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REVIEW

Midkine in nephrogenesis, hypertension and kidney diseases

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Midkine (MK; K; gene abbreviation, *Mdk*: mus musculus, *MDK*: homo sapiens) is a multifunctional heparin-binding growth factor that regulates cell growth, survival and migration as well as anti-apoptotic activity in nephrogenesis and development. Proximal tubular epithelial cells are the main sites of MK expression in the kidneys. The pathophysiological roles of MK are diverse, ranging from the development of acute kidney injury (AKI) to the progression of chronic kidney disease, often accompanied by hypertension, renal ischaemia and diabetic nephropathy. The obvious hypertension that develops in $Mdk^{+/+}$ mouse models of renal ablation compared with $Mdk^{-/-}$ mice eventually leads to progressive renal failure, such as glomerular sclerosis and tubulointerstitial damage associated with elevated plasma angiotensin (Ang) II levels. MK is also induced in the lung endothelium by oxidative stress and subsequently up-regulated by ACE, which hydrolyzes Ang II to induce further oxidative stress, thus accelerating MK generation; this leads to a vicious cycle of positive feedback in the MK-Ang II pathway. Kidney–lung interactions involving positive feedback between the renin-angiotensin system and MK might partly account for the pathogenesis of hypertension and kidney damage. MK is also involved in the pathogenesis of AKI and diabetic nephropathy through the recruitment of inflammatory cells. In contrast, MK plays a protective role against crescentic glomerulonephritis, by down-regulating plasminogen activator inhibitor-1. These diverse actions of MK might open up new avenues for targeted approaches to treating hypertension and various renal diseases.

LINKED ARTICLES
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Abbreviations
ACEi, ACE inhibitors; AGT, angiotensinogen; AKI, acute kidney injury; Ang, angiotensin; ARB, angiotensin receptor-blocking agents; AT_1 receptor, Ang type 1 receptor; ATRA, all-trans-retinoic acid; CKD, chronic kidney disease; ESRD, end-stage of renal disease; GBM, glomerular basement membrane; GN, glomerulonephritis; MCNS, minimal change nephrotic syndrome; MCP-1, macrophage chemotactic protein-1; MK, midkine; MOF, multiple organ failure; ODN, oligodeoxyribonucleotide; PAI, plasminogen activator inhibitor; PAN, puromycin aminonucleoside; RALDH2, retinaldehyde dehydrogenase type 2; RAS, renin-angiotensin system; ROS, Nox-mediated reactive oxygen species; STZ, streptozotocin

Introduction

Globally, the number of people suffering from hypertension is epidemic and continues to grow with the rapid spread of diabetes mellitus, excess salt intake and obesity (Johnson *et al.*, 2008). Indeed, hypertension is a major risk factor for cardiovascular and cerebral vascular diseases as well as nephrosclerosis (Hsueh and Wyne, 2011). Regardless of the primary cause of hypertension, renal dysfunction, including acute kidney injury (AKI) and chronic kidney disease (CKD), frequently accompany the various systemic diseases induced by endothelial dysfunction in the microcirculation. A further

complication is that the progression of moderate hypertension and renal dysfunction are usually asymptomatic. Early comprehensive management is therefore needed to prevent microvascular complications, such as coronary artery diseases, diabetic vasculopathy, cerebrovascular and renal diseases (Vinik *et al.*, 2006). The pathophysiology of systemic hypertension includes a complex interaction of multiple vascular effectors, such as catecholamines, the renin-angiotensin system (RAS), oxidative stress, NO, vascular endothelial growth factor, endothelin-1 and numerous inflammation-related cytokines (Kosugi *et al.*, 2009; Nakagawa *et al.*, 2011). A better understanding of any crosstalk that occurs among these reactions might help to prevent hypertension-related diseases. The kidney possesses all of the above components, and clarification of their interactions should thus lead to treatments that could inhibit or delay disease progression to multiple organ failure (MOF) and advanced CKD. Renal ischaemia, drug-induced nephropathy and crescentic glomerulonephritis (GN) are the major causes of AKI, and are strongly associated with damage in various other organs, induced by various injurious stimuli released from the damaged kidney, resulting in CKD, end-stage of renal disease (ESRD) and MOF. Diabetic nephropathy is one of the most significant long-term diseases in terms of morbidity and mortality for individuals with diabetes (Gross *et al.*, 2005). Longstanding hyperglycaemia induces severe endothelial dysfunction, oxidative stress and inflammation, leading to renal dysfunction.

