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Hepatocyte growth factor (HGF) modulates matrix turnover in human glomeruli[1]

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Hepatocyte growth factor (HGF) modulates matrix turnover in human glomeruli.

Background. The imbalance between synthesis and degradation of mesangial matrix causes glomerulosclerosis and leads to renal failure. Hepatocyte growth factor (HGF) has been shown to reduce the progression in murine models of chronic renal failure. The present study evaluated the effect of HGF on the extracellular matrix turnover and on c-met receptor in human glomeruli.

Methods. Human glomeruli microdissected from donor kidney biopsies before transplantation were incubated with culture media containing HGF (50 ng/mL). After 24 and 48 hours, the expression of c-met, (α2) IV collagen, transforming growth factor-β (TGF-β), metalloprotease (MMP) 2 and 9 and of the inhibitor of MMP-2, tissue inhibitors of metalloprotease-1 (TIMP-1), was evaluated by polymerase chain reaction (PCR). β-actin was used as housekeeping gene. The production of collagen type IV and TGF-β was evaluated by enzyme-linked immunosorbent assay (ELISA) and Western blotting and the activity of MMP by zymography.

Results. (α2) IV collagen, TGF-β, and TIMP-1 mRNA levels were markedly decreased in glomeruli treated with HGF at 24 and 48 hours. The expression of c-met was up-regulated by HGF treatment. HGF reduced the production of collagen type IV and TGF-β. MMP-2 but not MMP-9 mRNA level was increased in HGF-treated glomeruli, although the gelatinolytic activity of the supernatant was not changed. By light microscopic examination kidney biopsies neither showed glomerular hypercellularity nor mesangial expansion.

Conclusion. HGF reduced expression and synthesis of TGF-β and collagen type IV and increased MMP-2 mRNA level in normal human glomeruli. These results suggest an antifibrotic effect of HGF on glomerular cells and may explain its beneficial role in glomerulosclerosis.

[1]**See Editorial by Striker, p. 2500.**

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Hepatocyte growth factor (HGF) is a pleiotropic factor, which plays a central role in organogenesis and development [1]. HGF stimulates angiogenesis and cell migration, effects that are crucial in tumor invasiveness [2]. Morphogenic and motogenic properties are favored by the capacity of HGF to accelerate extracellular matrix turnover inducing the expression of metalloproteases (MMPs) [3]. The possibility to modulate extracellular matrix turnover has prompted several studies to evaluate the effects of HGF in animal models of chronic renal disease. HGF has been shown to reduce renal injury in rats with unilateral ureteral obstruction [4] and in the remnant kidney model of chronic renal disease [5]. However this antifibrotic effect has not been confirmed by Takayama, Larochelle, and Sabnis [6] who demonstrated that mice transgenic for HGF developed glomerulosclerosis. Contradictory results arise also from in vitro studies showing increased production of matrix in mesothelial and endothelial cells [7] [abstract; Esposito C et al, *J Am Soc Nephrol* 10:570A, 1999] or conversely inhibition of tubular epithelial to myofibroblast transition by HGF [8].

The aim of the present study was to evaluate the effect of HGF on the extracellular matrix turnover in isolated human glomeruli. We found that HGF reduced (α2) IV collagen and transforming growth factor-β (TGF-β) mRNA levels and proteins and increased the expression of MMP-2 but did not change gelatinolytic activity. Finally, HGF treatment up-regulated glomerular expression of the receptor c-met. These effects confirm a specific antifibrotic action of HGF at glomerular level.

METHODS

Isolation of human glomeruli

Human glomeruli were isolated by microdissection from renal cortex of five donor kidneys just before transplantation. At the same time a little core of tissue was obtained for histologic study, formalin fixed and paraffin embedded; 3 to 4 μm sections were stained with

Table 1. Primer sequences and PCR cycles

Gene	Sense primers	Antisense primers	Cycles
Collagen IV (α 2)	5′-TATTCCTTCCTCATGCACACGGCG-3′	5′-CCAATTTTTGGGTTGGCACC-3′	38
TGF-β	5′-GGAGGGGAAATTGAGGGCTTTCGC-3′	5′-TTATGCTGGTTGTACAGGGC-3′	38
MMP-2	5′-CCACATTCTGGCCTGAGCTCCC-3′	5′-GATTTGATGCTTCCAAACTTCAC-3′	40
MMP-9	5′-CACTGTCCACCCCTCAGAGC-3′	5′-GCCACTTGTCGGCGATAAGG-3′	40
TIMP-1	5′-AATTCCGACCTCGTCATCAGG-3′	5′-ACTGGAAGCCCTTTTCAGAGC-3′	38
c-Met	5′-GGAAACACCCATCCAGAATGTCATT-3′	5′-TGATATCGAATGCAATGGATGATCT-3′	35
β-actin	5′-CCCCAGGCACCAGGGCGTGAT-3′	5′-GGTCATCTTCTCGCGGTTGGCCTTGGGGT.	33

