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Localization of cyclooxygenases-1 and -2, and prostaglandin F synthase in human kidney and renal cell carcinoma ☆

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Abstract

Prostaglandin (PG)$F_{2\alpha}$ is one of the major prostanoids produced by the kidney, and its renal synthesis is regulated by sodium depletion, potassium depletion, and adrenal steroids. PGF synthase activity is detected in kidney of various mammals. Herein, we demonstrated immunochemically that PGF synthase was localized in proximal tubule of human kidney, together with cyclooxygenase (COX)-1, and that it was localized in human renal cell carcinoma, together with COX-2. These results suggest that PGF synthesized through COX-1 and PGF synthase plays an important physiological role in the kidney and that the expression of COX-2 in kidney is a useful maker for tumorigenesis of the renal call carcinoma in vivo.

Keywords: Cyclooxygenase; COX-1; COX-2; Prostaglandin; PGF; PGF synthase; Immunochemistry; Kidney

Prostglandins (PGs) play various physiological and pathophysiological roles. Among them, PGE_2, PGD_2, and PGF_2 are the predominant cyclooxygenase (COX) metabolites of arachidonic acid in the kidney. These 3 prostanoids play an important role in the tubular re-absorption of salt and water as well as in the control of renal vascular resistance and the maintenance of glomerular hemodynamics [1–5]. PGE_2 is known to be converted enzymatically to PGF_2 by PGE 9-ketoreductase, and this conversion is enhanced in pathophysiological settings including sodium depletion [2,5]. Moreover, PGD_2 is also converted to PGF_2 by PGF synthase, which also synthesizes PGF_2 from PGH_2 [6,7]. Although PGF synthase activity has been detected in the kidneys of various species [8], its role there is not yet known. First of all in the present study we determined the localization of PGF synthase in the kidney.

COX exists in 2 isoforms: COX-1 and COX-2 [9]. COX-1 is constitutively expressed and thought to function in normal cell physiology; whereas COX-2 is inducible, expressed in response to inflammatory stimuli, and is a known mediator of inflammation and regulator of cell growth [9]. Some canine renal cell carcinomas reportedly express high levels of COX-2 [10,11]. However, any difference between the normal kidney and renal cell carcinoma in the localization of COX-1 and COX-2, together with PGF synthase, has not yet been clarified. In this paper, we examined the localization of COX-1 and COX-2, as well as that of PGF synthase, in normal kidney and renal cell carcinoma.

Materials and methods

Materials. Horseradish peroxidase (HRP)-labeled anti-rabbit IgG was obtained from Jackson Immunoresearch (USA), and TRITC-labeled anti-rabbit IgG and FITC-labeled anti-goat IgG were from Daco (Denmark). Other materials and commercial sources were as follows: molecular mass markers for polyacrylamide gel electrophoresis (PAGE), from Bio-Rad (CA, USA); Protran nitrocellulose membrane (BA85) for Western blot,

☆ *Abbreviations:* PG, prostaglandin; COX, cyclooxygenase; HRP, horseradish peroxidase; PAGE, polyacrylamide gel electrophoresis; KPB, potassium phosphate buffer; hPGF synthase, human PGF synthase; DAB, 3,3′-diaminobenzidine tetrahydrochloride; PBS, phosphate-buffered saline; BSA, bovine serum albumin.

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from Schleicher & Schuell (Germany); DAB (3,3′-diaminobenzidine tetrahydrochloride), from Dojin (Japan). Other chemicals were at least of reagent grade.

Western blot analysis of human PGF synthase. Western blot analysis was done by the method described previously [12]. Human kidney tissues were collected from patients with renal cell carcinoma after their informed consent had been given. Human kidney and renal cell carcinoma specimens were homogenized in 3 vol. of 10 mM potassium phosphate buffer (KPB, pH 7.0) and then centrifuged at 10,000*g*. The supernatant fractions, as well as purified hPGF synthase (human PGF synthase), were subjected to SDS–PAGE and electrophoretically transferred to a Protran nitrocellulose membrane (BA85). Protein bands were immunostained with the anti-hPGF synthase antiserum (1:2000) in BlockAce followed by HRP-labeled anti-rabbit IgG (1:1000) in BlockAce and visualized with an Enhanced ChemiLuminescence Kit (Lumi-Light^{PLUS}, Roche Diagnostics, USA).

