

Expression of platelet-derived growth factor and its receptors in the developing and adult mouse kidney

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Background. Experimental analysis of gene function is increasingly being accomplished using mouse models. Glomerular malformations occur in mice in which the platelet-derived growth factor (PDGF) B-chain gene or the PDGF receptor β -subunit gene have been deleted. To understand potential PDGF signaling pathways in the kidney, we determined the expression pattern of PDGF ligand and receptor genes in mouse kidney during development and in the mature adult kidney.

Methods. We used *in situ* hybridization to map the expression of transcripts encoding the PDGF ligands (A-chain and B-chain) and PDGF receptors (PDGFR α and PDGFR β) in the developing and mature kidney of the mouse.

Results. PDGF A-chain transcripts are expressed by epithelial cells (especially in what appear to be the loop of Henle) and possibly in vascular smooth muscle cells. Its receptor, PDGFR α , is expressed by interstitial cells. PDGF B-chain transcripts are most highly expressed by vascular endothelial cells of developing and adult kidney and minimally by visceral epithelia of immature glomeruli. PDGFR β transcripts are expressed by fetal blastemal cells, interstitial cells, mesangial cells, and vascular smooth muscle cells and by adult mesangial and interstitial cells. PDGFR α and PDGFR β expression is especially prominent in lipid-laden interstitial cells in the adult kidney.

Conclusions. These patterns of expression are similar, but not identical, to those observed in rat and human and suggest that paracrine interactions mediated by the PDGF/PDGF receptor system may coordinate the development of the tubular, vascular, and interstitial components during kidney development and disease.

Platelet-derived growth factor (PDGF) is a widely expressed growth factor with both mitogenic and chemotactic activities for many connective tissue cell types. The PDGF family includes three proteins formed from two polypeptide chains, the PDGF A-chain and the PDGF B-chain [re-

viewed in 1]. These chains dimerize to form disulfide bonded homo- and heterodimers: PDGF-AA, -AB, and -BB. Similarly, the two PDGF receptor proteins (PDGFR α and PDGFR β) dimerize noncovalently to form high-affinity binding sites [2]. Not all forms of ligand bind to all forms of the receptor with high affinity. Notably, high-affinity binding of PDGF isoforms containing PDGF A-chain requires PDGFR α . PDGFR α and PDGFR β differ somewhat in the sequence of their intracellular domains [reviewed in 3], which suggests that they might generate different intracellular signals. Despite this potential, there are few, if any, processes evaluated in cell culture that have been demonstrated to be uniquely dependent on one of the PDGFR proteins in every cell type. A second (non-exclusive) possibility is that the differing specificity of the two receptor subunits, and the secretion of different isoforms of PDGF by different cells at different times, evolved to allow the PDGF system to function as a set of partially overlapping, and partially distinct, regulatory networks. For example, a cell expressing PDGFR α could respond to any isoform of PDGF. A second cell type that expressed only PDGFR β could respond to PDGF-BB but not to PDGF-AA or PDGF-AB. Testing the hypothesis that the biological functions of different forms of PDGF and PDGF receptors will be largely determined by their pattern of expression requires two types of information: (1) an accurate and complete description of when and where the component receptors and ligands are expressed, and (2) a systematic evaluation of the consequences of experimental manipulation of expression levels. In this report we provide the first category of information for kidney in the mouse, a species that is increasingly used for experimental manipulation by genetic techniques.

The importance of PDGF B-chain and PDGFR β in murine kidney development is demonstrated by the observation that inactivation of either the PDGF B-chain gene [4] or the PDGFR β gene [5] results in an absence of mesangial cell formation and failure to form the glomerular tuft. The importance of PDGF A-chain and PDGFR α is less clear. Disruption of the PDGF A-chain gene [6] and

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PDGFR α [7, 8] did not result in obvious abnormalities in the structure of the kidney.

