Kidney International, Vol. 48 (1995), pp. 146–154

CLINICAL INVESTIGATION

Platelet-derived growth factor A-chain expression in developing and mature human kidneys and in Wilms' tumor

CHARLES E. ALPERS, KELLY L. HUDKINS, MARINA FERGUSON, RICHARD J. JOHNSON, and JOE C. RUTLEDGE

Departments of Pathology, Medicine, and Laboratory Medicine, University of Washington School of Medicine and Childrens' Hospital and Medical Center, Seattle, Washington, USA

Platelet-derived growth factor A-chain expression in developing and mature human kidneys and in Wilms' tumor. Regulated expression of PDGF A-chain may be important in kidney development. We employed two polyclonal antisera to detect expression of PDGF A-chain in fetal and normal adult kidneys by immunohistochemistry. Specificity of the antisera was demonstrated by Western blots of fetal and adult kidneys, demonstrating monospecific bands at 10 to 15 kD, and by absorption studies with PDGF-A peptide. PDGF A-chain is uniformly expressed by visceral glomerular epithelial cells and the epithelial cells of the distal nephron, including collecting ducts and contiguous urothelium lining the renal pelvis, in both fetal and adult kidneys. Fetal kidneys also demonstrate expression of PDGF A-chain at the earliest stages of vesicle formation from the metanephric blastema; this expression is then only intermittently detectable in developing glomeruli until differentiation of visceral epithelial cells occurs. Fetal and mature arterial smooth muscle cells, and some neointimal smooth muscle cells in sclerotic arteries in adult kidneys also express PDGF A-chain. *In situ* hybridization with a riboprobe made from PDGF A-chain cDNA showed close correlation of mRNA expression with protein immunohistochemistry. PDGF A-chain expression was also identified in epithelial elements of 5/6 Wilms' tumors studied. These are the first studies to localize PDGF A-chain expression in human kidney and suggest sites of activity for PDGF A-chain in development, neoplasia, and in the renal arterial sclerosis of aging.

Platelet-derived growth factor (PDGF) exists as a dimer composed of two homologous but distinct peptides termed PDGF A and B chains. These peptides have distinctive functional qualities and are encoded by genes located on different chromosomes [1, 2]. The functional PDGF molecule can exist in each of the three possible isoforms: PDGF AA, AB, BB. The role of PDGF B-chain has been extensively studied in glomerular cells *in vitro* [3–6], in experimental models of glomerulonephritis [reviewed in 6–8], and in human renal development and disease [9–12]. The role of PDGF A-chain in renal disease is not as well known, due largely to the lack of useful reagents to evaluate expression of this molecule in normal and diseased states. We have previously shown by Northern blotting techniques that PDGF A-chain gene expression is up-regulated in rat glomeruli during the proliferative phase of mesangial proliferative glomerulonephritis induced by administration of anti-Thy-1 antibody [13]. Others have shown that PDGF A-chain gene transcription can be up-regulated in cultured mesangial cells exposed to phorbol ester, exogenous PDGF BB homodimer, and a number of peptide mitogens [14–16]. It is not known if other cell types within the rodent glomerulus express PDGF A-chain either constitutively or in response to specific stimuli.

In this study, we report additional characterization of two commercially available antibodies to PDGF A-chain and the use of these antibodies to define the expression of PDGF A-chain in human renal development and mature renal tissue by immunocytochemical methods. We further report the localization of corresponding mRNA production for PDGF A-chain by mature renal tissue using *in situ* hybridization techniques. These studies demonstrate PDGF A-chain expression in the visceral epithelial cells of differentiating fetal human glomeruli, and the persistent expression of this molecule by these cells in the mature glomerulus. PDGF A-chain is widely expressed by urothelial epithelium of the urinary tract both in developing and mature kidneys. This study also demonstrates the expression of PDGF A-chain by medial smooth muscle cells of the arterial vasculature in developing and mature human kidney, and its expression by a sub-population of intimal smooth muscle cells in the intimal proliferative sclerosing process characteristic of arterial vessels in aging kidneys. Finally, we also demonstrate PDGF A-chain expression in epithelial elements of Wilms' tumors reminiscent of the patterns of expression identified in normal kidney development.

Methods

Source of tissue

Human fetal kidneys (N = 12), estimated gestational age ranging from 54 to 122 days, were obtained fresh from tissue examined after therapeutic abortions. Tissues were fixed in methyl Carnoy's (methacarn) solution (60% methanol, 30% chloroform, 10% acetic acid) and processed and embedded in paraffin according to conventional techniques.

Normal human kidney (N = 21) was obtained fresh from uninvolved portions of kidneys surgically resected for localized renal cell carcinoma, or from cadaver donor kidneys unable to be utilized for transplantation. Portions of these tissues were fixed in either methacarn or 10% neutral buffered formalin and processed as above.

Received for publication December 8, 1994
and in revised form February 2, 1995
Accepted for publication February 2, 1995

Tissue from Wilms' tumors ($N = 6$) was obtained fresh after surgical excision and portions fixed in both methacarn and 10% neutral buffered formalin. Tissue remaining after all sections needed for medical diagnosis had been obtained was utilized for this study. None of the tumors were obtained from patients with familial occurrence and none were known to occur in concert with Wilms' tumor associated syndromes.

Antibodies

PDGF A-chain. Anti-PDGF A (Santa Cruz Biotechnology Inc, Santa Cruz, CA, USA) is an affinity-purified rabbit polyclonal antibody raised against a 30 amino acid peptide corresponding to the amino terminus of the human PDGF A-chain. The antibody specifically recognizes human PDGF A-chain under reducing and non-reducing conditions, and is non-reactive with either reduced or unreduced PDGF B-chain or unreduced human PDGF-AB. The second antisera utilized is a rabbit polyclonal protein A purified anti-human PDGF-AA (Upstate Biotechnology Inc., Lake Placid, NY, USA) which specifically recognizes PDGF-AA homodimer and PDGF-AB heterodimer. The immunogen for this antisera was purified human recombinant PDGF-AA homodimer [17], and this antisera neutralizes PDGF-AA and PDGF-AB in a PDGF stimulation of 3T3 cells bioassay.

Smooth muscle cell markers. Murine monoclonal antibody 1A4 (Dako Corp.) has been characterized by tissue immunohistochemistry and Western blotting [18], and has been previously demonstrated to recognize smooth muscle α-actin in methyl Carnoy's fixed tissues [19, 20].

Immunohistochemistry

Immunohistochemistry was performed on methyl Carnoy's fixed, paraffin embedded tissues following a standard avidin-biotin complex (ABC) method, as previously described [20, 21].

