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## *Review*

# Diabetic nephropathy: The central role of renal proximal tubular cells in tubulointerstitial injury

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**Summary.** Diabetic nephropathy is now the commonest cause of end stage renal disease and accounts for 30-40% of all patients requiring renal replacement therapy. Furthermore, the incidence of diabetic nephropathy continues to increase, in part due to the improved survival of type 2 diabetic patients as the cardiovascular mortality in this group declines (Ritz and Stefanski, 1996). Clinically incipient nephropathy is first manifest by the onset of persistent microalbuminuria, after which, overt diabetic nephropathy is heralded by the appearance of persistent proteinuria. Subsequently, there is a progressive decline in glomerular filtration rate (GFR) resulting, within 5 years, in end stage renal disease in 50% of patients (Hasslacher et al., 1989). The pathology of the renal lesions are similar in type I and II diabetes (Taft et al., 1994), although it has been suggested that there is more heterogeneity in type II diabetes (Chihara et al., 1986). Studies analysing structural-functional relationships have demonstrated that the development of proteinuria correlates with the degree of mesangial expansion (Mauer et al., 1984; White and Bilous, 2000). Although diabetic nephropathy was traditionally considered a primarily glomerular disease, it is now widely accepted that the rate of deterioration of function correlates best with the degree of renal tubulointerstitial fibrosis (Mauer et al., 1984, Bohle et al., 1991). This suggests that although in the majority of patients the primary event is a condition manifest by glomerular changes resulting in proteinuria, the long-term outcome is determined by events in the renal interstitium. With the increasing awareness of the importance of these pathological interstitial changes, interest has focused on the role of cells, such as the epithelial cells of the proximal tubule (PTC) or the interstitial myofibroblast, in the initiation of fibrosis. The aim of the present review is to analyse the available data supporting the role for the PTC in orchestrating renal interstitial fibrosis in diabetic nephropathy as a result of glucose-dependent alterations in PTC function. The potential for subsequent effects on PTC-fibroblast cross-talk will also be considered.

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### Glucose, PTC and adaptive changes

Hyperglycaemia has been implicated as a major factor in the aetiology of diabetic nephropathy. However, the molecular mechanisms linking hyperglycaemia to the development of nephropathy are not completely understood. Structural alterations related to diabetes mellitus may be divided into early adaptive alterations and the later pathological changes associated with progression of nephropathy. In the glomerulus this is exemplified by early glomerular hypertrophy and glomerular basement membrane thickening, neither of which signify progressive renal disease, and mesangial matrix expansion and alterations in mesangial cellularity, culminating in glomerulosclerosis. Similarly in the tubulointerstitium, tubular hypertrophy and associated basement membrane alterations precede tubulointerstitial fibrosis, which accompanies progressive renal dysfunction. Recent studies have clearly demonstrated that an increase in tubular basement membrane (TBM) width is an integral component of early nephropathy (Brito et al., 1998). Furthermore alterations in TBM width were more closely correlated with the levels of glycosylated haemoglobin than other structural parameters. This therefore suggests a direct effect of hyperglycaemia on the turnover of the TBM matrix components. Studies with a murine cortical tubular cell line have demonstrated induction of collagen gene transcription and secretion in response to high glucose concentrations (Ziyadeh et al., 1990, 1991). Other studies using primary cultures of human renal proximal tubular cells have also demonstrated that exposure to 25mM D-glucose increased the amount of type IV collagen and fibronectin in the culture supernatant, which was not dependant on *de novo* protein synthesis (Phillips et al., 1997a-c). The accumulation of matrix

proteins is the result of a change in the balance between their synthesis and degradation and the observed increases in collagen and fibronectin were found to be associated with an alteration in their degradative pathway. Although exposure of human proximal tubular cells (HPTC) to elevated glucose concentrations increased gelatinase A enzymatic activity, as a result of induction of TIMP1 and TIMP2, there was a net decrease in gelatinolytic activity which accounted for the accumulation of fibronectin and type IV collagen. In diabetes, proximal tubular cells may be exposed to conditions of elevated glucose either apically as a result of glycosuria, or basally as a result of elevated interstitial tissue concentrations of glucose. It has been demonstrated that glucose mediated alterations in the turnover of matrix components may be stimulated by elevated concentrations of glucose at either the apical or basolateral aspect of the cells, although the alterations in matrix turnover are manifest only on their basolateral aspect (Morrisey et al., 1999a,b).