Immunocytochemistry. Human kidney and renal cell carcinoma specimens were fixed by immersion in 4% paraformaldehyde for 1 day. After the tissues had been treated with ethanol/xylene series, they were dehydrated through an ethanol series and embedded in Paraplast. Sections were cut at 4 μm on a Microtome RP-50 (Yamato Kohki, Japan) and mounted on gelatin-coated slides. Immunohistochemistry was performed by the DAB method and the indirect immunofluorescence method. After deparaffinized sections had been treated with 0.3% hydrogen peroxide for 30 min to block endogenous peroxidase activity, they were incubated sequentially at room temperature with the following reagents: anti-hPGF synthase antiserum, anti-COX-1, or anti-COX-2 diluted to 1:200 in phosphate-buffered saline (PBS) containing 1% bovine serum albumin (BSA) for 6 h, followed by overnight incubation at room temperature with HRP-labeled anti-rabbit antibody diluted to 1:500 in PBS containing 1% BSA. The immunoreaction was visualized with 15% DAB and 0.1% hydrogen peroxide, and nuclei were stained with 0.02% hematoxylin. The stained sections were then observed, with a light microscope, an OLYMPUS DP70 (OLYMPUS, Japan). For the immunofluorescence method, after incubation with the anti-hPGF synthase antiserum, sections were reacted with TRITC-labeled anti-rabbit IgG. The sections were finally washed with PBS and then mounted in Entellan (Merck, Canada). The specificity of the immunostaining was examined by conducting a preabsorption test. The diluted antiserum was mixed with the antigen, purified hPGF synthase, at a final concentration of 10 μg/ml, and preabsorbed for 15 h at 4 °C before use in the specificity test.

Results

Specificity of antibody

The cross-reactivity and specificity of the anti-human PGF synthase antibody were examined by Western blot analysis. Fig. 1 shows that the antibody reacted specifically with the human enzyme, visualizing an ≈37-kDa major band in human kidney and renal cell carcinoma crude extracts corresponding to that for the purified enzyme. This band was not detected with the antigen-absorbed antibody or with serum from a non-immunized rabbit (data not shown).

Immunocytochemical localization of PGF synthase, and COX-1 and -2 in human normal kidney specimens

Fig. 2A shows a human kidney section stained with hematoxylin and eosin. The immunocytochemical localization of PGF synthase in the human kidney is shown in Figs. 2B and D by the DAB method and immunofluorescence method, respectively. Immunoreactivity for the enzyme was found in proximal tubule, but not in renal corpuscles or the brush border. This immunoreactivity in the proximal tubule was not found when the antigen-absorbed antibody (Figs. 2C and E) or serum from a non-immunized rabbit was used (data not shown).

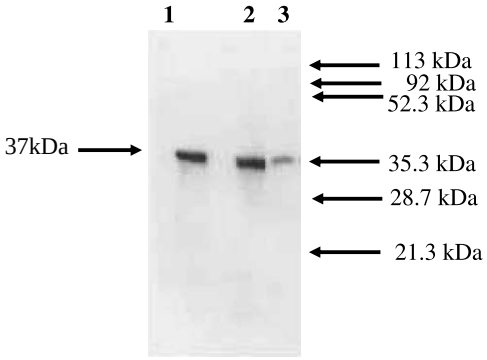

Fig. 1. Western blot analysis of human kidney using anti-hPGF synthase antibody. Lane 1, recombinant hPGF synthase purified from *Escherichia coli*; lane 2, human kidney cytosol fraction (10,000*g* supernatant); lane 3, human renal cell carcinoma (10,000*g* supernatant).

COX synthesizes the substrate for PGF synthase. The immunocytochemical localization of COX-1 and -2 was examined in human kidney specimens. COX-1 was also localized in proximal tubule (Fig. 3A), but this immunoreactivity was not found with non-immune serum (Fig. 3C). On the other hand, COX-2 was not localized in any region (Fig. 3B).