Briefly, sections were deparaffinized in xylene and rehydrated in graded ethanols. Endogenous peroxidase was blocked by incubation in 3% hydrogen peroxide and non-specific binding was blocked by incubation in 10% normal goat serum. The sections were then incubated overnight with the anti-PDGF-A or anti-PDGF-AA antisera in a humid chamber at 4°C. Following washes in PBS, the sections were sequentially incubated with biotinylated goat-anti-rabbit antisera (Vector Laboratories, Burlingame, CA, USA), the ABC-Elite avidin reagent (Vector Laboratories) and finally 3,3′-diaminobenzidine (with nickel chloride enhancement) as the chromogen. The sections were counterstained with methyl green, dehydrated and coverslipped.

Double labeling immunocytochemistry

Methyl Carnoy's fixed, paraffin embedded tissues were sectioned and mounted on aminopropylmethoxysilane (APTS) coated slides. After deparaffinization and rehydration, the slides were sequentially incubated with the anti-α-smooth muscle actin antibody; goat anti-mouse IgG-gold (Amersham, Arlington Heights, IL, USA) diluted in PBS plus 1% BSA and 0.1% gelatin for one hour at room temperature. Sections were washed, and the gold was visualized with an IntenSE M silver enhancement kit (Amersham). The sections were then incubated sequentially with: (1) rabbit-anti-PDGF A-chain diluted in PBS plus 1% BSA overnight at 4°C; (2) biotinylated goat-anti-rabbit IgG (Vector Laboratories); and (3) avidin-biotin-alkaline phosphatase complex (Vector Laboratories). The alkaline phosphatase was developed with a red substrate kit (Vector Laboratories) and the slides were counterstained with methyl green. Negative controls included substituting absorbed anti-PDGF A-chain antisera (see below), and substituting normal mouse IgG for the anti-α-smooth muscle actin.

Antibody absorption

Microtiter ELISA plates were coated with PDGF-A protein and PDGF-A control peptide (both from Santa Cruz Biotechnology, Inc.) CA) diluted in 50 mM carbonate buffer, pH 9.0, at concentrations ranging from 1 μg/ml to 100 μg/ml. After overnight incubation at 4°C, the antigen solution was removed and the plates were blocked with PBS containing 1% BSA and 0.02% sodium azide for two hours at room temperature. The plates were then washed with PBS and allowed to air dry. Rabbit anti-PDGF-A (Santa Cruz Biotechnology, Inc.) or rabbit anti-PDGF-AA (UBI) diluted 1/50 in PBS plus 2% BSA was added and the plates were incubated overnight at 4°C. The supernatant was removed from the wells and used as the absorbed primary antibody in a standard ABC immunohistochemistry procedure and was also used as a control in the Western blotting experiments detailed below. Positive controls (that is, repetition of the absorption procedure without antigen-specific absorption of the antisera) were done by using carbonate buffer only to coat the microtiter wells. This control antisera was used in procedures identical to those of the primary unabsorbed and absorbed antisera, and gave results similar to that of the original unmanipulated antisera (data not shown).

Western blotting

Homogenates of normal adult and fetal kidneys were electrophoresed on a 15% SDS polyacrylamide gel and then blotted onto nitrocellulose membranes. The blots were blocked with 5% BSA in PBS for one hour at 37°C and then incubated with anti-PDGF-A (Santa Cruz Biotechnology, Inc.) or anti-PDGF-AA (UBI) diluted 1/250 in 10 mM PBS containing 0.1% BSA and 10 mM sodium azide (PBS-BSA) for two hours at room temperature. After washing, the blots were incubated with a 1/100 dilution of gold conjugated goat-anti-rabbit (Amersham Corp.) for two hours. The gold signal was then enhanced by incubation in IntenSE BL (Amersham Corp.). In control experiments, reduced PDGF-AA protein (Santa Cruz Biotechnology, Inc.) and recombinant human PDGF-AA (UBI) and recombinant human PDGF-BB (UBI) were used to demonstrate specificity of the antisera. As a negative control, the primary antibody was replaced by normal rabbit IgG at an equivalent dilution.

Molecular probe

A 1280 bp human PDGF A-chain cDNA [22] was subcloned into SP64 vector. This probe was a gift of Dr. J.N. Wilcox, Emory University. The construct was linearized and transcribed into an antisense riboprobe using reagents obtained from Promega Biotec (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 A cDNA (either sense or antisense), 250 μCi ^{35}S-UTP (1,100 to 1,300 Ci/mmol), 500 μM each of ATP, CTP, and GTP, 40 U RNasin, 10 mM dithiothreitol, 40 mM Tris, and 10 U of either SP6 or T7 polymerase. After 75 minutes at 37°C, the DNA was digested by adding 1 U DNase (Promega) and incubation at 37°C for an additional 15 minutes.

Free nucleotides were then separated using a Sephadex G-50 column. Specific activity of the probes ranged from 5 to 30 $\times 10^7$ cpm/mg. Probes were used immediately.

In situ hybridization

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) 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 50°C. After hybridization, sections were washed with 0.5X 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, followed by several 2× SSC washes. After the tissue was air dried, it was dipped in NTB2 nuclear emulsion (Kodak) and exposed in the dark at 4°C for four weeks. After developing, the sections were counterstained with hematoxylin and eosin, dehydrated, mounted and viewed.

