Midkine and the kidney: health and diseases

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Abstract

Midkine (MK; gene name, Mdk), a heparin-binding growth factor, regulates cell growth, cell survival, migration and anti-apoptotic activity in nephrogenesis and development. In the kidney, MK is expressed mainly in proximal tubular epithelial cells and is induced by oxidative stress through the activation of hypoxia-inducible factor-1α. The pathophysiological roles of MK are diverse, ranging from the occurrence of acute kidney injury (AKI) to progression of chronic kidney disease, often accompanied by hypertension, renal ischemia and diabetic nephropathy. In particular, hypertension has indispensable implications for various vascular diseases, including cardiovascular and renal disorders. Mdk+/− mice exhibited marked hypertension in renal ablation model compared with Mdk−/− mice, eventually leading to more progressive renal failure such as glomerular sclerosis and tubulointerstitial injuries in association with elevated plasma angiotensin (Ang) II levels. MK is also induced in the lung endothelium by oxidative stress and subsequently up-regulated angiotensin-converting enzyme (ACE) in the lung. Ang II is hydrolyzed by ACE to induce further oxidative stress, accelerating MK generation and leading to a vicious cycle of positive feedback on the MK-Ang II pathway. The kidney–lung interaction involving positive feedback between the renin–angiotensin system and MK may in part account for the pathogenesis of hypertension and kidney injury. In addition to this pathway, MK is involved in the pathogenesis of diabetic nephropathy and AKI through the recruitment of the inflammatory cells. Such multidisciplinary findings may open new avenues for targeting therapies for hypertension and various renal diseases, including AKI and diabetic nephropathy.

Keywords: angiotensin II; diabetes mellitus; hypertension; inflammation; midkine

Introduction

The worldwide epidemic of hypertension continues to expand with the rapid spread of diabetes mellitus, excess salt intake and obesity [1, 2]. Indeed, hypertension is a major risk factor for cardiovascular and cerebrovascular diseases [3, 4]. Regardless of the primary disease process, renal dysfunction including acute kidney injury (AKI) and chronic kidney disease (CKD) is frequently accompanied by various systemic diseases induced by endothelial dysfunction in the microcirculation, eventually resulting in higher mortality and morbidity. Further complicating the situation is that individuals are often unaware of subsequent increases in these diseases, as moderate hypertension and renal dysfunction are usually asymptomatic. Early initiation of comprehensive management is therefore needed to prevent microvascular complications such as coronary artery disease, diabetic vasculopathy, cerebral vascular disease and renal disease [5]. In particular, the pathophysiology of systemic hypertension includes a complex interaction of multiple vascular effectors, such as catecholamines, the renin–angiotensin system (RAS), oxidative stress, nitric oxide (NO), vascular endothelial growth factor, endothelin-1 and a large number of inflammation-related cytokines [6–8]. An improved understanding of the crosstalk among these reactions might contribute to the prevention of multiple organ failure (MOF) with hypertension. In particular, the kidney possesses all the above components, and clarification of their interactions could thus be expected to lead to treatments inhibiting or delaying the progress of MOF.

Midkine (MK; gene name, Mdk) is a multi-functional heparin-binding growth factor with major biological roles which can be categorized into three areas: the nervous system, cancer and inflammation [9, 10]. To date, the neuronal cytoprotective effects of MK have been demonstrated in various in vivo models, including retinal degeneration, cerebral infarction and ischemia-induced neuronal death [11]. In human carcinoma, MK is markedly associated with poor prognosis [9]. In addition, MK is involved in inflammation, as revealed by in vivo studies such as arterial restenosis [12], rheumatoid arthritis, ischemic renal injury [13], cis-platin-induced tubulointerstitial injury [14] and diabetic nephropathy [15, 16]. The pathophysiological roles of MK are diverse, ranging from AKI to CKD accompanied by hypertension, ischemia and diabetes.

This review highlights various pathophysiological and inflammatory issues with an emphasis on the interactions among MK, the RAS and several inflammation-related cytokines in the kidney diseases.
Structure of MK and potential receptors

MK is involved in nephrogenesis and development, promoting cell growth, cell survival, migration of various cells, anti-apoptotic activity, fibrinolysis and oncogenesis [9, 10] (Figure 1). In the normal kidney, MK is expressed in both proximal and distal tubular epithelial cells [13] (TECs) and to a lesser extent in endothelial cells [17]. MK was first discovered as the product of a retinoic acid-responsive gene [9, 10]. MK protein is essentially composed of two domains tightly held by disulfide bridges, namely an N-terminally located N-domain and a C-terminally located C-domain. Most of its biological activities are carried out by the C-terminal half of the molecules of the C-domain. The receptor of MK is not fully elucidated; therefore, the pathological role of MK in pathological condition cannot be accounted for by the potential MK receptors that have been reported. The potential MK receptors are thought to form a molecular complex containing proteoglycans, including protein-tyrosine phosphatase ζ and members of the low-density lipoprotein receptor-related protein family [10, 18, 19]. The downstream signaling pathway includes mitogen-activated protein kinase and phosphatidylinositol 3-kinase (PI3K) [18, 19].