The renal reabsorption of glucose is trans-cellular. Glucose is concentrated across the luminal plasma membrane by $Na^+$-glucose co-transporters ($Na^+$-GLUT), which derive the energy for glucose transport by coupling glucose to the entry of $Na^+$. Glucose efflux from tubules into the interstitium is through basolateral facultative glucose transporters and is energised by the outwardly directed glucose gradient created by the $Na^+$-dependent influx. In diabetic rat tubules, adaptation of GLUT1 and GLUT2 expression and activity are associated with augmented facultative glucose flux, which results in a higher intracellular glucose load (Dominguez et al., 1994). Interestingly, accumulation of glucose is not a polarised phenomenon, and occurs following application of glucose to either the apical or basolateral aspect of the cell. One consequence resultant from intracellular glucose accumulation is activation of the polyol pathway. The metabolism of hexose or pentose sugars by this pathway is of great importance in tissues which do not require insulin for glucose uptake, such as the kidney, lens, retina and peripheral nerves. Importantly these organs are the major sites of diabetic complications. Numerous groups have demonstrated the importance of this pathway in glucose mediated alterations in proximal tubular cell matrix generation (Ziyadeh et al., 1991; Bleyer et al., 1994; Morrisey et al., 1999a,b). It has however been demonstrated that there is a significant amount of variability in the ability of cultured proximal tubular epithelial cells (PTC) from different individuals to activate the polyol pathway (Flath et al., 1992). This therefore may be one factor, of many, which explains the heterogeneous nature of diabetic nephropathy. It is clear however that this pathway is not the sole mediator of glucose induced changes in PTC function.

In addition to alterations in TBM width, tubular hypertrophy is an early renal structural feature of diabetes. In contrast to alterations in matrix generation, cell hypertrophy is not mediated by the polyol pathway (Ziyadeh et al., 1991). In contrast, the hypothesis has been put forward that high concentrations of filtered glucose contribute to cell hypertrophy by increasing proximal tubular sodium absorption (Kumar et al., 1988; Bank and Aynedjian 1990), as an increase in intracellular sodium concentration has been shown to play a pivotal role in the development of cellular hypertrophy (Stanton and Kaissling, 1989). It has been demonstrated that in isolated perfused tubules glucose added to the luminal fluid, but not the abluminal fluid/bath increases fluid absorption and transepithelial electrical potential (Burg et al., 1976). In addition, in both normal and diabetic rats, progressive increases in intra-luminal D-glucose concentration stimulate net sodium and water reabsorption (Bank and Aynedjian, 1990). A rise in intracellular sodium has also been reported *in vitro* when proximal tubular cells were exposed to an elevated glucose concentration (Kumar et al., 1988). As diabetes is also associated with an increase in Na/K ATPase activity (Pollock et al., 1991), it is likely that the increase in intracellular sodium relates to stimulation of apical sodium entry by brush border Na-glucose co-transporter (Bank and Aynedjian, 1990) beyond the rate of basolateral exit (Pollock et al., 1991). This enhanced tubular transport of sodium, is thought to be causally related to renal/tubular hypertrophy. Although the mechanism, by which cell hypertrophy is mediated is not entirely clear, several possible mechanisms have been put forward including alteration of alteration of intracellular calcium (Haworth et al., 1980), Protein kinase C activation (Takai, 1979), alteration of Na/H exchanger activity and alteration of cell alkalinisation (Fine et al., 1985).

## Glucose, PTC and alterations in renal structure associated with progressive renal disease

### *1. Influence of glucose on PTC Hyaluronan synthesis*

In addition to alterations in the turnover of "normal" matrix constituents, the diabetic state may also lead to the *de novo* induction of "abnormal" structural elements, such as hyaluronic acid (HA). HA is a water-soluble glycosaminoglycan, which is a key constituent of the pericellular matrix and has important structural functions in the extracellular matrix of all tissues. It has only recently been recognised that it can perform more subtle functions than just serving as a structural scaffold. HA may function as a cellular signalling molecule, following either binding to its cell surface receptors, (CD44 and RHAMM) or following internalisation via CD-44 mediated endocytosis (Hua et al., 1993). HA has therefore been implicated in a number of biological processes, including embryonic development, tumour growth, chronic inflammation and wound healing (Laurent and Fraser, 1992).

