MAP kinase in renal development

Midori Awazu, Sayu Omori and Mariko Hida

Department of Pediatrics, Keio University School of Medicine, Tokyo, Japan

Abstract

Background. Among mitogen-activated protein kinase (MAPK) family members, ERK promotes proliferation or differentiation, whereas JNK and p38 are thought to inhibit cell growth and induce apoptosis. During renal development, large-scale proliferation and apoptosis occur. We investigated the temporal and spatial expression patterns of MAPK and its phosphatase MKP-1 as well as the role of ERK and p38 during kidney development.

Methods. Western blot analysis and immunohistochemistry were performed in the developing and mature kidney of the rat. Rat metanephroi were cultured from 15-day-old embryos, and exposed to inhibitors of MEK, an activator of ERK, PD98059, U0126 or a p38 inhibitor, SB203580 24–120 h after the start of culture. Growth of metanephroi was measured by surface area and thymidine incorporation. Ureteric buds and glomeruli were identified by labelling with Dolichos biflorus lectin and peanut agglutinin, respectively.

Results. The expression of ERK and p38 was high in the developing kidney. On the other hand, JNK was expressed abundantly in the adult kidney. Immunohistochemical studies revealed that the spatial expression of ERK coincided with the maturation of the kidney. p38 and MKP-1 were expressed uniformly. Growth of metanephroi was significantly inhibited by SB203580 but not by PD98059 or U0126. Ureteric bud branching was not affected by SB203580 or MEK inhibitors. Glomerular number was markedly reduced by SB203580 and to a lesser extent by U0126.

Conclusions. ERK, p38 and MKP-1 are strongly expressed in the developing kidney, and JNK is detected predominantly in the adult kidney. ERK appears to play a role in nephrogenesis and p38 in kidney growth and nephrogenesis.

Keywords: extracellular signal-regulated kinase; c-Jun N-terminal kinase; p38 mitogen-activated protein kinase; mitogen-activated protein kinase phosphatase-1; nephrogenesis

Introduction

The mitogen-activated protein kinase (MAPK) family belongs to the serine/threonine kinases that comprise three major subgroups, namely extracellular signal-regulated protein kinase (ERK), p38 MAPK (p38) and c-Jun N-terminal kinase/stress-activated protein kinase (JNK) [1]. ERK is activated by various growth factors and promotes cell proliferation and differentiation. p38 and JNK, on the other hand, are activated by cytokines and cellular stresses, and are thought to inhibit cell growth and induce apoptosis [2]. MAPK phosphatase-1 (MKP-1) inactivates and modulates MAPKs. During renal development, large-scale proliferation and apoptosis are known to occur [3]. Growth factors, transcription factors and adhesion molecules that regulate proliferation or apoptosis have been shown to be involved during renal ontogeny. Little is known, however, about the intermediary signalling molecules that transmit extracellular stimuli to the nucleus and activate genes during kidney development. The MAPK family may play a role in these processes given their actions to regulate proliferation, differentiation and apoptosis. Thus, we investigated the temporal and spatial expression patterns of MAPKs and MKP-1 in rat kidney during development [4]. We also investigated the role of ERK and p38 in renal development using rat metanephric organ culture system [5].

Methods

Immunoblot analysis and immunohistochemistry were performed in the developing and mature kidney of the rat. Embryos were removed and decapitated on days 14 and 18 of gestation (E14 and E18). E14 embryos were fixed with neutral buffered formalin. Kidneys from E18 embryos were harvested and either fixed with neutral buffered formalin or homogenized. Kidneys from rats 1 (N1), 7 (N7) and 40 (A)
days after birth were treated in the same manner. Immunohistochemical staining was performed using the enzyme-labelled antibody method [4]. Proliferation and apoptosis were assessed by proliferating cell nuclear antigen (PCNA) and TUNEL staining, respectively. Rat metanephroi were cultured from 15-day-old embryos, and exposed to MEK inhibitors PD98059 (300 μM), U0126 (10 μM) or a p38 inhibitor SB203580 (30 μM) 24–120 h after the start of culture [5]. Growth of metanephroi was measured by surface area. Ureteric buds and glomeruli were identified by labelling with Dolichos biflorus lectin and peanut agglutinin, respectively.

Results

Immunoblot analysis

ERK was present in the kidney throughout all stages, but the abundance of both p44 and p42 isoforms was highest at E18, and decreased gradually after birth (Figure 1). p38 and MKP-1 were present in the embryonic kidney, with greatly diminished levels in N1 kidneys, and were undetectable in N7 and adult kidneys. The opposite pattern of expression was seen for JNK, which was strongly expressed in the adult kidney. JNK was present at lower levels in neonatal kidneys, and was barely detectable in embryos. The phosphorylated and activated form of MAPKs correlated with the protein levels of MAPKs. Thus, P-ERK was highest in the fetal kidney, with diminishing levels thereafter. P-p38 was detectable at E18 and with lower levels at N1. P-JNK was abundant in the adult kidney and slightly detectable in N7 kidneys.

Immunohistochemistry

PCNA. At E14, PCNA expression was detected in condensing mesenchyme and ureteric bud epithelial cells. In the E18 kidney, PCNA was most abundant in the nephrogenic zone. Ureteric bud branches and tubuloglomerular structures were variably stained. In the N1 kidney, PCNA-positive cells were also observed in the more differentiated middle cortical layer. They shifted to the deep cortical layer and medullary region at N7. In the adult kidney, PCNA staining was absent.