Results

Immunohistochemistry

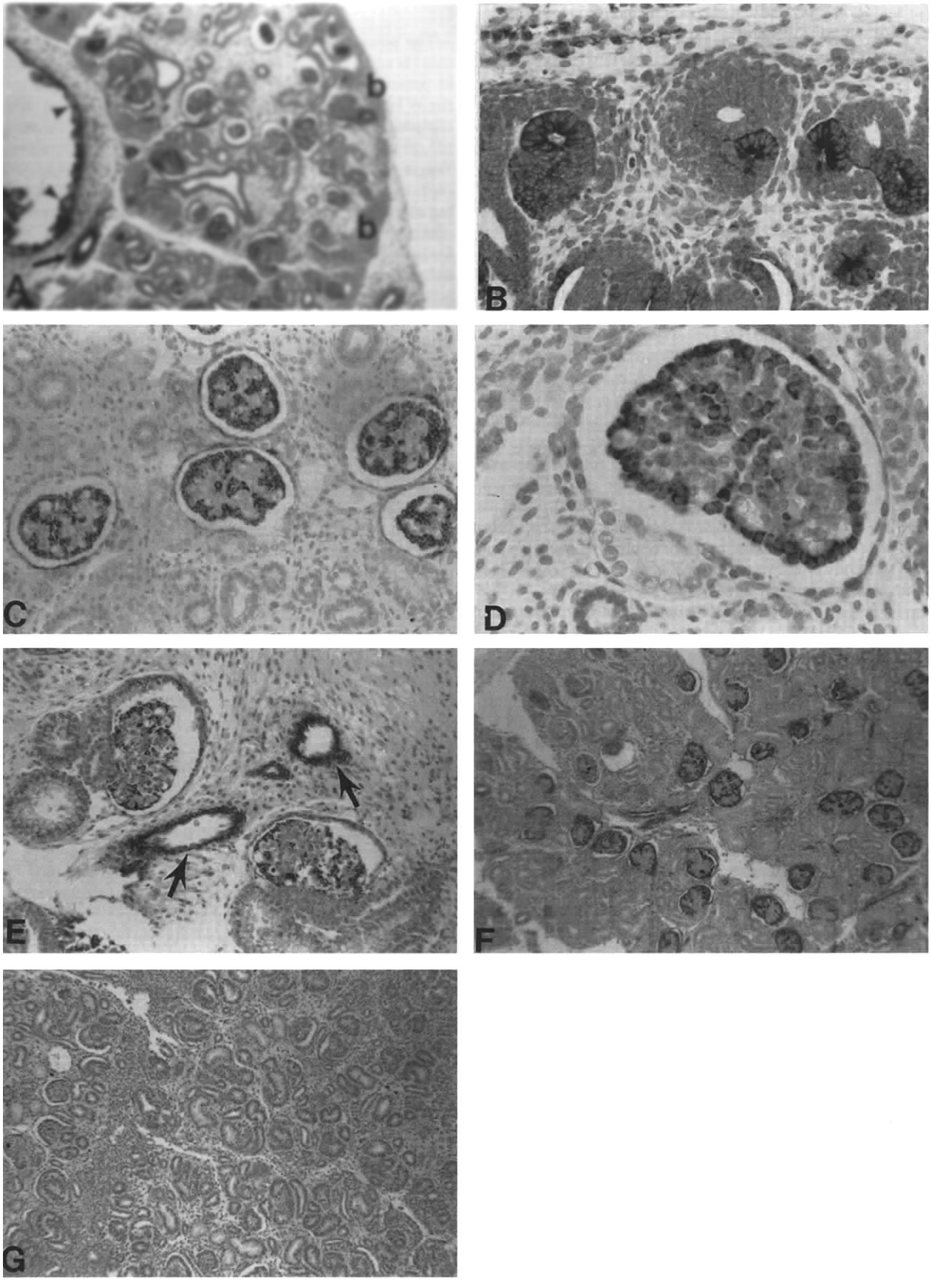
Two developmental stage-specific patterns of PDGF A-chain expression in developing nephrons were identified. PDGF A-chain could be first identified at the earliest stage of conversion of metanephric blastema into an epithelial vesicle, when this differentiating epithelium is still in close contact with the inductive differentiating stimulus, the ureteric bud (Fig. 1). There is no expression of PDGF A-chain by the adjacent blastema, ureteric bud, or interstitial tissue. As glomerular development proceeds through subsequent comma and S-shape stages, as they have been characterized by Saxén, Ekblom, and others [23–25], PDGF A-chain expression often becomes undetectable, although focal, intermittent expression by the epithelial cells comprising these primitive glomeruli is present. This variability was encountered not only between different kidney samples, but between glomerular structures of apparently identical morphologic development within the same tissue sections (Fig. 1B). In the last stages of glomerular development, PDGF A-chain is uniformly expressed by the visceral epithelial cells overlying glomerular capillaries (Fig. 1 C, D). The staining pattern is cytoplasmic, and appears to be of uniform intensity for glomeruli at all levels of maturation within developing kidneys (that is, superficial glomeruli as well as those near the cortico-medullary junction) once visceral epithelial cell differentiation has occurred. A minority of these differentiated glomeruli also show diffuse cytoplasmic expression of PDGF A-chain by parietal epithelial cells. Expression of PDGF A-chain by mesangial cells or endothelial cells is not detectable. Extraglomerular structures exhibiting widespread expression of PDGF A-chain included the urothelial lining of the ureter and renal pelvis, collecting duct epithelium in contiguity to the urothelium, and the smooth muscle cell layers of the developing renal arterial vasculature (Fig. 1 A, E).

The normal adult kidneys demonstrated persistence of patterns of PDGF A-chain expression exhibited late in renal development. Specifically, there is widespread expression by glomerular visceral epithelial cells and some parietal epithelial cells, but not other glomerular structures (Fig. 2A), widespread expression by collecting ducts and the urothelium lining the lower urinary tract (Fig. 2B), and diffuse expression by medial smooth muscle cells of the renal arterial tree (Fig. 2 A, C, D). In addition, many of the renal arteries sampled exhibited variable, most often mild to moderate, degrees of intimal sclerosis characteristic of aging kidneys. The majority of neointimal smooth muscle cells (shown by labeling with the α-smooth muscle actin antibody) present in these sclerosing arteries also exhibited prominent expression of PDGF A-chain (Fig. 2 C, D). PDGF A-chain expression by medial smooth muscle cells in these arteries was indistinguishable from that seen in arteries without prominent neointimal sclerosis.

The Wilms' tumor specimens exhibited the varied histopathologic features characteristic of these neoplasms, including blastemal, epithelial, and mesenchymal elements. All but one of these tumors exhibited focal expression of PDGF A-chain. PDGF A-chain expression was confined to more differentiated epithelial elements within these tumors, whereas neoplastic blastemal and mesenchymal structures had no detectable expression (Fig. 2E). Epithelial elements expressing PDGF A-chain often, but not invariably, assumed a tubular configuration; rarely, the PDGF A-chain expression in such structures appeared to be confined to the luminal surface, implying a degree of cell polarity. As expected from studies of normal kidneys, smooth muscle cells and/or pericytes comprising the non-neoplastic, infiltrating tumor vasculature also expressed PDGF A-chain.