In the normal kidney HA is expressed in the interstitium of the renal papilla only, however it is known to be expressed around proximal tubular cells

following renal injury (Lewington et al., 2000), and during progressive interstitial fibrosis (Wells et al., 1990, 1993; Sibalic et al., 1997). Although alterations in HA generation have been implicated in the pathogenesis of the glomerular lesions of diabetic nephropathy (Mahadevan et al., 1995, 1996), much less is known regarding its regulation in the interstitium in the diabetic state. Recent studies using cultured human proximal tubular cells, however, have demonstrated increased HA synthesis in response to either IL-1ß or elevated 25 mM D-glucose is associated with nuclear factor NF-κB activated transcription of the hyaluronan synthase gene HAS 2 (Jones et al., 2001). It has been shown that hyaluronan induces monocyte chemoattractant protein-1 expression (Beck-Schimmer et al., 1998) and up-regulation of ICAM-1 and VCAM-1 (Oertli et al., 1998) in renal tubular epithelial cells, the latter also involving activation of NF-kB. Although primarily a "metabolic" condition, macrophage influx, has been implicated in the pathogenesis of interstitial fibrosis associated with diabetic nephropathy. It is interesting to speculate therefore that the alteration in PTC HA generation may be a key factor which regulates leucocyte recruitment into the cortico-interstitium and also their interaction with resident cells in diabetic nephropathy.

### *2. Influence of glucose on PTC TGFß1 generation*

Transforming growth factor ß1 (TGFß1) has emerged as a mediator which is implicated in the pathogenesis of fibrosis in both glomerular and interstitial compartments of the kidney and has been particularly associated with diabetic nephropathy (Yamamoto et al., 1993). It is now clear that epithelial cells of the proximal tubule have the potential to contribute to the pathogenesis of renal fibrosis by the production of pro-fibrotic growth factors, including TGFß1 (Phillips et al., 1995, 1996, 1997a-c). Recent studies have demonstrated that TGFß1 production in PTC may be controlled at the levels of transcription, translation and secretion of pre-formed protein as well as at the level of activation of the latent protein (Phillips et al., 1995, 1996, 1997a-c). In the context of diabetic nephropathy the synergistic action of elevated concentrations of glucose with cytokines such as platelet derived growth factor (PDGF) or interleukin 1ß (IL-1ß) will stimulate TGF-ß1 synthesis by PTC (Phillips et al., 1995, 1996). In these studies, induction of TGF-ß1 synthesis resulted from the stimulation of translation of glucose-induced TGF-ß1 mRNA by either cytokine, and was associated with an alteration in its inherent stability. From these studies, it is interesting to speculate that although glucose primes PTC for TGF-ß1 synthesis, that glucose stimulated leucocyte recruitment via hyaluronan generation is necessary for TGF-ß1 protein synthesis. In contrast, studies utilising SV40 transformed proximal tubular epithelial cells, demonstrated that exposure to high glucose alone was sufficient to increase their generation of TGF-ß1 (Rocco et al., 1992). Transformation by SV40 may be accompanied by alterations in response to cytokines, which may explain discrepancies in results between transformed and non-transformed cells (Wang et al., 1996). The results obtained in primary cultures of HPTC, however, are similar to those observed in studies using non-transformed endothelial cells. These demonstrated inhibition of cell proliferation and increased gene expression of basement components and TGF-ß1, on addition of high concentrations of D-glucose (Cagliero et al., 1988a,b, 1991). Significantly, however, there was no increase in active TGF-ß1 production, as assessed by bioassay. Furthermore the effects of elevated D-glucose on cell proliferation and basement membrane component gene expression, were not antagonised by the addition of neutralising antibodies to TGF-ß1 (Cagliero et al., 1995). These results therefore suggest that glucose alone may be insufficient to induce TGF-ß1. This is consistent with the clinical observation that hyperglycaemia alone is not sufficient to initiate progressive renal disease in all patients.

### *3. Functional role of PTC-derived TGF-ß1*

(a) Effect on PTC

Many laboratories, including our own, have begun to investigate the role of the interstitial fibroblast in the progression of fibrosis. In the normal interstitium there are relatively few of these cells and they lie in close apposition to the PTC basement membrane. In progressive disease, however, their numbers increase and they take on the phenotypic appearance of activated myofibroblasts. These cells are believed to be the major source of the expanded extracellular matrix and their presence has been suggested as the best marker for progressive disease. The cellular origin of the myofibroblast, however, remains largely unknown. Hypotheses put forward include proliferation and activation of resident interstitial cells, migration of fibroblasts into the site of injury or the transdifferentiation of PTC. Of these the latter has particularly received increasing attention in recent years.