In addition to the proposed roles during renal development, PDGF has also been proposed to mediate some of the cellular changes that follow injury to the adult kidney, including some manifestations of mesangial proliferative disorders. Fellstrom et al first reported that glomerular PDGFR β expression was elevated in a variety of human inflammatory and proliferative kidney diseases [9], and similar observations have been made by others [10, 11]. A role for PDGF in mesangial proliferative kidney disease is supported by results from cell culture studies and animal models of renal disease. Cultured mesangial cells express PDGF receptors and are both mitogenically [12] and chemotactically [13] stimulated by PDGF. Mouse models of mesangial proliferative glomerulonephritis demonstrate up-regulation of PDGF expression [14]. Immune-mediated mesangiolysis in rats results in initiation of glomerular production of PDGF B-chain and increased expression of PDGFR- β by mesangial cells [11], which is closely correlated with mesangial cell proliferation. A neutralizing antibody to PDGF markedly diminishes the cellular proliferation normally observed in this rat model of mesangial proliferative disease [15]. Mesangial cells proliferate in response to infused PDGF B-chain if first "primed" by a sublytic immunological challenge [16], indicating that mesangial cells can respond to PDGF *in vivo* as well as *in vitro*, but that responsiveness may require additional alterations in the state/environment of the target cells. PDGF B-chain expression *in vivo* has also been increased experimentally via direct transfection of rat glomeruli *in vivo* and results in cell proliferation in glomeruli [17]. PDGF may contribute to other renal diseases in addition to mesangial proliferative disease. Increased expression of PDGF A-chain is observed in the endothelium of arteries in kidneys undergoing transplant rejection [18], and the proliferative responses of glomerular visceral epithelial cells are associated with increased expression of PDGF B-chain [19].

Despite the significant number of studies on the role of PDGF/PDGFR during kidney development and disease, there has not been a comprehensive examination of PDGF or PDGFR expression patterns during development and in the normal adult mouse kidney. In this study, we examine the expression pattern of all four components of the PDGF ligand/receptor system sequentially through the late stages of murine embryogenesis and in the mature metanephric kidney, and identify patterns of localization from which potentially important epithelial/stromal interactions may be inferred. Knowledge of murine patterns of PDGF expression should be particularly useful in view of the intense efforts of many investigators to develop murine models of renal injury that resemble human diseases and well-studied models of kidney disease in rats, so that the tools of mouse genetics can be brought to bear on mechanistic questions of pathophysiology.

METHODS

Source of tissue

Time-mated pregnant Swiss-Webster mice were ordered from Simonsen Laboratories (Gilroy, CA, USA) and housed by the Department of Comparative Medicine until tissues were taken for processing. The mice were euthanized by CO₂ asphyxiation and adult and embryonic tissues (E14.5-E17.5) fixed in 4% paraformaldehyde, 0.1 M sodium phosphate buffer pH 7.4 for 3 to 7 days prior to embedding in paraffin. Adult kidneys were transversely bisected before fixation. Embryonic kidneys were fixed *in situ* after opening the back and abdominal cavity to allow penetration of the fixative.

Molecular probes

Antisense riboprobes were produced from the T7 promoter after the plasmid (described below) had been linearized with *Hind*III. All the probes were labeled by incorporation of [α -³²P]UTP (New England Nuclear, Boston, MA, USA) using an *in vitro* transcription kit (Promega Biotec, Madison, WI, USA) and hydrolyzed to an average length of 150 nt.

PDGFR α . A 1636 bp *Eco*RI fragment of the mouse cDNA (GenBank Acc. # M57683; nt 201-1837) was cloned into the *Eco*RI site of pGEM-3Zf+ (Promega Biotec). The full length probe is 1692 nt and corresponds to the extracellular domain, the transmembrane domain, and the first 40 aa of the intracellular domain.

PDGFR β . A 2075 bp *Pst*I fragment of the mouse cDNA (GenBank Acc. # X04367; nt 1667 to 3742) was cloned into the *Pst*I site of pGEM-3Zf+ (Promega Biotec). The full length probe is 2131 nt and corresponds to 20 aa of extracellular, all of the transmembrane and intracellular domains, and 310 nt of 3' untranslated cDNA.

PDGF A-chain. A 536 bp *Sma*I fragment of the mouse cDNA (GenBank Acc. M29464; nt 1-906) was cloned into the *Sma*I site of pGEM-3Zf+ (Promega Biotec). The full length probe is 592 nt and corresponds to most of the mature PDGF A-chain molecule and 25 nt of 3' non-translated cDNA.

