Short Communication

Immunohistochemical Expressions of Main PGE₂ Biosynthesis-related Enzymes and PGE₂ Receptor in Rat Nephrogenesis

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Abstract: Endogenous prostaglandin (PG) E₂ plays important roles in renal homeostasis. Immunoexpressions of PGE₂ biosynthesis-related enzymes, cyclooxygenase (COX)-2 and microsomal PGE₂ synthetase (mPGES)-1 and EP4 (a PGE₂ receptor), were investigated in renal development. Kidney tissues were obtained from fetuses on gestation days 18 and 21 and neonates on days 1 to 18. In fetuses and early neonates, the expressions of COX-2, mPGES-1 and EP4 were observed in developing renal tubules, indicating that COX-2 and its product, PGE₂, play important roles in renal tubular development via EP4. Cyclin D1 expression was seen in both the nucleus and cytoplasm of the developing tubules. These findings differed from the decreased COX-2 expression and exclusive nuclear expression of cyclin D1 seen in abnormal epithelial regeneration of injured renal tubules in cisplatin-treated rats in our previous articles. Collectively, PGE₂ induced by COX-2, regulates renal tubular epithelial formation via EP4. (DOI: 10.1293/tox.24.257; J Toxicol Pathol 2011; 24: 257–261)

Key words: rat, nephrogenesis, PGE₂, COX-2, EP4

Prostaglandin (PG), mainly PGE₂, plays important roles in renal hemodynamics, renin release and salt and water homeostasis¹,². The PGE₂ is synthesized from arachidonic acid; first, arachidonic acid is converted to an unstable intermediate PGH₂ by cyclooxygenase (COX). PGH₂ is converted to PGE₂ by prostaglandin E synthase (PGES)³,⁴. COX consists of two isofoms, COX-1 and COX-2; COX-1 is constitutively expressed and responsible for basal PG production in relation to homeostasis, whereas COX-2 is an inducible form that may be primarily associated with inflammatory reaction⁵. In the kidney, however, the expressions of COX-1 and COX-2 are constitutively seen, and they play important roles in renal homeostasis⁵,⁶. The activity of PGE₂ is mediated through four different receptor subtypes: EP1, EP2, EP3 and EP4. EP1 is associated with calcium mobilization, EP2 and EP4 are associated with stimulation of adeny- late cyclase (AC) and EP3 is associated with inhibition of AC or with stimulation of phosphoinositol turnover⁷.

Previously, we showed that endogenous PGE₂ participated in abnormal regeneration of renal tubular epithelial cells after injury induced in rats by cisplatin (CDDP) exclusively through EP4⁸. We considered that regeneration of renal tubules after injury recapitulates development processes in nephrogenesis⁹. To shed some light on renal tubular regeneration after CDDP injury, in the present study, we investigated expression patterns of main PGE₂ biosynthesis-related enzymes, COX-2 and microsomal PGES (mPGES)-1, as well as EP4 in rat nephrogenesis. PGE₂ may affect the G₁ phase of the cell cycle of renal tubules through EP4⁹. We also examined the expression of cyclin D1, a marker of the G₁ phase in the cell cycle⁹.

The following experiments conformed to our institutional guidelines for animal care. Pregnant female F344/DuCrj rats were obtained from Charles River Laboratories Japan (Hino, Shiga, Japan). They were housed in an animal room controlled to 22 ± 3 °C with a 12:12-h light-dark cycle and were allowed free access to a standard commercial diet (MF, Oriental Yeast Co., Ltd., Tokyo) and tap water. The day of birth was designated postnatal day 0 (P0). Animals were euthanized under deep anesthesia, and kidney tissues were obtained from fetuses on gestation days (GDs) 18 and 21, as well as neonates on days 1, 3, 6, 9, 12 15 and 18 (at each examination point, at least three rat samples were used).

Kidneys were fixed in 10% neutral buffered formalin and periodate-lysine-paraformaldehyde (PLP) fixatives. Formalin-fixed samples were processed routinely and em-

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The Expression of Endogenous PGE₂ in Rat Nephrogenesis

Fig. 1.

Fig. 2A.

Fig. 2B.

Fig. 3.

Fig. 4.

Fig. 5.
bedded in paraffin; PLP solution-fixed specimens were embedded in paraffin by the AMeX method (PLP-AMeX method)\textsuperscript{[10]}. Formalin-fixed, paraffin-embedded samples were cut at a thickness of 3–4 μm, and stained with hematoxylin-

For immunohistochemistry, the specimens were cut at a thickness of 4-μm, deparaffinized with xylene, rehydrated with graded ethanol and washed in water. These sections were boiled with microwave for 5 minutes for antigen retrieval. They were then treated with 3% H$_2$O$_2$, for 10 minutes at room temperature for blocking of endogenous peroxidase. Tissue sections were incubated with polyclonal anti-COX-2 (1:300, Cayman Chemical Company.), polyclonal anti-mPGES-1 (1:200, Cayman Chemical Company.), polyclonal anti-EP4 (1:500, Upstate Biotechnology Inc.) and monoclonal anti-cyclin D1 (1:200, Upstate Biotechnology Inc.) for 12–14 hours at 4 °C. Thereafter, sections were washed three times with phosphate-buffered saline (PBS) and incubated for 45 minutes with the secondary antibody (Histofine Sample Stain MAX PO, Nichirei Corporation, Tokyo, Japan). Positive reactions were visualized with 3, 3′-diaminobenzidine (DAB). Sections were lightly counterstained with hematoxylin.

Fig. 1. Histology of nephrogenesis in a neonate on day 1. Developing renal tubules (arrows) and maturing glomeruli (arrowhead) are present. HE stain. Bar=100 μm.

