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Localization of PDGF α-receptor in the developing and mature human kidney

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Localization of PDGF α-receptor in the developing and mature human kidney. Using *in situ* hybridization and immunocytochemistry we describe the renal localization of the PDGF α-receptor. PDGF α-receptor mRNA was uniformly present in human metanephric kidney in interstitial cells and vascular arcades that course through the blastema. PDGF α-receptor mRNA was present in some mesangial structures in early glomeruli, but was largely lost as glomeruli matured. It was present in adventitial fibroblasts, but usually not in vascular smooth muscle cells or endothelial cells of the fetal vasculature. This pattern persisted in adult kidneys, with extensive expression of mRNA by interstitial cells and only occasional expression by mesangial cells. All *in situ* hybridization findings were corroborated by immunocytochemistry. Double immunolabeling confirmed the rare expression of the PDGF α-receptor protein by vascular smooth muscle cells and the absence of its expression by endothelial cells. Given that both PDGF A- and B-chain can promote smooth muscle cell and fibroblast migration and proliferation and that both signal through the PDGF α-receptor, these data suggest that PDGF α-receptor may play important roles in the early vasculogenesis of the fetal kidney as well as in the pathogenesis of renal interstitial fibrosis.

There is now a considerable body of evidence that PDGF is an important mediator of many aspects of renal pathophysiology. Studies of cultured cells, animal models of disease, mammalian development, and human biopsy material have shown that PDGF mediates important mesangial responses to injury including: cell proliferation and migration [1–6]; it is essential for normal development of the mesangium [7, 8]; it may regulate proliferation of interstitial fibroblasts and modify the phenotype and activity of these cells during interstitial injury [9–11]; and PDGF is implicated in the development of arterial neointimal development in chronic vascular rejection, analogous to its apparent role in atherosclerotic and restenotic arterial injury [12–14].

The bulk of the studies in the kidney have focused on activity and localization of PDGF B-chain and the PDGF β-receptor [10, 15–19]. Biologically active PDGF exists as a dimer composed of any of the three possible combinations of two isoforms of this molecule: PDGF A and B chain [reviewed in 20, 21]. The receptor for PDGF also functions as a dimer, and can be composed of any of three possible combinations of two isoforms known as PDGF α- and β-receptor. While PDGF β-receptor subunit binds only PDGF B-chain with high affinity, the PDGF α-receptor subunit may bind either chain of PDGF [20, 21]. Data about the sites of expression of both the PDGF α- and β-receptor are necessary to achieve a comprehensive understanding of how PDGF-AA, PDGF-AB, or PDGF-BB may function in renal development and renal pathophysiology.

The localization of PDGF A and B chains and PDGF β-receptor have been previously characterized in developing and mature human kidneys as well as in renal diseases [10, 16–19, 22, 23]. In contrast, little information is currently available on the localization of PDGF α-receptor in adult kidney and none on its expression in metanephric renal development. The only studies to investigate PDGF α-receptor in normal human mature kidney reported that expression of PDGF α-receptor could either not be detected [24] or strictly mirrored that of PDGF β-receptor but was rather low compared to the β-receptor [23]. In the current study, we have re-assessed these controversial data and describe the localization of PDGF α-receptor mRNA and protein in the developing and mature human kidney. Our data provide a basis for further mechanistic understanding of the role of the PDGF system in human development and disease.

Methods

Tissue selection

Human fetal kidneys ($N = 26$) were obtained fresh from tissue examined after therapeutic abortions. Twelve of these (estimated gestational age 54 to 81 days) were fixed in 10% neutral buffered formalin and processed and embedded in paraffin according to conventional techniques. Specimens of twenty of the fetal kidneys (estimated gestational age 75 to 105 days) were fixed in methyl Carnoy's solution [16] and paraffin embedded. In addition to these fetal kidneys, we also investigated normal appearing human mature kidneys ($N = 22$), obtained fresh from uninvolved portions of kidneys surgically resected for localized renal cell carcinoma. Portions of these tissues were fixed in methyl Carnoy's solution, buffered formalin and other portions were fixed in paraformaldehyde or acetone and then snap frozen as described [16]. Four micrometer sections of all specimens were stained with the PAS reagent for light microscopy and evaluation of the overall morphology.

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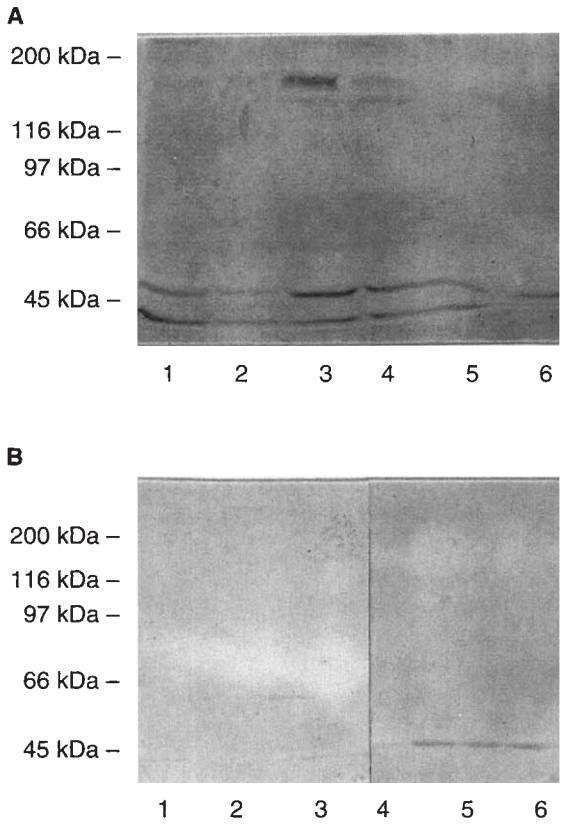


Fig. 1. *Western blotting of cell lysates for expression of the PDGF α-receptor using (A) the PDGF (R)-A 951 antibody or (B) an equivalent concentration of irrelevant rabbit IgG.* Cell lysates are: Lane 1, growth arrested human fetal arterial smooth muscle cells; lane 2, growth arrested smooth muscle cells from adult human aorta; lane 3, growth arrested human lung fibroblasts; lane 4, growth arrested human lung fibroblasts stimulated for six hours with PDGF-AA; lane 5, growth arrested human microvascular neonatal endothelial cells; lane 6, proliferating microvascular neonatal endothelial cells.

Table 1. Expression of PDGF α-receptor in fetal human kidney

Site	Immunohistochemistry	*In situ* hybridization
Metanephric blastema	neg.	neg.
Glomerular vesicles	neg.	neg.
Early glomerular differentiation (comma, S-stage)	mesangial stalk +, otherwise neg.	+ in hilar and mesangial regions
Differentiated glomeruli	neg. to rare +	occasionally +
Ureteric buds, tubules	neg.	neg.
Interstitial cells	+ (incl. adventitial cells and prominently around glomerular capsules and tubules)	+
Vasculature	+ in vascular arcades, + occasionally in vascular smooth muscle cells, + occasional in internal elastic membrane; neg. in endothelial cells	+ in vascular arcades, occasionally + in vascular smooth muscle cells, neg. in endothelial cells

Antibodies

PDGF α-receptor. PDGF (R)-A 951 (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) is an affinity-purified rabbit polyclonal antibody raised against a GST fusion protein construct containing PDGF α-receptor sequences corresponding to amino acid residues 951-1089 of the carboxy terminus of the human PDGF α-receptor. This antibody recognized PDGF α-receptor in acetone-fixed, frozen and methyl Carnoy's fixed, paraffin-embedded sections.