MK and nephrogenesis

MK induced by retinoic acids is highly expressed in the mid-gestation period in embryogenesis, whereas its expression is very restricted in adult normal tissues. Generation of embryonic kidney development depends on mesenchymal–epithelial interactions. Retinoic acids modulate nephrogenesis in a dose-dependent manner both in vivo and in vitro [20, 21]. When several tissues interact to form an organ, MK is intensely expressed in the epithelial tissue. In the kidney, the epithelium is derived from the mesoderm. MK gene product may thus be involved in the generation of epithelial tissues or in their interactions with other tissues. Indeed, MK is expressed uniformly in both the ureteric bud and metaneprogenic mesenchyme in 11-day-old mouse embryos [22]. The immature metanephros expresses MK more strongly than the mature metanephros, whereas MK expression in the kidney is very weak after birth.

Furthermore, in vitro nephrogenesis is strongly inhibited by neutralizing antibodies for MK, resulting in ~50% reductions in the number of nephrons formed in vitro without changes in ureteric bud branching morphogenesis [20]. In metanephalic organ culture experiments, MK selectively promotes the overgrowth of Pax-2- and N-CAM-positive nephrogenic mesenchymal cells, fails to stimulate expansion of the stromal compartment and suppresses branching morphogenesis of the ureteric bud [23]. These data suggest that MK regulates the balance of epithelial and stromal progenitor cell populations of the metanephric mesenchyme during renal organogenesis.

Pathophysiological roles of the RAS

The necessary RAS components are distributed in several organs, and dysregulation of this system is associated with the pathogenesis of hypertension, cardiovascular and renal disorders [1]. In particular, angiotensin (Ang) II mediates systemic hypertension by regulating the releases of catecholamines and aldosterone from the adrenal gland and prejunctional nerve endings. In contrast to established theory, recent investigations have focused on the intrarenal RAS in hypertension [24, 25]. In vitro studies have shown that Ang II induces intrarenal angiotensinogen (AGT) expression in proximal TECs [26]. With in vitro studies, chronic Ang II infusion was shown to induce an increased intrarenal Ang II concentration [27], and local de novo Ang II formation due to enhanced intrarenal AGT induction contributed to higher Ang II levels in the whole kidneys [28]. Based on this evidence, Navar et al. [25] suggested that inactive renin in the distal nephrons and AGT secreted in proximal tubules coordinate to increase the formation of Ang II in the distal nephrons. Under physiological conditions, Ang II also inhibits renin release, thus providing the above system with a negative feedback loop. However, this hypothesis provides a possible explanation for positive feedback in the RAS. Furthermore, Matsusaka et al. contributed an elegant study in which circulating AGT filtered from injured glomeruli was absorbed and increased in the renal proximal tubules [29]. With regard to the controversy over normal feedback regulation, Ang II might play an amplifying role in the uncontrolled feedback seen in the kidney diseases.

More interestingly, RAS overactivation can suppress the phosphorylation of protein kinase B in the PI3K pathway, resulting in the inhibition of insulin secretion and an increase in insulin resistance via the Ang Type I receptor (AT1R) [3]. Inhibition of the PI3K pathway further reduces NO induction in vascular endothelial cells, leading to endothelial dysfunction in various organs. These responses...
cause diabetes mellitus, metabolic syndrome, atherosclerosis and CKD, eventually resulting in uncontrollable hypertension.

Currently, however, we have encountered clinical scenarios in which the effect of RAS blockade is insufficient. This phenomenon, known as ‘aldosterone breakthrough’, has not been completely elucidated [30]. The recent literature indicates that this phenomenon can be induced in ~40% of diabetic patients treated by RAS blockade [6]. This treatment can initially suppress serum aldosterone levels during the earlier stage of the treatment period but subsequently fails to sufficiently reduce serum aldosterone. Indeed, an animal study has also shown that spironolactone can prevent the development of hypertensive and diabetic nephropathy in diabetic mice lacking endothelial NO despite incomplete suppression by RAS blockade [8]. Thus, we might also need to elucidate the mechanisms regulating the RAS in order to treat uncontrollable hypertension.

