

The role of stress-activated protein kinase signaling in renal pathophysiology

F.Y. Ma, J. Liu and D.J. Nikolic-Paterson

Department of Nephrology and Monash University Department of Medicine, Monash Medical Center, Clayton, Victoria, Australia

Correspondence to: D.J. Nikolic-Paterson, Department of Nephrology, Monash Medical Center, 246 Clayton Road, Clayton, Victoria 3168, Australia
Fax +61-3-9594-6530. E-mail: david.nikolic-paterson@med.monash.edu.au

Two major stress-activated protein kinases are the p38 mitogen-activated protein kinase (MAPK) and the c-Jun amino terminal kinase (JNK). p38 and JNK are widely expressed in different cell types in various tissues and can be activated by a diverse range of stimuli. Signaling through p38 and JNK is critical for embryonic development. In adult kidney, p38 and JNK signaling is evident in a restricted pattern suggesting a normal physiological role. Marked activation of both p38 and JNK pathways occurs in human renal disease, including glomerulonephritis, diabetic nephropathy and acute renal failure. Administration of small molecule inhibitors of p38 and JNK has been shown to provide protection from renal injury in different types of experimental kidney disease through inhibition of renal inflammation, fibrosis, and apoptosis. In particular, a role for JNK signaling has been identified in macrophage activation resulting in up-regulation of pro-inflammatory mediators and the induction of renal injury. The ability to provide renal protection by blocking either p38 or JNK indicates a lack of redundancy for these two signaling pathways despite their activation by common stimuli. Therefore, the stress-activated protein kinases, p38 and JNK, are promising candidates for therapeutic intervention in human renal diseases.

Key words: p38; c-Jun amino terminal kinase; Inflammation; Fibrosis; Apoptosis; Renal pathophysiology

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Introduction

The stress-activated protein kinases (SAPK) are a group of serine/threonine kinases belonging to the mitogen-activated protein kinase (MAPK) family. The two major SAPK are c-Jun amino terminal kinase (JNK) and p38 MAPK, which play an essential role in mediating the cellular response to various stresses by modulating transcription factor function and thereby changing the pattern of gene transcription. This operates by direct phosphorylation of transcription factors, or by an indirect mechanism involving the phosphorylation of MAPK-activated protein kinases (MAPKAPK). A diverse range of biological functions can be induced through activation of the p38 and JNK pathways, including differentiation, proliferation, inflammation, apoptosis, and fibrosis (1).

p38 and JNK are activated in response to a wide range of stimuli such as pro-inflammatory cytokines, Toll-like receptor activation in the innate immune response, a variety of growth factors, and stresses such as reactive oxygen species, osmotic stress, hyperglycemia and UV irradiation (1) (see Figure 1). Ligand-based activation of cell surface receptors is linked to activation of a three-tiered MAPK cascade via the p21-activated kinase family of enzymes that bind to and are activated by small GTPases of the Cdc42 and Rac families. Phosphorylation of the activation loop in the JNK and p38 kinases operates via a cascade of a MAP kinase kinase kinase (MAP3K), a MAP kinase kinase (MAP2K), and then a MAPK (see Figure 1). There is a considerable redundancy in the different MAP3Ks that can lead to activation of p38 and JNK, whereas direct activation of p38 and JNK by MAP2K is restricted to MAPK

kinase (MKK) 3 and 6 or MKK4 and 7, respectively (see Figure 1). Scaffolding proteins, such as the family of JNK interacting proteins (JIP1-4), facilitate the three-tiered phosphorylation cascade by binding these kinases in close proximity. These scaffolding proteins may also be important in determining the specificity of SAPK signal transduction (2).