PDGF B-chain. A 806 bp *Eco*RI fragment of a mouse cDNA (cut from pMBSK which contains a 752 bp fragment of mouse B-chain, 26 bp of cloning vector DNA on the 3' end and 28 bp of cloning vector DNA on the 5' end; a kind gift from C. Stiles, Dana-Farber Cancer Institute, Boston, MA, USA) was cloned into the *Eco*RI site of pGEM-3Zf+ (Promega Biotec). The full length probe is 862 nt and corresponds to the whole PDGF B-chain molecule (752 nt), 30 nt of untranslated B-chain cDNA (8 nt 3' and 22 nt 5') and 80 nt of cloning vector DNA.

In situ hybridization

Six to 8 micron sections were deparaffinized, hydrated, and fixed for 10 minutes with 4% paraformaldehyde, 0.1 M

sodium phosphate, pH 7.4. The slides were then washed five minutes in 0.1 M sodium phosphate, pH 7.4, followed by 7.5 minutes in 20 μ g/ml proteinase K, 0.05 M Tris pH 7.1, 5 mM EDTA at 37°C, five minutes in 4% paraformaldehyde, 0.1 M sodium phosphate, pH 7.4, and then five minutes in 0.1 M sodium phosphate, pH 7.4. The sections were immersed in 0.1 M triethanolamine, pH 8.0, for five minutes, and then in 0.1 M triethanolamine, 0.25% acetic anhydride, pH 8.0, for 10 minutes with agitation for the first minute. The slides were next dipped 20 times in $2 \times$ SSPE, dehydrated through a graded series of ethanol, and air dried at room temperature. Hybridization buffer (50% formamide, $2 \times$ SSPE, $1 \times$ Denhardt's, 10% dextran sulfate, 20 mM Tris, pH 7.4, 5 mM EDTA, 100 μ g/ml yeast tRNA) containing 5×10^7 cpm/ml of ^{33} P-labeled RNA probe was added to the tissue sections and a coverslip applied. The sections were incubated overnight at 60°C. The slides were soaked for 15 minutes at room temperature in $2 \times$ SSC, the coverslips removed, and the slides soaked in fresh $2 \times$ SSC for 15 minutes. The slides were washed at high stringency, 10 minutes at 65°C in 50% formamide, 5 mM EDTA, 20 mM Tris, pH 7.4, and rinsed in $2 \times$ SSC. The sections were exposed to 20 μ g/ml RNase A in 10 mM Tris, pH 7.1, 1 mM EDTA, 0.5 M NaCl for 30 minutes at 37°C. They were then rinsed at room temperature in the same buffer but without RNase A. A high stringency wash at 65°C (as described above) was repeated and the sections were washed for 30 minutes in $2 \times$ SSC with gentle agitation. The sections were then dehydrated in a graded ethanol series, air dried at room temperature and dipped in Kodak NBT-2 emulsion. The emulsion coated slides were stored in the dark for three to six weeks, developed in Kodak D19 developer and counterstained with hematoxylin and eosin.

Probe specificity

The specificity of the riboprobes were first tested by Northern blotting using as a target either the plasmid DNA template from which the respective riboprobes were generated or total RNA isolated from 13-day mouse placenta RNA (previously performed Northern blots had shown that placenta expresses all four of the PDGF and PDGF receptor genes). After RNase treatment to reduce non-specific hybridization, both sense and antisense P-33 riboprobes labeled the double-stranded plasmid DNA, but only the anti-sense probes labeled transcript bands appropriate for each of the target transcripts in placental RNA. The sense and anti-sense probes were then tested on sections of 13-day mouse placenta and sagittally sectioned 13-day mouse embryos. Each of the anti-sense probes labeled an array of cell types depending on the probe and the tissue type examined, and no labeling was seen with any of the sense probes in any of the tissues. In subsequent experiments the sense probes were not always included. The unique labeling pattern of the four probes served as a

controls for each other; that is, every cell type that was labeled by one of the probes was not labeled by one or more of the other probes.