The second antibody utilized was a murine monoclonal IgG_1 antibody to human PDGF α-receptor (Genzyme Diagnostics, Cambridge, MA, USA). The antibody specifically binds to PDGF α-receptor subunit and is non-reactive with PDGF β-receptor [25]. This antibody was non-reactive with paraffin-embedded material but recognized PDGF α-receptor in acetone-fixed, frozen sections.

Further antibodies against the PDGF α-receptor utilized in this study included rabbit polyclonal antibodies 1890 and 2025 against the extracellular domain of the mouse PDGF α-receptor, expressed as a fusion protein with glutathione-S-transferase. Both antibodies recognized PDGF α-receptor in methyl Carnoy's fixed, paraffin-embedded sections as well as acetone-fixed frozen sections.

Smooth muscle cell marker. Murine monoclonal antibody 1A4 (Dako Corp., Carpinteria, CA, USA) has been characterized by tissue immunohistochemistry and Western blotting [26], and has been previously demonstrated to recognize smooth muscle α-actin in methyl Carnoy's fixed tissues [16, 27].

Endothelial cell marker. Ulex europaeus agglutinin I (Vector Labs, Burlingame, CA, USA) is a lectin that labels human endothelial cell surfaces, type O red blood cells and cells of collecting ducts and has been utilized previously to delineate renal vascular endothelium [16, 28, 29].

Immunohistochemistry

Immunohistochemistry was performed on methyl Carnoy's fixed, paraffin embedded tissues following a standard avidin-biotin complex (ABC) method. Briefly, sections were deparaffinized in xylene and rehydrated in graded ethanols. Endogenous peroxidase was blocked by incubation in 3% hydrogen peroxide. The sections were then incubated for one hour with the primary antibody diluted in PBS plus 1% bovine serum albumin. Following washes in PBS, the sections were sequentially incubated with biotinylated goat-anti-rabbit or horse anti-mouse antibody (Vector Labs), the ABC-Elite reagent (Vector Labs) and finally 3,3′-diaminobenzidine (DAB; with nickel chloride enhancement) was used as the chromogen. Sections were counterstained with methyl green, dehydrated and coverslipped.

Frozen sections of acetone or paraformaldehyde fixed tissue were hydrated in PBS, blocked with 0.3% hydrogen peroxide and

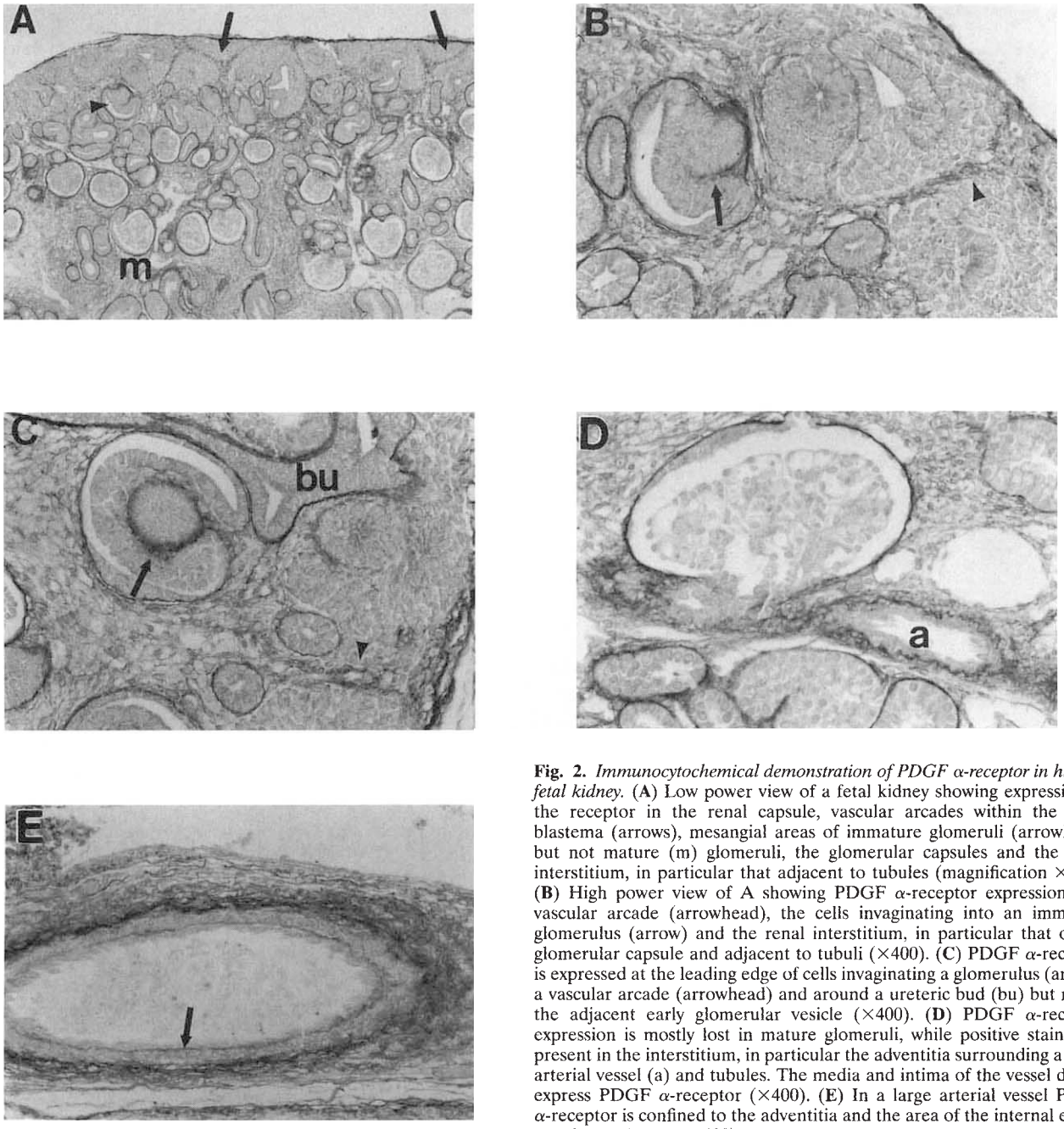


Fig. 2. *Immunocytochemical demonstration of PDGF α-receptor in human fetal kidney.* (**A**) Low power view of a fetal kidney showing expression of the receptor in the renal capsule, vascular arcades within the renal blastema (arrows), mesangial areas of immature glomeruli (arrowhead) but not mature (m) glomeruli, the glomerular capsules and the renal interstitium, in particular that adjacent to tubules (magnification ×100). (**B**) High power view of A showing PDGF α-receptor expression in a vascular arcade (arrowhead), the cells invaginating into an immature glomerulus (arrow) and the renal interstitium, in particular that of the glomerular capsule and adjacent to tubuli (×400). (**C**) PDGF α-receptor is expressed at the leading edge of cells invaginating a glomerulus (arrow), a vascular arcade (arrowhead) and around a ureteric bud (bu) but not in the adjacent early glomerular vesicle (×400). (**D**) PDGF α-receptor expression is mostly lost in mature glomeruli, while positive staining is present in the interstitium, in particular the adventitia surrounding a small arterial vessel (a) and tubules. The media and intima of the vessel do not express PDGF α-receptor (×400). (**E**) In a large arterial vessel PDGF α-receptor is confined to the adventitia and the area of the internal elastic membrane (arrow; ×400).

washed in PBS. The sections were then incubated with the primary antibody, and subsequently processed as above using a streptavidin-biotin immunoperoxidase method, counterstained with methyl green, dehydrated and cover-slipped.