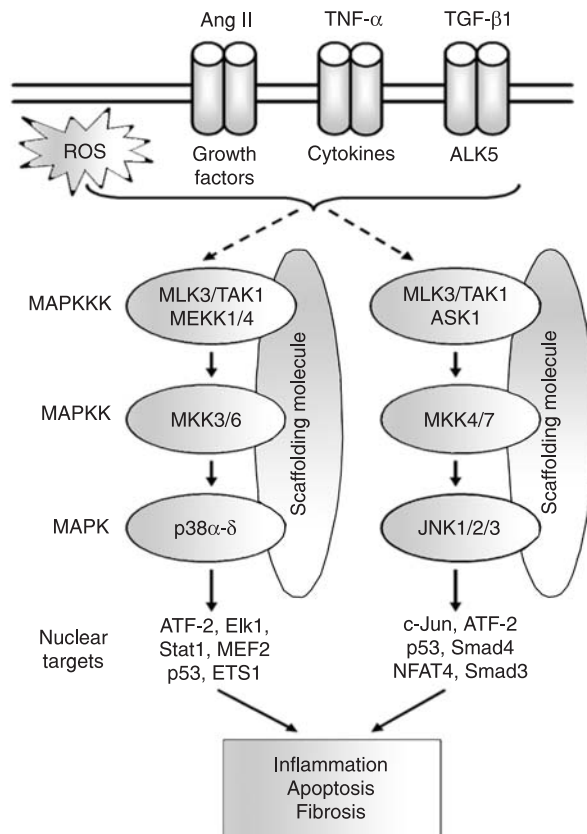


Figure 1. Overview of the stress-activated protein kinase (SAPK) signaling pathways, which is a subset of the mitogen-activated protein kinase (MAPK) family. Activation of different receptor types, such as G-protein coupled receptors for angiotensin II (ANG II) and the receptor tyrosine kinases for tumor necrosis factor alpha (TNF- α) or transforming growth factor-beta 1 (TGF- β 1), leads to the activation of MAP kinase kinase kinases (MAPKKK). These kinases can also be activated by reactive oxygen species (ROS), osmotic stress and UV irradiation through poorly defined intracellular mechanisms. The MAPKKK-MAPKK-MAPK phosphorylation cascade is facilitated by scaffold proteins, which bind members of the MAPKKK, MAPKK and MAPK families in close proximity. There is a variety of different MAPKKK, whereas there are only two MAPKK, which are usually involved in phosphorylating the terminal JNK or p38 kinases. The stress-activated protein kinases can then translocate to the nucleus and modulate the function of a variety of transcription factors through phosphorylation, which induces a change in the pattern of gene transcription resulting in a biological response.

Inactivation of SAPK signaling is achieved by removal of phosphate groups from the activation loop in the JNK and p38 kinases. This is performed by members of a structurally distinct subfamily of dual-specificity (Thr/Tyr) protein phosphatases in a process regulated in two ways. First, the specificity of action by MAPK phosphatases (MKP) is achieved by interaction with their substrate kinases through a modular kinase interaction motif (KIM) located within the N-terminal non-catalytic domain of the protein. In addition, MAPK binding is often accompanied by enzymatic activation of the C terminal catalytic domain of the MKP, thus ensuring specificity of phosphatase action. Second, MKP levels are tightly controlled at the transcriptional level, providing a rapid negative feedback in response to SAPK signaling (3).

Further selectivity and regulation of SAPK signaling comes from the presence of several isoforms of both p38 and JNK in mammalian cells. The p38 SAPK has four distinct isoforms (p38 α , β , γ and δ), which are encoded by individual genes. Of these isoforms, p38 α and p38 β are ubiquitously expressed in most tissues; whereas p38 γ and p38 δ have more restricted expression pattern (4). Genetic deletion of p38 α induces embryonic death of mice due to placental defects. In contrast, mice with p38 β , γ , or δ knockout show a normal phenotype (5). Most of our understanding of p38 kinase function comes from the use of pharmacological inhibitors of the p38 α and p38 β isoforms. Thus, it is difficult to discriminate between the function of p38 α and p38 β , although reciprocal effects of p38 α and p38 β on iNOS expression in mesangial cells have been described (6). In contrast, the role of p38 γ and p38 δ in health and disease is poorly understood.