RESULTS

PDGF and PDGFR expression during mouse renal development

Kidney development results from inductive interactions between ureteric bud epithelia and metanephric blastemal mesenchyme. The tip of ureteric bud branches and induces the kidney blastema to condense and form vesicles. These vesicles cavitate, elongate and undergo regional differentiation to form both glomeruli and tubules (proximal and distal tubules and the loop of Henle). The extensions of the branched ureteric bud fuse with the tubular system of the developing nephron and contribute the collecting tubules and ducts of the fully formed nephron. Our analysis of PDGF/PDGF receptor expression follows these developmental pathways; that is, expression in the ureteric bud epithelia, blastema, vesicle, glomerulogenesis, tubulogenesis and the kidney interstitium. Table 1 summarizes the expression data in both the embryonic and adult kidney.

Overview

We evaluated PDGF/PDGFR expression between days E14.5 through E17.5. We illustrate the findings with Figures taken from E17.5 embryos because kidneys at this developmental stage include examples of all stages of renal development. Figure 1 provides a low magnification overview of PDGF/PDGFR transcript expression in E17.5 mouse kidney. It is immediately apparent that the four PDGF/PDGFR genes are expressed in distinctly different patterns. PDGFR α transcripts are diffusely expressed throughout the interstitium, especially near the renal pelvis, while PDGFR β transcripts are also broadly expressed throughout the interstitium but are most highly expressed immediately adjacent to epithelial structures. PDGF A-chain transcripts are expressed in a punctate pattern at very high level near the renal pelvis and at lower level in more cortical regions. PDGF B-chain transcripts are broadly expressed throughout the kidney but also appear to be more highly expressed in a punctate pattern. The following sections describe these patterns of expression in greater detail. The locations from which subsequent high-magnification photos were taken are indicated by boxes in the H & E stained section in Figure 1.

Ureteric bud derivatives

Figure 2 shows collecting duct epithelium (derived from the ureteric bud) emptying into the renal pelvis. None of the PDGF/PDGFR genes are detected in the collecting duct epithelium nor in the tall transitional epithelium lining the renal pelvis immediately adjacent to the origin of the ureteric buds. This contrasts with the strong expression of

Table 1. Platelet-derived growth factor (PDGF) and its receptor expression in embryonic and adult mouse kidney

	PDGFR α	A-chain	PDGFR β	B-chain
Embryonic kidney				
Blastema and derivatives				
Blastema	++++	+/-	++	+/-
Vesicle epithelia	-	+	-	+/-
Comma epithelia	-	+	-	+/-
S-shaped epithelia	-	+	-	+
Glomerular epithelia	-	-	-	++
Proximal tubules	-	-	-	-
"Loop of Henle"	-	++++	-	+/-
Distal tubules	-	-	-	-
Ureteric bud and derivatives				
Ureteric bud	-	+/-	-	-
Collecting tubules	-	-	-	-
Urothelium	-	+	-	-
Mesenchyme and derivatives				
Mesenchyme	++++	+/-	++	+/-
Mesangial cells	+/-	-	++++	-
Endothelial cells	-	-	-	++
Smooth muscle cells	-	++	+++	-
Adult kidney				
Blastema-derived epithelia				
Glomerular epithelia	-	-	-	-
Proximal tubules	-	-	-	-
"Loop of Henle"	-	+++	-	-
Distal tubules	-	-	-	-
Ureteric bud-derived epithelia				
Collecting tubules and ducts	-	-	-	-
Urothelium	-	-	-	-
Mesenchyme-derived cells				
Tubular interstitial cells	+++	-	++++	+/-
Lipid-laden interstitial cells	+++	-	+++	-
Mesangial cells	+/-	-	++++	-
Endothelial cells	-	-	-	++
Smooth muscle cells	-	+/-	-	-
Vascular adv. fibroblasts	+++	-	+++	-

This table summarizes the PDGF ligand and receptor expression data in the embryonic and adult mouse. The number of pluses is based on a visual estimate of expression is meant only to represent relative differences of expression for each probe. The data should not be viewed as quantitative nor providing comparison of expression levels between the different molecules. A +/- entry indicates that transcripts, if present, produce a signal that is barely above background silver grain density.