For all samples, negative controls for the immunohistochemical procedures consisted of substitution of the primary antibody with irrelevant murine monoclonal antibodies or non-immune rabbit antibody or PBS.

Double labeling immunohistochemistry

Methyl Carnoy's fixed, paraffin embedded tissues were prepared for immunocytochemistry as described above. The slides were then sequentially incubated with the PDGF (R)-A 951 antibody against PDGF α-receptor, biotinylated goat anti-rabbit IgG (Vector), the ABC-Elite reagent (Vector Labs) and finally DAB with nickel enhancement to give a black reaction product. Peroxidase activity was then blocked again with 3% hydrogen peroxide. Following this, the sections were incubated sequentially with murine monoclonal antibody against α-smooth muscle actin, alkaline phosphatase conjugated rabbit anti-mouse IgG antibody and the alkaline phosphatase substrate kit I (Vector) plus 1 mM levamisole (Sigma) which yields a red reaction product. After washing and counterstaining with methyl green, the sections were dehydrated, cover-slipped and viewed.

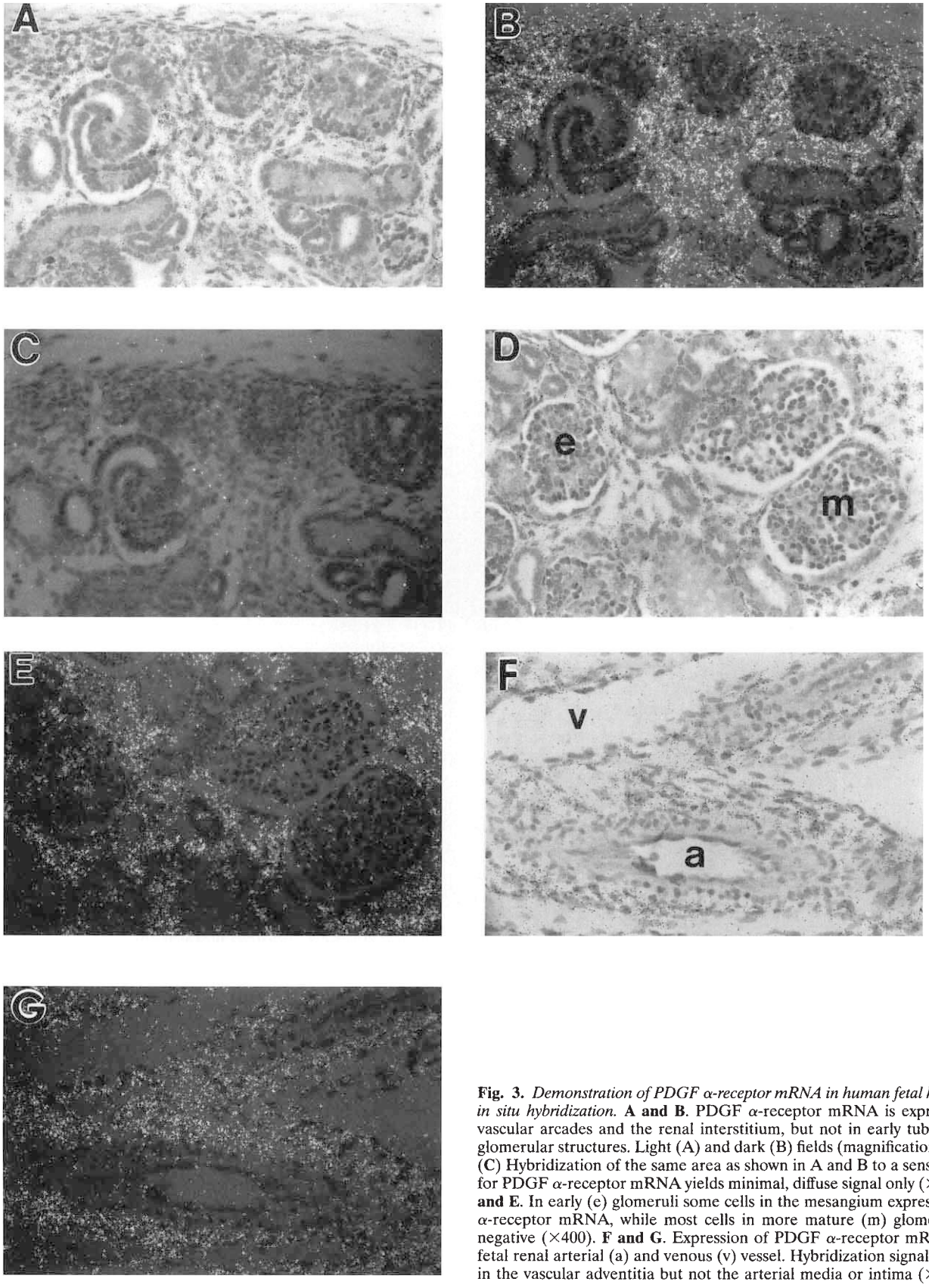


Fig. 3. *Demonstration of PDGF α-receptor mRNA in human fetal kidney by in situ hybridization.* **A and B.** PDGF α-receptor mRNA is expressed in vascular arcades and the renal interstitium, but not in early tubular and glomerular structures. Light (A) and dark (B) fields (magnification ×100). (**C**) Hybridization of the same area as shown in A and B to a sense cRNA for PDGF α-receptor mRNA yields minimal, diffuse signal only (×100). **D and E.** In early (e) glomeruli some cells in the mesangium express PDGF α-receptor mRNA, while most cells in more mature (m) glomeruli are negative (×400). **F and G.** Expression of PDGF α-receptor mRNA in a fetal renal arterial (a) and venous (v) vessel. Hybridization signal is noted in the vascular adventitia but not the arterial media or intima (× 400).

