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Glomerular ultrafiltration and apical tubular action of IGF-I, TGF-β, and HGF in nephrotic syndrome

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Glomerular ultrafiltration and apical tubular action of IGF-I, TGF-β, and HGF in the nephrotic syndrome. In nephrotic glomerulopathies, there is ultrafiltration of high molecular weight forms of insulin-like growth factor-I (IGF-I), hepatocyte growth factor (HGF), and transforming growth factor-β (TGF-β), which are bioactive in tubular fluid and act through apical tubular receptors. Experimental evidence indicates that ultrafiltered IGF-I, HGF, and TGF-β may contribute to increased tubular phosphate and sodium absorption, synthesis of extracellular matrix proteins, and secretion of chemokines such as monocyte chemoattractant protein-1 (MCP-1). Through these mechanisms, glomerular proteinuria may contribute to tubulointerstitial pathobiology in nephrotic syndrome.

In nephrotic glomerular diseases, there is a strong association between the degree of proteinuria and the onset and rate of progression of chronic renal failure. Moreover, the onset and progression of renal failure are tightly linked to tubulointerstitial injury such as tubular atrophy, interstitial fibrosis, and a macrophage infiltrate [1–5]. This chain of events suggests that the glomerular ultrafiltration of proteins in nephrotic syndrome causes or contributes to tubulointerstitial pathobiology. Glomerular protein ultrafiltration causes translocation of bioactive molecules from plasma into tubular fluid. These include growth factors that are present in serum in high molecular weight, largely inactive forms and are not ultrafiltered in normal glomeruli. Moreover, functioning growth factor receptors are abundantly expressed in the kidney and are also distributed in apical tubular membranes in some segments of the nephron. Glomerular ultrafiltration of these growth factors and their tubular action through respective apical receptors may convey important mechanisms of tubulointerstitial injury and may be an important avenue in the nephrotic syndrome by which glomeruli and tubules become pathophysiologically linked.

This and other laboratories have investigated different aspects of such growth factor ligand–receptor interactions, as they may apply to glomerular proteinuria. Such ligand-receptor pairs where the ligand is present in ultrafiltrate and the receptor is expressed in apical membranes in tubular cells include insulin-like growth factor-I (IGF-I)–IGF-I receptors, hepatocyte growth factor (HGF)–p190^{met}, transforming growth factor-β (TGF-β)–TGF-β type I/II receptors, and possibly HGF-like growth factor (HGFL–Ron).

Key words: apical tubular receptors, tubulointerstitial nephritis, proteinuria, progressive renal disease.

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INTERACTION OF ULTRAFILTERED INSULIN-LIKE GROWTH FACTOR-I WITH TUBULAR CELL INSULIN-LIKE GROWTH FACTOR-I RECEPTORS

Insulin-like growth factor-I (7.6 kDa) is present in serum at considerable levels (20 to 40 nM). However, more than 99% of serum IGF-I circulates in high molecular weight protein complexes due to binding to specific binding proteins [6]. Most (approximately 75%) of the peptide is bound to IGF-binding protein (IGFBP)-3, which associates with an acid labile subunit (75 kDa) and the ligand to form a circulating complex of 150 kDa. Most of the remainder of serum IgI is present in complexes of approximately 50 kDa, where the peptide is bound to IGFBP-4, -2, or -1.

Insulin-like growth factor-I receptors are expressed in the nephron in basolateral but also in apical tubular membranes. Apical IGF-I receptor expression has been demonstrated in proximal tubules, thick ascending limbs of the loop of Henle, and cortical collecting ducts [7, 8]. Although IGF-I is expressed in some nephron segments, it does not appear to be secreted into tubular fluid [9]. IGF-I is normally not expressed in proximal tubules [10]. Hence, the natural ligand for apical (proximal) tubular IGF-I receptors is most likely and exclusively IGF-I that originates from glomerular ultrafiltration.

Glomerular ultrafiltration of IGF-I/IGFBP-complexes in the nephrotic syndrome

Insulin-like growth factor-I is either absent from normal glomerular ultrafiltrate or is present at extremely

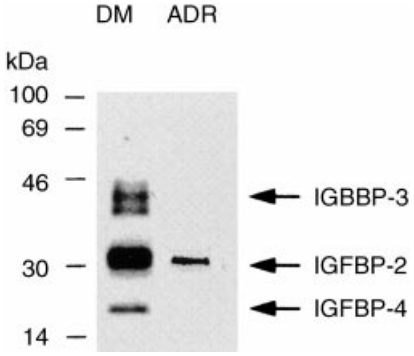

Fig. 1. Western ligand blot of insulin-like growth factor (IGF)-binding proteins in glomerular ultrafiltrate in rats with diabetic (DM) or adriamycin (ADR) nephropathy.