Transdifferentiation is defined as a shift in the phenotype of a differentiated cell to that of another cell type. Transdifferentiation of epithelial cells into mesenchymal cells has been widely described in other cell systems. In the kidney, the work of Strutz et al. suggested that PTC may express fibroblast specific markers *in vitro* and *in vivo* in a murine model of anti-TBM and anti-GBM model of nephritis (Strutz, 1995). More recent studies in 5/6 nephrectomised rats have demonstrated *de novo* expression of α-smooth muscle actin (α-SMA), a marker of myofibroblast phenotype, by PTC, associated with disruption of the tubular basement membrane (Ng et al., 1998). Furthermore more recent studies have identified a murine fibroblast specific marker, FSP1, which when transfected into murine PTC, led to a marked alteration in cell phenotype suggestive

of transdifferentiation (Strutz et al., 1995). Expression of this protein in murine proximal tubular epithelial cells can be induced by growth factors, such as TGF-ß1, and alterations in the extracellular matrix suggesting a role for these factors as potential modulators of renal epithelial transdifferentiation (Okada et al., 1997).

Recent studies have shown that stimulation of renal PTC with TGF-ß1 led to marked alteration in cell morphology, such that cells acquired an elongated, spindle shape, "fibroblastic" appearance associated with rearrangement of their actin cytoskeleton. In addition "activation" of the epithelial cell by the addition of TGF-ß1 was associated with induction of α-SMA mRNA and recruitment of actin into polymerisation of stress fibres. Despite these changes cells remained cytokeratin positive and desmin negative. Furthermore these changes were reversible on removal of TGF-ß1, suggesting that TGF-ß1 did not induce a stable alteration in cell phenotype. Although PTC expressed mRNA for type I collagen, alteration in cell morphology was not associated with changes in type I collagen mRNA expression. Similarly no stimulation of type I collagen protein synthesis could be demonstrated. In contrast alteration in PTC morphology in response to TGF-ß1 was associated with induction of type IV collagen mRNA and stimulation of collagen synthesis and its incorporation into the extracellular matrix. These studies therefore suggest that PTC are not the source of interstitial myofibroblasts, and also that TGF-ß1-activated PTC are not the source of the interstitial collagens which accumulate in the renal interstitium during progressive interstitial fibrosis.

In addition to its effect on PTC "activation" and matrix turnover PTC derived TGF-ß1 may also affect PTC cytokine generation. It has been demonstrated that TGF-ß1 stimulates FGF-2 generation by PTC is by stimulation of the secretion of pre-formed cytokine from within the cells (Jones et al., 1999a,b). FGF-2 in turn may also stimulate the secretion of pre-formed, latent TGF-ß1 by PTC suggesting a positive feedback loop involving TGF-ß1 and FGF-2, which may be involved in the progressive nature of renal fibrosis *in vivo*.

*(b) Effect on fibroblast*

Based on histological evidence that PTC injury precedes interstitial fibrosis (Knecht et al., 1991), and that regions of active interstitial fibrosis predominantly exhibit a peritubular rather than perivascular distribution (Alpers et al., 1994; Fine et al., 1995), it has been postulated that PTC relay fibrogenic signals to cortical fibroblasts in diseased kidneys. There is clear evidence to support this hypothesis, in that conditioned medium collected form non-stimulated PTC, may alter fibroblast mitogenesis and matrix synthesis (Lewis et al., 1996; Johnson et al., 1998; Lewis and Norman, 1998). In addition, TGF-ß1 regulates the terminal differentiation of fibroblasts and this is associated with induction of type I, type III and type IV collagen gene expression and protein deposition into the extracellular matrix. It is interesting to speculate therefore that PTC derived TGF-ß1 may be the key to the generation of the myofibroblast in the renal cortex in diabetes which subsequently mediates the progressive deposition of interstitial collagens.