Midkine (MK; gene abbreviation, *Mdk*: mus musculus, *MDK*: homo sapiens) along with pleiotrophin (HB-GAM) (Deuel *et al.*, 2002) are members of the same family, of which only two have been identified in humans. The MK sequence is highly conserved in humans and mice: 87% of amino acids are identical and all amino acid changes are conserved except for a single insertion (Tsutsui *et al.*, 1991). Retinoic acid induces the expression of MK and the promoter region has a functional retinoic acid-responsive element (Matsubara *et al.*, 1994). Glucocorticoid suppresses the expression of MK through its nuclear receptor (Kaplan *et al.*, 2003). The promoter region of MK also has a binding site for WT-1, the product of the Wilms' tumour suppressor gene (Adachi *et al.*, 1996). Furthermore, hypoxia induces the expression of MK through the binding of hypoxia inducible factor-1α (HIF-1α) to a hypoxia responsive element in the MK promoter (Reynolds *et al.*, 2004). Receptors for MK have not been fully elucidated and there are no known MK receptors that can account for the roles of MK in pathological conditions. Candidate MK receptors are considered to form a molecular complex containing proteoglycans that includes protein-tyrosine phosphatase ζ and members of a low-density lipoprotein receptor-related protein family (Muramatsu, 2002; Chen *et al.*, 2007; Sakamoto *et al.*, 2011). MAPK and PI3K are included in the downstream signalling pathway (Chen *et al.*, 2007; Sakamoto *et al.*, 2011). MK is a multifunctional heparin-binding growth factor with major biological roles that can be categorized into three areas: the nervous system, cancer and inflammation (Muramatsu, 2002; Kadomatsu and Muramatsu, 2004). To date, neuronal cytoprotective effects of MK have been shown in various models *in vivo*, including retinal degeneration, cerebral infarction and ischaemia-induced neuronal death (Takada *et al.*, 2005). MK is closely associated with a poor prognosis for humans with carcinoma (Kadomatsu and Muramatsu, 2004). In addition, MK is involved in inflammation, as revealed by studies *in vivo* such as arterial restenosis (Horiba *et al.*, 2000), rheumatoid arthritis, ischaemic renal injury (Sato *et al.*, 2001), *cis*-platin-induced tubulointerstitial injury (Kawai *et al.*, 2004), diabetic nephropathy (Kosugi *et al.*, 2006; 2007) and crescentic glomerular nephropathy (Kojima *et al.*, 2013). The diverse pathophysiological roles of MK range from AKI to CKD accompanied by hypertension, ischaemia and diabetes.

This review highlights the various pathophysiological and inflammatory effects of MK, with emphasis on its involvement in nephrogenesis, hypertension through the RAS and several kidney diseases based on findings derived from experimental animal models.

MK and nephrogenesis

The expression levels of MK induced by retinoic acids are high during the mid-gestation period of embryogenesis, but very low in normal adult tissues. Development of the embryonic kidney depends on mesenchymal-epithelial interactions. Retinoic acids dose-dependently modulate nephrogenesis *in vivo* and *in vitro* (Vilar *et al.*, 2002). When several tissues interact to form an organ, very high levels of MK are expressed in epithelial tissues. The epithelium of the kidney is derived from the mesoderm. Thus, the MK gene product might be involved in the generation of epithelial tissues or their interactions with other tissues. Indeed, MK is uniformly expressed in both the ureteric bud and the metanephrogenic mesenchyme in 11-day-old mouse embryos (Sato *et al.*, 2002). The expression of MK is very high in the immature, compared with the mature metanephros, but very low in the kidney after birth.

A vitamin A deficiency *in vivo* did not modify the specific spatial and temporal expression profiles of the MK gene in the metanephros, although mRNA expression was decreased. Neutralizing antibodies for MK inhibit nephrogenesis *in vitro* by reducing the number of nephrons formed *de novo* by about 50% without affecting the ureteric bud branching morphogenesis (Vilar *et al.*, 2002). It has recently been reported that MK expression is up-regulated in neuronal differentiation; this was shown with stem cells (Jarmalavičiūtė *et al.*, 2013). MK might play an important role in the regeneration of a kidney using stem cells. The current understanding of how renal branching morphogenesis is regulated *in vivo* has been derived from analyses of genetic mutations that disrupt the development of collecting duct systems in mice and humans. The biological effects of purified recombinant MK have been analysed in metanephric organs cultured in serum-free media. These studies showed that MK selectively promotes the overgrowth of Pax-2 and N-CAM positive nephrogenic mesenchymal cells, does not stimulate expansion of the stromal compartment and suppresses branching morphogenesis of the ureteric bud. MK suppresses apoptosis, stimulates the cellular proliferation of nephrogenic mesenchymal cells and maintains the viability of isolated mesenchyme cultured without the ureteric bud (Qiu *et al.*, 2004).

Based on these findings, MK is thought to regulate the balance between epithelial and stromal progenitor cell

populations of the metanephric mesenchyme during renal organogenesis.