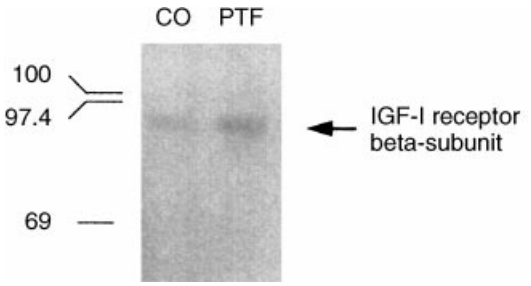

Fig. 2. Activation of IGF-I receptors in proximal tubular cells by glomerular ultrafiltrate. Suspended cells that were labeled with ^{32}P were incubated with proximal tubular fluid from control (CO) or rats with adriamycin-induced nephrotic syndrome (ADR). Cells were lyzed, and IGF-I receptors were immunoprecipitated. Precipitates were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The autoradiogram demonstrates increases in phosphorylation of IGF-I receptor β-subunits upon incubation with nephrotic rat tubular fluid.

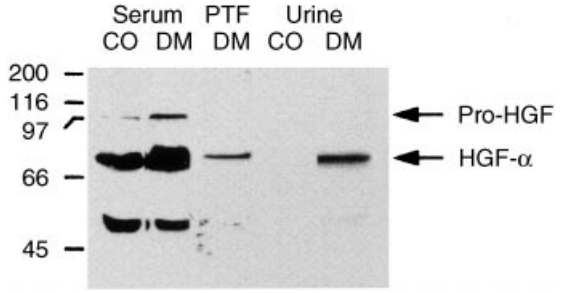

Fig. 3. Western blot analysis of hepatocyte growth factor (HGF) in serum, proximal tubular fluid (PTF), and urine under reducing conditions in normal control animals (CO) and rats with diabetic nephropathy (DM).

low levels, which do not allow for detection with available assays. However, in rat models of glomerular diseases such as the adriamycin-induced nephrotic syndrome or streptozotocin-induced diabetic nephropathy, IGF-I/IGFBP complexes are ultrafiltered into proximal tubular fluid (Fig. 1). In studies from our laboratory, IGF-I levels were measured by radioimmunoassay in early proximal tubular fluid that was collected by nephron micropuncture from rats with adriamycin or diabetic nephropathy and were on average 1.35 and 2.54 nM, respectively (abstract; Wang and Hirschberg, *J Am Soc Nephrol*, 9:124, 1998) [11].

Bioactivity of proximal tubular fluid IGF-I was examined *in vitro* in suspended mouse proximal tubular cells (mPTCs) after labeling of the cell ATP pool with ^{32}P-orthophosphate. The incubation of labeled cells for two minutes with proximal tubular fluid that was obtained from nephrotic rats by renal micropuncture activates IGF-I receptors (Fig. 2) and initiates recruitment of phosphatidylinositol-3-kinase to activated insulin receptor substrate (IRS)-1 (unpublished observation). Moreover, in proximal tubular cells that were incubated in 96-well plates, nephrotic rat glomerular ultrafiltrate increases the uptake of ^{3}H-thymidine into DNA, and this activity of tubular fluid is, in part, blocked by coincubation with a neutralizing IGF-I receptor monoclonal antibody [11].

Other studies have provided direct or indirect experimental evidence that ultrafiltered tubular fluid IGF-I increases proximal tubular phosphate absorption and distal tubular sodium absorption and tubular collagen secretion (abstract; Wang and Hirschberg, *J Am Soc Nephrol*, ibid) [8, 11, 12]. These studies exemplify that a growth factor, namely IGF-I, undergoes glomerular ultrafiltration in nephrotic syndrome, activates specific apical tubular receptors, initiates signal transduction, and confers various pathophysiological consequences to tubular cells.

HEPATOCYTE GROWTH FACTOR AND p190^{met}

Hepatocyte growth factor is synthesized and released from tissues, mainly liver, as a biologically inactive, monomeric precursor molecule. Pro-HGF (approximately 95 kDa) is cleaved by a specific serine protease (HGF activator) in the extracellular environment into a αβ-dimeric, active molecule (approximately 82 kDa) consisting of an α-chain (54 to 69 kDa, dependent on glycosylation) and a 34 kDa β-chain.

Hepatocyte growth factor is present in serum as both the Pro- and mature molecule. In rats with diabetic nephropathy, HGF is ultrafiltered into proximal tubular fluid (abstract; Wang and Hirschberg, *J Am Soc Nephrol* 8:632, 1997). Western blot analysis of diabetic rat proximal tubular fluid demonstrates the presence of only mature HGF (Fig. 3). Either Pro-HGF is ultrafiltered at low rates not allowing for visualization by Western blot, or ultrafiltered Pro-HGF is quickly activated by proteolysis. Indeed, urokinase, which is abundantly present in tubular fluid, has been shown to activate Pro-HGF [13].