Table 2. Expression of PDGF α-receptor in mature human kidney

Site	Immunohistochemistry	*In situ* hybridization
Glomeruli	occasionally + in mesangial areas neg. in epithelial cells	occasionally + in mesangial areas, neg. in epithelial cells
Tubules	neg. in all segments	neg. in all segments
Interstitium	+ in cortical and medullary interstitial cells (occasional prominent staining around the glomerular capsule and tubules)	occasionally + in cortical and medullary interstitial cells
Vessels	+ in adventitia, occasionally + in media neg. in intima	+ in adventitia, neg. in media and intima

In the case of double labeling for PDGF α-receptor and endothelial cells, incubation with the PDGF (R)-A 951 antibody was followed by peroxidase-conjugated goat anti-rabbit antibody (Zymed, San Francisco, CA, USA) and DAB without nickel enhancement yielding a brown reaction product. Following a repeat peroxidase block, sections were sequentially incubated with Ulex europaeus agglutinin I, biotinylated goat anti-Ulex europaeus agglutinin I antibody (Vector), ABC Elite reagent (Vector) and the Vector VIP substrate kit, which yields a purple reaction product. The final steps were identical to those described above.

Controls for the double labels were done by substitution of the first or second primary antibody with PBS in which cases no double label was observed.

Western blotting

To confirm the specificity of the antibody PDGF (R)-A 951 employed for immunohistochemistry, Western blotting was performed using lysates from various cell lines (see below). All cell lines were grown under similar conditions. In each case, 5 million cells were centrifuged and the resulting cell pellet was lysed in 1 ml of SDS containing sample buffer. Thirty microliters of lysate were used for each of the Western blots. Lysates were electrophoresed on a 6% SDS gel and then blotted onto polyvinylidene difluoride membranes. The blots were blocked with 5% BSA in PBS for one hour at 37°C and then incubated with PDGF (R)-A 951 antibody or an equal concentration of normal rabbit IgG diluted in PBS containing 0.1% BSA for two hours at room temperature. After washing, the blots were incubated with alkaline phosphatase conjugated goat anti-rabbit IgG antibody (Dako) for one hour. The blots were then visualized with 5-bromo-4-chloro-3-indolylphosphate-nitro blue tetrazolium.

The following cell lines were used:

(1) Human lung fibroblasts (a kind gift from S. Narayanan, Dept. Pathology, University of Washington, Seattle, WA, USA) were grown in RPMI medium (Gibco, Gaithersburg, MD, USA), supplemented with 10% fetal calf serum, 100 U/ml penicillin-streptomycin, 2 mM glutamine, 10 mM HEPES and 5 μg/ml insulin (all from Sigma). After reaching confluency, the cells were growth arrested in medium with 0.5% fetal calf serum and incubated 24 hours. Finally, the cells were stimulated with either 100 ng/ml PDGF-AA (a kind gift of C. Hart, ZymoGenetics, Seattle, WA, USA; dissolved at 1 mg/ml in 0.1 N acetic acid) or vehicle for further six hours. The cells were then scraped off the tissue culture flasks, washed once with PBS and the content of each 75 ml tissue culture flask was lysed in 1 ml sample buffer (0.05 M Tris, pH 6.8, 2% SDS, 10% glycerol, 0.025% bromphenol blue and 5% β-mercaptoethanol). Cell lysates were sheared ten times through a 25G needle, placed in a 95°C heating block for five minutes, centrifuged for 20 minutes at 12,000 rpm and 30 μl were then electrophoresed on the SDS gel.

(2) Human fetal arterial smooth muscle cells and smooth muscle cells obtained from an adult human aorta were kind gifts of C. Giachelli (Dept. of Pathology, University of Washington). The cells were grown to confluency, growth arrested for 24 hours and then lysed as described for the fibroblasts.

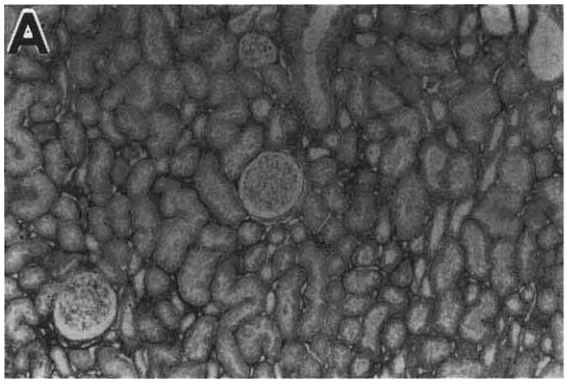
(3) Human microvascular neonatal endothelial cells were purchased from Clonetics (San Diego, CA, USA). The cells were grown to confluency in MCDB 131 medium, supplemented with 10% fetal calf serum, 100 U/ml penicillin-streptomycin, 250 μg/ml amphotericin B, 2 mM glutamine (all from Gibco), 20 μg/ml heparin (Sigma) and 50 μg/ml endothelial cell growth supplement (Biomedical Technologies Inc., Stoughton, MA, USA) using gelatine-coated tissue culture flasks. Twenty-four hours prior to lysis cells were either switched to serum-free medium or continued to be maintained in medium with 10% fetal calf serum. Lysis was carried out as described above, except that the content of two 75 ml flasks was lysed in 0.5 ml buffer. Again, 30 μl of these lysates were electrophoresed on the SDS gel.

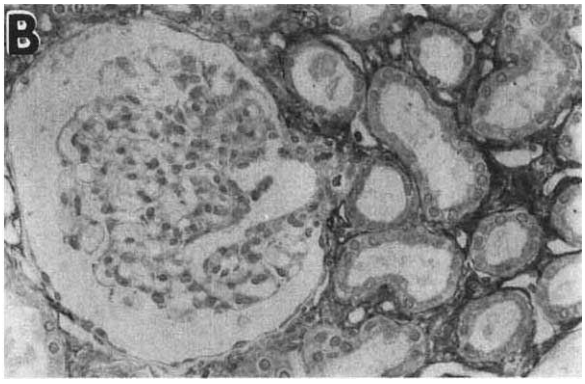
Molecular probe

A 1718-bp fragment (nucleotides 76 to 1794 of the human PDGF α-receptor sequence) was cloned in both directions into pGEM3zf (+). This construct was linearized and transcribed into an antisense or sense riboprobe using reagents from Promega (Madison, WI, USA), except [^{35}S]-UTP, which was obtained from New England Nuclear (Boston, MA, USA). The transcription reaction mixture contained 1 μg of PDGF α-receptor cDNA (sense or antisense), 250 μCi of [^{35}S]-UTP, 500 μmol/liter each of ATP, CTP and GTP, 40 U of RNAsin, 10 mmol/liter dithiothreitol, 40 mmol/liter Tris and 10 U of either SP6 or T7 polymerase. After 75 minutes at 37°C, the template DNA was digested by adding 1 U of DNAse (Promega) and incubating at 37°C for an additional 15 minutes. Free nucleotides were separated with a Sephadex G-50 column. The collected fraction containing labeled probe was then ethanol precipitated and subjected to limited alkaline hydrolysis [30] to obtain fragments of approximately 200 bp. After hydrolysis, the probe was again precipated, resuspended in nuclease free water containing 10 mmol dithiothriotol and stored at −20°C. Probes were used within 48 hours.