Immunocytochemical localization of PGF synthase and COX-1 and -2 in human renal cell carcinoma

Expression of both isoforms of COX (COX-1 and COX-2), and of PGF synthase was evaluated in kidneys bearing renal cell carcinoma. Fig. 4A shows a human renal cell carcinoma stained with hematoxylin/eosin. The immunoreactivity of PGF synthase was detected in it (Fig. 4B), but this immunoreactivity was not found when the antigen-absorbed antibody was used in the place of antibody (Fig. 4C). The renal cell carcinoma also showed the immunoreactivity for COX-2 (Fig. 4E), but none for COX-1 (Fig. 4D) and none when non-immune serum was used in the place of antibody (Fig. 4F). As described above, the normal kidney showed the reverse results for COX, and the expression of COX-2 in the renal cell carcinoma was a remarkable result.

Discussion

$PGF_{2\alpha}$ is a major product of cyclooxygenase-mediated arachidonate metabolism in the kidney [1–5], and renal synthesis of PGF_2 is regulated by sodium depletion, potassium depletion, and adrenal steroids [1–5]. Infusion of exogenous PGF_2 modulates renal salt excretion and urine flow [1–5]. Rathaus et al. [13] reported that $PGF_{2\alpha}$

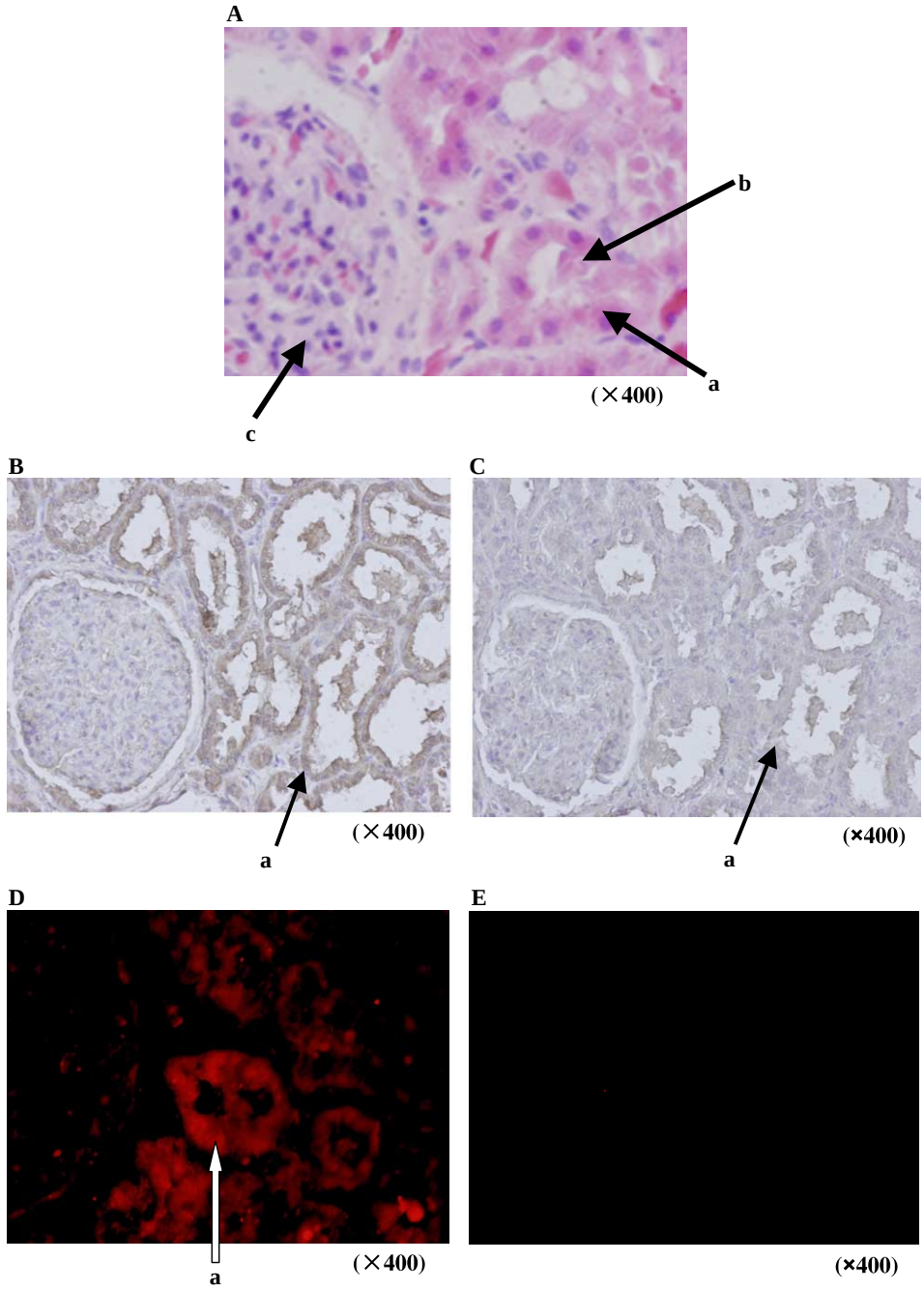