There are also multiple isoforms of JNK (JNK1, 2, and 3), which are encoded by individual genes and alternative mRNA splicing can give rise to even greater enzyme heterogeneity (7). While the expression of JNK3 is largely confined to the central nervous system, both JNK1 and JNK2 are widely expressed in many cell types in most tissues. Genetic deletion of a single JNK gene has little impact upon mouse development and survival. However, combined deletion of JNK1 and JNK2 is fetal lethal (5), indicating considerable redundancy between these kinases.

SAPK in renal physiology

p38 MAPK and JNK signaling is evident during kidney development in the rat (8). This appears to be important in kidney development since the addition of a p38 inhibitor to cultured metanephroi impaired cell proliferation and nephrogenesis (9). In the adult rodent and human kidney, there is limited p38 MAPK activation as assessed by

immunostaining and Western blotting for phosphorylated-p38 (p-p38) (10,11). p38 activation is evident in a small number of podocytes, parietal epithelial cells, cells of the macula densa and some tubular epithelial cells (10,11). This limited activation of p38 in tubular cells may be related to osmotic stress, as *in vitro* studies have shown that hypertonic stress activates p38 MAPK signaling in tubular epithelial cells (12), and this activation may be linked to reorganization of the actin cytoskeleton in response to hypertonic cell shrinkage (13). An interesting recent finding is that administration of the p38 MAPK inhibitor, SB202190, induced a reduction in mean arterial blood pressure, glomerular filtration rate, and filtration fraction in both normal and diabetic rats (14). Whether this operates by modulating p38 function in cells of the macula densa, or whether p38 regulates endothelium-dependent vasodilation has yet to be established (11,14). One other piece of data suggesting a role for p38 signaling in renal physiology comes from the finding that mice heterozygote for the p38 α gene (p38 α +/-) appear normal but with age exhibit increased water intake and a higher kidney weight. These kidneys develop dilation of proximal convoluted tubules, vacuolar degeneration, focal interstitial fibrosis/inflammation, and enlargement of Bowman's capsule with advancing age (15).

Signaling via the JNK pathway is restricted to cells of the collecting duct and occasional parietal epithelial cells in normal kidney (16). Examination of gene-deficient mice has shown that JNK signaling in normal kidney can operate via JNK1 or JNK2 (16). This low level JNK activation may also represent a response to osmotic stress since JNK activation can be induced in rat renal epithelial cells by hypotonic stimuli (17). In addition, sodium reabsorption from tubules may also be affected by JNK activation through regulation of the synthesis of Na-K-ATPase γ -subunit (17,18). Administration of JNK inhibitor drugs to rats has shown no overall or renal toxicity (16,19), suggesting that JNK signaling is not critical for renal physiology - although this needs to be rigorously examined.

SAPK in human kidney disease

Immunostaining of human biopsy specimens identified a marked increase in the number of p-p38+ cells in glomeruli and tubules in non-proliferative and proliferative forms of glomerulonephritis and a substantial increase in interstitial p-p38+ cells in proliferative glomerulonephritis (10). p38 activation was identified in intrinsic renal cells (podocytes, endothelial and tubular cells), infiltrating macrophage and neutrophils, and in alpha smooth muscle actin positive (α -SMA+) myofibroblasts. The number of p-

p38+ glomerular cells correlated with segmental proliferative and necrotic lesions, crescent formation, and macrophage accumulation. Meanwhile, interstitial p38 activation correlated with the degree of interstitial inflammation. There was also a correlation between renal failure and the number of p-p38+ glomerular, tubular, and interstitial cells; while proteinuria correlated with the number of p-p38+ podocytes, tubular, and interstitial cells (10,20). A significant increase in p38 activation is also evident in human and experimental diabetic nephropathy (20,21), with the number of p-p38+ tubulointerstitial cells reflecting the severity of tubulointerstitial lesions (22). These studies suggest that p38 activation may play an important pathogenic role in human renal diseases.

Activation of JNK signaling has also been examined in human kidney disease. Immunostaining for phosphorylated-c-Jun, a well-defined marker of JNK signaling, identified a striking increase in JNK activation in kidney diseases including various glomerulonephritis, hypertension and diabetic nephropathy (23). p-c-Jun staining was evident in many glomerular and tubular cells. Indeed, the number of p-c-Jun+ glomerular cells correlated with the degree of glomerulosclerosis, while tubulointerstitial p-c-Jun staining correlated with interstitial fibrosis and renal dysfunction, implicating JNK signaling in the pathogenesis of different forms of human kidney disease (23).