The specific receptor for HGF is the protein product of the met proto-oncogene $p190^{met}$. The expression of this heterodimeric tyrosine kinase receptor in the nephron was recently examined in this laboratory in kidneys from normal and streptozotocin diabetic rats by immunohistology (abstract; Wang and Hirschberg, *J Am Soc Nephrol* 8:632, 1997). In diabetic rat kidneys, $p190^{met}$ is expressed in apical membranes in S3 segments of proximal tubules, thick ascending limbs of the loop of Henle, distal tubules, and cortical and medullary collecting ducts.

TRANSFORMING GROWTH FACTOR-β AND TRANSFORMING GROWTH FACTOR-β RECEPTORS

Transforming growth factor-β (25 kDa) is a homodimeric growth factor peptide that is released from cells as a precursor molecule bound to latency-associated peptide (LAP) as a complex of 100 kDa, or bound to LAP-LTBP (latent TGF-β binding protein, approximately 220 kDa) or to α2-macroglobulin (approximately 900 kDa). Most of the body content of TGF-β is present in platelets, but TGF-β1 is also present in plasma at considerable levels (60 to 100 ng/ml), but in high molecular weight, inactive forms.

Transforming growth factor-β acts through two related transmembrane serine-threonine kinase receptor proteins, type I and II receptors [14]. Type II receptors bind the ligand, resulting in recruitment of type I receptors into a heterodimeric I/II complex. In this ligand-activated complex, type II receptor kinase activates type I receptors by phosphorylation on ser-thr residues and causes subsequent transmission of signals to intracellular substrates such as Smads [14].

Transforming growth factor-β receptor expression in the kidney has been examined by Ando et al [15], and this laboratory has specifically studied the distribution of type I and II receptors in apical and basolateral membranes in the nephron in normal and diabetic rats (abstract; Wang and Hirschberg, *J Am Soc Nephrol* 8:648, 1997). In general, type I and II receptors are coexpressed in the nephron in glomerular arterioles, cortical and medullary collecting ducts, and papillary collecting ducts. In rats with streptozotocin-induced diabetic nephropathy, there is also moderate TGF-β receptor expression in some glomeruli (mesangium) and in apical membranes of distal tubules and cortical and medullary collecting ducts.

The glomerular ultrafiltration of active and total TGF-β has been examined in tubular fluid that was collected by micropuncture from normal rats and from rats with streptozotocin-induced diabetic nephropathy in this laboratory using a highly sensitive and specific assay (abstract; Wang and Hirschberg, *J Am Soc Nephrol* 8:648, 1997). In this assay, mink lung epithelial cells that were stably transfected with a TGF-β–sensitive truncated plasminogen activator inhibitor-1 (PAI-1) promoter fused to a luciferase reporter (kindly donated by Dan Rifkin, New York University, New York, NY, USA) were incubated in 96-well plates with medium containing rhTGF-β1 (1 to 500 pg/ml) as standards or diluted normal or diabetic rat proximal tubular fluid (25 μl/well). After 16 hours, cells were washed and lyzed, and luciferase activity in lysates was measured by luminescence assay.

In native or heat-activated (80°C, 10 min) proximal tubular fluid from normal rats, TGF-β levels were less than 10 pg/ml. In contrast, in rats with diabetic nephropathy, the early proximal tubular fluid levels of active TGF-β were 76 ± 43 pg/ml, and the total (bioactivatable) TGF-β levels were 729 ± 272 pg/ml ($N = 5$). These data not only indicate that TGF-β undergoes glomerular ultrafiltration, but they also demonstrate that at least a portion of TGF-β is bioactive in tubular fluid. It is possible that further activation of ultrafiltered TGF-β occurs such as with downstream acidification of tubular fluid [16].

INDUCTION AND BASOLATERAL SECRETION OF MONOCYTE CHEMOATTRACTANT PROTEIN-1 BY ULTRAFILTERED HGF AND TGF-β

Ultrafiltered HGF and TGF-β may induce several biological events to apical receptor-bearing tubular segments that cause or contribute to tubulointerstitial pathobiology in proteinuric glomerulopathies. These may include increased expression of extracellular matrix proteins. TGF-β may contribute to tubular atrophy because it can induce cell cycle arrest through activating cyclin dependent kinase (CDK) inhibitors and induce proapoptotic signals [17].