MK regulates hypertension through the RAS activation

Ezquerra et al. [31] have shown that AGT and renin messenger RNA (mRNA) expression are significantly elevated in the aorta of Mdk−/− mice, whereas angiotensin-converting enzyme (ACE) mRNA expression is significantly suppressed. However, our study revealed that systolic and mean blood pressure are comparable between Mdk+/+ and Mdk−/− mice, and Mdk−/− mice develop without apparent disturbances [32]. The biological meanings of changes in the RAS molecules in the aorta of Mdk−/− mice are thus obscure. To investigate the molecular mechanisms regulating the RAS, we evaluated the relationship between MK and RAS activation in renal ablation mice. This model is a popular and useful model of glomerular sclerosis since the remnant kidney model of progressive renal injury is generally characterized by systemic hypertension and glomerular hyperfiltration, the latter eventually causing glomerular sclerosis [33, 34]. In this model, both systolic and mean blood pressures are strikingly higher in Mdk+/+ mice than in Mdk−/− mice during the experimental period [32]. In addition, Mdk+/+ mice exhibit more progressive renal failure, such as glomerular sclerosis and tubular interstitial injuries in association with elevated plasma Ang II levels. The development of these pathophysiological injuries is prevented by ACE inhibitor or angiotensin receptor blocker treatment but not by the vasodilator hydralazine. This evidence indicates that renal dysfunction in the remnant kidney model is due to the induction of Ang II. Inevitably, MK expression is associated with the activation of the RAS.

So how is MK involved in the pathogenesis of hypertension? MK expression, particularly in the renal tubular epithelium, is induced by 5/6 nephrectomy. Nonetheless, reductions in RAS components have been reported in a rat remnant kidney model [35, 36], which is consistent with our results. 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 lung reveals marked induction of ACE after renal ablation [32]. MK expression in the lung and plasma is also elevated. Both MK and ACE expression are located on the endothelium of microvessels in the lung. In an in vivo study, Mdk−/− mice with supplementary administration of exogenous MK exhibited systemic hypertension and induction of ACE in the lungs. Furthermore, administration of exogenous MK protein to primary cultured human lung microvascular endothelial cells strikingly enhances ACE expression, suggesting ACE as one of the consequent targets of MK in the lung. The renoprotective benefit of RAS blockade may partially involve an increase in endothelial NO bioavailability. MK may thus also be involved in the pathogenesis of aldosterone breakthrough via NO regulation.

What regulates MK expression in systemic hypertension by 5/6 nephrectomy?

Other investigators have indicated various reasons why marked mortality and morbidity should no longer be suggested to be caused by pulmonary complications following acute or subacute kidney injury [37]. In addition to MK and RAS, most of these injuries are accompanied by the production of several cytokines, such as transforming growth factor (TGF)-β, interleukin (IL)-1β, IL-6, IL-18, nuclear factor kappa-light-chain enhancer of activated B cells and tumor necrosis factor-α in the kidney–lung interactions [37, 38]. What kinds of factors mediate the interactions upstream of the MK-RAS cascade? We have reported that oxidative stress induces MK expression [16]. Reynaud et al. also documented that MK is regulated by hypoxia via hypoxia-inducible factor-1α and causes pulmonary vascular remodeling [39]. We postulate the importance of reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidases (Nox), superoxide-generating enzymes that release superoxide by electron transfer from NADPH to oxygen. Expressions of Nox1, 2 and 4 in the lungs of Mdk+/+ mice are significantly enhanced by renal ablation but are not affected by the procedure in Mdk−/− mice [32]. Furthermore, the production of Nox-mediated reactive oxygen species (ROS) has been shown to induce MK expression in the lungs. In addition, a cell membrane-permeable radical scavenger 4-hydroxy-2,2,6,6-tetramethylpiperidine-N-oxyl (Tempol) reduces MK induction and plasma Ang II to normal levels in the lungs, resulting in the improvement of blood pressure and amelioration of renal dysfunctions such as glomerular sclerosis and tubular interstitial injury. ROS have very short half-lives, and are thus unlikely to travel between the kidney and lung. Ang II itself is well known to induce Nox expression [40]. Taking these facts together, we conclude that the initial MK induced in the endothelium in response to oxidative stress up-regulates ACE expression in the lung, leading to a vicious cycle of Ang II overexpression (Figure 2). In other words, RAS might intensively promote the positive feedback caused by oxidative stress.

MK and acute kidney injury

AKI develops as an important and potentially devastating complication with severe hypertension, and its incidence
has been reported to vary from 5% in hospitalized patients to 30–50% in intensive care units in the past two decades [41–43]. Renal ischemia, one of the major causes of AKI, has been intensely linked with damage in various organs through the interorgan interactions involving the kidney by several chemokines, resulting in MOF.