In situ hybridization

Fetal and adult kidney tissue which had been fixed in 10% formalin and embedded in paraffin was deparaffinized following standard protocol. The sections were washed with 0.5 × standard saline citrate (SSC) (1 × SSC = 150 mM NaCl, 15 mM Na citrate, pH 7.0) and digested with proteinase K (1 μg/ml; Sigma Chemical Co., St. Louis, MO, USA) in RNase A (Promega) buffer for 40 minutes at 37°C. Several 0.5 × SSC washes were followed by prehybridization for two hours in 50 μl of prehybridization buffer (0.3 M NaCl, 20 mM Tris pH 8.0, 5 mM EDTA, 1 × Denhardt's solution, 10% dextran sulfate, 10 mM DTT). The hybridizations were started by adding 500,000 cpm of ^{35}S-labeled riboprobe in 50 μl of prehybridization buffer and allowed to proceed overnight at





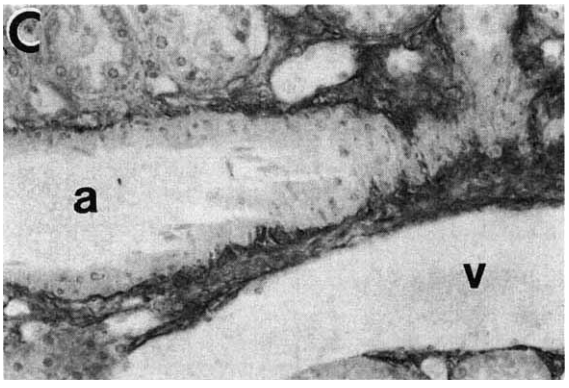
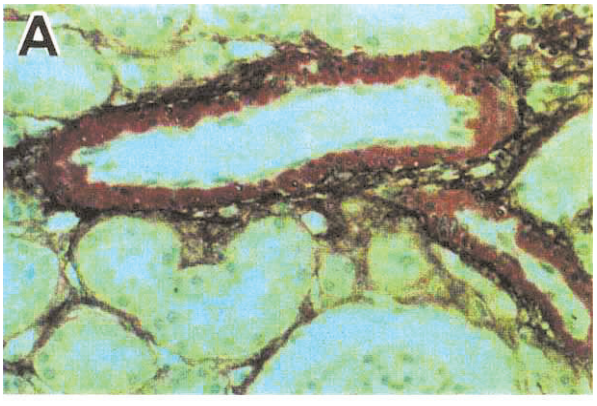


Fig. 4. *Immunocytochemical demonstration of PDGF α-receptor in human mature kidney.* **A.** Low power view of a mature kidney showing expression of the receptor in renal interstitial tissue only (magnification ×100). **B.** No glomerular expression of PDGF α-receptor is present in tubules or in the glomerulus illustrated, while prominent interstitial staining is noted (×400). **C.** No expression of PDGF α-receptor is present in the endothelium or media of an artery (a) or in endothelial cells of a small vein (v). Prominent expression of the receptor is noted in the arteriolar adventitia and the interstitium (×400).



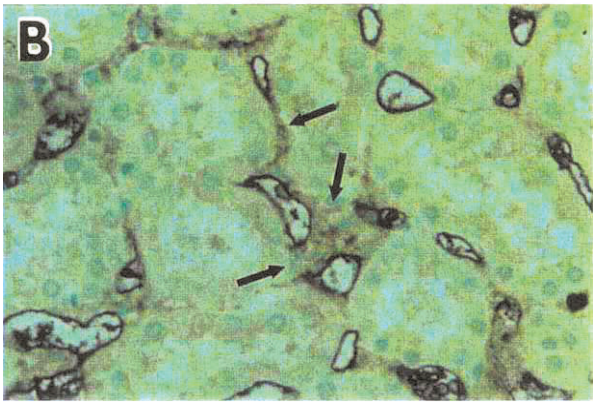


Fig. 5. A. *Double immunolabeling for PDGF α-receptor expression (brown stain) and α-smooth muscle actin expression (red stain) in human mature kidney.* In the arterial media α-smooth muscle actin but not PDGF α-receptor is expressed. In the interstitium most PDGF α-receptor positive cells do not express α-smooth muscle actin, but some double labeling can be noted in myofibroblasts (top right corner) (magnification ×400). **B.** Double immunolabeling for PDGF α-receptor expression (brown stain) and binding of Ulex europeaus (dark purple stain) in human mature kidney. Interstitial cells expressing PDGF α-receptor (arrows) are negative for Ulex europeaus binding (×400).

50°C. After hybridization, sections were washed with 0.5 × SSC, treated with RNase A (20 μg/ml, 30 min room temperature), washed in 2 × SSC (2 × 2 min), followed by three high-stringency washes in 0.1 × SSC/Tween 20 (Sigma) at 37°C, and several 2 × SSC washes. After the tissue was dehydrated and air dried, it was dipped in NTB2 nuclear emulsion (Kodak, Rochester, NY, USA) and exposed in the dark at 4°C for two weeks. After developing, the sections were counterstained with hematoxylin and eosin, dehydrated, mounted and viewed. Positive cellular labeling was defined as five or more silver grains concentrated over a single cell.

Results

Antibody characterization

Usage of the four different antibodies against PDGF α-receptor generally yielded similar staining patterns (see below) under the various tissue storage and fixation conditions described in the **Methods** section. In single specimens or under particular staining conditions, we occasionally observed irregular staining of tubular cells in mature and fetal kidneys or visceral glomerular epithelial cells in some fetal kidneys. Given the inconsistency of these findings and the lack of corresponding observations during *in situ* hybridization, we considered them to represent nonspecific staining. However, we cannot fully exclude that in some of the locations, PDGF α-receptor antigen or cross reacting antigens may have been detected by the various antibodies. Since the polyclonal PDGF (R)-A 951 (Santa Cruz Biotechnology) antibody yielded the most consistent results and reacted with the antigen in paraffin-embedded sections, that is, provided better morphological resolution, we chose this antibody for an extended study.

Western blotting using the PDGF (R)-A 951 antibody detected a single 180 kDa band, corresponding to the 180 kDa PDGF α-receptor, in growth arrested human lung fibroblasts (Fig. 1). Following a six hour pretreatment with PDGF-AA, the fibroblast expression of the PDGF α-receptor was greatly diminished (Fig. 1). In contrast, no PDGF α-receptor expression could be detected in lysates of fetal or mature arterial smooth muscle cells or neonatal microvascular endothelial cells (both under conditions of growth arrest and FCS-induced proliferation; Fig. 1).

Fetal kidneys

The overall morphology of the fetal kidneys as observed during light microscopy corresponded to that described previously [16]. Immunohistochemical findings and the findings of *in situ* hybridization in the fetal kidneys are summarized in Table 1.