**Potential for cross-talk**

Glucose also has the potential to stimulate the interstitial fibroblast to increase its synthesis of extracellular matrix and fibrillar collagens. This may be mediated by a direct effect on the cell (Jones et al., 1999a,b) or through an autocrine loop involving the induction of TGF-ß1 (Han et al., 1999). Furthermore the fibroblasts may also release factors which in turn stimulate PTC (Johnson et al., 1998). Thus TGF-ß1 and PDGF released from PTC enhanced the secretion of insulin-like growth factor-1 (IGF-1) and IGF binding protein-3 (IGFBP-3) from cortical fibroblasts. IGF-1 is believed to have an important role in modulating the progression of diabetic nephropathy (Sayed-Ahmed et al., 1993; Wang et al., 1999, 2000; Rossert et al., 2000). It has also been shown to have a direct effect on PTC, including increasing phosphate transport into the cells (Silverstein et al., 2000). The conditioned medium from TGF-ß1-treated fibroblasts was subsequently shown to induce the proliferation and increased $Na^+/H^+$ exchange activity of PTC (Johnson et al., 1999). The active agent in the supernatants was identified as IGF-1 and the response could be reproduced by the addition of exogenous IGF-1 to the PTC. Furthermore the effect of IGF-1 was enhanced by IGFBP-3 implying a further method of control on this paracrine system. These observations not only implicate the PTC in the modulation of the function of adjacent fibroblasts but also demonstrate that the fibroblasts have the potential for a reciprocal effect on the function of the PTC. Furthermore we have recently reported that insulin affects the production of TGF-ß1 by PTC an effect mediated by signalling via the IGF-1 receptor (Morrisey et al., 1999a,b). This was a post-transcriptional effect involving a cytoplasmic protein binding to the 5' UTR of the TGF-ß1 mRNA transcript. There is therefore a great potential for reciprocal paracrine activation of PTC and fibroblasts.

The PTC is the predominant cell type in the normal renal intersititium, in which there are few resident interstitial fibroblasts. Adaptive changes in the renal cortex such as tubular cell hypertrophy and tubular membrane expansion, are a direct consequence of the alteration in the "glycaemic environment". We hypothesise that the pathological changes in the diabetic renal intersitium associated with progressive renal insufficiency are initiated by glucose, which acts in combination with cytokines and pro-inflammatory stimuli. These cyokine-activated cells, however, are not a source of intersitial collagens. Furthermore the evidence supporting human PTC-myofibroblst

transdifferentiation is still inconclusive. Activated PTC, however do contribute to interstitial fibrosis indirectly by activation of interstitial fibroblast. Stimulated fibroblasts and myofibroblasts in turn may further amplify this response by their reciprocal action on the PTC. Further research in this area of PTC-interstitial fibroblast cross-talk may therefore hold the key to the understanding of the mechanisms that underlie the development of progressive renal fibrosis in diabetes mellitus.

## References

Alpers C.E., Hudkins K.L., Floege J. and Johnson R.J. (1994). Human renal cortical interstitial cells with some features of smooth muscle cells participate in tubulointerstitial and crescentic glomerular injury. J. Am. Soc. Nephrol. 5, 210-210.

Bank N. and Aynedjian H.S. (1990). Progressive increases in luminal glucose stimulate proximal sodium adsorption in normal and diabetic rats. J. Clin. Invest. 86, 309-316.

Beck-Schimmer B., Oertli B., Pasch T. and Wüthrich R.P. (1998). Hyaluronan induces monocyte chemoattractant protein-1 expression in renal tubular epithelial cells. J. Am. Soc. Nephrol. 9, 2283-2290.

Bleyer A.J., Fumo P., Snipes E.R., Goldfarb S., Simmons D.A. and Ziyadeh F.N. (1994). Polyol pathway mediates high glucose induced collagen synthesis in proximal tubule. Kidney Int. 45, 659-666.

Bohle A., Wehrmann M., Bogenschutz O., Batz C. and Muller G.A. (1991). The pathogenesis of chronic renal failure in diabetic nephropathy. Pathol. Res. Pract. 187, 251-259.

Brito P.L., Fioretto P., Drummond K., Kim Y., Steffes M.W., Basgen J.M., Sisson-Ross S. and Mauer M. (1998). Proximal tubular basement membrane width in insulin-dependent diabetes mellitus. Kidney Int. 53, 754-761.

Burg M., Patlak N., Green N. and Villey D. (1976). Organic solutes in fluid absorption by renal proximal convoluted tubules. Am. J. Physiol. 231, 2:627-637.

Cagliero E., Maiello M., Boeri D. and Lorenzi M. (1988a). High glucose increases TGF-ß mRNA in endothelial cells: a mechanism for the altered regulation of basement membrane components? Diabetes 37 (Suppl 1), 97A.