Fig. 2. Immunohistochemical findings for cyclooxygenase (COX)-2 in fetuses on gestation days (GDs) 18 (A) and 21 (B). COX-2 expressions are observed in developing renal tubules (arrowhead) and S-shaped bodies (arrow, A) on GD 18 and in the macula densa (arrowhead) of the developing tubules (Fig. 2A, 3, 4 and 5) in fetuses and early neonates. Except cyclin D1, which was expressed in the developing tubules (Fig. 2A, 3, 4 and 5) in fetuses and early neonates. Except cyclin D1, which was expressed in both the nucleus and cytoplasm in the developing tubules (Fig. 5), the positive reactions for other antibodies were seen in the cytoplasm. As renal development proceeded, the expression patterns in the renal tubules were close to those of adult animals; COX-2 was restricted in the straight tubules in the cortex and macula densa (Fig. 2B); mPGES-1 was limited in the renal tubules in the medulla, which develop into the thin limb of the loop of Henle; cyclin D1 was seen only in the nuclei of renal tubular epithelial cells; EP4 was restricted in the distal tubules.

In the S-shaped body, immunoreactions for COX-2 (Fig. 2A), EP4 (Fig. 4) and cyclin D1 (Fig. 5) were observed, but that of mPGES-1 was not observed (Fig. 3 inset). In the interstitium, mesenchymal cells reacted to EP4 (Fig. 4) and cyclin D1 (Fig. 5) in varying degrees.

In metanephric development, mutual induction between the blastemal mesenchymal cells and epithelial cells of the ureteric bud occurs initially; after that, some blastemal cell-derived mesenchymal cells form epithelial cells of the renal tubules and glomeruli through mesenchymal-epithelial transition in the cortex, whereas others persist in the interstitium and maintain the features of mesenchymal cells\textsuperscript{[11]}. PGE$_2$, which is synthesized from arachidonic acid converted by COX-1 or COX-2, has important roles in nephrogenesis and homeostasis in the kidneys\textsuperscript{[2,12,13]}. However, COX-1 seems not to be essential for renal development because no alterations were observed in renal structures in COX-1 deficient mice\textsuperscript{[4]}. On the contrary, COX-2 deficient mice exhibit abnormal renal development\textsuperscript{[5,6]}. As demonstrated in this study, COX-2 expression was seen in the developing renal tubules in fetuses and early neonates, indicating participation of COX-2 in development of renal epithelial cells from blastemal cells in fetuses. Moreover, we investigated the expression patterns of mPGES-1, a PGE$_2$ biosynthesis-related enzyme, and EP4 in developing rat kidneys. In agreement with the expression of COX-2, mPGES-1 and EP4 were expressed in developing renal tubules of fetuses and early neonates. These findings indicate that PGE$_2$ can regulate renal tubular epithelial development (in

Fig. 4. Immunohistochemical findings for PGF$_2$ receptor (EP) 4 in a fetus on GD 18. EP4 expressions are observed in the developing renal tubules (arrowheads) and S-shaped bodies (arrow), as well as occasional interstitial mesenchymal cells. Immunohistochemical staining, counterstained with hematoxylin. Bar=100 μm.

Fig. 5. Immunohistochemical findings for cyclin D1 in a neonate on day 1. Cyclin D1 expressions are observed in both the nucleus and cytoplasm of the developing renal tubule (arrowheads) and S-shaped bodies (arrow), as well as mesenchymal cells. Immunohistochemical staining, counterstained with hematoxylin. Bar=100 μm.
developing nephrons) via EP4 and that the PGE₂ is induced mainly by COX-2. In the S-shaped body, the expressions of COX-2, EP4 and cyclin D1 were observed; however, that of mPGES-1 was not observed. Because PGES has multiple forms, namely mPGES-1, mPGES-2 and cPGES, other forms of PGES might participate in glomerular formation from S-shaped bodies. In addition to mesenchymal cells reacting to EP4 and cyclin D1, the significance of S-shaped body development remains to be pursued further.

Previously, we showed that COX-2 expression was decreased in the stage of renal tubule regeneration after CDDP injury. This finding differed from the increased expression of COX-2 seen in developing renal tubules in the present study. Cyclin D1 is a marker of the G₁ phase in the cell cycle; the expression is seen in the nucleus in cells at the G₁ phase and the cytoplasm in cells at the S phase. The epithelial cells in developing renal tubules showed a positive reaction to cyclin D1 in both the nucleus and cytoplasm, which is indicative of normal cell cycle regulation. Because the expressions of COX-2 and EP4 were also observed at the same site as cyclin D1 expression, COX-2 and its product, PGE₂, are considered to be involved in normal proliferation and cellular differentiation of blastemal cell-derived renal tubules through EP4. On the other hand, cyclin D1 expression was restricted within the nucleus of regenerating renal epithelia in CDDP-treated kidneys, suggesting abnormal regeneration of the affected renal tubules.

In conclusion, it was found that endogenous PGE₂ plays important roles in nephrogenesis, particularly blastemal cell-derived renal tubular development in the cortex, via EP4 expression. During development, COX-2 acted as an important inducer. Additionally, cyclin D1 expressions seen in the epithelial nucleus and cytoplasm indicated normal proliferation and cellular differentiation in the developing renal tubules. These findings were different from those of CDDP-induced renal lesions, which showed decreased COX-2 expression and exclusive nuclear expression of cyclin D1. We had expected that developing renal tubules might reflect epithelial regeneration of CDDP-injured renal tubules. The differences between developing renal tubules and CDDP-injured renal epithelia might give us some clues to pursue the pathogenesis of renal fibrosis after tissue injury.

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