Immunohistochemistry

As shown in Figure 2 A and B, expression of PDGF α-receptor protein was not observed in cells of the undifferentiated metanephric blastema or glomerular vesicles. However, within the metanephric blastema, positive staining for PDGF α-receptor was noted in the vascular arcades that course through these regions (Fig. 2 A, B). Specific identification of the cell type expressing this molecule was not achieved. PDGF α-receptor was occasionally expressed in the invaginating mesangium or the hilar or stalk region of early glomerular differentiation stages, such as the comma or S-stage (Fig. 2 B, C). In some cases the invaginating PDGF α-receptor expressing cells were in unbroken continuity with the vascular arcades coursing down from the blastemal region. In differentiated glomeruli, positive staining for PDGF α-receptor was largely lost. No staining of tubular cells or cells of the ureteric bud could be demonstrated (Fig. 2 A-C). However, outside of the metanephric blastema, we frequently noted a staining pattern which was reminiscent of tubular basement membrane staining and staining of the glomerular capsule (Fig. 2 B-C). Whether this staining indeed represented basement membrane staining or staining of interstitial fibroblast extensions could not be resolved at the light microscopic level. PDGF α-receptor was uniformly expressed in interstitial cells in all specimens (Fig. 2 A-D). Strong staining was also observed in the adventitia of the fetal arterial vasculature, while staining was negative or trace in the media and absent in intimal cells (Fig. 2E). In occasional large vessels, apparent staining of the internal elastic membrane was noted (Fig. 2E). As in the case of the tubular basement membrane, we were unable to determine by light microscopy whether this latter staining was extracellular or staining of cellular extensions.

In situ hybridization

PDGF α-receptor mRNA was not detected in cells of the undifferentiated metanephric blastema or glomerular vesicles but was notably present in the vascular arcades coursing through the blastema (Fig. 3 A, B). At later stages of glomerular development (comma or S-stage), positive signal could be detected in the glomerular mesangial areas and hilar regions (Fig. 3 D, E). This signal was largely lost in differentiated glomeruli, in which only rare cells in mesangial areas expressed PDGF α-receptor mRNA (Fig. 3 D, E). No signal overlying glomerular visceral or epithelial cells was noted. Tubular cells and ureteric buds consistently failed to express PDGF α-receptor mRNA, while in all specimens the surrounding interstitial cells as well as cells comprising the vascular adventitia showed a strong hybridization signal (Fig. 3 F, G). Other vascular cells, such as those of the arterial media or intima were negative by *in situ* hybridization (Fig. 3 F, G).

Mature kidneys

Immunohistochemical findings and the findings of *in situ* hybridization in the mature kidneys are summarized in Table 2.

Immunohistochemistry

As shown in Figure 4 A and B, PDGF α-receptor staining in glomeruli was detectable in a small minority of the glomeruli examined. Such glomeruli were occasionally encountered in 70% of the kidney specimens examined; the remaining specimens had no glomerular staining whatsoever. Glomerular staining was confined to mesangial areas. Visceral or parietal glomerular epithelial cells consistently failed to exhibit positive staining for PDGF α-receptor. Tubular staining was also generally absent at the antibody concentrations employed (Fig. 4 A and B). In the renal interstitium, PDGF α-receptor was constitutively expressed in all specimens, both in the cortex and medulla (Fig. 4 A-C). PDGF α-receptor expression was particularly prominent around vessels and in the adventitia of small arteries. Also, in rare instances, a staining pattern reminiscent of tubular basement membrane staining or of the glomerular capsule (similar to that in fetal kidneys and shown in Fig. 2 A-C) was present. Within the media of small arteries, most cells were not stained, but rare

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As shown in Figure 2 A and B, expression of PDGF α-receptor protein was not observed in cells of the undifferentiated metanephric blastema or glomerular vesicles. However, within the metanephric blastema, positive staining for PDGF α-receptor was noted in the vascular arcades that course through these regions (Fig. 2 A, B). Specific identification of the cell type expressing this molecule was not achieved. PDGF α-receptor was occasionally expressed in the invaginating mesangium or the hilar or stalk region of early glomerular differentiation stages, such as the comma or S-stage (Fig. 2 B, C). In some cases the invaginating PDGF α-receptor expressing cells were in unbroken continuity with the vascular arcades coursing down from the blastemal region. In differentiated glomeruli, positive staining for PDGF α-receptor was largely lost. No staining of tubular cells or cells of the ureteric bud could be demonstrated (Fig. 2 A-C). However, outside of the metanephric blastema, we frequently noted a staining pattern which was reminiscent of tubular basement membrane staining and staining of the glomerular capsule (Fig. 2 B-C). Whether this staining indeed represented basement membrane staining or staining of interstitial fibroblast extensions could not be resolved at the light microscopic level. PDGF α-receptor was uniformly expressed in interstitial cells in all specimens (Fig. 2 A-D). Strong staining was also observed in the adventitia of the fetal arterial vasculature, while staining was negative or trace in the media and absent in intimal cells (Fig. 2E). In occasional large vessels, apparent staining of the internal elastic membrane was noted (Fig. 2E). As in the case of the tubular basement membrane, we were unable to determine by light microscopy whether this latter staining was extracellular or staining of cellular extensions.

In situ hybridization

PDGF α-receptor mRNA was not detected in cells of the undifferentiated metanephric blastema or glomerular vesicles but was notably present in the vascular arcades coursing through the blastema (Fig. 3 A, B). At later stages of glomerular development (comma or S-stage), positive signal could be detected in the glomerular mesangial areas and hilar regions (Fig. 3 D, E). This signal was largely lost in differentiated glomeruli, in which only rare cells in mesangial areas expressed PDGF α-receptor mRNA (Fig. 3 D, E). No signal overlying glomerular visceral or epithelial cells was noted. Tubular cells and ureteric buds consistently failed to express PDGF α-receptor mRNA, while in all specimens the surrounding interstitial cells as well as cells comprising the vascular adventitia showed a strong hybridization signal (Fig. 3 F, G). Other vascular cells, such as those of the arterial media or intima were negative by *in situ* hybridization (Fig. 3 F, G).

Mature kidneys

Immunohistochemical findings and the findings of *in situ* hybridization in the mature kidneys are summarized in Table 2.

Immunohistochemistry

As shown in Figure 4 A and B, PDGF α-receptor staining in glomeruli was detectable in a small minority of the glomeruli examined. Such glomeruli were occasionally encountered in 70% of the kidney specimens examined; the remaining specimens had no glomerular staining whatsoever. Glomerular staining was confined to mesangial areas. Visceral or parietal glomerular epithelial cells consistently failed to exhibit positive staining for PDGF α-receptor. Tubular staining was also generally absent at the antibody concentrations employed (Fig. 4 A and B). In the renal interstitium, PDGF α-receptor was constitutively expressed in all specimens, both in the cortex and medulla (Fig. 4 A-C). PDGF α-receptor expression was particularly prominent around vessels and in the adventitia of small arteries. Also, in rare instances, a staining pattern reminiscent of tubular basement membrane staining or of the glomerular capsule (similar to that in fetal kidneys and shown in Fig. 2 A-C) was present. Within the media of small arteries, most cells were not stained, but rare