Cagliero E., Maiello M., Boeri D., Roy S. and Lorenzi M. (1988b). Increased expression of basement components in human endothelial cells cultured in high glucose. J. Clin. Invest. 82, 735-738.

Cagliero E., Roth T., Maiello M. and Lorenzi M. (1991). Expression of genes related to the extracellular matrix in human endothelial cells. J. Biol. Chem. 266, 22:14244-14250.

Cagliero E., Roth T., Taylor A.W. and Lorenzi M. (1995). The effects of high glucose on human endothelial cell growth and gene expression are not mediated by TGF-ß. Lab. Invest. 73, 667-673.

Chihara J., Takegayashi S., Taguchi T., Yokatama K., Horada T. and Naito S. (1986). Glomerulonephritis in diabetic patients and its effect on the prognosis. Nephron 43, 45-49.

Dominguez J.H., Camp K., Maianu L., Feister H. and Garvey T. (1994). Molecular adaptations of GLUT1 and GLUT2 in renal proximal tubules of diabetic rats. Am. J. Physiol. 266, F283-F290.

Fine L.G., Bradley-Dezfooly B., Lowe A.G., Hamzeh A., Wells J. and Saaehmoghaddam S. (1985). Stimulation of the Na/H antiport is an early event in hypertrophy of renal proximal tubule cells. Proc. Nat. Acad. Sci. 82, 1736-1740.

Fine L.G., Norman J.T. and Ong A. (1995). Cell-cell cross-talk in the pathogenesis of renal interstitial fibrosis. Kidney Int. 49, S48-S50.

Flath M.C., Bylander J.E. and Sens D.A. (1992). Variation in sorbitol accumulation and polyol-pathway activity in cultured human proximal tubular cells. Diabetes 41,1050-1055.

Han D.C., Isono M., Hoffman B.B. and Ziyadeh F.N. (1999). High glucose stimulates proliferation and collagen type I synthesis in renal cortical fibroblasts: mediation by autocrine activation of TGF-beta. J. Am. Soc. Nephrol. 10, 1891-1899.

Hasslacher C., Ritz E., Wahl P. and Michael C. (1989). Similar risks of nephropathy in patients with Type I or Type II diabetes mellitus. Nephrol. Dial. Transplant. 4, 859-863.

Haworth R.A., Hunter D.R. and Berkoff H.A. (1980). Na releases Ca from the liver, kidney and lung mitochondria. FEBS Lett. 110, 216-218.

Hua Q., Knudson C.B. and Knudson W. (1993). Internalisation of hyaluronan by chondrocytes occurs via receptor mediated endocytosis. J. Cell Sci. 106, 365-375.

Johnson D.W., Saunders H.J., Baxter R.C., Field M.J. and Pollock C.A. (1998). Paracrine stimulation of human renal fibroblasts by proximal tubule cells. Kidney Int. 54, 747-757.

Johnson D.W., Saunders H.J., Johnson F.J., Huq S.O., Field M.J. and Pollock C.A. (1999). Cyclosporin exerts a direct fibrogenic effect on human tubulointerstitial cells: roles of insulin-like growth factor I, transforming growth factor beta1, and platelet-derived growth factor. J. Pharmacol. Exp. Ther. 289, 535-542.

Jones S.C., Saunders H.J. and Pollock C.A. (1999a). High glucose increases growth and collagen synthesis in cultured human tubulointerstitial cells. Diabet Med. 16, 11, 932-938.

Jones S.G., Morrisey T., Williams J.D. and Phillips A.O. (1999b). TGF-ß1 stimulates release of pre-formed bFGF from renal proximal tubular cells. Kidney Int. 56, 83-91.

Jones S.G., Jones S. and Phillips A.O. (2001). Regulation of renal proximal tubular epithelial cell hyaluronan generation - implications for diabetic nephropathy. Kidney Int. (in press).

Knecht A., Fine L.G., Kleinman K.S., Rodermann H.P., Muller G.A., Woo D.D.L. and Norman J.T. (1991). Fibroblasts of rabbit kidney in culture II: paracrine stimulation of papillary fibroblasts by PDGF. Am. J. Physiol. 261, F292-F299.

Kumar A.M., Gupta R.K. and Spitzer A. (1988). Intracellular sodium in proximal tubules of diabetic rats: Role of glucose. Kidney Int. 33, 792-797.

Laurent T.C. and Fraser R.E. (1992). Hyaluronan. FASEB J. 6, 2397-2404.