No significant differences in staining patterns were identified between either of the two primary antisera utilized, although a stronger signal was obtained with the antisera raised against the 30 amino acid terminus of PDGF A-chain monomer. No specific cellular staining was seen with substitution of control rabbit IgG or PBS for the primary antibody. Staining patterns were uniform in both adult and fetal kidneys except for the variability in detectable expression of PDGF A-chain by early differentiating

Fig. 1. *Expression of PDGF A-chain in developing kidneys.* **A.** Low power photomicrograph of 59 day gestational age kidney showing expression of PDGF A-chain in developing glomerular structures, muscular arteries (arrows) and urothelium lining the renal pelvis (arrowheads). There is no expression in the rim of metanephric blastema (b) at the outer cortex of the kidney, or in interstitial cells. **B.** Same kidney as A. PDGF A-chain is expressed by epithelia of early differentiating structures (S and comma shaped/folded nephrons) but not by adjacent undifferentiated blastema. **C.** Differentiated fetal glomeruli uniformly demonstrate PDGF A-chain expression by visceral epithelial cells and irregularly by parietal epithelial cells lining Bowman's capsules. **D.** High magnification of a fetal glomerulus demonstrating PDGF A-chain expression in visceral epithelial cells. **E.** PDGF A-chain expression by smooth muscle cells of arteries (arrows) in a fetal kidney. **F.** Low power view of PDGF A-chain expression in fetal kidney of 81 days gestational age, demonstrating patterns described in A-E. **G.** Adjacent tissue section to that shown in F reacted with absorbed antisera. There is complete abolition of staining, providing strong evidence of antibody specificity.


A
b
b
B
C
D
E
F
G

vesicular and glomerular structures as indicated above. Replication of the immunohistochemical procedures with the absorbed antisera removed the specific staining of arterial smooth muscle cells, urothelium, and glomerular epithelial cells (Fig. 1 F, G).

In situ hybridization

In situ hybridization studies of fetal kidneys could not be performed due to degradation of the mRNA transcripts in these tissues. Studies of the adult kidneys showed a pattern of PDGF A-chain mRNA production corresponding to the patterns of protein expression described above. Hybridization signal was strongest overlying the smooth muscle cells comprising the media of arteries (Fig. 2F). Discrete signals were also identified in some neointimal smooth muscle cells, in some glomerular visceral epithelial cells and, rarely, parietal epithelial cells (Fig. 2G). While the distribution of mRNA expression revealed no discrepancies with the immunolabeling results (that is, no cell types appeared to produce PDGF A-chain mRNA that did not express identifiable PDGF A-chain protein), there was much less uniformity of mRNA production by positive cell types within each individual tissue section when assessed by our hybridization techniques as compared to the immunolabeling results. This may not represent a meaningful biological difference as hybridization incubation times were relatively short and other technical details (such as time of fixation after surgical removal of the specimen) for which hybridization studies are more sensitive than protein immunocytochemistry could not always be rigorously controlled. All positive hybridization results were obtained with use of the "antisense" strand probe; concomitant procedures utilizing the "sense" strand probe were uniformly negative (data not shown).

Western blotting

Both the anti-PDGF-A and anti-PDGF-AA antisera demonstrated discrete bands corresponding to proteins of 10 to 15 kD which correspond to the size of the PDGF A-chain monomer as indicated by simultaneous blotting of a control preparation of purified PDGF-AA (Fig. 3). Identical bands were found in both fetal and mature kidneys. In separate blotting experiments, both antisera recognized reduced and unreduced recombinant human PDGF-AA protein, with discrete bands at 14 to 18 kD and 30 to 32 kD, respectively. The antisera did not recognize reduced or unreduced PDGF-BB (data not shown). In separate blotting experiments, the identification of the 10 to 15 kD band could be abolished by the use of the antiserum pre-absorbed with PDGF-A peptide (data not shown).