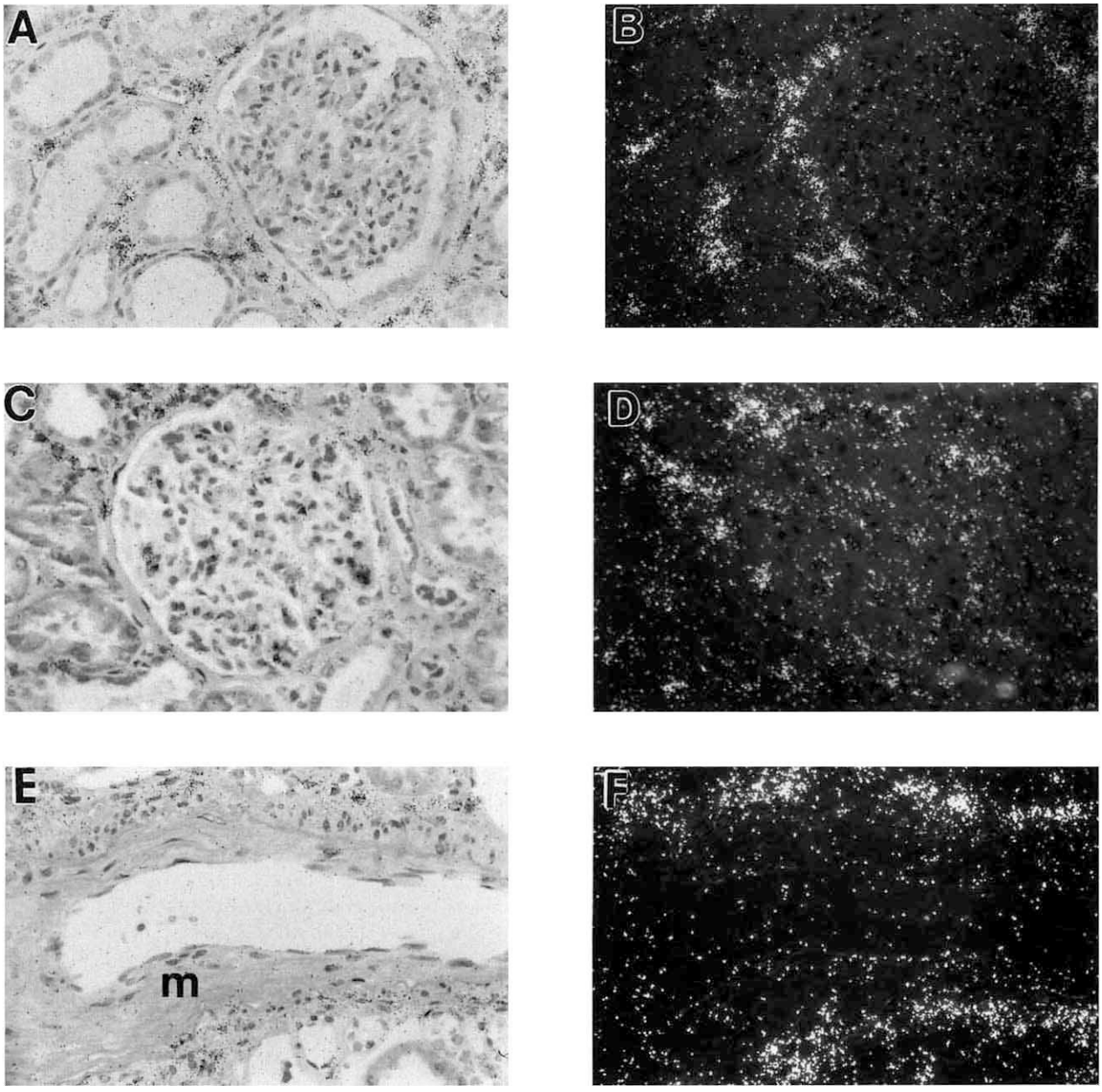

Fig. 6. *Demonstration of PDGF α-receptor mRNA in human mature kidney by in situ hybridization.* **A** and **B**. Most interstitial cells express PDGF α-receptor mRNA, while no significant hybridization signal is observed in the glomerulus or tubular cells (magnification ×400). **C** and **D**. At the other end of the spectrum, occasional glomeruli showed mild and even moderate expression of PDGF α-receptor mRNA, as demonstrated in this glomerulus (×400). **E** and **F**. In an arterial vessel, adventitial cells express PDGF α-receptor mRNA, while no significant signal is present in the intima or media (m) (×400).

weakly stained cells could be observed (Fig. 4C). No PDGF α-receptor expression was detectable on vascular endothelial cells or in the area of the internal elastic membrane of arterial vessel walls (Fig. 4C).

Expression of α-smooth muscle actin in normal mature kidney was similar to that described previously in that vascular smooth muscle cells stained positive as did occasional cells in mesangial areas and some interstitial cells and [31]. Double immunostaining for α-smooth muscle actin and PDGF α-receptor revealed occasional doubly labeled cells in mesangial and interstitial locations, while the large majority of α-smooth muscle actin positive vascular smooth muscle cells failed to express the PDGF α-receptor (Fig. 5A).

Ulex europeaus lectin bound to endothelial cells of all vessels, collecting ducts and erythrocytes, similar to previous descriptions [16, 28, 29]. In this case double immunostaining for Ulex europeaus and PDGF α-receptor failed to show co-localization of the two antigens (Fig. 5B).

Table 3. Comparative expression of PDGF α and β in developing and mature kidney

	PDGFRα	PDGFRβ
Developing kidney		
Metanephric blastema	−	+
Early mesangial structures	+	+
Differentiated mesangium	−	+
Glomerular epithelial cells	−	−
Glomerular endothelial cells	−	−
Tubules	−	−
Interstitial cells	+	+
Vascular adventitial cells	+	+
Vascular arcades	+	+
Ureteric buds	−	−
Mature kidney		
Glomerular mesangium	occasional +	+
Visceral epithelium	−	−
Parietal epithelium	−	+
Interstitial cells	+	+
Vascular adventitial cells	+	+
Tubules	−	−

In situ hybridization

No or only weak expression of PDGF α-receptor mRNA was detectable in most glomeruli of 7 of 15 (47%) of the specimens examined (Fig. 6 A, B). In the remaining eight specimens a weak to moderate signal was sometimes noted within the glomerular tuft (Table 2). As in the case of immunohistochemistry, PDGF α-receptor mRNA expression by visceral or parietal glomerular epithelial cells was not observed. Tubular cells consistently failed to show hybridization with the probe (Fig. 6 A, B). In the cortical and medullary interstitium, expression of PDGF α-receptor mRNA was always detectable but was focal and ranged from absent to strong within all specimens (Fig. 6 A, B). Small arteries exhibited weak to moderate hybridization in the adventitia but no detectable PDGF α-receptor mRNA expression by medial or intimal cells (Fig. 6 C, D).

Discussion

In both developing and mature human kidneys, immunohistochemistry with four different antibodies against the PDGF α-receptor, as well as *in situ* hybridization for PDGF α-receptor mRNA, consistently demonstrated prominent synthesis and expression of the receptor in interstitial fibroblasts. A second cell type expressing PDGF α-receptor, albeit to a lower and more variable extent than fibroblasts, were glomerular mesangial cells, in particular the probable mesangial cell precursors in fetal kidneys. Possible expression of the receptor was identified in a third mesenchymal cell type, vascular smooth muscle cells, but at an even lower and more variable degree than in mesangial cells. PDGF α-receptor was also detected in the vascular arcades of the fetal metanephric blastema, but it is not certain which cell type expressed the receptor in these early vessels. In contrast to these cell types, we found no conclusive evidence for expression of PDGF α-receptor mRNA or protein in glomerular epithelial cells, tubular cells or endothelial cells of large intrarenal vessels or the microvasculature in fetal and mature kidney. Finally, by immunocytochemistry we noted occasional staining along the tubular basement membrane, the glomerular capsule and the internal elastic membrane of large vessels, which were more common in fetal than in mature kidney. Light microscopy did not allow us to localize these sites of receptor expression to extracellular structures with certainty. However, the findings could conceivably relate to circulating extracellular domains of the PDGF α-receptor [32] binding to extracellular matrix proteins.