Fig. 2. Hematoxylin and eosin staining (A) and immunostaining of hPGF synthase (B–E) in the human kidney. Arrows labeled "a" in (A–D) mark proximal tubules. The arrow labeled "b" in (A) points to a brush border; and that labeled "c" in (A) indicates a renal corpuscle (glomerulus, Bowman's capsule). In (B) immunostaining was done with anti-hPGF synthase antibody; and in (C), with antigen-absorbed antibody. Positive reactions were visualized by the DAB method. In (D,E) the immunofluorescence method was used with anti-hPGF synthase antibody and antigen-absorbed antibody, respectively.

participates in the renal adaptation to KCl-loading but not when K is accompanied by non-Cl anions. Moreover, 9α,11β-PGF_2 also elevated urine flow, urinary sodium/potassium, the hematocrit value, and urinary sodium excretion [14]. PGF synthase synthesizes $PGF_{2\alpha}$ from PGH_2, and 9α,11β-PGF_2 from PGD_2 [8]. PGF synthase activity was detected in rat or bovine kidney as well as lung and liver [5]. Moreover, PGF_2 and PGE_2 play an important role in the tubular reabsorption of salt and water as well as in the control of renal vascular resistance and in the maintenance of glomerular hemodynamics [3]. In this study, we demonstrated that PGF synthase was localized in proximal tubule of the normal human kidney and that COX-1 was also co-localized with PGF synthase. These results suggest that PGF synthase contributes the synthesis of PGF_2 through COX-1 in proximal tubule.

