Midkine expression in the course of nephrogenesis and its role in ischaemic reperfusion injury

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Abstract
Midkine (MK) is a multifunctional heparin-binding growth factor with migration-promoting activity for neutrophils, macrophages and neurones. Since enhanced expression of MK is observed in the tubular epithelial cells of the diseased kidney, it has been suggested that MK plays important roles in the pathogenesis of tubulointerstitial injury. The aim of this study was to determine the contribution of MK in nephrogenesis and in a murine model of ischaemic renal reperfusion injury (IRI). In the 11 day embryo, MK was expressed uniformly in both ureteric bud and metanephrogenic mesenchyme. The immature metanephros expressed both MK mRNA and MK protein more strongly than the mature metanephros. We studied the extent of tubulointerstitial injuries in MK wild-type [Mdk(+/-)] and knockout [Mdk(-/-)] mice 90 min after IRI. MK was expressed weakly in the proximal tubules in Mdk(+/-) mouse kidneys. After IRI, MK expression in proximal tubules increased and the new expression was observed in the distal tubules in Mdk(+/-) mice. Immediate induction of MK expression was observed when cultured tubular epithelial cells (TEpiCs) were exposed to 5 mM H2O2. Recombinant mouse MK (10 ng ml) induced the increased expression of macrophage inflammatory protein 2 (MIP-2) mRNA in TEpiCs. Shortly after IRI, there were significantly fewer inflammatory leukocytes such as neutrophils and macrophages in Mdk(-/-) mice than in Mdk(+/-) mice. Marked up-regulation of monocyte chemoattractant protein 1 (MCP-1) and MIP-2 expression was detected in Mdk(+/-) mouse kidneys. Tubulointerstitial damage observed after IRI was significantly more suppressed in Mdk(-/-) mice than in Mdk(+/-) mice. These results suggest an important role for MK in the molecular cascade that regulates nephrogenesis. The present work also indicates that MK induces the chemotaxis of inflammatory leukocytes into the tubulointerstitium at least partly through the induction of MCP-1 and MIP-2, and that MK contributes to the aggravation of ischaemia-induced tubulointerstitial damage.

Keywords: chemokine; inflammatory; midkine; nephrogenesis; tubulointerstitial injury

Introduction
Midkine (MK) is induced by retinoic acid and is a novel multifunctional heparin-binding growth factor [1–3]. Its molecular weight is 13 kDa and it is rich in many basic amino acids and cysteine residues. MK is structurally unrelated to fibroblast growth factors or other heparin-binding growth factors, and is the initial member of this molecular family [4,5]. MK has been implicated in diverse biological processes, e.g. embryogenesis, angiogenesis and oncogenesis. Furthermore, MK has been implicated in diverse biological processes, e.g. embryogenesis, angiogenesis and oncogenesis. Furthermore, MK has been confirmed to promote chemotaxis of neutrophils and macrophages [6-8], and is proposed to be an important molecule regulating inflammatory responses. According to our recent data, the most relevant MK receptor is LRP1; namely, low-density lipoprotein (LDL) receptor-related protein type 1. Our preliminary data show that MK could activate mitogen-activated protein (MAP) kinase after binding to LRP1. It is very interesting that megarin (LRP2), which is another member of the LDL receptor family, can bind to MK in vitro, but much more weakly than LRP1. LRP1 is a multiligand receptor, and MK is supposed to be one of the important ligands [9].
Materials and methods

In the present study, we investigated MK expression during nephrogenesis by in situ hybridization and immunohistochemistry. We explored the role of MK in pathological conditions in the mature kidney, and performed in vivo experiments using MK knockout mice. Embryos were obtained from 129SV mice; the day of the first appearance of a vaginal plug was designated as day E0 of pregnancy; the gestational age of embryos in embryonic days (E) was also confirmed by morphological criteria. We also studied a human embryo. In the renal injury model, the right kidneys were removed 7 days before ischaemic renal injury and left renal arteries were clamped by special clips for 90 min, and then the clips were removed. The left kidneys were examined at 1, 2, 3 and 7 days after renal ischaemia.

Results

In the 11 day embryo, MK was expressed uniformly in both ureteric bud and metanephrogenic mesenchyme. The immature metanephros expressed both MK mRNA and MK protein more strongly than the mature metanephros. MK expression was detected in the mesonephric tubules, but barely in the glomeruli in the 17 day embryo. In the neonatal kidney, MK mRNA and MK protein decreased day by day, while the adult kidney weakly expressed MK mRNA and MK protein in the tubules. MK is strongly expressed in the glomeruli and tubules in the human fetal kidney at 30 weeks. When we studied the fetal kidney of congenital polycystic kidneys, MK expression was very weak. At present, we do not know the causative relationship between decreased MK expression and cyst formation. There is no difference in phenotype between Mdk(+/+) and Mdk(-/-).

MK is one of the important ligands for LRP, which receives signals from multiligands and transducers. Therefore, the normal development of the kidney might not be altered by MK deficiency alone. In adult kidneys, MK is expressed mainly in the proximal tubules. MK expression was significantly increased 2 days after renal ischaemia, peaked on day 2 and returned to normal levels within 7 days. An immediate increase in MK expression was observed when cultured tubular epithelial cells (TEpiCs) were exposed to 5 mM H2O2. We then compared histological damage between wild-type and MK knockout mice after renal ischaemia. Histological damage was significantly milder in MK knockout mice. In a semi-quantitative analysis of the tubulointerstitial damage, tubular injury observed in the wild-type mice was more pronounced than in MK knockout mice (Figure 1).

To explore the mechanisms of MK-mediated renal injuries, we studied the influence of MK on leukocyte infiltration. In the time course study of CD45-positive cells infiltrating into interstitial tissue in the wild-type mice, the leukocyte infiltration peaked at day 2. The infiltration of CD45-positive leukocytes was remarkably suppressed in the kidneys from MK knockout mice after renal ischaemia. Since neutrophils and macrophages were the main cell types in ischaemic renal injuries, we then studied the expression of chemokines involved in the chemotaxis of neutrophils and macrophages. mRNAs of two chemokines, MIP-2 and MCP-1, were markedly increased after renal ischaemia in wild-type mice. However, this increase was not observed in MK knockout mice. In order to clarify whether or not MK induces chemokine induction, we performed in vitro studies using cultured proximal tubular epithelial cells from wild-type mice. Both macrophage inflammatory protein 2 (MIP-2) and monocyte chemoattractant protein 1 (MCP-1) were significantly increased after MK stimulation. In an attempt to suppress MK expression in vivo, we constructed antisense oligonucleotides which were administered intravenously to mice 24 h after ischaemic renal injuries. Antisense oligonucleotide was localized in the proximal tubules. In these tubules, MK expression was inhibited successfully when compared with controls administered control sense oligonucleotide on day 2. When we studied the histology...
at day 2, mice injected with antisense oligonucleotide showed much milder damage than the controls injected with sense oligonucleotide.

**Conclusion**

These results suggest an important role for MK in the molecular cascade that regulates nephrogenesis. The present work also indicates that MK induces the chemotaxis of inflammatory leukocytes into the tubulointerstitium at least partly through the induction of MCP-1 and MIP-2, and that MK contributes to the aggravation of ischaemia-induced tubulointerstitial damage.

**References**