Lewington A.J.P., Padanilam B.J., Martin D.R. and Hammeramn M.R. (2000). Expression of CD44 in kidney after acute ischemic injury in rats. Am. J. Physiol. 278, R247-R254.

Lewis M.P., Fine L.G. and Norman J.T. (1996). Pexicrine effects of basement membrane components on paracrine signalling by renal tubular cells. Kidney Int. 49, 48-58.

Lewis M.P. and Norman J.T. (1998). Differential response of activated versus non-activated renal fibroblasts to tubular epithelial cells; A model of initiation and progression of fibrosis? Exp. Nephrol. 6, 132-143.

Mahadevan P., Larkins R.G., Fraser J.R.E., Fosang A.J. and Dunlop M.E. (1995). Increased hyaluronan production in the glomeruli from diabetic rats: link between glucose induced prostaglandin production and reduced sulphated proteoglycans. Diabetologia 38, 298-305.

Mahadevan P., Larkins R.G., Fraser R.E. and Dunlop M.E. (1996). Effect of prostaglandin E2 and hyaluronan on mesangial cell proliferation. Diabetes 45, 44-50.

Mauer S.M., Steffes M.W., Ellis E.E., Sutherland D.E.R., Brown D.M. and Goetz F.C. (1984). Structural-functional relationships in diabetic nephropathy. J. Clin. Invest. 74, 1143-1155.

Morrisey K., Steadman R., Williams J.D. and Phillips A.O. (1999a). Renal proximal tubular cell fibronectin accumulation in response to glucose is polyol pathway dependent. Kidney Int. 55, 160-167.

Morrisey K., Williams J.D. and Phillips A.O. (1999b). Modulation of renal proximal tubular cell TGF-ß1 production by insulin. J. Am. Soc. Nephrol. 10, 554A.

Ng Y.Y., Huang T.P., Yang W.C., Chen Z.P., Yang A.H., Mu W., Nikolic-Paterson D.J., Atkins R.C. and Lan H.Y. (1998). Tubular epithelial myofibroblast transdifferentiation in progressive tubulointerstitial fibrosis in 5/6 nephrectomized rats. Kidney Int. 54, 864-876.

Oertli B., Beck-Schimmer B., Fan X. and Wüthrich R.P. (1998). Mechanisms of hyaluronan-induced up-regulation of ICAM-1 and VCAM-1 expression by murine kidney tubular epithelial cells: Hyaluronan triggers cell adhesion molecule expression through a mechanism involving activation of nuclear factor-κB activating protein-1. J. Immunol. 161, 3431-3437.

Okada H., Danoff T.M., Kalluri R. and Neilson E.G. (1997). Early role of Fsp1 in epithelial mesenchymal transformation. Am. J. Physiol. 273, F563-F574.

Phillips A.O., Steadman R., Topley N. and Williams J.D. (1995). Elevated D-glucose concentrations modulate TGF-ß1 synthesis by human cultured renal proximal tubular cells: the permissive role of platelet derived growth factor. Am. J. Pathol. 147, 362-374.

Phillips A.O., Topley N., Steadman R., Morrisey K. and Williams J. (1996). Induction of TGF-ß1 synthesis in D-glucose primed human proximal tubular cells: differential stimulation by the macrophage derived pro-inflammatory cytokines IL-1ß and TNFα. Kidney Int. 50, 1546-1554.

Phillips A.O., Morrisey K., Martin J., Williams J.D. and Steadman R. (1997a). Exposure of human renal proximal tubular cells to glucose leads to accumulation of type IV collagen and fibronectin by decreased degradation. Kidney Int. 52, 973-984.

Phillips A.O., Morrisey R., Steadman R. and Williams J.D. (1997b). Polarity of stimulation and secretion of TGF-ß1 by proximal tubular cells. Am. J. Pathol. 150, 3:1101-1111.

Phillips A.O., Topley N., Morrisey K., Williams J.D. and Steadman R. (1997c). Basic fibroblast growth factor stimulates the release of pre-formed TGF-ß1 from human proximal tubular cells in the absence of de-novo gene transcription or mRNA translation. Lab. Invest. 76,4:591-600.

Pollock C.A., Field M.J., Bostrom T.E., Dyne M., Gyory A.Z. and Cockayne D.J.H. (1991). Proximal tubular cell sodium concentration in early diabetic nephropathy assessed by electron microprobe analysis. Eur. J. Physiol. 418, 14-17.