In the process of renal ischemia, depletion of energy in renal epithelial cells affects various beneficial and deleterious life systems, directly causing disruption of the cytoskeleton and cell polarity, and cell death, or indirectly inducing chemotaxis through the activation of various kinds of cells such as endothelial cells and leukocytes. Necrosis and autophagy occur after ischemic reperfusion injury. Severe depletion of ATP is well-known to favor necrotic cell death, whereas GTP depletion tends to promote apoptotic cell death [44]. In this setting, vascular endothelial cell dysfunction results in vascular congestion and edema, reductions in blood flow and the migration of inflammatory cells including neutrophils and macrophages [45]. Infiltrated inflammatory cells in the injured kidney can release cytokines, ROS, proteases, myeloperoxidase and other chemokines to trigger further damage. This concept has been overwhelmingly supported by many nephrologists and has been well demonstrated in the animal model of renal reperfusion. After ischemic reperfusion in vivo, MK is immediately induced in the proximal tubules, leading to the up-regulation of macrophage inflammatory protein-2 for neutrophils and monocyte chemotactic protein (MCP)-1 for macrophages [13, 46]. Eventually, infiltrated inflammatory cells cause severe tubulointerstitial injury. Inhibition of MK can prevent the migration of inflammatory cells to the injured epithelial layer, reducing the severity of renal damage. These results indicate that MK enhances migration of inflammatory cells on ischemic injury of the kidney directly and also through induction of chemokines and contributes to the augmentation of ischemic tissue damage.

**MK and diabetic nephropathy**

Longstanding hyperglycemia induces severe endothelial dysfunction, oxidative stress and inflammation, leading to changes in renal morphology and hemodynamics. These processes involve the participation of a wide variety of chemokines, including Ang II, NO, TGF-β, IL-1β, plasminogen activator inhibitor-1 and MCP-1 [47]. Diabetic nephropathy thus warrants a multidisciplinary approach to elucidate the molecular mechanisms underlying this disease.

In the streptozotocin (STZ)-induced 129SV diabetic model, MK induces glomerular sclerosis accompanied by infiltrated macrophages [16], which is likely to resemble pathological changes due to hypertension. Hyperglycemia enhances MK expression in mesangial cells, which promotes the accumulation of extracellular matrix through the phosphorylation of protein kinase C (PKC) and extracellular single-regulated kinase (ERK). In this setting, TGF-β interacts with MK indirectly via the PKC–ERK pathway. In the early stage of diabetic nephropathy, MK in TECs also activates macrophage recruitment through MCP-1 induction, eventually resulting in tubulointerstitial injury [15]. In addition, Diamond-Stanic et al. [48] demonstrated that menopause aggravates diabetic nephropathy and MK is up-regulated on DNA microarrays. Interestingly, endothelial NO synthase expression is strikingly decreased in this model (T. Kosugi and W. Sato, unpublished data). Basically, endothelial NO is produced by endothelial cells in afferent arterioles and glomerulus and to a lesser extent in efferent arterioles. Under physiological conditions, endothelial NO regulates intraglomerular pressure, whereas in settings in which NO is decreased, increased systolic blood pressure coupled with altered arteriolar autoregulation results in the increased transmission of pressure to the glomerulus [6]. Likewise, Shibata et al. [49] suggested that endothelial dysfunction could also be responsible for the tubulointerstitial injury in STZ-induced diabetic rat. As guessed by Temm’s group, plasma leakage from peritubular capillaries due to endothelial dysfunction could be a mechanism for tubulointerstitial injury in diabetes [50]. MK may also be involved in the pathogenesis of glomerular hypertension and endothelial dysfunction.

These data identify MK as a key molecule in diabetic nephropathy and suggest that MK accelerates the intracellular signaling network evoked by hyperglycemia in diabetic nephropathy.

**Conclusions**

With increased ROS production and inflammatory cell recruitment, MK has been implicated in various pathologies, including hypertension, renal ischemia and diabetes. The mechanisms underlying these diseases in multiple organs involve several complicated pathways and numerous related molecules. Among the various complexities, hypertension in the kidney–lung interaction involves positive feedback from the RAS regulated by MK. In addition to oxidative stress, inflammation with macrophage recruitment and endothelial dysfunction 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 remain incompletely understood. In the near future, we hope to further elucidate the mechanisms of endothelial dysfunction mediated by MK. As a result, MK might prove to be important as a candidate mediator for pulmonary and other organ complications associated with the kidney disease. These findings
would be helpful in understanding the high mortality rates associated with MOF following AKI. Finally, such multidisciplinary knowledge could open a new avenue for the development of targeted therapies against MK in the kidney diseases.

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