Pathophysiological roles of the RAS

The RAS plays a pivotal role in the homeostatic control of arterial pressure and the maintenance of sodium concentration and water excretion. The components of the RAS are distributed in systemic organs, and its dysregulation causes the pathogenesis of many diseases, including hypertension, cardiovascular and renal disorders (Johnson *et al.*, 2008). Angiotensin II in particular mediates systemic hypertension by regulating the release of catecholamines and aldosterone from the adrenal gland and from prejunctional nerve endings. Recent studies of hypertension have examined the intrarenal RAS (Qiu *et al.*, 2004; Navar *et al.*, 2011). Angiotensin (Ang) II induces intrarenal angiotensinogen (AGT) expression in proximal tubular epithelial cells (TECs) *in vitro* (Prieto-Carrasquero *et al.*, 2009). Chronic Ang II infusion increases intrarenal Ang II concentrations *in vitro* and local *de novo* formation of Ang II, enhances intrarenal AGT and contributes to higher Ang II levels in whole kidneys (Kobori *et al.*, 2003). Based on these finding, inactive renin in the distal nephrons and AGT secreted in proximal tubules might coordinate to increase Ang II formation in distal nephrons (Navar *et al.*, 2011). Ang II also inhibits renin release under physiological conditions, thus providing the above system with a negative feedback loop, which could explain positive feedback in the RAS. Furthermore, one study has shown that circulating AGT filtered from injured glomeruli is absorbed and increased in the renal proximal tubules (Okubo *et al.*, 1998). Thus, Ang II might play an amplifying role in the feedback associated with kidney diseases.

Overactivation of the RAS can suppress the phosphorylation of PKB in the PI3K pathway, which results in inhibition of insulin secretion and an increase in insulin resistance via the Ang type 1 receptor (AT_1) (Hsueh and Wyne, 2011). Inhibition of the PI3K pathway further reduces the induction of NO in vascular endothelial cells, leading to endothelial dysfunction in various organs. These responses cause diabetes mellitus, metabolic syndrome, atherosclerosis and CKD, eventually resulting in refractory hypertension.

Clinical effect of RAS blockade

Ang II overexpression and AT_1 receptor activation cause an increase in aldosterone levels, thus creating a vicious cycle that impairs various organs. Blocking the RAS using ACE inhibitors (ACEi) and angiotensin receptor-blocking agents (ARB) breaks this cycle and benefits several organs including the heart and kidneys (Brenner *et al.*, 2001; Ruggenenti *et al.*, 2010). Inhibition of the RAS is presently a first-choice therapy for patients with CKD. However, although serum aldosterone levels are initially suppressed by imposing a RAS blockade using ACE inhibitors or ARBs in the clinical setting, subsequently, the levels become too high again. This phenomenon, known as 'aldosterone breakthrough,' has not been completely elucidated (Nobakht *et al.*, 2011). Both selective and non-selective aldosterone antagonists reduce the risk of death and hospitalization for patients with congestive heart failure and CKD. The renoprotective benefit of RAS blockade might partially involve an increase in endothelial NO bioavailability. We confirmed that a reduction in NO caused by endothelial dysfunction participates in aldosterone breakthrough (Kosugi *et al.*, 2009; Nakagawa *et al.*, 2011). Because ACE regulates NO, ACE activity is likely to be involved in the pathogenesis of this phenomenon.

MK regulates hypertension through RAS activation

Both AGT and the expression of renin mRNA are significantly elevated, whereas the expression of ACE mRNA is significantly suppressed in the aorta of $Mdk^{-/-}$ mice (Ezquerra *et al.*, 2005). However, we demonstrated that systolic and mean blood pressure are comparable between $Mdk^{+/+}$ and $Mdk^{-/-}$ mice, and that $Mdk^{-/-}$ mice appear to develop normally (Hobo *et al.*, 2009). The biological significance of these changes in RAS molecules in the aorta of $Mdk^{-/-}$ mice is obscure. We evaluated the relationship between MK and RAS activation in mice with renal ablation to determine the molecular mechanisms involved in RAS regulation. The remnant kidney model of advanced renal injury is generally characterized by systemic hypertension and glomerular hyperfiltration, and causes glomerular sclerosis eventually (Ferrari, 2007). Systolic blood pressure and mean blood pressure are significantly higher in the $Mdk^{+/+}$ mice than in the $Mdk^{-/-}$ mice with a remnant kidney (Hobo *et al.*, 2009). In addition, $Mdk^{+/+}$ mice exhibit a more pernicious renal failure, with glomerular sclerosis and tubular interstitial injuries in association with elevated plasma Ang II levels. The development of these pathophysiological injuries can be prevented by ACEi or ARB, but not by the vasodilator hydralazine.