Abbreviations are: TGF-β, transforming growth factor-β; MMP-2, metalloprotease 2; MMP-9, metalloprotease 9; TIMP-1, tissue inhibitors of metalloprotease-1.

hematoxylin-eosin, periodic acid-Schiff (PAS) and Masson's trichrome. In order to isolate glomeruli a small piece of renal cortex was placed immediately at 4°C in saline solution containing RNase inhibitors. Glomeruli were microdissected and washed three times in cold saline solution; pools of five glomeruli were then transferred to sterile Eppendorf tubes and cultured with RPMI medium containing fetal calf serum (FCS) (10%) and HGF (50 ng/mL). Glomeruli incubated with medium without HGF were used as control. Since glomerular size varied, in preliminary experiments several pools of five glomeruli were prepared from the same kidney, individually reverse transcribed and fractions of cDNAs were amplified before and after pooling the cDNA in a single tube. No difference was observed between the pools of five glomeruli and the pools of cDNA suggesting that pooling five glomeruli blunted the differences in RNA yield.

Extracellular matrix turnover and c-met expression

Extracellular matrix turnover is a continual process deriving from the balance between deposition of matrix components and removal by enzymatic degradation. This process is further regulated by molecules that modulate the activity of collagenase such as tissue inhibitors of metalloproteases (TIMPs). One of the most important factors in the accumulation of extracellular matrix is TGF-β, which plays a central role in fibrosis. In order to evaluate the effect of HGF on matrix turnover we measured the expression of (α2) IV collagen, MMP-2 and MMP-9, TIMP-1, and TGF-β in human glomeruli treated with HGF for 24 and 48 hours. The effect of HGF on the expression of its receptor was also evaluated. Briefly, isolated glomeruli (five glomeruli/tube) were incubated with HGF-containing medium for 24 and 48 hours. At the end of the incubation time glomeruli were washed, freeze/thawed three times in presence of Triton X-100 (2%) and centrifuged (20,000*g*). Supernatant (9 μL) was reverse transcribed in situ using a First Strand cDNA Synthesis Kit (AMV) (Roche Diagnostic GmbH, Mannheim, Germany). Oligo(dT) was used to prime the reverse transcription (RT) for subsequent mRNA amplification. The reaction mixture was incubated at 60°C for 60 minutes and the reverse trascriptase was inactivated by heating the reaction at 90°C for 7 minutes. cDNA (2 μL) was amplified by polymerase chain reaction (PCR) using the primers and the cycles indicated in Table 1 [9]. PCR was performed using the cDNA Amplification Kit (Laboratoires Eurobio, Toulouse, France) and an initial reaction was carried out for each gene to determine the final magnesium concentration. To determine the number of cycles required for each gene and ascertain that amplification was in the logarithmic linear phase preliminary reactions were performed using different amplification cycles and different amount of starting material. A linear correlation between signal intensity and amplification cycles indicated that amplification was in the logarithmic part of PCR reaction (Fig. 1). Amplification program consisted of a first incubation of 3 minutes at 94°C followed by cycles of the following steps: denaturation at 94°C for 1 minute, annealing at 60°C for 1 minute, and extension at 72°C for 3 minutes. The final incubation was performed at 72°C for 7 minutes. PCR products were separated by agarose (2%) gel electrophoresis, stained with ethidium bromide, identified by ultraviolet light and measured by densitometry. To further prevent variations due to different RNA yield between samples, RNA degradation and cDNA synthesis, we also used β-actin as a standard to assure equal synthesis of cDNA in different samples and the results were analyzed as ratios sample gene/β-actin.

Zymography for 72 and 92 kD (MMP-2 and MMP-9)

MMP activity was evaluated by zymography as described previously [10]. Briefly glomeruli were incubated with medium containing HGF for 24 and 48 hours. At the end of the incubation time they were further incubated in serum-free medium containing 0.1% bovine serum albumin (BSA) for 24 hours. The supernatant was then collected, concentrated 10 times using a 22 kD cut-off filter (Millipore, Milan, Italy). An aliquot (32 μL) was loaded onto a 10% gelatin polyacrylamide gel. After electrophoresis the gel was incubated in Triton X-100 buffer and activated in development buffer for 24 hours at 37°C. Gelatinase activity was visualized by staining the gel with Coomassie brilliant blue. The level of MMPs was quantified by densitometry.