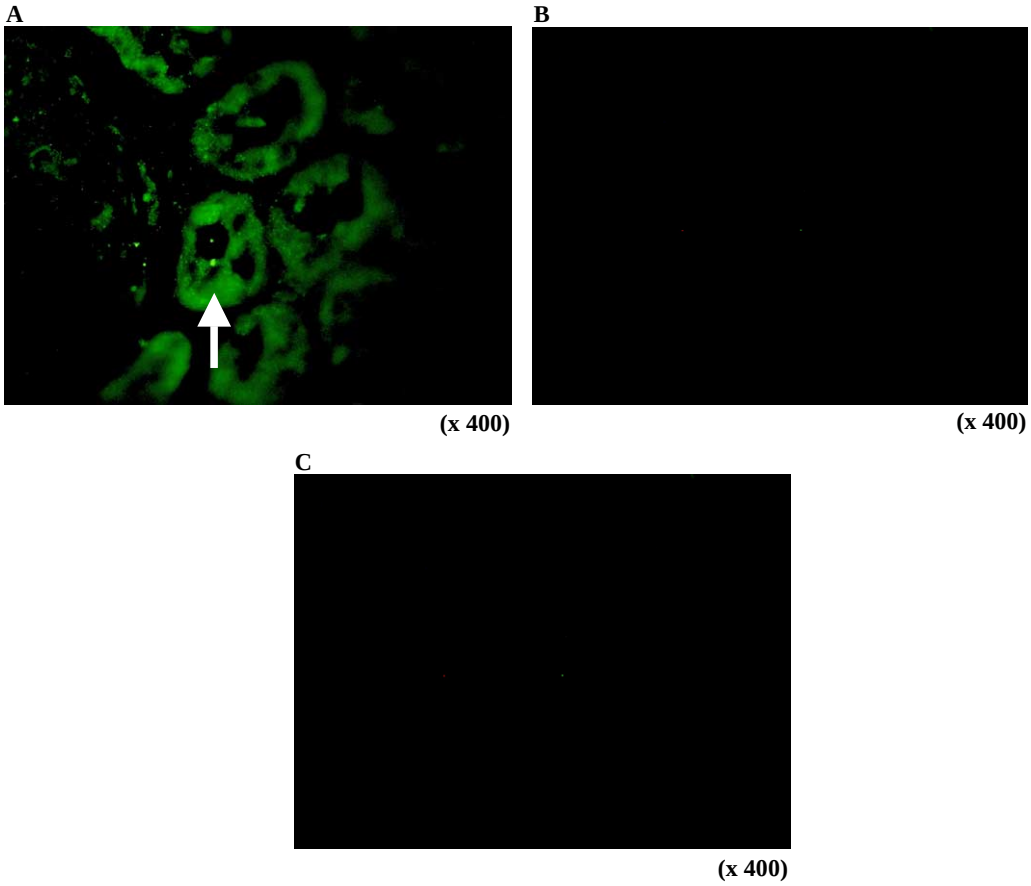

Fig. 3. Immunostaining of COX-1 and -2 in human kidney specimens. Arrow in (A) indicates a proximal tubule positively immunostained with anti-COX-1 antibody. In (B,C) immunostaining was done with anti-COX-2 antibody and non-immune serum, respectively.

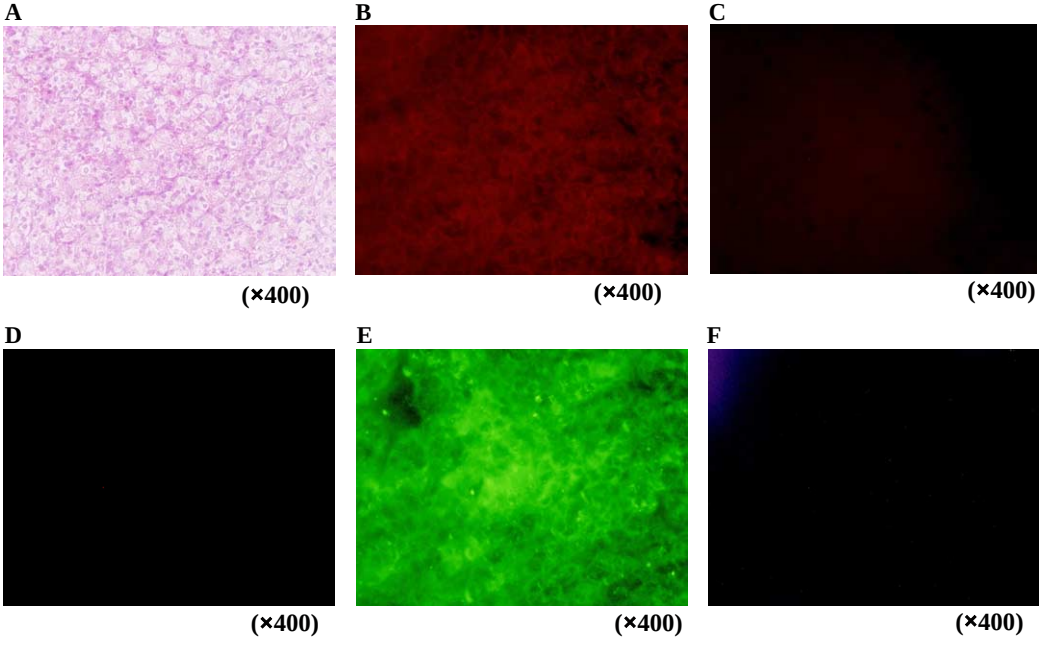

Fig. 4. Hematoxylin and eosin staining (A) and immunostaining of hPGF synthase (B,C) and of COX-1 (D) and -2 (E) in a specimen of renal cell carcinoma. In (B, C, and F), immunostaining was done with anti-hPGF synthase antibody, antigen-absorbed antibody, and non-immune serum, respectively.

COX-2 plays an important role in the modulation of neoplastic cell growth [10], and in the development of various cancers due to its angiogenic function [15]. It is reported that the expression of COX-2 is to be up-regulated in human renal cell carcinoma, bladder tumor, and prostate cancer [15]. However, the comparison between COX-1 and COX-2 in renal cell carcinomas and the relationship of PGF synthase with these COXs are not yet well known. We demonstrated herein that COX-2, not COX-1, was expressed in renal cell carcinoma, together with PGF synthase. These results suggest that the expression of COX-2 in the kidney is a useful maker for tumorigenesis of the renal cell carcinoma in vivo and that COX-2-mediated production of PGF_2 may also play a pathophysiological role in angiogenesis, modulation of neoplastic cell growth, and so forth.

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