FEATURING OF RENAL TUBULOINTERSTITIAL DISEASE

Introduction to Guest Editor - Dr. Mai-Szu Wu

I wish to thank Dr. Mai-Szu Wu for serving as the guest editor. The group of authors who have contributed articles to this issue on “Featuring of Renal Tubulointerstitial Disease” are outstanding experts in their field.


Dr. Mai-Szu Wu

Dr. Wu graduated from Taipei Medical University in 1985 and completed his residency in internal medicine at Chang Gung Memorial Hospital in 1989. Subsequently he had a clinical fellowship in nephrology in the same institute and one more year fellowship at Institut Nationale de la Santé et de la Recherche Médicale (INSERM) Unité 246 Paris between 1993 and 1994.

Currently Dr. Wu is a full professor at College of Medicine, Chang Gung University and has been serving as the director of the division of nephrology in the department of internal medicine at Keelung Chang Gung Memorial Hospital since 2003.

Dr. Wu’s research interest mainly focuses on Renal Cellular Physiology and Biology, Fluid and Electrolytes disorders, Clinical Nephrology and Renal Replacement Therapy. He has been quite enthusiastic in academic pursuit and published more than 100 articles in various Journals and read more than 50 abstract in various conferences. As the result of his outstanding performance, he has been awarded many times both domestically and internationally including Asian Young Investigator Award by international society of peritoneal dialysis, Young Investigator Award by European Renal Association-European Dialysis and Transplant Association and Investigator Award of international forum on Angiotensin II receptor antagonist twice in the year of 1999 and in the year of 2000.

Fu-Chan Wei, MD
Editor-in-Chief

The Role of Proximal Tubular Cells in Interstitial Fibrosis: Understanding TGF-β1

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In PTC, it is clear that TGF-β1 synthesis may be controlled independently at the levels of transcription and translation. In the context of diabetic nephropathy glucose is a potent stimulus of TGF-β1 promoter activity. The resultant transcript is however poorly translated such that stimulation of PTC with glucose does not increase de novo TGF-β1 protein synthesis. Although diabetes is a "metabolic" disease, in the kidney, nephropathy is associated with an inflammatory cell infiltrate. For example using the GK rat model of type II diabetes, we have demonstrated that progressive renal disease is associated with a prominent macrophage influx. This led us to examine TGF-β1 regulation when the effects of macrophage derived cytokines such as platelet derived growth factor and interleukin-1 are combined with exposure to elevated glucose concentrations. These studies have demonstrated that such cytokines specifically facilitate translation of glucose induced TGF-β1 transcripts.

In addition, direct interaction between monocyte-macrophage CD18 and PTC cell surface ICAM-1 stimulates TGF-β1 synthesis. Recent data from numerous experimental systems have suggested that the extracellular matrix component hyaluronan (HA) may be involved in the regulation of the inflammatory process. We have now identified HA based structures synthesised on the surface of PTC, which act to prevent PTC-macrophage interaction through ICAM-1 thus preventing macrophage driven TGF-β1 synthesis. Disease promoting cytokines such as IL-1β down-regulate these structures whilst potential therapeutic agents such as BMP-7 increase their assembly, that HA may possess disease limiting activity. *(Chang Gung Med J 2007;30:2-6)*

Key words: fibrosis, epithelial cells, TGF-β1, macrophages, hyaluronan

Why are we interested in the proximal tubular cell?

Renal interstitial fibrosis is the common end result caused by diverse clinical entities such as obstruction, chronic inflammation and diabetes, resulting in end stage renal failure.[1,2] With the realisation that the degree of interstitial fibrosis is the best correlate with the rate of progression of renal dysfunction,[3-8] interest has focused on the possible mechanisms which may drive this process. The most prominent cell type in the renal cortex is the proximal tubular epithelial cell (PTC), responsible in health for the maintenance of fluid and electrolyte balance. This cell type may influence the fibrotic process in the renal interstitium both by the generation of pro-fibrotic cytokines and also through the process of epithelial-mesenchymal transformation. PTC may be one source of the interstitial fibroblast

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which is a key direct mediator of the fibrotic process.

