http://www.kidney-international.org

Toll-like receptor activation: from renal inflammation to fibrosis

Wai Han Yiu[1], Miao Lin[1] and Sydney C.W. Tang[1]

[1]*Division of Nephrology, Department of Medicine, Queen Mary Hospital, The University of Hong Kong, Hong Kong*

Toll-like receptors (TLRs) are a conserved family of pattern recognition receptors that play a fundamental role in the innate immune system by triggering proinflammatory signaling pathways in response to microbial pathogens through exogenous pathogen-associated molecular patterns or tissue injury through endogenous danger-associated molecular patterns. In the kidney, TLRs are widely expressed in a variety of cell types. Emerging evidence demonstrates the participation of TLRs in the activation of these cells during renal fibrosis. This review highlights the role of TLRs and their endogenous ligands in the pathogenesis of renal fibrosis using ureteral obstruction and diabetic nephropathy as models of chronic kidney disease.

Kidney International Supplements (2014) **4,** 20–25; doi:10.1038/kisup.2014.5

KEYWORDS: diabetic nephropathy; pro-fibrotic; proinflammatory; renal fibrosis; Toll-like receptors; unilateral ureteral obstruction

Correspondence: *Sydney C.W. Tang, Division of Nephrology, Department of Medicine, Queen Mary Hospital, The University of Hong Kong, 102 Pokfulam Road, Hong Kong. E-mail: scwtang@hku.hk*

Renal fibrosis is a wound healing/scarring response following kidney injury that occurs in many forms of chronic kidney disease (CKD). It is also a crucial determinant underlying the progression from CKD to end-stage renal disease. After over a decade of intense study, it is now known that the interstitial accumulation of extracellular matrix (ECM) proteins is not only the aftermath of fibroblast activation, but also a process contributed to by other intrinsic renal cells, including tubular epithelial cells (TECs), mesangial cells, endothelial cells and infiltrating macrophages.[1,2] Following kidney injury, resident fibroblasts are activated by various pro-inflammatory and pro-fibrotic stimuli. Activated fibroblasts, also called myofibroblasts, produce excessive ECM proteins that accumulate in the interstitium, and therefore they are considered as the key mediator of renal fibrosis.[3]

Despite the fact that most myofibroblasts are derived from local resident fibroblasts, recent studies demonstrated that these ECM-producing cells might originate from bone marrow through differentiation, and from TECs and endothelial cells via a process of epithelial-to-mesenchymal transition and endothelial-to-mesenchymal transition, respectively.[4,5] Although there is an ongoing debate about the existence of epithelial-to-mesenchymal transition *in vivo*,[6] these studies illustrated the contribution of TECs in fibrogenesis through a paracrine mechanism. TECs in co-culture with cortical fibroblasts secreted transforming growth factor-β (TGF-β) and the AB-heterodimer of platelet-derived growth factor (PDGF-AB), which in turn stimulated fibroblast proliferation and total collagen synthesis.[7] The production of insulin-like growth factor binding protein from fibroblasts was also enhanced in the presence of TEC-conditioned medium; thus, these cells could modulate the proliferative response during repair.[8]

Regardless of the primary insult leading to renal fibrosis, chronic inflammation appears to be a critical process heralding fibrogenesis. Elevated levels of inflammatory markers were associated with an increased risk of developing CKD.[9] Induction of various pro-inflammatory cytokines (interleukin (IL)-6, IL-8, IL-10, chemokine (C-C motif) ligand 2, and tumor necrosis factor-α) and adhesion molecules (intercellular adhesion molecule-1 and vascular cell adhesion molecule-1) attracted the transmigration of macrophages and T cells from the circulation to the interstitium, thereby further enhancing the inflammatory state.[10]

Toll-like receptors (TLRs) are a conserved family of pattern recognition receptors that play a fundamental role in innate immunity. TLRs mediate host cell inflammatory response by recognizing pathogen-associated molecular patterns with an extracellular domain comprising leucine-rich repeats and triggering the intracellular signal transduction through a cytoplasmic Toll/IL-1 receptor-like domain. In addition, TLRs are involved in non-infectious inflammatory disease, in which they are activated by endogenous danger-associated molecular patterns that are released from injured tissue.[11] Activation of TLR pathways has been implicated in various renal diseases, including acute kidney injury, ischemia–reperfusion injury, allograft rejection and immune complex nephritis,[12,13] in which they participate in the induction of acute inflammation and early tubular injury. Emerging evidence suggests that TLRs may participate in the pathogenesis of renal fibrosis.