SAPK in experimental kidney disease

The functional role of p38 and JNK signaling in a variety of animal models of kidney disease has been examined by administration of small molecular weight kinase inhibitors, the results of which are listed in Table 1. An important consideration is that p38 and JNK are activated in both intrinsic renal cell types as well as in inflammatory cells. Thus, it remains difficult to identify, in most situations, which cell types are the main target in the beneficial effects of these kinase inhibitor drugs.

p38 MAPK in glomerular disease

Anti-glomerular basement membrane (GBM) glomerulonephritis is an aggressive inflammatory disease in which renal injury is macrophage dependent (24). Rat models of anti-GBM disease exhibit a dramatic increase in p38 activation in glomeruli, which is followed by up-regulation of p38 in the tubulointerstitium, being evident in infiltrating macrophages, neutrophils, glomerular endothelial cells, podocytes, tubular epithelial cells, and myofibroblasts (25,26). Blocking p38 α / β signaling in several independent studies using different kinase inhibitors successfully suppressed proteinuria and histological damage in this model (25-27). Specific

effects of p38 blockade included inhibition of endothelial P-selectin expression resulting in a diminished neutrophil infiltrate (25), and the suppression of up-regulation of monocyte chemoattractant protein-1 (MCP-1), which was associated with inhibition of renal macrophage infiltration and suppression of glomerular damage (26,27). Furthermore, delayed treatment with a p38 inhibitor was able to suppress estab-

lished anti-GBM disease (27).

In MRL-*Fas*^{lpr} mice, a model of lupus nephritis, treatment with the p38 inhibitor FR167653 suppressed renal pathology and prolonged survival (28). These protective effects were associated with reduced renal macrophage and T cell infiltration, MCP-1 and TNF- α expression, and glomerular IgG deposition (28).

Table 1. A summary of p38 and c-Jun amino terminal kinase (JNK) blockade studies in experimental kidney disease.

Disease model	Inhibitor	Outcome	Reference
p38 inhibitor studies			
Anti-glomerular basement membrane glomerulonephritis	NPC 31145	↓ proteinuria	25
	FR167653	↓ inflammation ↓ proteinuria	26
	RO4399247	↓ inflammation ↓ proteinuria	27
Lupus nephritis	FR167653	↓ inflammation ↓ autoantibody ↓ proteinuria	28
Puromycin aminonucleoside nephrosis	FR167653	↓ proteinuria ↓ podocyte injury	29
Adriamycin nephropathy	FR167653	↓ proteinuria ↓ inflammation	29
	SB203580	↓ proteinuria ↓ inflammation	30
Spontaneously hypertensive-stroke-prone rat model	FR167653	↓ proteinuria ↓ inflammation	58
	SB239063	↓ glomerulosclerosis ↑ survival ↓ proteinuria	59
High-renin Ren-2 rats	SB239063	↓ renal damage	60
Heymann's nephritis	FR167653	↑ proteinuria	33
Subtotal nephrectomy	NPC 31169	↑ proteinuria	34
		↓ renal function ↑ inflammation	
Obstructed kidney	NPC 31169	↓ fibrosis	42
Renal ischemia-reperfusion injury	FR167653	↑ renal function	48
		↓ tubular damage	
		↓ inflammation	
JNK inhibitor studies			
Rat anti-glomerular basement membrane glomerulonephritis	CC-401	↓ proteinuria ↓ inflammation	16
Obstructed kidney	CC-401	↓ fibrosis	19
Renal ischemia-reperfusion injury	SP600125	↑ renal function	47
		↓ tubular damage	
		↓ inflammation	
Cisplatin-induced acute renal failure	SP600125	↑ renal function	50
		↓ tubular damage	
		↓ inflammation	