PDGF A-chain by nearby epithelial cells in the renal papilla. PDGF A-chain transcripts are expressed at low level in the transitional epithelial cells which line the rest of the renal pelvis in E16.5-E17.5 embryos (seen at low magnification in Fig. 1 but not apparent in Fig. 2). Figure 3 shows the branching tips of the ureteric bud which project into the blastemal mesenchyme. Again, these epithelial cells do not express detectable PDGFR α , PDGFR β , or PDGF B-chain, but there does appear to be low level expression of PDGF A-chain. Expression of PDGF A-chain by these epithelial cells is not much above the limit of detection at this time point, but is more highly and broadly expressed in ureteric bud epithelia of E14.5 embryos (data not shown). This suggests that as the ureteric bud branches and differentiates, expression of PDGF A-chain decreases. The low level expression by epithelial cells in Figures 2 and

3 contrasts with the striking expression of PDGFR α , PDGFR β , and PDGF B-chain within the interstitium (see below).

Blastema and glomeruli

The ureteric bud epithelium contacts the blastemal mesenchyme and induces vesicle formation at the outer edge of the embryonic kidney cortex. The blastema appears to express low levels of PDGFR α and PDGFR β transcripts but not PDGF A-chain or PDGF B-chain (Figs. 1 and 3). PDGF B-chain transcripts are found in the interstitium surrounding the vesicular epithelium, usually associated with blood vessel profiles. As the vesicular epithelium progresses to later (comma and S-phase) stages of glomerular development (Fig. 4), both PDGF A-chain and B-chain are expressed at low levels by epithelial cells. PDGF A-chain is expressed at the earliest stages (vesicle) whereas PDGF B-chain expression occurs at later stages (S-phase) of glomerular development (Fig. 4e). PDGFR α is expressed by cells surrounding the glomerulus and PDGFR β , and PDGF B-chain transcripts are present in the mesenchymal/interstitial cells that are recruited into the glomerular cleft and that will form the vascular tuft of the mature glomerulus. These cells express PDGFR β and PDGF B-chain transcripts at especially high levels during the later stages of glomerular development when capillaries are beginning to fill the glomerular tuft (Fig. 5). By this stage, PDGFR α transcripts are barely detectable and PDGF A-chain transcripts are undetectable.

Tubules

The distal portion of the vesicular epithelium elongates and forms the proximal and distal tubules, with the intervening ascending and descending tubules and the loop of Henle. Proximal tubules are readily identified by the large eosinophilic cytoplasm and do not express any of the PDGF/PDGFR transcripts (Fig. 6). Distal tubules are harder to distinguish in the cortical region but presumably do not express any of the PDGF/PDGFR genes either, since none of the tubular structures in the cortical region are labeled above a background level. PDGF A-chain transcripts are very highly expressed in tubules with a tightly packed cuboidal epithelium that are found near the renal pelvis (Figs. 2 and 7). The tight restriction of PDGF A-chain expression to a region near the border of the renal pelvis is very obvious in the low magnification overview (Fig. 1). These cells appear to be negative for PDGFR α , PDGFR β and PDGF B-chain.

Interstitium and vascular structures

Both PDGFR α and PDGFR β are broadly expressed in the embryonic kidney interstitium but in distinctly different patterns (Fig. 1). PDGFR α transcripts are expressed by most interstitial cells, with a gradient of expression level from highest expression near the renal pelvis and lowest

expression near the outer surface of the kidney. PDGFR β expression does not have a similar cortico-medullary gradient of expression and its pattern of expression is less uniform than that of PDGFR α (Fig. 1). PDGFR β is most highly expressed in association with blood vessels, including large and small interstitial blood vessels and the capillary-filled glomerular tuft (such as, Fig. 5). PDGF A-chain is not detectably expressed by interstitial cells. PDGF B-chain expression is mostly restricted to the endothelium of blood vessels, presumably expressed by endothelial cells. PDGF B-chain expression by endothelial cells is difficult to discern due to the high cell density and histological complexity of the embryonic kidney. Vascular/endothelial expression of PDGF B-chain is much more readily seen in the embryonic mid-brain (Fig. 5 e, f) where clusters of silver grains are nearly always associated with small blood vessels.