The most common cause of renal failure in our patient population in the UK is now diabetic nephropathy in which hyperglycaemia is thought to play a key role. Many of our studies are focused on identifying the mechanisms which stimulate PTC transforming growth factor β1 (TGF-β1) synthesis in the context of diabetic nephropathy,[9-13] as TGF-β1 plays a pivotal role in accumulation of extracellular matrix during renal fibrosis and the transition of renal tubular epithelial cells to myofibroblasts.[14,15]

TGF-β1 regulation

A single TGF-β1 transcript 2.5 KB in length has been described in human cells. Many studies have shown that this is inherently poorly translated, leading to tissue specific disparity between mRNA and protein expression. Our studies have confirmed that this is also true of TGF-β1 mRNA expressed by PTC. Exposure of PTC to elevated D-glucose concentrations increased the expression of a poorly translated TGF-β1 transcript without any associated change in TGF-β1 protein synthesis.[9] More recently we have demonstrated synergistic effects on TGF-β1 synthesis following PDGF stimulation of glucose pre-treated cells.[11] Without a prior glucose- induced increase in the amount of TGF-β1 transcript, PDGF did not stimulate significant TGF-β1 protein synthesis. PDGF at low doses did not influence TGF-β1 transcription, but led to alteration in glucose induced TGF-β1 mRNA stability and translation. We have also demonstrated that prolonged culture of PTC under conditions of high glucose stimulated de-novo TGF-β1 synthesis, a process that involved a p38MAP kinase mediated glucose dependent increase in TGF-β1 transcription, and stimulation of PDGF alpha receptor signalling which facilitated TGF-β1 mRNA translation, the latter being dependent upon activation of the ERK MAP kinase pathway.[12] Translational regulation of TGF-β1 synthesis is not, however, confined to PDGF. Stimulation of glucose pre-treated PTC with IL-1β also facilitates TGF-β1 mRNA translation, a process that is accompanied by an increase in TGF-β1 mRNA stability.[10] The 2.5 kb TGF-β1 transcript has unusually long, GC rich 5' untranslated regions (UTR),[16] a feature suggestive of translational regulation.[17] This region has been demonstrated to inhibit translation *in vitro*, and deletion analysis of the 5'UTR using heterologous reporter gene constructs suggests that the region between nucleotides +11 and +147 (termed the D region) is the key part of this sequence with respect to inhibition of translation.[18] Our recent work has highlighted the importance of the 5' untranslated region of TGF-β1 mRNA in translational regulation of TGF-β1, and has identified the Y-box protein-1 (YB-1) as an important regulator of translation through interaction with the 5'UTR +11-+147 region of TGF-β1 mRNA.[19]

Macrophages as the "second stimulus"

Our work, therefore, suggests that elevated glucose concentrations alone may be insufficient to initiate pathological changes, but may prime the kidney for enhanced responses when exposed to other insults. This is consistent with the clinical observation that only 30% of all diabetic patients develop diabetic nephropathy, and that its pathogenesis is multifactorial in aetiology, rather than the sole result of hyperglycaemia. Identification of the source of factors which may act synergistically with glucose to initiate pathological changes in diabetic nephropathy is therefore an important goal.

Although considered primarily to be a metabolic abnormality, macrophage infiltration has been implicated in recent studies in the pathogenesis of diabetic nephropathy. In *vivo* studies of streptozotocin-induced diabetic rats demonstrated prominent macrophage infiltration.[20, 21] A role for macrophages is further supported by our studies in the Goto-Kakizaki model of type II diabetes. This is a model of prolonged type 2 diabetes with no overt renal disease.[22] Induction of hypertension in these animals, however, led to prominent tubulo-interstitial macrophage influx and significant interstitial fibrosis[23] In addition to work in animal models of diabetic nephropathy, studies of renal biopsies taken from patients with non-insulin-dependent diabetes mellitus also suggested that macrophages and their products are involved in the initiation of the pathological changes of human diabetic nephropathy.[24] Clearly macrophages are a rich source of cytokines such as IL-1β and PDGF, which *in vitro*, act synergistically with glucose to stimulate generation of the pro-fibrotic cytokine TGF-β1as discussed above. How then could macrophages be recruited into the renal interstitium and what is the role of PTC in the process?

Hyaluronan - regulator of macrophage recruitment, TGF-β1 generation and signalling?

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), the accumulation of which may be associated with inflammatory diseases.[25,26] HA is a water-soluble glycosaminoglycan, which is a key constituent of the peri-cellular 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 CD44 mediated endocytosis.[27] The functional significance of altered generation of HA in the kidney, remains unclear.