THE EXPRESSION OF TLRs DURING CHRONIC KIDNEY INJURY

TLRs are important in innate immunity and are widely distributed on myeloid cells. To date, 10 human TLRs (TLR1–10) have been identified and demonstrated to initiate proinflammatory signaling pathways via the adaptor protein MyD88,[11] except TLR3, which triggers via TRIF/TICAM-1. Other TLR adaptors include TRIF (TIR-domain-containing adaptor-inducing interferon-β), TIRAP (TIR-domain-containing adaptor protein), and TRAM (TRIF-related adaptor molecule).[14] TLRs utilize these adaptor proteins to transmit signals downstream, leading to the activation of nuclear factor-κB, mitogen-activated protein kinases, JNKs (c-Jun NH2-terminal kinases), p38, ERKs and IRF (Figure 1).[15]

So far, most of the TLR studies associated with chronic renal injury have focused on TLR2 and TLR4 (Table 1). In the kidney, interstitial and glomerular macrophages express TLR1, 2, 4, and 6, and dendritic cells express TLR4, 7, 8, and 9. TLR2 and TLR4 are upregulated in monocytes, and TLR4 is upregulated in neutrophils of end-stage renal disease patients. The alteration of TLR expression in immune cells might contribute to the increased susceptibility to microbial infection and prevailing inflammation in these patients.[16] This notion is supported by the observation that TLR2 expression on monocytes was associated with the inflammatory response of patients with stage 3–4 CKD.[17]

In addition to myeloid cells, TLRs are also expressed in intrinsic renal cells. TECs and mesangial cells express TLR1, 2, 3, 4, and 6, and podocytes express TLR1, 2, 3, 4, 5, 6, and 10. Immunohistochemical studies on human renal biopsies demonstrated the upregulation of TLR2[18] in the glomerular endothelial and mesangial area and TLR4[19] expression in the tubules of patients with diabetic nephropathy (DN) compared with normal renal sections. Increased expression of TLR2 was also observed in interstitial myofibroblasts, tubules, and macrophages of the kidney sections from patients with obstructive hydronephrosis and IgA nephropathy.[20] Although TLRs are present in both immune and renal cells, it is likely that TLR signaling predominates in intrinsic renal cells rather than leukocytes. For example TLR4-deficient mice engrafted with competent leukocytes showed less tubular damage than wild-type mice reconstituted with TLR4-deficient bone marrow cells.[21]

ENDOGENOUS TLR LIGANDS RELEASED DURING CHRONIC KIDNEY INJURY

Over the last decade, numerous endogenous ligands have been identified for the activation of TLRs during kidney injury, and some of them have been shown to be closely associated with renal fibrosis.

High-mobility-group box 1 (HMGB1)

HMGB1 protein belongs to a family of non-histone chromosomal proteins that were first identified to bind to DNA and regulate gene transcription. It is also a secreted protein from activated macrophages and dendritic cells during infection and from damaged cells during tissue injury. Increasing evidence supports that extracellular HMGB1 is a key endogenous ligand of TLR signaling that is involved in the initiation of renal inflammation and the subsequent development of progressive renal fibrosis.

Both experimental and clinical models of renal fibrosis have demonstrated that a high HMGB1 level is associated with the development of progressive CKD, including unilateral ureteral obstruction (UUO) injury,[20] 5/6 nephrectomy,[22] and DN.[18,19,23] Extracellular HMGB1 binds to TLR2 and TLR4[24] to elicit inflammatory responses via a nuclear factor-κB-dependent pathway. Anti-HMGB1 treatment has been shown to attenuate the severity of sepsis in CKD.[22] HMGB1 has also been demonstrated to induce epithelial-to-mesenchymal transition in human proximal TECs.[25]

Heat shock proteins (HSPs)

HSPs were originally characterized as intracellular chaperone proteins that are involved in protein folding and stabilization. Interestingly, HSPs also interact with TLRs during the maturation of immune cells[26] and during induction and termination of cytokine secretion. HSP60 and HSP70 are the two best-known HSPs that bind to TLR2 and TLR4 in inflamed tissue. An early study on DN revealed that HSP60 and HSP70 proteins were significantly induced in lymphocytes of type 2 diabetic patients.[27] Together, the serum level of HSP60 and HSP70 was shown to correlate with the increase of TLR2 and TLR4 expression in monocytes of type 2 diabetic patients.[23] This supports the role of HSPs as endogenous ligands for TLR signaling.

ECM degradation product

Progressive renal fibrosis results in increasing matrix turnover and excessive production of ECM degradation products such as fibrinogen, biglycan, heparin sulfate, hyaluroran, and fibronectin, which have been shown to interact with TLR4.[28,29]