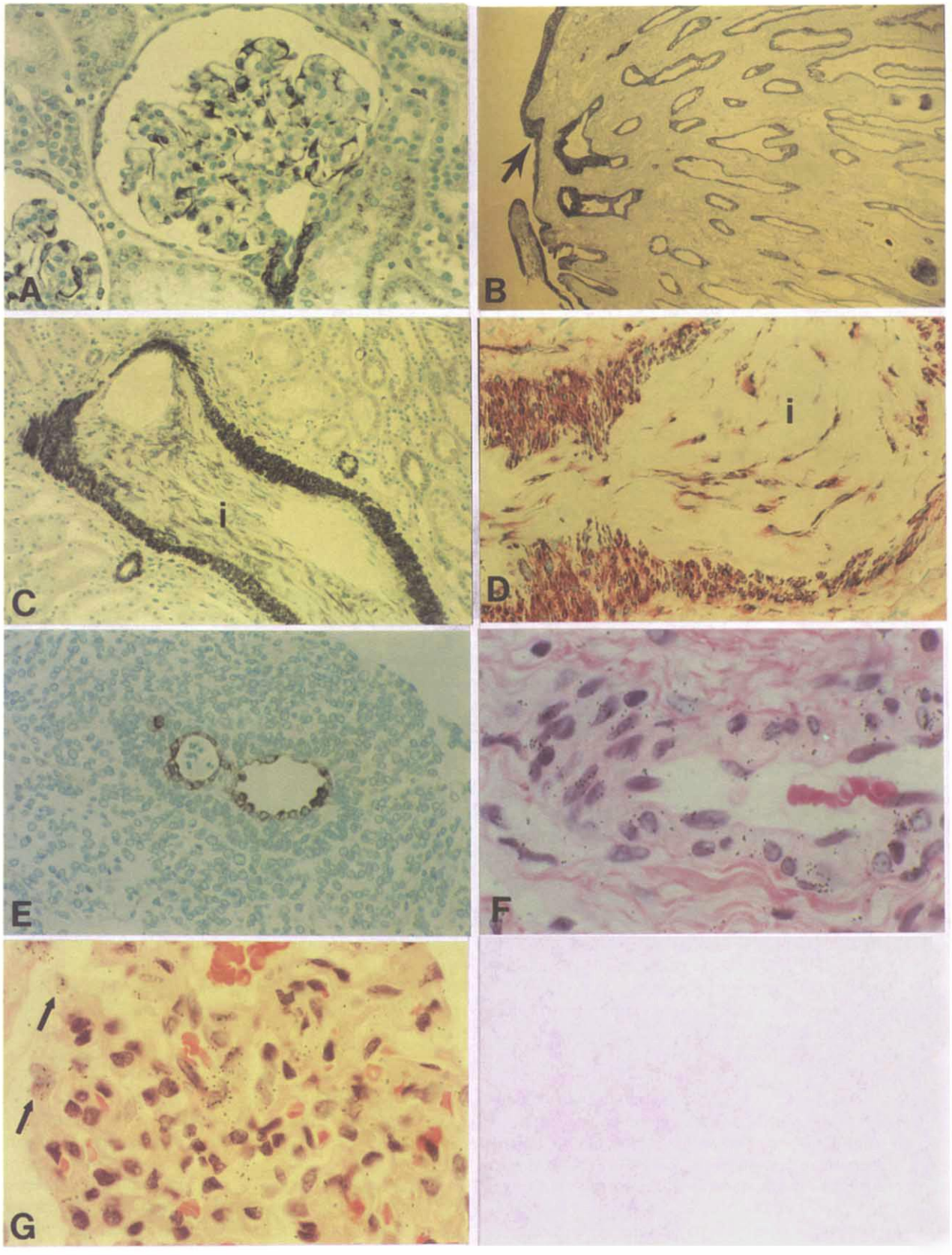
Discussion

The potential significance of PDGF A-chain in renal development is suggested by studies identifying a Wilms' tumor gene and its protein product WT1 [26]. One of the few known activities of the WT1 protein is that it has been shown to bind and repress the transcription activating sequence of the PDGF A-chain gene [27–29]. Mutations in this gene, which lead to the development of Wilms' tumor, might then be expected to lead to increased expression of PDGF A-chain, possibly in the Wilms' tumor itself, but also possibly within the non-tumorous renal parenchyma. The effects of such expression are not predictable, given how little is known of the function or sites of expression of PDGF A-chain and its receptor in either kidney development or normal physiologic activity.

This study demonstrates expression of PDGF A-chain in Wilms' tumors, albeit in a pattern restricted to tumor elements demonstrating epithelial and most often tubular differentiation. Those portions of Wilms' tumors exhibiting features more like undifferentiated blastema or primitive mesenchyme (in distinction from the non-neoplastic mesenchymal structures comprising the infiltrating tumor vasculature) were never found to express PDGF A-chain. Wilms' tumors typically demonstrate composite histologic features comprising blastema and mesenchymal elements as well as more differentiated epithelial elements [30]; the significance of PDGF A-chain expression by only one of these elements is of unknown clinical or biologic significance. However, this finding in Wilms' tumors does correspond to the pattern of PDGF A-chain expression by vesicular structures at the earliest stage of epithelial differentiation from the blastema and by tubular structures of the distal nephron in developing metanephric kidneys; tumor expression of PDGF A-chain may then be both a function and potential histologic marker of tumor differentiation. The differentiating elements of these tumors in this instance appear to maintain fidelity of growth factor expression with normal renal development.

Our studies also demonstrate expression of PDGF A-chain in the developing metanephric kidney in humans (Table 1). They show expression that is predominantly localized to differentiating visceral epithelial cells, collecting duct structures and the contiguous urothelial lining of lower urinary tract structures such as renal pelvis and ureter, and the smooth muscle cells of the arterial vasculature. These relatively differentiated structures bear no obvious relation to the metanephric blastema from which Wilms' tumors arise. However, as noted above, PDGF A-chain is also expressed in a restricted manner to vesicular structures which

Fig. 2. **A.** Adult normal human glomerulus showing persistent expression of PDGF A-chain in visceral epithelial cells, parietal epithelial cells, and the smooth muscle cells of a hilar arteriole. **B.** Adult normal human kidney shows persistent, widespread expression of PDGF A-chain by urothelium lining the renal pelvis (arrow) as well as by the contiguous epithelium of the renal collecting ducts. **C.** Arcuate artery from adult human kidney with intimal sclerosis. PDGF A-chain is expressed by smooth muscle cells comprising both the vessel media and intima (i). Several smaller arteries also demonstrate PDGF A-chain expression by medial smooth muscle cells. **D.** Double immunolabeling of an intrarenal artery from an adult human kidney also demonstrating intimal (i) sclerosis. PDGF A-chain expression by smooth muscle cells is identified by red alkaline phosphatase label, while the smooth muscle cell identity of intimal and medial cells is confirmed by co-localization of a black immunogold label for α-smooth muscle actin. **E.** Wilms' tumor. There is focal expression of PDGF A-chain by portions of the tumor exhibiting epithelial differentiation, while less well differentiated (blastemal) elements do not express detectable levels of this peptide. **F.** *In situ* hybridization of adult human kidney, with PDGF A-chain mRNA expression demonstrable in smooth muscle cells of renal arterial structures, in accordance with immunohistochemical results. Adventitia and adjacent matrix cells show no expression. **G.** Same kidney as F, with glomerulus showing PDGF A-chain mRNA expression which can generally be localized to visceral epithelial cells at periphery (arrows) and central portions of glomerular tuft.


A
B
C
i
D
i
E
F
G

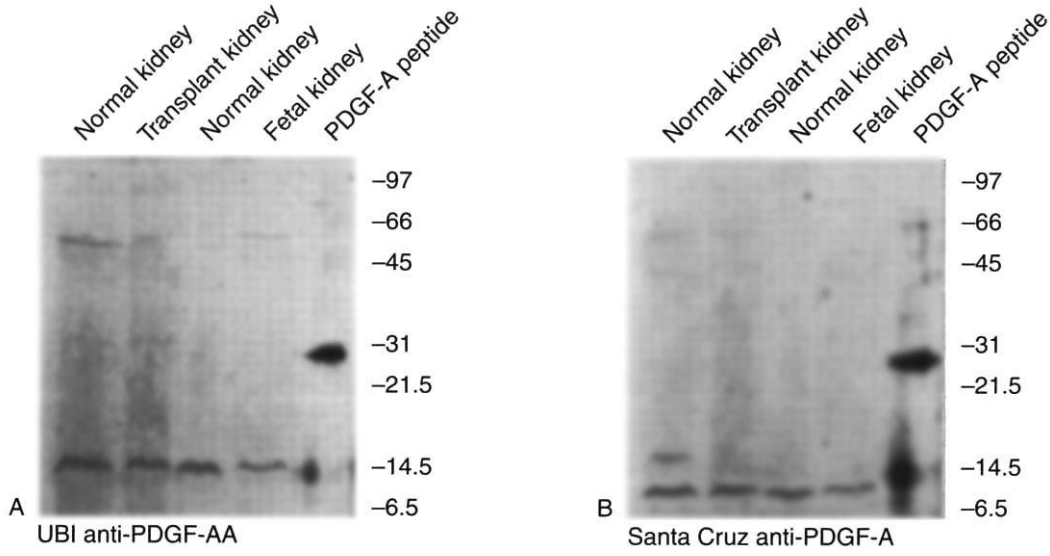


Fig. 3. *Western blots utilizing each of the PDGF A-chain antisera employed in this study, reacted with tissue obtained from adult (lanes 1–3) and fetal (lane 4) human kidneys.* Lane 5 contains PDGF A-chain peptide. Monospecific bands are obtained with the anti-PDGF-AA antisera in **A**, and with antisera directed against the 30 amino acid terminus of PDGF A-chain in **B**. Higher molecular weight bands recognized in lane 5 indicate likely dimerization of the peptide.