How MK is involved in the pathogenesis of hypertension still needs to be determined. The expression of MK particularly in the renal tubular epithelium is increased in a model of chronic renal failure, 5/6 nephrectomy. This finding is consistent with the reductions in RAS components identified in a rat remnant kidney model (Pupilli *et al.*, 1992). These data suggest that hypertension after 5/6 nephrectomy might not be due to activation of the intrarenal RAS. In contrast to the kidney, the expression of ACE is obviously increased in the lungs after renal ablation (Hobo *et al.*, 2009) and levels of expression of MK are elevated in the lungs and plasma. Both MK and ACE are expressed on the endothelium of microvessels in the lung. Administration of MK to $Mdk^{-/-}$ mice *in vivo* resulted in systemic hypertension and an increase in ACE in the lungs. Furthermore, the addition of exogenous MK protein to primary cultured human lung microvascular endothelial cells enhanced ACE expression, suggesting that ACE is a subsequent target of MK in the lungs. The renoprotective effect of inhibiting the RAS might partially involve an increase in endothelial NO bioavailability. Thus, MK, by inducing the activation of RAS in the endothelium, might also be involved in the pathogenesis of hypertension.

How MK expression is regulated in systemic hypertension after 5/6 nephrectomy

Various hypotheses have been proposed to explain why pulmonary complications following acute or subacute kidney injury should no longer be considered as causes of mortality and morbidity. In addition to MK and the RAS, most of such damage is accompanied by the production of various cytokines, including TGF-β, IL-1β, IL-6, IL-18, NF-κB and TNF-α, that mediate the pulmonary injury associated with ARF (Hoke *et al.*, 2007). Various factors mediate interactions upstream of the MK-RAS cascade. We demonstrated that oxidative stress induces the expression of MK (Kosugi *et al.*, 2006) and others have found that hypoxia, via HIF-1α, affects the expression of MK and causes pulmonary vascular remodelling (Ruggenenti *et al.*, 2010). We postulate that reduced NADPH oxidases (Nox), which are superoxide-generating enzymes that release superoxide upon electron transfer from NADPH to oxygen, are significantly involved in the deleterious effects associated with kidney injury. The expression of Nox1, 2 and 4 is significantly enhanced by renal ablation in the lungs of $Mdk^{+/+}$, but not $Mdk^{-/-}$ mice (Hobo *et al.*, 2009), and the production of Nox-mediated reactive oxygen species (ROS) induces MK expression in the lungs. Furthermore, 4-hydroxy-2,2,6,6-tetramethylpiperidine-N-oxyl, a cell membrane-permeable radical scavenger, reduces the expression of MK induced in the lungs and restores plasma Ang II to normal levels in the lungs, resulting in improved blood pressure, and the amelioration of renal pathologies such as glomerular sclerosis and tubular interstitial injury. The half-lives of ROS are very short, and they are thus unlikely to travel between the kidneys and the lungs. However, Ang II can induce the expression of Nox (Mollnau *et al.*, 2002). Based these findings, we conclude that the expression of MK in the endothelium is initially increased in response to oxidative stress and this up-regulates ACE expression in the lung, leading to a vicious cycle of Ang II overexpression. That is, the RAS might intensively promote positive feedback caused by oxidative stress (Figure 1).

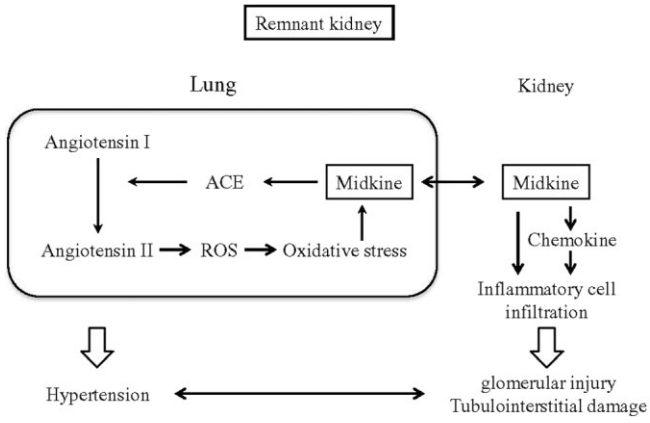

Figure 1
Schematic diagram of the pathomechanisms involved in hypertension induced through activation of the RAS and the lung–kidney interactions. ROS, Nox-mediated reactive oxygen species.

MK and renal ischaemic reperfusion injury

AKI accompanied by severe hypertension is an important and potentially devastating complication, and the incidence reported over the past two decades varies from 5% of hospitalized patients to 30–50% of those in intensive care units (Schrier *et al.*, 2004). Renal ischaemia, which is a major cause of AKI, has been closely linked with damage to various organs caused by cytokines released in response to injury of the kidney, resulting in MOF.