TUNEL. In the E14 kidney, TUNEL staining was found in uninduced cells surrounding condensing mesenchyme. In the E18 and N1 kidney, apoptosis was most intense in comma- and S-shaped bodies, with mesenchymal cells surrounding them within the nephrogenic zone subjacent to the uninduced mesenchyme. TUNEL-positive nuclei were also found less frequently in epithelial cells within maturing glomeruli and tubular epithelial structures. No apoptosis was evident in the N7 or adult kidney.

MAPKs and MKP-1. Immunohistochemical studies revealed that ERK was ubiquitously expressed in metanephric kidneys at E14. In the E18 kidney, ERK expression was confined to the nephrogenic zone. ERK was strongly expressed in condensing mesenchyme, ureteric bud tips and undifferentiated mesenchyme. In neonatal kidneys, ERK shifted to the deep cortical layer and medullary region, correlating with PCNA expression. Thus, the spatial expression of ERK coincided with the maturation processes of nephrons. While ERK expression was reduced but maintained in the adult kidney, expression of p38 and MKP-1 was confined to the embryonic kidney. p38 and MKP-1 were detected uniformly in mesenchymal cells, mesangial cells and ureteric bud epithelia of the fetal kidney without an obvious correlation with the occurrence of apoptosis. JNK was expressed by tubular cells and podocytes of the adult kidney. These results strongly suggest that ERK and p38 may play important roles in kidney organogenesis.

Organ culture

Growth of metanephroi assessed by surface area during 5-day culture was significantly inhibited by SB203580 but not by PD98059 or U0126 (70 ± 7, 90 ± 6 and 92 ± 8% of controls, respectively, n = 9). Similarly, thymidine incorporation was not affected by MEK.
MAP kinase in renal development

inhibitors, but was slightly but significantly decreased by SB203580. Ureteric bud branching was not affected by MEK inhibitors or SB203580. Glomerular number was markedly reduced by SB203580 and to a lesser extent by U0126 (14 ± 2 and 48 ± 10% of controls, respectively, \( n = 3 \)).

On histological examination, the number of tubuloglomerular structures was reduced in MEK inhibitor-treated metanephros compared with controls. Very few mesenchymal condensates were observed in kidneys incubated with SB203580. PCNA-positive cells were reduced in SB203580-treated metanepros compared with control and PD98059-treated kidneys. TUNEL-positive cells were increased in SB203580-treated kidneys and to a lesser extent in PD98059-treated cultures. The WT1 Wilms' tumour suppressor gene expression is low in uninduced mesenchyme, and increases during mesenchymal–epithelial conversion. In control explants, WT1 expression was observed in condensing mesenchyme, vesicles and pre-podocytes of comma- and S-shaped bodies. In PD98059-treated kidneys, the distribution of WT1-positive cells was similar to that in controls. In SB203580-treated cultures, WT1-positive cells were distributed loosely in mesenchyme. Cells around ureteric buds, which show some signs of condensation of mesenchyme, tended to have stronger WT1 expression.

Conclusions

ERK, p38 and MKP-1 are strongly expressed in the developing kidney, and JNK is detected predominantly in the adult kidney. Both the temporal and spatial expression of ERK coincide with the maturation of the kidney. The distribution of p38, on the other hand, was diffuse. Contrary to previous belief, spatial expression of p38 correlated with proliferation rather than apoptosis. JNK was expressed differently from p38, and might participate in the differentiation at a later stage or in the maintenance of the integrity of tubular epithelia. MKP-1 expression was localized in cells which expressed ERK and p38, suggesting that MKP-1 may regulate activities of ERK and/or p38 in the embryonic kidney.

Organ culture studies demonstrated that ERK and p38 are required for renal development. ERK inhibition attenuated nephron formation with minimal effect on kidney growth. On the other hand, both kidney growth and nephron formation were suppressed by p38 inhibition. Together with the immunohistochemical data, ERK may be involved in differentiation, whereas p38 may play a role in the maintenance of the stromal cell population necessary for metanephros growth and nephrogenesis. Importantly, ERK or p38 inhibition suppressed nephron formation despite normal ureteric bud branching. Thus, p38 and ERK probably mediate the signal of molecules responsible for proliferation of mesenchymal cells, as well as mesenchymal-epithelial transformation. These molecules include fibroblast growth factor 2 [6], Wnt-4 [7], bone morphogenetic protein-7 [8], leukaemia-inhibiting factor [9] and others. Further studies examining the role of ERK and p38 in renal development may provide a better understanding of the mechanisms that lead to kidney malformations.

Acknowledgements. This study was supported by grants from the Ministry of Education, Science and Culture, Japan (10670757, 12770610, 12770401), Pharmacai-Upjohn Fund for Growth and Development Research, Keio Gijuku Academic Development Fund and Keio University Grant-in-Aid for Encouragement of Young Medical Scientists.

References

9. Barasch J, Yang J, Ware CB et al. Mesenchymal to epithelial conversion in rat metanephros is induced by LIF. Cell 1999; 99: 377–386