Gesualdo et al [23] have recently analyzed the expression of PDGF α-receptor protein in frozen sections of normal mature human kidneys. They reported but did not illustrate receptor protein expression in interstitial fibroblasts and mesangial areas as well as in glomerular epithelial cells. By *in situ* hybridization, again performed on frozen tissue, PDGF α-receptor mRNA localized prominently to mesangial, to a lower degree to interstitial areas and apparently also to glomerular epithelial cells [23]. Vascular expression of PDGF α-receptor was not described. Apart from the findings in glomerular epithelial cells, the data of Gesualdo et al [23] and those of the present study are qualitatively similar. On a quantitative basis the two studies differ in that we regularly observed stronger expression of PDGF α-receptor mRNA and protein in interstitial as compared to mesangial areas. In a second recent study, Stein-Oakley et al [24] described their failure to detect any renal expression of PDGF α-receptor in frozen biopsies from eight normal mature human kidneys. In the light of our data and those of Gesualdo et al [23], these findings possibly represent a technical limitation, since that study exclusively relied on immunohistochemistry with a single antibody.

Our findings are corroborated by a variety of *in vitro* observations: Cultured fibroblasts of dermal and pulmonary origin express PDGF α-receptor and respond to stimulation with PDGF AA [33–37], but cultured renal fibroblasts have not been investigated so far. In isolated rat glomeruli, PDGF α-receptor mRNA and protein could not be detected [15], but cell culture studies have demonstrated the ability of human and rat mesangial cells to synthesize PDGF α-receptor [3, 4, 38]. Finally, expression of PDGF α-receptor and/or responsiveness to PDGF-AA has also been noted in macrovascular arterial and venous smooth muscle cells [37, 39–41]. However, in all three cell types, that is, fibroblasts, mesangial cells and smooth muscle cells, the responses of smooth muscle cells to PDGF-AA have been variable, ranging from absent to pronounced [3, 4, 33, 34, 37, 39–42]. These latter findings likely result from the fact that PDGF α-receptor expression is highly regulated via many factors, including endogenous PDGF A-chain production, cytokines, lipopolysaccharide and α_2-macroglobulin [3, 33, 34, 37, 38, 40, 43]. These *in vitro* data can also explain some of the variability of our mesangial PDGF α-receptor expression *in vivo*, and in particular the *in vitro* data showing PDGF α-receptor synthesis in vascular smooth cells but little or no expression in normal vascular smooth muscle cells *in vivo*.

Responsiveness of human glomerular epithelial cells or tubular cells to PDGF AA, implying the presence of α-receptors, has not been described so far. In agreement with our *in vivo* observations, most investigators have also failed to demonstrate PDGF α-receptors available for PDGF AA-responsiveness on endothelial cells [44, 45]. However, others have reported that PDGF α-receptor expression can occur on rat micro- or macrovascular endothelial cells under cell culture conditions mimicking injury [46] or following endothelial injury *in vivo* [47]. Consequently, we cannot exclude that human renal endothelial cells possess the ability to synthesize PDGF α-receptors, since under normal conditions the receptors may either not be expressed or expression may be below the detection threshold of immunocytochemistry and *in situ*

hybridization. The potential *de novo* expression or up-regulation of PDGF α-receptors on human renal endothelial cells following injury might be of pathophysiological importance, given that neointimal smooth muscle cells and injured endothelium produce PDGF A-chain [12, 22, 47, 48], that is, paracrine and autocrine loops would be constituted.

The functional relevance of renal PDGF α-receptor expression, as of renal PDGF A-chain production, is largely undefined. Mice genetically deficient for the PDGF A-chain showed a defective pulmonary alveolar myofibroblast development and died either prenatally or within 60 days after birth of emphysema [49]. Kidneys and the renal vasculature developed a normal morphology (C.E. Alpers, unpublished observation). Similarly, PDGF α-receptor knockout mice demonstrated no gross morphological abnormalities in their kidneys (P. Soriano, personal communication). Thus, the lack of signalling via the PDGF α-receptor in these mice apparently is compensated by PDGF-independent pathways [50].

In the mature kidney, the constitutive and ubiquitous expression of the receptor on renal interstitial cells suggests that it might contribute to the pathogenesis of renal fibrosis, the common final pathway of progressive renal diseases. In lung fibroblasts, in particular those stimulated with endotoxin, PDGF α-receptors mediate mitogenic and chemotactic responses [33], supportive of a potential role in fibrogenesis. The developmental abnormalities of pulmonary myofibroblasts in PDGF A-chain knockout mice [49] further strengthen this assumption. Similar to fibroblasts, mesangial cells can exhibit a proliferative response to PDGF-AA [3, 4] and PDGF A-chain is overexpressed in glomeruli during mesangioproliferative nephritis [15], suggesting a possible involvement of the α-receptor in mesangioproliferative diseases. Finally, in smooth muscle cells, stimulation with PDGF-AA also results in chemotaxis, hyperplasia and/or hypertrophy [42, 43, 51]. A third potential role of renal PDGF α-receptors may thereby lie in the pathogenesis of vascular sclerosis and/or renal transplant rejection.

A noteworthy finding in this study were both the differences and similarities observed between localization of PDGF α-receptor and that of PDGF β-receptor, the only receptors known to bind PDGF. As previously detailed [10, 16] and summarized in Table 3, PDGF β-receptor is present in metanephric blastema, interstitial cells, mesangial cells, and adventitial cells in developing human kidneys, and in mesangial areas, parietal epithelial cells, interstitial cells, and vascular adventitial cells in the mature kidney. PDGF α-receptor co-localizes with PDGF β-receptor in interstitial cells and adventitial cells in the developing and mature kidney. As we have demonstrated in this study, prominent expression of PDGF α-receptor by more differentiated mesangial cells is not seen in the developing kidneys, and only focal, generally low levels of this receptor are detected in mature mesangial cells of uninjured human glomeruli. Prominent expression of PDGF α-receptor by the metanephric blastema is also not apparent, although it is expressed by cells that comprise at least in part the vascular arcades that course through this tissue compartment. Neither PDGF α-receptor nor PDGF β-receptor are present in the ureteric bud structures.

In summary, the current study shows widespread expression of PDGF α-receptor in fetal and mature renal fibroblasts and developing renal vessels as well as lesser degrees of expression in glomerular mesangial cells. Although yet undefined, this location of the receptor suggests roles in renal development and in the pathogenesis of renal fibrosis, mesangioproliferative diseases, and vascular diseases. The present study, which completes the localization of the normal and developing human renal PDGF ligand and receptor system [10, 16–19, 22, 23], lays the basis for further investigations in renal diseases and for functional studies on the role of the PDGF α-receptor in the kidney.

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