Energy depletion in renal epithelial cells during the process of renal ischaemia affects various life systems, directly causing cell death and disruption of the cytoskeleton and cell polarity, or indirectly inducing chemotaxis by activating various types of cell, such as endothelial cells and leukocytes. Necrosis and autophagy occur after ischaemic reperfusion injury. The depletion of ATP favours necrotic cell death, whereas GTP depletion tends to promote apoptotic cell death (Padanilam, 2003). In such a setting, vascular endothelial cell dysfunction results in vascular congestion and oedema, reduced blood flow, and the migration of inflammatory cells including neutrophils and macrophages to the kidney (Lauriat and Linas, 1998). In the damaged kidney, these infiltrating inflammatory cells can release cytokines, ROS, proteases, myeloperoxidase and other chemokines to trigger further damage. Many nephrologists overwhelmingly support this concept, which has been demonstrated experimentally in animal models of renal reperfusion.

MK promotes the migration of neutrophils and macrophages but is expressed at very low levels in proximal tubules. After ischaemic reperfusion, MK is up-regulated in the proximal tubules. The absence of MK protects against renal ischaemic reperfusion injury by reducing the infiltration of leukocytes. Furthermore, the up-regulation of chemokines such as macrophage inflammatory protein-2 for neutrophils and macrophage chemotactic protein-1 (MCP-1) for macrophages induced by ischaemic reperfusion injury is diminished in MK-deficient mice (Figure 2) (Sato *et al.*, 2001). The effects of antisense MK for treating ischaemic renal failure have been evaluated by determining the effects of an i.v. administration of MK antisense phosphorothioate oligodeoxyribonucleotide (ODN) to mice before and after ischaemic reperfusion injury. After its rapid incorporation into proximal tubular epithelial cells, ODN inhibited MK synthesis, which reduced the migration of inflammatory cells to the injured epithelial layer. Consequently, renal damage was less severe in the mice treated with MK antisense ODN compared with mice that were untreated or treated with MK sense ODN (Sato *et al.*, 2005).

These results indicate that MK directly enhances inflammatory cell migration after ischaemic injury of the kidney and contributes to the augmentation of ischaemic tissue damage by inducing the production of chemokines.

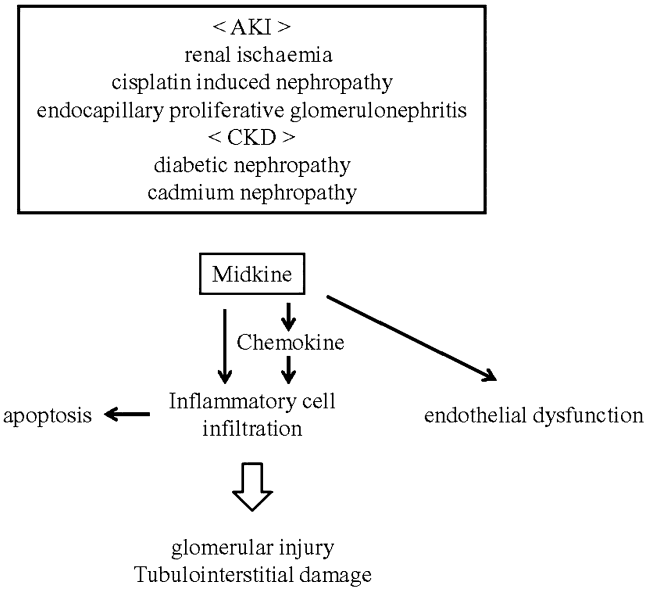

Figure 2

Schematic diagram of the pathomechanisms involved in acute kidney injuries and chronic kidney diseases with regard to midkine, inflammation cell infiltration endothelial dysfunction and apoptosis. AKI, acute kidney injuries; CKD, chronic kidney diseases.

MK and cisplatin-induced nephropathy

Cisplatin is one of the most effective antineoplastic drugs in the chemotherapeutic arsenal. However, the antitumour properties of cisplatin, like many other antitumour agents, are associated with undesirable physiological side effects such as nephrotoxicity, ototoxicity, neurotoxicity and bone marrow suppression. In particular, the renal toxicity of cisplatin is dose-limiting. Altered kidney functions induced by cisplatin are characterized by signs of damage such as changes in urine volume, body weight and glutathione status, increased amounts of lipid peroxidation products in the urine and kidneys and significantly elevated levels of serum creatinine and urea (Antunes and Neville, 2000).