Blockade of p38 signaling has also been directly implicated in preventing podocyte damage and associated proteinuria. Consistent with human studies (10), p38 activation in podocytes was evident just prior to the onset of proteinuria in models of puromycin aminonucleoside (PAN) nephrosis and adriamycin nephropathy (29). Pre-treatment with a p38 inhibitor prevented podocyte damage and suppressed proteinuria in both models (29,30), arguing that p38 signaling is involved in podocyte injury leading to proteinuria. This postulate is supported by a number of studies. Albumin overload *in vivo* and *in vitro* induced podocyte injury and proteinuria via a transforming growth factor-beta 1 (TGF- β 1)/p38 pathway (31), while p38 signaling was shown to be required for PAN-induced actin reorganization in cultured podocytes (29). In addition, p38 signaling is required for TGF- β 1- and angiotensin II-mediated podocyte apoptosis (32). However, a quite different result was found in passive Heymann's nephritis, a rat model of complement-dependent podocyte damage. Blockade of p38 signaling was found to exacerbate podocyte damage and proteinuria *in vivo*, while antibody and complement mediated damage of cultured podocytes was also shown to be dependent upon p38 activation. In addition, increased p38 activation induced by over-expression of a constitutively active mutant of TGF- β 1-activated kinase 1 (TAK1), a kinase upstream of p38, was shown to suppress antibody and complement mediated podocyte damage (33).

Another setting in which p38 blockade was found to be detrimental was in 5/6 subtotal nephrectomy in rats, in which administration of a p38 inhibitor accelerated renal disease (34). This exacerbation of disease was attributed to an up-regulation of extracellular signal-regulated kinase (ERK) signaling.

JNK in glomerular disease

There is also a marked increase in JNK activity in rat anti-GBM glomerulonephritis, with immunostaining for p-JNK and p-c-Jun showing JNK activation in glomerular macrophages, podocytes, tubular epithelial cells and α -SMA+ myofibroblasts (16). The role of JNK in macrophage-mediated renal injury in this model was addressed using an adoptive transfer approach. Administration of a JNK inhibitor to cultured macrophages before their transfer into leukocyte-depleted rats given anti-GBM serum did not prevent macrophage recruitment into glomeruli, but it profoundly inhibited the induction of proteinuria and mesangial cell proliferation indicating that the macrophage response had been modified (35). Indeed, JNK blockade markedly blunted the TNF- α response in cultured macrophages (35).

In subsequent studies, oral administration of a highly selected JNK inhibitor (CC-401) was used in a standard model of rat anti-GBM disease (16). CC-401 treatment was effective in suppressing JNK activity without modifying the increase in p38 and ERK activation. JNK blockade suppresses proteinuria and reduced the severity of glomerular and tubulointerstitial lesions despite a marked glomerular macrophage infiltrate. Analysis showed that markers of macrophage activation (iNOS and TNF- α) were suppressed by JNK blockade, whereas the T cell and humoral immune response were unaltered, leading to the conclusion that the beneficial effects of JNK blockade were due to inhibition of the macrophage pro-inflammatory response (16). These findings are supported by a recent study in which the genetic susceptibility of WKY rats to anti-GBM disease was attributed to up-regulation of JunD leading to increased AP-1 activity and a macrophage pro-inflammatory phenotype (36).

We examined JNK activation in a non-immune model of glomerular injury. A single bolus injection of PAN induces podocyte damage and apoptosis, which is associated with heavy proteinuria. We identified a striking induction of JNK signaling in glomerular podocytes on day 4, the time at which proteinuria first becomes evident in this disease model (Figure 2), suggesting a potential role for JNK activation in podocyte damage. Functional studies are now required to determine whether JNK signaling directly promotes podocyte damage in disease models.

SAPK in interstitial fibrosis

All progressive forms of kidney disease involve a pathological process in which the accumulation of extracellular matrix replaces the cellular structure resulting in progressive fibrosis leading to end-stage renal failure. The role of growth factors, such as TGF- β 1 and angiotensin II, is well established in renal fibrosis. However, we are beginning to appreciate that SAPK signaling plays an important role in promoting fibrosis through stimulating fibroblast proliferation (37), fibroblast transition into α -SMA+ myofibroblasts (38), TGF- β 1 production (19), TGF- β 1-induced collagen and fibronectin production (39), and in facilitating TGF- β 1-induced transition of tubular epithelial cells into myofibroblasts (40).