comprise the earliest stages of differentiation of the nephron from the blastema. This localization of PDGF A-chain suggests the possibility that PDGF A-chain could play a role critical in the early events in the differentiating metanephric kidney. Of particular note is that this pattern of PDGF A-chain expression in early and differentiated glomerular epithelial structures is similar, if not identical, to that reported for the WT1 gene product in developing human kidneys [31, 32]. That PDGF A-chain expression as well as that of a protein that represses PDGF A-chain expression should co-localize to the same cell types presents a paradox that is currently unexplainable. However, this finding does indicate that expression of the WT-1 gene product alone is insufficient to repress PDGF A-chain expression by at least some cell types of the developing kidney. Alternatively, it is possible that the WT1 protein may also function as a gene activator under conditions that still need to be defined [29]. We cannot exclude the possibility that even earlier in development, a further program(s) of PDGF A-chain expression and/or suppression are required for initial formation of metanephric kidney structures, but that such programs are no longer essential or detectable in the process of conversion of metanephric blastema to differentiated nephronal structures which was a focus for this study. This possibility is supported by studies in mice using knockout techniques which show that deletion of the WT1 gene results in failure to develop differentiated metanephric structures concomitant with absence of the ureteric bud which induces such differentiation [33].

Additional considerations of these findings in developing and mature kidneys are in order. PDGF B-chain has previously been localized to developing nephronal epithelial structures, at a stage slightly later than that of PDGF A-chain expression (Table 1) [9]. Co-localization of PDGF β receptor (which binds only the PDGF B-chain isoform) to the metanephric blastema and interstitial tissues suggested local paracrine interactions by which PDGF B-chain activity could promote further glomerular differentiation [9]. In the absence of reagents that would allow localization of the PDGF α receptor, which binds the PDGF A-chain isoform, we are unable to define the significance of PDGF A-chain expression at these stages of development to a similar degree. One possibility, suggested by the known ability of PDGF A-chain to stimulate proliferation and migration of vascular smooth muscle cells, is that PDGF A-chain may promote the local migration of interstitial vascular elements into the glomerulus in the process of establishing the glomerular vascular architecture [2, 34, 35].

Secondly, as glomerular differentiation proceeds, there is clear divergence of the sites of PDGF-A and B chain production. PDGF B-chain, and the PDGFβ receptor which binds only PDGF B-chain, are produced by mesangial cells in health and disease in both humans and rodents [6, 7, 10, 12, 13, 36], although in the rat injured visceral epithelial cells also have been shown to produce PDGF B-chain [37]. Once the developing human glomerular tuft has differentiated, PDGF A-chain production appears confined to visceral epithelial cells throughout the maturation process and into adulthood. Other growth factors with a similar localization in the human glomerulus are vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) [38–40]. It is known that there is a limited homology between the genes encoding VEGF and PDGF [41, 42], but other structural, interactive, or molecular relationships among these three peptide growth factors have not been identified. Although VEGF, bFGF, and PDGF do share mitogenic, angiogenic and chemotactic properties to a varying degree [2, 41–45], it is not clear how such properties may contribute to visceral epithelial cell function and injury.

Thirdly, PDGF A-chain demonstrates widespread expression by the smooth muscle cells of the renal vasculature, including smooth muscle cells in the neointima of arteries demonstrating intimal sclerosis. PDGF A-chain protein and mRNA has been localized previously to human atherosclerotic plaques [35, 46, 47], and up-regulated expression of this molecule has also been demonstrated in the neointima of rat arteries subjected to balloon

Table 1. Phenotypic characterization of stages of glomerulogenesis in human fetal kidney

	Metanephric blastema	Vesicle	Early glomerular differentiation (comma, S-stage)	Differentiated glomerulus
• PDGF B-chain	−	+/−	−	Mesangium
• PDGFRβ	+	−	−	Mesangium
• PDGF A-chain	−	focal	+	Visceral epithelial cell
• WT1	−	focal	+	Visceral epithelial cell

Expression of members of the PDGF system and the potentially regulatory protein WT1 during human glomerulogenesis. Data on PDGF B-chain and PDGFRβ are from [9]; data on WT1 expression are from [31, 32].

catheter injury [34]. Based on the sequence of pathologic events occurring in experimental balloon catheter injury and on studies of vascular smooth muscle cells *in vitro*, evidence has accumulated that PDGF A-chain may function as a smooth muscle cell mitogen and promoter of vascular smooth muscle cell migration. In this study, the arterial localization of PDGF A-chain provides the first direct demonstration of the potential of PDGF A-chain to participate in human renal vascular injury. The identification of PDGF A-chain in neointimal smooth muscle cells in particular suggests the potential for both paracrine and autocrine activity involving these mesenchymal cells within the vessel neointima, as well as the potential to stimulate the neighboring smooth muscle cells of the arterial vessel media to replicate or migrate and hence promote further neointimal expansion. It has even been suggested that PDGF A-chain may have non-mitogenic trophic functions that are otherwise unspecified but nonetheless important in the maintenance of arterial structures [47]. The apparently constitutive expression of PDGF A-chain mRNA and protein by medial smooth muscle cells in both developing and mature arteries is evidence for such a trophic function in renal arteries. Since virtually nothing is known of the processes which cause the renal arterial sclerosis that is a hallmark of renal aging, nor of those causing sclerosis as a consequence of more discreet forms of vascular injury, we are unable to more specifically identify the specific functional roles of PDGF A-chain in these processes as yet. However, *in situ* identification of expression of molecules at both the transcriptional and protein levels that could mediate such processes is an initial approach towards understanding the pathogenetic basis of human renovascular disease. Some evidence in support of a role for PDGF A-chain in such processes is provided in our recent study of allograft kidneys demonstrating widespread expression of PDGF A-chain by the neointimal cells of arteries demonstrating chronic vascular rejection in these kidneys [48].