Thus, cisplatin acts directly on proximal tubule epithelial cells and causes cell death. However, the biological significance of its secondary effects, such as inflammation is not understood in detail. High MK levels expressed in the proximal tubule exert conflicting anti-apoptotic and chemotactic effects with regard to cisplatin nephrotoxicity. Survival rates after cisplatin treatment are significantly higher in MK-deficient, than in wild-type mice and blood levels of urea nitrogen, tubular degeneration and apoptosis are higher in wild-type mice despite the anti-apoptotic activity of MK. We hypothesize that, *in vivo*, the anti-apoptotic effect of endogenous MK is eroded by the enhanced leukocyte migration induced by MK. Neutrophil recruitment is enhanced more in wild-type mice, which is consistent with the chemotactic activity of MK (Figure 2). MK expression in wild-type mice persists for 24 h, and then conspicuously decreases. The administration of MK antisense ODN to wild-type mice suppresses the expression of MK and consequently neutrophil infiltration (Kawai *et al.*, 2004).

Based on these findings, the circuits of early molecular events involving MK can be considered to induce an inflammatory response and eventually enhance the death of proximal tubule epithelial cells in cisplatin-induced renal damage.

MK and endocapillary proliferative glomerulonephritis

Acute progressive renal injury is a key event that occurs either as the result of a primary glomerular disease or as a consequence of various systemic diseases. It is caused by immune-mediated mechanisms such as the cytokine-driven inflammation and immune complex formation (Naicker *et al.*, 2007). For example, glomerular endothelial dysfunction caused by infectious inflammation and an increase in capillary pressure that promotes glomerular permeability results in excessive protein infiltration into the lumen of proximal tubules (Remuzzi *et al.*, 1997). The re-absorption of filtered protein activates the tubular epithelium to generate inflammatory mediators including chemokines that are secreted into the basolateral side of the tubules where a tubulointerstitial inflammatory reaction is initiated (Remuzzi *et al.*, 1997).

In contrast to MK-deficient mice with glomerular endothelial dysfunction, wild-type mice with this dysfunction demonstrate diffuse cellular proliferation; mesangial and circulating inflammatory cells proliferate in mesangial areas and capillary lumens, respectively, resulting in both glomerular and tubulointerstitial injuries. Electron microscopy has confirmed that glomerular capillary lumens in these wild-type mice, when compared with the MK-deficient mice, become narrower and obstructed by the marked increase in expanded mesangial cells, the various proliferated cells and the deposition these cells in mesangial areas. The depositions of IgG and C3, detected on the capillary walls and mesangial areas in glomeruli, were significantly increased in the wild-type mice compared with the MK-deficient mice. Furthermore, the number of infiltrating neutrophils into glomeruli was more significant in the wild-type mice than in the MK-deficient mice. These pathological findings could be attributed to neutrophil infiltration induced by stimulation of the MK–MIP-2 pathway (Figure 2), but they appear to be associated with MK-related IgG deposition and C3 activation. These findings are common in infection-related glomerular damage and resemble endocapillary proliferative GN (Zhang *et al.*, 2005). Furthermore, the profile of MK expression is consistent with that of clinical glomerular and tubulointerstitial damage (Kato *et al.*, 2011).

These findings define the deleterious role of MK in endocapillary proliferative glomerulonephritis induced by protein overload.

MK and diabetic nephropathy

Diabetic nephropathy is a life-threatening major complication of diabetes mellitus, which in turn is the most common cause of end-stage renal failure worldwide. Persistent hyperglycaemia is known to induce severe endothelial dysfunction,

oxidative stress and inflammation, leading to changes in renal morphology and haemodynamics. These processes involve various chemokines, including Ang II, NO, TGF-β, IL-1β, plasminogen activator inhibitor-1 (PAI-1) and MCP-1 (Diamond-Stanic *et al.*, 2011). Diabetic nephropathy thus warrants a multidisciplinary approach to elucidate the underlying molecular mechanisms.

