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*Rapid communication*

# High glucose and hyperosmolality stimulate hepatocyte growth factor secretion from cultured human mesangial cells

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**Summary** Hepatocyte growth factor is a recently cloned potent mitogen to hepatocytes, but its extrahepatic roles are not completely defined. It causes proliferation of endothelial and epithelial cells implicating potential action in the glomerulus. We aimed to determine whether cultured human mesangial cells secrete hepatocyte growth factor and the effect of high glucose conditions. Mesangial cells were isolated from the normal cortex of a child's kidney. After differential glomerular sieving and trypsin digestion of glomeruli, mesangial cells were cultured in 20% fetal calf serum/RPMI. Glucose concentration in the medium was adjusted to 5 mmol/l, 11 mmol/l, 25 mmol/l or 5 mmol/l/20 mmol/l mannitol to correct for osmolality. After 0, 24, 48, 72 h incubation, hepatocyte growth factor was measured in the supernatant by enzyme immuno assay using recombinant hepatocyte growth factor and monoclonal antibodies to human hepatocyte growth factor. Hepatocyte growth factor was secreted by cultured mesangial cells. High glucose and hyperosmolar conditions caused a 100–200% increase in hepatocyte growth factor secretion at 48–72 h ($p = 0.001$). Hepatocyte growth factor secretion at 48 h in 5 mmol/l glucose was 16.46 ± 1.09 ng/ml (mean ± SEM), 11 mmol/l glucose: 32.98 ± 4.54, 25 mmol/l glucose: 33.32 ± 7.89, 5 mmol/l glucose/20 mmol/l mannitol: 34.05 ± 3.64; at 72 h in 5 mmol/l glucose: 23.92 ± 2.85 ng/ml, 11 mmol/l glucose: 28.26 ± 2.03, 25 mmo/l glucose: 62.04 ± 12.2, 5 mmol/l glucose/20 mmol/l mannitol: 45.76 ± 6.25. Trypan blue exclusion demonstrated membrane integrity. These findings demonstrate for the first time that cultured human mesangial cells secrete hepatocyte growth factor and there is stimulation by high glucose and hyperosmolar conditions. Hepatocyte growth factor may have a renotropic role in the pathogenesis of diabetic nephropathy. [Diabetologia (1994) 37: 533–535]

**Key words** Hepatocyte growth factor, mesangial cells, diabetic nephropathy.

HGF is a recently cloned novel growth factor [1]. It is a potent hepatocyte mitogen and is produced by Ito cells in the liver. Ito cells are perisinusoidal cells, which produce collagen I, III, IV, fibronectin and laminin. However, the extrahepatic functions of HGF are not completely defined. It is known to be a mitogen and motility factor for cultured endothelial cells [2] and a mitogen for glomerular epithelial cells [3], suggesting a potential role in the glomerulus. Rat mesangial cells have recently been shown to express HGF mRNA [4], but it is not known whether mesangial cells produce HGF in vivo.

We aimed to investigate whether human mesangial cells secrete HGF in vivo and the effect of high glucose conditions.

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*Abbreviations:* HGF, hepatocyte growth factor; FCS, fetal calf serum; PDGF, platelet derived growth factor; ANOVA, analysis of variance

**Table 1.** HGF (ng/ml) secretion from human mesangial cells

| | 0 h | 24 h | 48 h | 72 h |
|---|---|---|---|---|
| 5 mmol/l glucose | 0 | 6.56 ± 0.26 [a] | 16.46 ± 1.09 | 23.92 ± 2.85 |
| 11 mmol/l glucose | 0 | 10.62 ± 1.37 | 32.98 ± 4.54 | 28.26 ± 2.03 |
| 25 mmol/l glucose | 0 | 7.22 ± 0.31 | 33.32 ± 7.89 | 62.04 ± 12.2 |
| 5 mmol/l glucose + 20 mmol/l mannitol | 0 | 6.28 ± 0.52 | 34.05 ± 3.64 | 45.76 ± 6.25 |
| 20% FCS/RPMI | 0 | | | |

ANOVA showed a significant increase in HGF secretion at 25 mmol/l glucose and 5 mmol/l glucose/20 mmol/l mannitol at 48 h and 72 h ($p = 0.001$). [a] mean ± SEM for four experiments

## Materials and methods

Normal renal cortex was obtained from a child undergoing a nephrectomy for Stage 1 Wilms' tumour. Informed parental consent was obtained and the study was approved by the Adelaide Children's Hospital Human Ethics Committee.

*Isolation and culture of mesangial cells:* Glomeruli were isolated under sterile conditions using differential sieving of cortical tissue, free of tubular fragments. Isolated glomeruli were suspended in 0.2% trypsin in phosphate buffered saline at 37°C, 5% $CO_2$ for 20 min to remove capsules and cultured at approximately 30 glomerular cores per dish in medium (20% FCS/RPMI 1640/20 mmol/l HEPES/l glutamine/penicillin streptomycin) at 37°C, 5% $CO_2$. At the first passage mesangial cells were identified by morphology and positive fibronectin immunofluorescent staining (Biodesign, Kennebunkport, Me., USA). Endothelial denudation was confirmed by lack of staining for factor VIII. Experiments were performed at passages 3–6.