Ritz E. and Stefanski A. (1996). Diabetic nephropathy in type II diabetes. Am. J. Kidney Dis. 27, 167-194.

Rocco M.V., Chen Y., Goldfarb S. and Ziyadeh F.N. (1992). Elevated glucose stimulates TGF-ß gene expression and bioactivity in proximal tubule. Kidney Int. 41, 107-114.

Rossert J., Terraz-Durasnel C. and Brideau G. (2000). Growth factors, cytokines, and renal fibrosis during the course of diabetic nephropathy. Diabetes Metab. 26 (Suppl 4), 16-24.

Sayed-Ahmed N., Muchaneta-Kubara E.C., Besbas N., Shortland J., Cope G.H. and El Nahas A.M. (1993). Insulin-like growth factor-I and experimental diabetic kidney disease. Exp. Nephrol. 1, 364-371.

Sibalic V., Fan X., Loffing J. and Würthrich R.P. (1997). Upregulated renal tubular CD44, hyaluronan, and osteopontin in kdkd mice with interstitial nephritis. Nephrol. Dial. Transplant. 12, 1344-1353.

Silverstein D., Spitzer A. and Barac-Nieto M. (2000). Hormonal regulation of sodium-dependent phosphate transport in opossum kidney cells. Horm. Res. 54, 38-43.

Stanton B.A. and Kaissling B. (1989). Regulation of renal ion transport and cell growth by sodium. Cell 257, F1-F10.

Strutz F. (1995). Novel aspects of renal fibrogenesis. Nephrol. Dial. Transplant. 10, 1526-1532.

Strutz F., Okada H., Lo C.W., Danoff T., Carone R.L., Tomaszewski J.E. and Neilson E.G. (1995). Identification and characterisation of a fibroblast marker: FSP1. J. Cell Biol. 130, 393-405.

Taft J.L., Nolan C.J., Yeung S.P., Hewitson T.D. and Martin I.R. (1994). Clinical and histological correlations of decline in renal function in diabetic patients with proteinuria. Diabetes 43, 1046-1051.

Takai Y. (1979). Calcium-dependent activation of a multi-functional protein kinase by membrane phospholipids. J. Cell Biol. 254, 3692-3695.

Wang J.L., Nister M., Rudloff E.B., Ponten J. and Westermark B. (1996). Suppression of platelet derived growth factor α- and ß-receptor mRNA levels in human fibroblast by SV40 T/t antigen. J. Cell. Physiol. 166, 12-21.

Wang S.N., LaPage J. and Hirschberg R. (1999). Pathophysiologic glomerulotubular growth factor link. Miner. Electrolyte Metab. 25, 234-241.

Wang S.N., LaPage J. and Hirschberg R. (2000). Role of glomerular ultrafiltration of growth factors in progressive interstitial fibrosis in diabetic nephropathy. Kidney Int. 57, 1002-1014.

Wells A., Larsson E., Hanas E., Laurent T., Hallgren R. and Tufvesson G. (1993). Increased hyaluronan in acutely rejecting human kidney grafts. Transplantation 55, 1346-1349.

Wells A.F., Larsson E., Tengblad A., Fellstrom B., Tufvesson G., Klareskog L. and Laurent T.C. (1990). The localisation of hyaluronan in normal and rejected human kidneys. Transplantation 50, 240-243.

White K.E. and Bilous R.W. (2000). Type 2 diabetic patients with nephropathy show structural-functional relationships that are similar to type 1 disease. J. Am. Soc. Nephrol. 11, 1667-1673.

Yamamoto T., Nakamura T., Noble N.A., Ruoslahti E. and Border W.A. (1993). Expression of Transforming Growth Factor-ß is elevated in human and experimental diabetic nephrophathy. Proc. Natl. Acad. Sci. 90, 1814-1818.

Ziyadeh F.N., Simmons D.A., Snopes E.R. and Goldfarb S. (1991). Effect of Myo-inositol on cell proliferation and collagen transcription in proximal tubule cells cultured in elevated glucose. J. Am. Soc. Nephrol. 1, 1220-1229.

Ziyadeh F.N., Snipes E.R., Watanabe M., Alverez R.J., Goldfarb S. and Haverty T. (1990). High glucose induces cell hypertrophy and stimulates collagen gene transcription in proximal tubule. Am. J. Physiol. 259, F704-F714.

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