In mice with streptozotocin (STZ)-induced 129SV diabetes, MK induces glomerular sclerosis accompanied by macrophage infiltration (Kosugi *et al.*, 2006), which apparently resembles the pathological changes induced by hypertension. Hyperglycaemia can enhance MK expression in mesangial cells, thus promoting the accumulation of extracellular matrix through the phosphorylation of PKC and ERK. In this setting, TGF-β indirectly interacts with MK via the PKC-ERK pathway. Macrophage recruitment activated by MK in TECs through MCP-1 induction at the early stage of diabetic nephropathy eventually results in tubulointerstitial injury (Kosugi *et al.*, 2007). In addition, menopause aggravates diabetic nephropathy and MK is up-regulated (demonstrated using DNA microarrays; Diamond-Stanic *et al.*, 2011). Endothelial NO synthase expression is strikingly decreased in this model (Figure 2). Endothelial NO is produced by endothelial cells in afferent arterioles and glomeruli, and to a lesser extent in efferent arterioles. Endothelial NO regulates intraglomerular pressure under physiological conditions, whereas in the presence of decreased NO, increased systolic blood pressure coupled with altered arteriolar autoregulation results in an increased transmission of pressure to the glomerulus (Nakagawa *et al.*, 2011). Another study has, likewise, suggested that endothelial dysfunction could also be responsible for the tubulointerstitial injury found in STZ-induced diabetic rats (Shibata *et al.*, 2009). Plasma leakage from peritubular capillaries due to endothelial dysfunction could be a mechanism of tubulointerstitial injury in diabetes. MK might also be involved in the pathogenesis of glomerular hypertension and endothelial dysfunction. Diabetes in a mouse model of menopause treated with 4-vinylcyclohexene diepoxide was induced using STZ and then post-menopausal changes in gene expression in the diabetic kidney were assessed using DNA microarrays. Real-time PCR, for confirmation, randomly identified the *Mdk* gene and the immediate early response gene 3 (Diamond-Stanic *et al.*, 2011).

Thus, MK plays key roles in diabetic nephropathy and the above findings suggest that MK accelerates the intracellular signalling network evoked by hyperglycaemia in diabetic nephropathy and plays a critical role in the tubulointerstitial inflammation associated with diabetic nephropathy through activation of the MCP-1 pathway.

MK and crescentic GN

Crescentic GN, a rapidly progressive kidney disease characterized by inflammatory cell infiltration, fibrin deposition, and thrombotic microangiopathy, is mostly diagnosed as pulmorenal syndrome with acute onset and a high likelihood of progression towards end stage renal disease (ESRD) without intense immunosuppressive therapy. Crescents form in the glomerulus as a result of segmental breaks in the glomerular basement membrane (GBM), often in association with fibrinoid necrosis (Eddy and Fogo, 2006); this also includes fibrin deposition, inflammatory cell infiltration and extracellular matrix accumulation. Among these, fibrin deposition not only interrupts glomerular blood flow and leads to irreversible ischaemia and glomerular obsolescence, but also promotes inflammatory cell infiltration and epithelial cell proliferation in Bowman's space (Hertig and Rondeau, 2004). Glomerular fibrin deposition is a consequence of activating the coagulation system and an imbalance between fibrin deposition and removal by the plasminogen/plasmin system (Kitching *et al.*, 1997). Macrophage and neutrophil recruitment triggered by inflammatory mediators such as fibrin, oxidative stress and various chemokines also induces coagulation during crescentic GN and thrombotic microangiopathy that is accompanied by severe endothelial dysfunction (Eddy and Fogo, 2006). Breaking the vicious cycle of these processes is therefore critical for reducing mortality rates associated with aggressive kidney diseases.

Our previous studies *in vivo* revealed that MK, by inducing the recruitment of inflammatory cells, plays deleterious roles in both glomerular damage and tubulointerstitial injury (Sato *et al.*, 2001). In contrast to these results, the study of crescentic GN model demonstrated that despite the lack of significant differences in autologous or heterologous reactions, mice deficient in MK develop more necrotizing glomerular and tubulointerstitial damage than wild-type mice and that these two conditions positively correlate. High levels of PAI-1 induced in damaged glomeruli of MK-deficient mice, particularly in crescents and endothelial cells, are associated with an increase in inflammatory cell infiltration and matrix deposition in the glomerulus and interstitium of these mice. In line with these findings *in vivo*, primary cultured endothelial cells derived from MK-deficient mice express more PAI-1 mRNA upon fibrin challenge and demonstrate less fibrinolysis than wild-type mice, whereas the expression of plasminogen activator is not affected (Figure 3) (Kojima *et al.*, 2013).

These findings indicate that MK helps to protect against crescentic GN and that the pathological features of crescentic GN are attributable to an imbalance in the coagulation-fibrinolysis system.

MK and puromycin aminonucleoside-induced nephrosis

Disruption of the slit diaphragm is histologically recognized as effacement of the foot processes projected by podocytes, and it typically occurs in patients with nephrotic syndromes, such as minimal change nephrotic syndrome (MCNS). In response to appropriate therapy including the administration of glucocorticoids, proteinuria in MCNS is ameliorated along with recovery of the slit diaphragm. In the sense that it presents as temporal massive proteinuria with subsequent complete remission and with no scarring in the kidney, puromycin aminonucleoside-induced (PAN) nephrosis accompanied by foot process effacement and the focal detachment of podocytes from GBM (Caulfield *et al.*, 1976) mimics human MCNS.