*HGF secretion studies:* Cells were plated at $4 \times 10^4$ cells/well, incubated for 48 h in 20% FCS/RPMI, and wells then washed (Hanks) and replaced with fresh medium. Glucose concentration in the medium (20% FCS/RPMI 1640) was adjusted to 5 mmol/l, 11 mmol/l, or 25 mmol/l, or 5 mmol/l with the addition of 20 mmol/l mannitol to correct osmolality. After 0, 24, 48 and 72 h incubation, the supernatant was collected and stored at –70°C.

HGF was measured by an enzyme immuno assay (EIA), which is available as a standard kit from the Institute of Immunology Co. Ltd., Tokyo, Japan. Microtitre plates were coated with a mouse monoclonal antibody specific for human HGF. The test samples or standards (recombinant HGF) diluted in FCS were added. After a further incubation the wells were washed and horse radish peroxidase conjugated mouse monoclonal antibody specific to HGF (but to a different epitope) was added and after a further incubation the enzyme substrate (sodium perborate, tetrahydrate) containing the colour developer (O-phenylendiamine, dihydrocloride) was added. The reaction was stopped by adding 4N-sulphuric acid. Absorbance was measured at 492 nm in a microtitre plate reader (Dynatech, Guernsey, Channel Islands, UK). Quantitative determination of serum HGF was made by referring to the calibration curve prepared using the HGF standard solution provided with the kit. Standard curves using this method display linearity between 0.1–1.6 ng/ml. The assay has no cross reactivity to plasmin, lipoprotein(a), epidermal growth factor, fibroblast growth factor, PDGF and transforming growth factor $\beta$.

*Statistical analysis:* Results are expressed as mean ± SEM. Data were compared using ANOVA (two way analysis).

## Results

### *HGF secretion studies*

The results of four experiments performed in triplicate showed that cultured human mesangial cells secreted hHGF. High glucose (25 mmol/l) conditions caused a 100–200% increase in hHGF secretion from 48 to 72 h. Hyperosmolality (5 mmol/l glucose/20 mmol/l mannitol) caused a comparable increase, that was not significantly different from the effect of 25 mmol/l glucose. (Table 1). These findings were significant and independent of changes over time using ANOVA ($p = 0.001$). Trypan blue exclusion (> 90%) at 72 h demonstrated membrane integrity under normal glucose, high glucose, and hyperosmolar conditions.

## Discussion

We have shown for the first time that cultured human mesangial cells secrete HGF. Furthermore, both high glucose and hyperosmolality stimulated secretion of HGF indicating that the effect of high glucose was not specific. HGF is known to be produced by other mesenchymal cells including Ito cells in the liver and is the most potent mitogen known for hepatocytes [1]. It is also a potent mitogen to human endothelial cells [2] and rat glomerular epithelial cells [3, 4] but not to rat mesangial cells [4]. These findings and ours suggest a paracrine effect in the glomerulus.

However, it is not known whether mesangial cells produce HGF in vivo. There is a marked early increase in HGF expression in unilateral nephrectomy and carbon tetrachloride rat models. This production has been localized using in situ hybridization techniques to endothelial cells in the renal medulla, rather than tubular epithelial cells [5]. While this finding does not exclude in vivo production in the mesangium future studies could now be directed to examining this question in the glomerulus.

Glomerular mesangial, endothelial and epithelial cells increase collagen IV production, the major component of mesangial matrix, under high glucose conditions. PDGF may be an important intermediate factor [6], but otherwise the mechanisms of increased collagen IV production and putative growth factors are not established. PDGF is the most potent mitogen of mesangial cells [7], while it appears the major biologic effect of other growth factors, IGFI and transforming growth factor-$\beta$, may be to promote matrix expansion. Whether HGF is involved in mesangial matrix expansion in vitro or in vivo is yet to be explored. Rat mesangial cells do not express the HGF receptor, the c-met proto-oncogene [4], and we are currently investigating this in human mesangial cells. It is also possible that expression of the receptor is altered under pathological conditions.

HGF cDNA has been cloned and sequenced and has close homology to plasminogen, tissue plasminogen activator and lipoprotein(a), sharing common kringle domains [1]. Furthermore HGF and scatter factor are identical ligands for the receptor encoded by the c-met proto-oncogene and both stimulate tyrosine kinase activity of the receptor [8]. In addition to this specific high affinity receptor, low affinity/high capacity binding sites of HGF have been detected. HGF binds to heparin [9] and therefore it is possible that heparan sulphate proteoglycans of the extracellular matrix provide these additional binding sites. HGF is known to be sequestered in the extracellular matrix in the liver [10].

Our findings suggest that this interesting growth factor should now be included in work exploring the role of growth factors in the mesangium in diabetes. We are currently extending our in vitro work to examine intracellular processes relating to HGF action and production by mesangial cells. The significance of HGF in the glomerular hypertrophy of experimental diabetes is another avenue now relevant to future research.

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