Unilateral ureter obstruction is a model widely used to study renal fibrosis. This model has a rapid onset of fibrosis in response to tubular mechanical stretch, which is independent of the immune response (41). There is a striking increase in p38 activation in both tubular epithelial cells and in interstitial α -SMA+ myofibroblasts (42) in the obstructed kidney. Administration of a p38 α inhibitor was effective in reducing myofibroblast accumulation and col-

lagen deposition in the obstructed kidney. Interestingly, p38 blockade did not prevent up-regulation of TGF- β 1 mRNA or protein, indicating that p38 signaling acts downstream of TGF- β 1 (42). In further studies, the contribution of the upstream kinase, MKK3, to p38 activation and renal fibrosis was examined in the obstructed kidney. *Mkk3*^{-/-} mice were found to have a compensatory up-regulation of MKK6 in the kidney, although p38 activation was still suppressed in both normal and obstructed kidney compared to wild-type mice (43). *Mkk3*^{-/-} mice were not protected from developing renal fibrosis indicating redundancy between MKK3 and MKK6 in this response. However, *Mkk3*^{-/-} mice were protected from tubular cell apoptosis, identifying a specific role for MKK3-p38 signaling in this response (43).

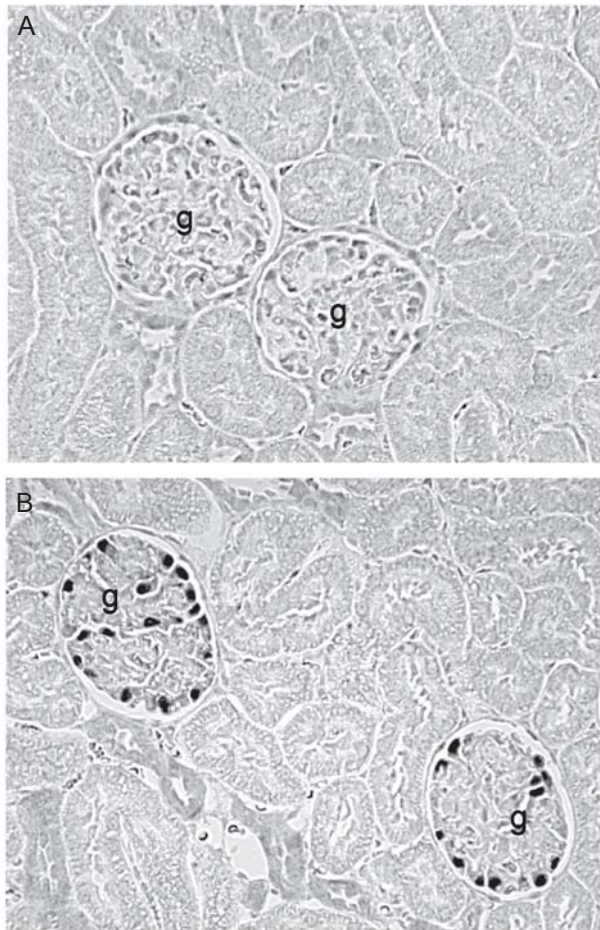


Figure 2. c-Jun amino terminal kinase (JNK) activation in rat puromycin aminonucleoside (PAN) nephrosis. JNK signaling was assessed by immunostaining at phospho-serine 63 in c-Jun. *A*, Normal rat kidney lacks phospho-c-Jun staining. *B*, Clear p-c-Jun staining is seen in glomerular podocytes on day 4 after PAN administration, the time when proteinuria is first evident prior to the peak of proteinuria on day 8. g = glomerulus.