MK is a target gene for all-*trans*-retinoic acid (ATRA) (Muramatsu, 1993) and it has been examined in relation to

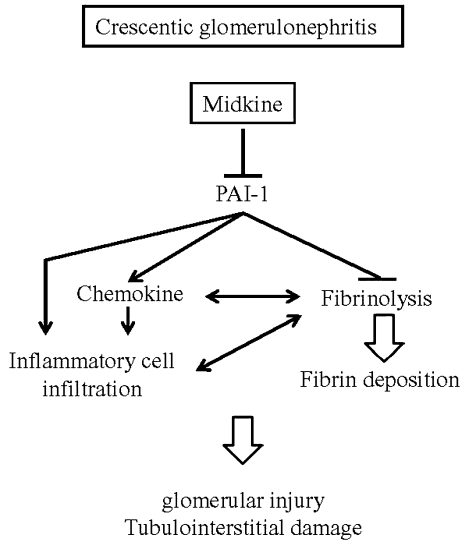

Figure 3
Schematic diagram of the pathomechanisms involved in crescentic glomerulonephritis with regard to midkine and plasminogen activator inhibitor. PAI-1: plasminogen-activator inhibitor.

local ATRA concentrations. A key enzyme in the production of ATRA, retinaldehyde dehydrogenase type 2 (RALDH2), is significantly up-regulated in the podocytes of rats with PAN nephrosis. On day 5 of PAN nephrosis, RALDH2 markedly increases the levels of ATRA, whereas glomerular expression levels on the slit diaphragm of MK and nephrin, of which a gene mutation causes the Finnish type of congenital nephrotic syndrome, are down-regulated. Daily doses of ATRA ameliorate proteinuria and this is accompanied by improvements in effacement of the foot processes and increases in MK and nephrin (Suzuki *et al.*, 2003).

These findings suggest that MK can be a biomarker of progress or repair in nephrotic syndrome, because ATRA regulates both genes through a common or similar mechanism.

MK and cadmium nephropathy

The general population is constantly exposed to cadmium usually at low levels through cigarette smoking and dietary sources. However, cadmium is also one of the most toxic environmental and industrial pollutants and its accumulation results in chronic damage to multiple organs, such as the liver, heart and kidneys of humans and other animals (Satarug *et al.*, 2000).

Cadmium concentrations in rat kidney tissues after 8 weeks of exposure increase ~90-fold compared with those in control rat kidneys. More cadmium accumulates in the liver, than in the kidney and heart. Levels of tissue TNF-α and caspase-3 are significantly higher, and MK mRNA and protein are significantly up-regulated in cadmium-treated compared to control rats (Figure 2) (Satarug *et al.*, 2000).

The results indicate that chronic cadmium administration induces inflammation and apoptosis in the rat kidney, and that MK is involved in the mechanisms of cadmium-induced tissue damage.

MK and haemodialysis (HD)

Various abnormalities of the bioregulatory system have been identified in patients on maintenance haemodialysis (HD). Many complications, such as anaemia, secondary hyperparathyroidism (Hory and Drueke, 1997) and dialysis-related amyloidosis can be attributed to the dysregulation of hormones, cytokines and growth factors.

Although serum MK levels do not significantly differ between controls and patients before starting HD, levels significantly increase in patients during early HD sessions, when heparin is used, and gradually decrease after dialysis. Heparin administered i.v. to normal control individuals induces a similar sudden increase in MK, but levels then rapidly decrease. MK is released mainly from endothelial cells immediately after heparin administration to patients during HD and gradually disappears from the bloodstream as a result of renal impairment (Fujisawa *et al.*, 1998).

These findings suggest that understanding the diverse pathologies in which MK is involved will help to clarify endothelial functions and provide new insights into some of the complications associated with HD.

Conclusions

MK is a double-edged sword that acts according to the type of renal disease. With increased ROS production, inflammatory cell recruitment and PAI-1 down-regulation, MK has been implicated in various pathologies, including hypertension, renal ischaemia, diabetes and crescentic GN. Several complicated pathways and numerous related molecules are involved in the mechanisms underlying these diseases in multiple organs. Among the various complexities, hypertension associated with a kidney–lung interaction involves positive feedback from the RAS regulated by MK. In addition to oxidative stress, inflammation with macrophage recruitment and endothelial dysfunction caused by Ang II-mediated NO down-regulation might be involved. This mechanism could also be involved in the pathogenesis of diabetic nephropathy. However, these mechanisms are not fully understood. We hope to further elucidate the mechanisms of endothelial dysfunction mediated by MK in the near future. MK might prove to be an important candidate mediator of pulmonary and other organ complications associated with kidney disease. Such findings would be helpful in understanding the high mortality rates associated with MOF that follows AKI. Finally, such multidisciplinary knowledge could open up new avenues for the development of therapies that target MK for patients with kidney diseases.

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Conflict of interest

The authors have no conflict of interest regarding this study.

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