In summary, our studies indicate regulated expression of PDGF A-chain in developing kidney glomerular epithelial cells, tubules, and blood vessels and persistence of these patterns into adulthood. However, they do not identify the role of PDGF A-chain in human kidney development or answer the question of whether the PDGF A-chain expression observed is essential for normal kidney maturation and function. The critical role for PDGF B-chain in this process has recently been demonstrated in the PDGF B-chain knockout mouse which revealed that PDGF B-chain is required for normal development of the glomerular mesangium [49]; the importance of PDGF A-chain may be determined by a similar experimental approach. Knowledge about the sites of activity for PDGF A-chain within the kidney awaits better localization of its receptor. It has been reported recently that in the adult kidney PDGFα receptor co-localizes in a distribution similar to PDGF β receptor [12]; however, none of this was illustrated and we remain cautious in our understanding of the distribution of PDGF α receptor. Some indication of a role for PDGF A-chain in the pathogenesis of renal vascular disease is already provided by demonstration of its expression in atherosclerosis [35, 47], renal arteriosclerosis, and renal allograft rejection [48]. Finally, we have identified a pattern of glomerular expression of PDGF A-chain similar to that of the Wilms' tumor gene product and further identified PDGF A-chain expression in a portion of Wilms' tumors, adding to the evidence associating these molecules in the processes of renal development and neoplasia.

Acknowledgments

This work was supported by grants HL42270, HL47151, DK43422 and a George O'Brien Kidney Research Center grant DK47659 from the National Institutes of Health. The authors thank Drs. Christer Betscholtz and Cy Wilcox for provision of the PDGF A-chain probe, the Central Laboratory for Human Embryology at the University of Washington (supported by grant HD00836 from the National Institutes of Health) for assistance in providing fetal tissue, and Sheri Storey for secretarial assistance.

Reprint requests to Dr. Charles E. Alpers, Department of Pathology, RC-72, University of Washington Medical Center, Seattle, Washington 98195, USA.

References

1. Ross R, Raines EW, Bowen-Pope DF: The biology of PDGF. *Cell* 46:155–169, 1986
2. Raines EW, Ross R: Platelet-derived growth factor in vivo, in *Cytokines* (vol 5), edited by Westermark B, Sorg C, Basel, Karger, 1993, pp 74–114
3. Silver PJ, Jaffer FE, Abboud HE: Platelet-derived growth factor synthesis in mesangial cells: Induction by multiple peptide mitogens. *Proc Natl Acad Sci USA* 86:1056–1060, 1988
4. Shultz PJ, Dicorleto PE, Silver BJ, Abboud HE: Mesangial cells express PDGF mRNAs and proliferate in response to PDGF. *Am J Physiol* 255(Renal Fluid Electrol Physiol. 24):F674-F684, 1988
5. Floege J, Topley N, Hoppe J, Barrett TB, Resch K: Mitogenic effect of platelet-derived growth factor in human glomerular mesangial cells: Modulation and/or suppression by inflammatory cytokines. *Clin Exp Immunol* 86:334–341, 1991
6. Abboud HE: Growth factors in glomerulonephritis. *Kidney Int* 43: 252–267, 1993
7. Johnson RJ, Floege J, Couser WG, Alpers CE: Role of platelet-derived growth factor in glomerular disease. *JASN* 4:119–128, 1993
8. Johnson RJ: The glomerular response to injury: Progression or resolution? *Kidney Int* 45:1769–1782, 1994
9. Alpers CE, Seifert RA, Hudkins KL, Johnson RJ, Bowen-Pope DF: Developmental patterns of PDGF B-chain, PDGF-receptor, and α actin expression in human glomerulogenesis. *Kidney Int* 42:390–399, 1992
10. Gesualdo L, Pinzani M, Floriano JJ, Hassan MO, Nagy NU, Schena FP, Emancipator SN, Abboud HE: Platelet-derived growth factor expression in mesangial proliferative glomerulonephritis. *Lab Invest* 64:160–167, 1991
11. Nakajima M, Hewitson TD, Mathews DC, Kincaid-Smith P: Platelet-derived growth factor mesangial deposits in mesangial IgA glomerulonephritis. *Nephrol Dial Transplant* 6:11–16, 1991
12. Gesualdo L, Di Paolo S, Milani S, Pinzani M, Grappone C, Ranieri E, Pannarale G, Schena FP: Expression of platelet-derived growth factor receptors in normal and diseased human kidney: An

