondary GN (Pearson's chi-square p=0.023, p=0.038 respectively). Patients with non proliferative GN were characterized by severe proteinuria more frequently than patients with proliferative GN (p=0.003); the latter patients demonstrated macroscopic hematuria more often than patietns with non-proliferative GN (p=0.03).

In all normal kidney tissue sections examined, c-met protein was mainly detected in the epithelial cells of distal renal tubules, collecting ducts, less commonly in the epithelial cells of proximal tubules and the epithelial cells of the Bowman capsule.

In GN specimens, the percentages for c-met immunopositivity incidence with regard to the various histologic structures were as follows: 73%, 43.9%, 29%, 73%, 11% and 58% as far as parietal epithelium, podocytes, mesangial cells, proximal tubules, distal tubules and collecting tubules are concerned, respectively. With regard to the pathologic lesions detected, we noticed that c-met was often detected in epithelial cells around microadhesions (16/25, 64%) as well as in epithelial cells of atrophic tubules (30/41, 73.2%) (Fig.) and, less often, in segmental sclerotic and hyperplastic areas (11/33, 33.4% and 13/67, 19.4% respectively). No statistical differences emerged among the various GN groups with regard to c-met immunopositivity and no statistical association was observed between the clinical severity of GN and c-met immunoexpression either.

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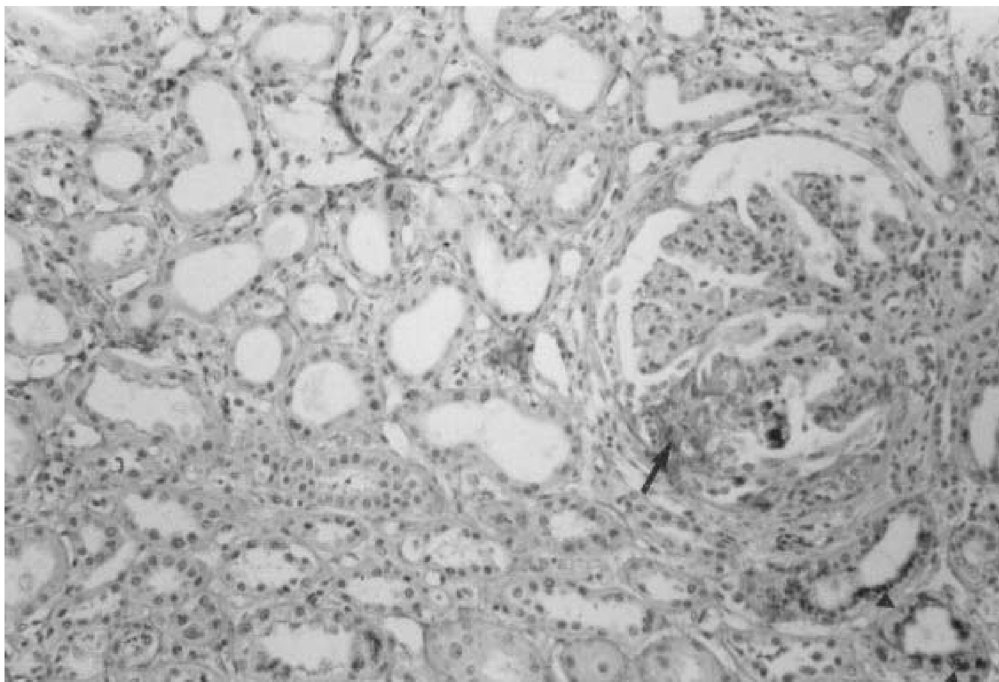
Figure 1. C-met immunoreactivity in microadhesions, segmentally in an area with mesangial hyperplasia (mesangial cells' cytoplasm) (arrow) and tubular epithelial cells (arrow heads) (strept ABC-HRP, X200).



Discussion

In the examined samples, c-met was detectable in various types of renal cells including glomerular and tubular epithelial cells in GN specimens and normal controls. There has been some controversy in the presence of c-met in mesangial cells which may be due to the sensitivity of detectable methods (Northern blot vs RT-PCR); using RT-PCR, c-met has been indeed detected in rat mesangial and human mesangial cells (7). In a previous immunohistochemical study, c-met was expressed in epithelial cells (while immunoreactive HGF was observed in the conditioned medium of cultured mesangial cells, but not epithelial cells) (8). Immunohistochemistry has detected c-met in the vascular stalk within the crevices of primitive glomeruli, an area that contains endothelial and putative mesangial cells (1). In 29% of the pathological samples of the present study and in just a few normal controls, c-met was detectable in mesangial cells. Neverthelles, HGF arguably does not stimulate growth of mesangial cells, despite the presence of c-met (7). C-met in mesangial cells is considered a functional receptor for at least HGF secretion. Mesangial cells are known to bind various growth factors that transduce signals via receptor tyrosine kinases. C-met was expressed in mesangial cells, at a low incidence though; it was mainly expressed in glomerular epithelial cells in both normal and GN specimens. These observations may be related to the view that HGF from mesangial cells could have paracrine effects on the growth of glomerular epithelial cells, which are well known to express c-met (1).

It is noteworthy that in the examined GN specimens, c-met was commonly expressed in microadhesions and atrophic tubules and so it appears to be involved in some early stage of the fibrotic procedure. This finding warrants further investigation since HGF is supposed to be a potent antifibrogenic factor, both in vivo and in vitro. Endogenous activation of HGF tends to preserve kidney structure and function in rats with chronic renal disease by activating matrix degradation pathways (9). On the other hand, it has been suggested that chronically elevated HGF contributes to the progression of chronic renal disease in diabetes by decreasing the glomerular filtration rate and possibly promoting the accumulation of extracellular matrix (10). Consequently, the role of the HGF/c-met interaction in the fibrotic procedure of GNs remains to be clarified.

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