The association of an increase in HA in the renal cortical interstitium and the rate of progression of IgA nephropathy has led to the hypothesis that HA has a disease promoting activity. This is supported by in vitro studies, as addition of exogenous low molecular weight hyaluronan induced monocyte chemoattractant protein-1 expression[28] and up-regulation of ICAM-1 and VCAM-1[29] in renal tubular epithelial cells, suggesting a potential pro-inflammatory effect for HA in the renal cortex. In contrast to these potential pro-inflammatory effects of low molecular weight HA, we have demonstrated that addition of exogenous HA of a high molecular weight (2×10^6) and crosstalk between CD44 and the TGF-β receptor leads to attenuation of TGF-β1 signalling,[30] increased trafficking of TGF-β receptors to lipid raft-associated pools[31] and increased receptor turnover. Significantly however HA of lower molecular weight (65000) did not antagonise the effect of TGF-β1.[31] These observations are consistent with the assumption that in general, high molecular weight HA represents the normal homeostatic state whereas the generation of low molecular weight HA fragments signals a disruption of the normal homeostatic environment, which may have disease promoting activity.

Several cell types *in vitro* surround themselves with HA in an organised peri-cellular matrix or "coat"[32,33] in which HA may be anchored to the surface of cells via an interaction with CD44.[34] We have demonstrated that organisation of HA into peri-cellular coats is associated with enhanced migration in PTC. In PTC this effect is mimicked by CD44 activation achieved by addition of exogenous HA in an epithelial cell scratch wound model. As epithelial cell migration is a critical step in epithelial-fibroblast transdifferentiation,[14] we have postulated that enhanced coat formation may be an important component of this process. We have used our PTC model in order to further examine the factors which regulate assembly of peri-cellular HA. In this cell type, stimulation of HA synthesis by factors generally considered to promote renal injury, such as elevated glucose concentrations (diabetic nephropathy) or the pro-inflammatory cytokine IL-1β, is associated with transcriptional activation of HA synthase 2 (HAS2). We have generated stable PTC/HK2 lines over-expressing the inducible and constitutive HAS isoforms HAS2 and HAS3. Over-expression of HAS2 cDNA increased HA synthesis and increased the peri-cellular HA matrix and a corresponding increase in cell migration. This is therefore consistent with a disease promoting function for inducible HA synthesis.

In addition to HA "coats", we have demonstrated that PTC form peri-cellular HA cable-like structures that bind mononuclear leukocytes *via* their cell surface CD44 receptors,[35] and that binding of monocytes to these structures attenuates monocyte dependent PTC generation of TGF-β1.[36] Generation of HA cables is a regulated process. Bone morphogenic protein-7, a member of the TGF-β super-family which is down-regulated in renal disease, is a stimulus which facilitates cable formation, while the pro-inflammatory cytokine IL-1β, a factor associated with acute injury, markedly decreases cable formation. From these data we have postulated that HA-cables represent a mechanism which under normal conditions prevents tissue leukocytes initiating tissue injury, while the loss of the cables associated with acute or chronic renal injury removes this protective mechanism, and allows leukocytes to interact directly with resident epithelial cells triggering a cascade of events leading to progressive fibrosis. In contrast to HAS2, the HAS3 isoform of HA synthase is constitutively expressed by PTC.[37] In a HAS-3 over-expressing cell line, we demonstrated an increase in HA cables. This was associated with enhanced HA dependent leukocyte binding and a reduction in ICAM-1 dependent TGF-β1 synthesis. This is there-

fore consistent with the hypothesis that HAS3, constitutive HA generation, may be a mechanism to maintain normal homeostasis. What is now apparent however is that the significance of alteration in HA synthesis is not solely the result of HAS isoform expression, as the expression and function of hyaladherins which regulate the "packaging" of HA also contribute to its functional consequence. These results suggest that the context in which HA is generated in the kidney will dictate if it has a disease promoting or disease limiting function. This concept is further supported by studies in which we have demonstrated that HA may down-regulate TGF-β1 signalling, which is in contrast to stimuli such as glucose, which enhance signalling. To further clarify the significance of alterations in HA, we have examined the expression of HA in renal biopsies of a cohort of patients with diabetic nephropathy. In these studies we have shown that although HA expression increases at all stages of disease, its presence does not predict outcome. In contrast, outcome is well predicted with the degree of inflammatory infiltrate, which in turn does not correlate with HA expression. This therefore suggests that HA is not driving the inflammatory/fibrotic response, which is consistent with our *in vitro* data.

In conclusion these studies support the concept that PTC contribute to fibrotic changes in the renal intersititum. In addition it is clear that the regulation of TGF-β1 synthesis in the renal cortex is a complex process in which interactions between PTC, infiltrating inflammatory cells and the extracellular environment/matrix all play important roles.

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