immunohistochemistry and in situ hybridization study. *J Clin Invest* 94:50–58, 1994
13. IIDA H, SEIFERT R, ALPERS CE, GRONWALD RGK, PHILLIPS PE, PRITZL P, GORDON K, GOWN AM, ROSS R, BOWEN-POPE DF, JOHNSON RJ: Platelet-derived growth factor (PDGF) and PDGF receptor are induced in mesangial proliferative nephritis in the rat: An effect mediated by platelets and complement. *Proc Natl Acad Sci USA* 88:6560–6564, 1991
14. BHANDARI B, ABBOUD HE: Platelet derived growth factor-A chain gene expression in cultured mesangial cells: Regulation by phorbol ester at the level of mRNA abundance, transcription and mRNA stability. *Mol Cell Endocrinol* 91:185–191, 1993
15. BHANDARI B, GRANDALIANO G, ABBOUD HE: Platelet-derived growth factor (PDGF) BB homodimer regulates PDGF A- and PDGF B-chain gene transcription in human mesangial cells. *Biochem J* 297:385–388, 1994
16. SILVER BJ, JAFFER FE, ABBOUD HE: Platelet-derived growth factor (PDGF) synthesis in mesangial cells: Induction by multiple peptide mitogens. *Proc Natl Acad Sci USA* 86:1056–1060, 1989
17. HART CE, BAILEY M, CURTIS DA, OSBORN S, RAINES E, ROSS R, FORSTROM JW: Purification of PDGF-AB and PDGF-BB from human platelet extracts and identification of all three PDGF dimers in human platelets. *Biochem* 29:166–172, 1990
18. SKALLI O, ROPRAX P, TRZECIAK A, BENXONANA G, GILLESSEN D, GABBIANI G: A monoclonal antibody against α-smooth muscle actin: A new probe for smooth muscle differentiation. *J Cell Biol* 103:2787–2796, 1986
19. JOHNSON RJ, IIDA H, ALPERS CE, MAJESKY MW, SCHWARTZ SM, PRITZL P, GORDON K, GOWN AM: Expression of smooth muscle cell phenotype by rat mesangial cells in immune complex nephritis: α-smooth muscle actin is a marker of mesangial cell proliferation. *J Clin Invest* 87:847–858, 1991
20. O'BRIEN KD, ALLEN MD, MCDONALD TO, CHAIT A, HARLAN JM, FISHBEIN D, MCCARTY J, FERGUSON M, HUDKINS K, BENJAMIN CD, LOBB R, ALPERS CE: Vascular cell adhesion molecule-1 is expressed in human coronary atherosclerotic plaques: Implications for the mode of progression of advanced coronary atherosclerosis. *J Clin Invest* 92:945–951, 1993
21. GOWN AM, VOGEL AM: Monoclonal antibodies to intermediate filaments proteins of human cells. II. Distribution of filament proteins in normal human tissues. *Am J Pathol* 114:309–321, 1984
22. BETSHOLTZ C, JOHNSSON A, HELDIN C-H, WESTERMARK B, LIND P, URDEA MS, EDDY R, SHOWS TB, PHILPOTT K, MELLOR AL, KNOTT TJ, SCOTT J: cDNA sequence and chromosomal localization of human platelet-derived growth factor A-chain and its expression in tumour cell lines. *Nature* 320:695–699, 1986
23. SAXEN L: *Organogenesis of the Kidney*. Cambridge, Cambridge University Press, 1987
24. EKBLOM P: Developmentally regulated conversion of mesenchyme to epithelium. *FASEB J* 3:2141–2150, 1989
25. ABRAHAMSOM DR: Glomerulogenesis in the developing kidney. *Semin Nephrol* 11:375–389, 1991
26. RAUSCHER FJ III: The WT1 Wilms tumor gene product: A developmentally regulated transcriptional factor in the kidney that functions as a tumor suppressor. *FASEB J* 7:896–903, 1993
27. GASHLER AL, BONTHRON DT, MADDEN SL, RAUSCHER FJ III, COLLINS T, SUKHATME VP: Human platelet-derived growth factor A chain is transcriptionally repressed by the Wilms Tumor Suppressor WT1. *Proc Natl Acad Sci USA* 89:10984–10988, 1992
28. WANG ZY, MADDEN SL, DEUEL TF, RAUSCHER FJ III: The Wilms' tumor gene product, WT1, represses transcription of the platelet-derived growth factor A-chain gene. *J Biol Chem* 267:21999–22002, 1992
29. WANG ZY, QIU QQ, DEUEL TF: The Wilms' tumor gene product WT1 activates or suppresses transcription through separate functional domains. *J Biol Chem* 268:9172–9175, 1993
30. BECKWITH JB, PALMER NF: Histopathology and prognosis of Wilms tumors: Results from the First National Wilms' tumor study. *Cancer* 41:1937–1948, 1978
31. PRITCHARD-JONES K, FLEMING S, DAVIDSON D, BICKMORE W, PORTEUS D, GOSDEN C, BARD J, BUCKLER A, PELLETIER J, HOUSMAN D, VAN HEYNINGER V, HASTIE N: The candidate Wilms' tumour gene is involved in genitourinary development. *Nature* 346:194–197, 1990
32. MUNDLOS S, PELLETIER J, DARVEAU A, BACHMANN M, WINTERPACHT A, ZABEL B: Nuclear localization of the protein encoded by the Wilms' tumor gene WT1 in embryonic and adult tissues. *Development* 119:1329–1341, 1993
33. KREIDBERG JA, SARIOLA H, LORING JM, MAEDA M, PELLETIER J, HOUSMAN D, JAENISCH R: WT-1 is required for early kidney development. *Cell* 74:679–691, 1993
34. MAJESKY MW, REIDY MA, BOWEN-POPE DF, HART CE, WILCOX JN, SCHWARTZ SM: PDGF ligand and receptor gene expression during repair of arterial injury. *J Cell Biol* 111:2149–2158, 1990
35. REKHTER MD, GORDON D: Does platelet-derived growth factor-A chain stimulate proliferation of arterial mesenchymal cells in human atherosclerotic plaques? *Circ Res* 75:410–417, 1994
36. ALPERS CE, SEIFERT RA, HUDKINS KL, JOHNSON RJ, BOWEN-POPE DF: PDGF-receptor localizes to mesangial, parietal epithelial, and interstitial cells in human and primate kidneys. *Kidney Int* 43:286–294, 1993
37. FLOEGE J, JOHNSON RJ, ALPERS CE, FATEMI-NAINIE S, RICHARDSON CA, GORDON K, COUSER WG: Visceral glomerular epithelial cells can proliferate in vivo and synthesize PDGF B-chain. *Am J Pathol* 142:637–650, 1993
38. BROWN LF, BERSE B, TOGNAZZI K, MANSEAU EJ, VAN DE WATER L, SENGER DR, DVORAK HF, ROSEN S: Vascular permeability factor mRNA and protein expression in human kidney. *Kidney Int* 42:1457–1461, 1992
39. ALPERS CE, JOHNSON RJ: Growth factors and the glomerulus: Relations between development and injury. *Adv Nephrol* 24:33–52, 1995
40. ALPERS CE, SCHELLING ME, HUDKINS KL, REIDY MA, LINDNER V: Localization of basic fibroblast growth factor (bFGF) and its receptor (FGFR1. *flg*) in fetal, mature, and transplanted human kidneys (abstract) *Lab Invest* 70:156A, 1994
41. LEUNG DW, CACHIANES G, KUANG W-J, GOEDDEL DV, FERRARA N: Vascular endothelial growth factor is a secreted angiogenic mitogen. *Science* 246:1306–1309, 1989
42. KECK PJ, HAUSER SD, KRIVI G, SANZO K, WARREN T, FEDER J, CONNOLLY DT: Vascular permeability factor, an endothelial cell mitogen related to PDGF. *Science* 246:1309–1312, 1989
43. RISAU W, DREXLER H, MIRONOV V, SMITS A, SIEGBAHN A, FUNA K, HELDIN C-H: Platelet-derived growth factor is angiogenic *in vivo*. *Growth Factors* 7:261–266, 1992
44. KLAGSBRUN M, EDELMAN ER: Biological and biochemical properties of fibroblast growth factors. *Arteriosclerosis* 9:269–278, 1989
45. JUDAH F, SHING Y: Angiogenesis. *J Biol Chem* 267:10931–10934, 1992
46. WILCOX JN, SMITH KM, WILLIAMS LT, SCHWARTZ SM, GORDON D: Platelet-derived growth factor mRNA detection in human atherosclerotic plaques by *in situ* hybridization. *J Clin Invest* 82:1134–1143, 1988
47. BARRETT TB, BENDITT EP: Platelet-derived growth factor gene expression in human atherosclerotic plaques and normal artery wall. *Proc Natl Acad Sci USA* 85:2810–2814, 1988
48. ALPERS CE, HUDKINS K: Platelet-derived growth factor (PDGF) A-chain expression in normal and rejecting renal arteries (abstract). *Lab Invest* 72:157A, 1995
49. LEVEEN P, PEKNY M, GEBRE-MEDHIN S, SWOLIN B, LARSSON E, BETSHOLTZ C: Mice deficient for PDGF B show renal, cardiovascular, and hematological abnormalities. *Gene Develop* 8:1875–1887, 1994