Hepatocyte growth factor and TGF-β may also stimulate basolateral secretion of chemokines that subsequently act on the interstitium. One such molecule is monocyte chemoattractant protein-1 (MCP-1). MCP-1 has been associated with renal interstitial macrophage accumulation. Indeed, interstitial macrophages play a role in the progression of proteinuric renal diseases [18]. For example, in streptozotocin-induced diabetic nephropathy at week 20 to 30, the number of $ED\text{-}1^+$ interstitial macrophages is increased twofold compared with control (abstract; Wang and Hirschberg, *J Am Soc Nephrol*, 9:644, 1998). These cells secrete other growth factors and cytokines, including TGF-β, that activate interstitial fibroblasts, resulting in increased extracellular matrix protein deposition and interstitial fibrosis.

Recent studies from our laboratory suggest important contributions of ultrafiltered HGF and TGF-β in MCP-1–

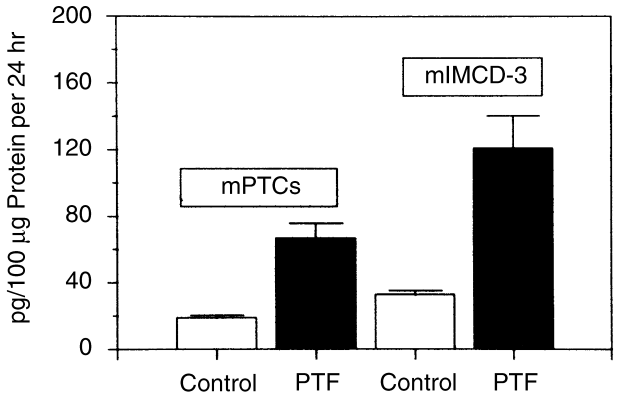

Fig. 4. Secretion of monocyte chemoattractant protein-1 (MCP-1) by mouse proximal tubular cells (mPTCs) or mouse inner medullary collecting duct cells (mIMCD-3) upon incubation with proximal tubular fluid (PTF) from rats with diabetic nephropathy.

induced recruitment of interstitial macrophages. The incubation of mPTCs or mouse inner medullary collecting duct cells (mIMCD-3) in 96-well plates with diluted proximal tubular fluid that was collected by micropuncture from rats with diabetic nephropathy increases MCP-1 mRNA levels approximately twofold as measured by quantitative reverse transcription-polymerase chain reaction ($P < 0.05$; abstract; Wang and Hirschberg, *J Am Soc Nephrol*, 9:644, 1998). Moreover, the incubation of mPTC or mIMCD-3 with rhHGF or rhTGF-β (1 nM) increases MCP-1 mRNA threefold to fourfold compared with control ($P < 0.05$). In contrast, neither rhIGF-I nor elevated glucose levels increase MCP-1 mRNA in these cells.

The secretion of MCP-1 peptide into the medium upon incubation of mPTCs and mIMCD-3 cells with diluted proximal tubular fluid from diabetic rats was examined by enzyme-linked immunosorbent assay (ELISA) using a commercially available kit (R&D Systems, Minneapolis, MN, USA). This highly sensitive assay is specific for mouse MCP-1/JE and does not recognize rat MCP-1. Proximal tubular fluid from diabetic rats at a 1:5 dilution induces a threefold to fourfold increase in MCP-1 that is secreted during 24 hours (Fig. 4). The HGF-induced stimulation of MCP-1 in mPTCs is partially blocked by coincubation with Genistein or Wortmannin, suggesting that this bioactivity of the growth factor is mediated through tyrosine kinase signaling pathways, more specifically, through pathways employing phosphatidylinositol-3 kinase.

It is conceivable that interstitial macrophages are attracted *in vivo* by MCP-1 that is specifically secreted through basolateral tubular membranes. Hence, additional experiments were performed to demonstrate that MCP-1 is secreted through basolateral membranes upon apical stimulation with HGF or TGF-β. Indeed, in mPTCs and mIMCD-3 cells that were grown to confluence on Anopore support membranes and were incubated with rhTGF-β or rhHGF (1 nM) selectively in the apical compartment, approximately two thirds of the MCP-1 accumulated in the basolateral compartment.

In summary, these studies provide experimental evidence that the glomerular ultrafiltration of high molecular weight growth factors in the nephrotic syndrome or other glomerulopathies such as diabetic nephropathy leads to an interaction with specific receptors in apical tubular membranes. Specifically, ultrafiltered IGF-I may induce increased tubular collagen production and may raise proximal tubular phosphate and distal tubular Na absorption. Ultrafiltered HGF and TGF-β increase expression and basolateral secretion of MCP-1, which may contribute to the interstitial macrophage infiltrate in these diseases. It is likely that there are other growth factor-receptor pairs, such as HGFL–Ron, where the ligand is present in glomerular ultrafiltrate and receptors are expressed in apical tubular membranes. Furthermore, other tubular effects of these ultrafiltered growth factors may also contribute to tubulointerstitial pathobiology in the nephrotic syndrome.

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