The JNK signaling pathway also shows dramatic activation in tubular epithelial cells and myofibroblasts in the obstructed kidney (19). Administration of a JNK inhibitor was effective in preventing c-Jun phosphorylation and significantly reducing interstitial myofibroblast accumulation and collagen synthesis and deposition. In contrast to p38 blockade, inhibition of JNK signaling in the obstructed kidney caused a substantial reduction in TGF- β 1 mRNA, indicating that JNK but not p38 signaling is required for up-regulation of TGF- β 1 expression (19,42). As both JNK1 and JNK2 isoforms are expressed in the kidney, interstitial fibrosis in *Jnk1*^{-/-} and *Jnk2*^{-/-} mice was examined in the obstructive kidney. Genetic deletion of either *Jnk1* or *Jnk2* did not prevent the marked activation of JNK signaling seen in the obstructed kidney, or affect the development of renal fibrosis, demonstrating redundancy between the two JNK isoforms. However, tubular epithelial cell apoptosis was significantly reduced in *Jnk1*^{-/-} mice, identifying a specific role for this isoform in one aspect of renal injury (19). The identification of both MKK3 and JNK1 in tubular apoptosis in the obstructed kidney is supported by studies in which stretch-induced apoptosis of cultured tubular cells is dependent upon p38 and JNK signaling (44).

SAPK in tubular damage

There is a striking activation of the p38 and JNK pathways in acute renal failure induced by ischemia/reperfusion injury or administration of cisplatin. This is a rapid response that occurs primarily in tubular epithelial cells (45). The administration of p38 or JNK inhibitors has been shown to reduce acute renal failure, tubular apoptosis and histologic damage in models of renal ischemia/reperfusion (46-48). Similarly, blockade of p38 or JNK is protective against acute renal failure, tubular cell apoptosis and histologic damage in cisplatin-induced acute renal failure (49,50). These findings are supported by *in vitro* studies in which blockade of p38 or JNK signaling can suppress tubular epithelial cell apoptosis induced by hypoxia or H₂O₂ (51). Furthermore, an antisense oligonucleotide approach has been used to demonstrate that JNK1 is required for hypoxia-induced apoptosis of human kidney cells (52), which is consistent with the protection of *Jnk1*^{-/-} mice from tubular cell apoptosis in the obstructed kidney (19).

In a different form of tubular damage, that of autosomal dominant polycystic kidney disease (PKD), immunohistochemistry staining identified phosphorylation of c-Jun and activating transcription factor 2 (ATF2) in tubular cysts in human and mouse PKD (53), suggesting that JNK/p38 MAPK signaling may be involved in cyst formation. In addition, the proteins PKD1 and PKD2 can both activate AP-1 via

JNK (54). However, whether this has any relation to the increase in JNK signaling seen in PKD, in which PKD1 and/or PKD2 are mutated, is not clear.

Clinical potential for SAPK blockade

The human and experimental studies described above make a strong argument for clinical trials of p38 and JNK inhibitors in human kidney disease. A number of p38 and JNK inhibitors have moved into clinical trials in non-renal fields. VX-702, an orally active p38 kinase inhibitor, has completed two phase II trials involving 130 patients with rheumatoid arthritis (55). Another p38 inhibitor, BIRB 796, has completed a multi-center trial in 284 patients with Crohn's disease (56). The PRECEPT trial tested the mixed lineage kinase inhibitor CEP-1347, which blocks JNK and p38 signaling, in patients with Parkinson's disease. The trial was stopped early (after 21 months) because of a lack of efficacy (57). However, all of these drugs have been well tolerated, apart from a mild and transient increase in serum transaminase levels in the BIRB 796 study (56). Thus, the safety profile of p38 and JNK inhibitors appears to be suitable for clinical trials in kidney disease, the question remains as to whether they will be efficacious.

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Conclusions

Human studies and functional blockade in experimental models have identified an important role for p38 and JNK signaling in a variety of renal diseases. Several important pathogenic mechanisms including renal inflammation, fibrosis and tubular damage can all be suppressed by targeting p38 or JNK signaling. Limited data suggest a role for SAPK in normal renal physiology, but this has not proven to be of concern in clinical trials using p38 or JNK inhibitors. Therefore, blockade of p38 and JNK signaling is a promising therapeutic strategy for the treatment of various forms of kidney disease.

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