PDGF and PDGFR expression in adult kidney

Overview

Figure 8 provides a low magnification overview of the expression patterns of PDGF/PDGFR genes in the adult mouse kidney. The H&E stained section (top panel) shows that this section of kidney extends from the edge of the renal cortex down through the medullary region to the tip of the renal papilla. At this magnification it is apparent that PDGFR α expression is barely detectable in the cortex, is expressed at higher levels in the medulla, and is most highly expressed in the renal papilla. PDGFR α is also highly expressed by cells in the renal capsule and in the connective tissue lining the renal pelvis. PDGF A-chain expression is highest in the renal papilla with a gradient of expression ranging from high at the medullary-papillary border to low at the tip of the papilla. PDGFR β transcripts are expressed throughout the kidney, with the highest interstitial expression in the papilla and additional punctate expression in the cortex. PDGF B-chain expression is less readily detectable than the other PDGF/PDGFR members. However, PDGF B-chain does seem to be expressed focally in all layers of the kidney, sometimes by cells arranged in linear arrays oriented in a cortical to papillary direction. The following sections describe these patterns of expression in greater detail. The locations from which subsequent high-magnification photos were taken are indicated by boxes in the H & E stained section in Figure 8.

Glomerulus

Both PDGFR β and PDGF B-chain transcripts are expressed in the mature glomerulus (Fig. 9). PDGFR β is expressed in a mesangial cell pattern and PDGF B-chain might be expressed by either glomerular endothelial cells or podocytes. Neither PDGFR α nor PDGF A-chain are expressed at detectable levels.

Cortex

None of the PDGF/PDGFR genes are expressed by cortical tubular cells and PDGF A-chain transcripts are not detectably expressed anywhere in the renal cortex (with the possible exception of vascular smooth muscle cells, see below). PDGF B-chain is not highly expressed in the cortex (Fig. 8) but positive cells (probably endothelial cells; see below) are occasionally seen in the cortical interstitium (Fig. 10). Both PDGFR α and PDGFR β are expressed by interstitial cells surrounding the tubular epithelial cells of the renal cortex (Fig. 10). Based on a similar pattern of expression in human kidney that we evaluated by immunoelectron microscopy [20], the interstitial cells expressing PDGFR β are interstitial fibroblasts rather than capillary endothelial cells.

Medulla

None of the PDGF/PDGFR genes are detectably expressed in tubular or ductal epithelial cells in the medulla. Both PDGFR α and PDGFR β are expressed by interstitial cells surrounding the tubules and collecting ducts in the medullary region (Fig. 11). PDGF B-chain transcripts are present in cells arranged in linear arrays passing from the medullary into the cortical region. These cells are probably endothelial cells of the vasa recta.

Papilla

The relatively poor preservation of morphological detail in both the medulla and papilla after the *in situ* hybridization protocol makes recognition of cell types particularly difficult in these regions. Nonetheless, it appears that the expression of both PDGFR α and PDGFR β in the papillary region is in interstitial cells (not tubular epithelial cells), consistent with the expression pattern in the kidney cortex and medulla. In particular, expression of both PDGFR α and PDGFR β is highest in cells with elongated nuclei arranged in palisades (Fig. 12) having the appearance of the lipid-laden interstitial cells described by Lemly and Kriz [21]. This identification is based on two observations. First, PDGFR α expressing cells and lipid-laden interstitial cells have a similar distribution pattern in the adult kidney: they are absent from the cortex and increase in number as one proceeds from the medulla to the papilla. Second, the PDGFR α expressing cells have the same appearance as lipid-laden interstitial cells; that is, they form columnar palisades of nuclei between tubules. PDGF B-chain is also expressed by interstitial cells of unknown type. The papilla is the region of the kidney in which PDGF A-chain is most highly expressed, both in embryonic and adult kidneys (Figs. 1 and 8). Expression of PDGF A-chain in the adult kidney is largely restricted to tubular epithelial cells with large round nuclei at the medullary-papillary border. The number of cells expressing PDGF A-chain transcripts diminish to very low levels near the papillary tip.

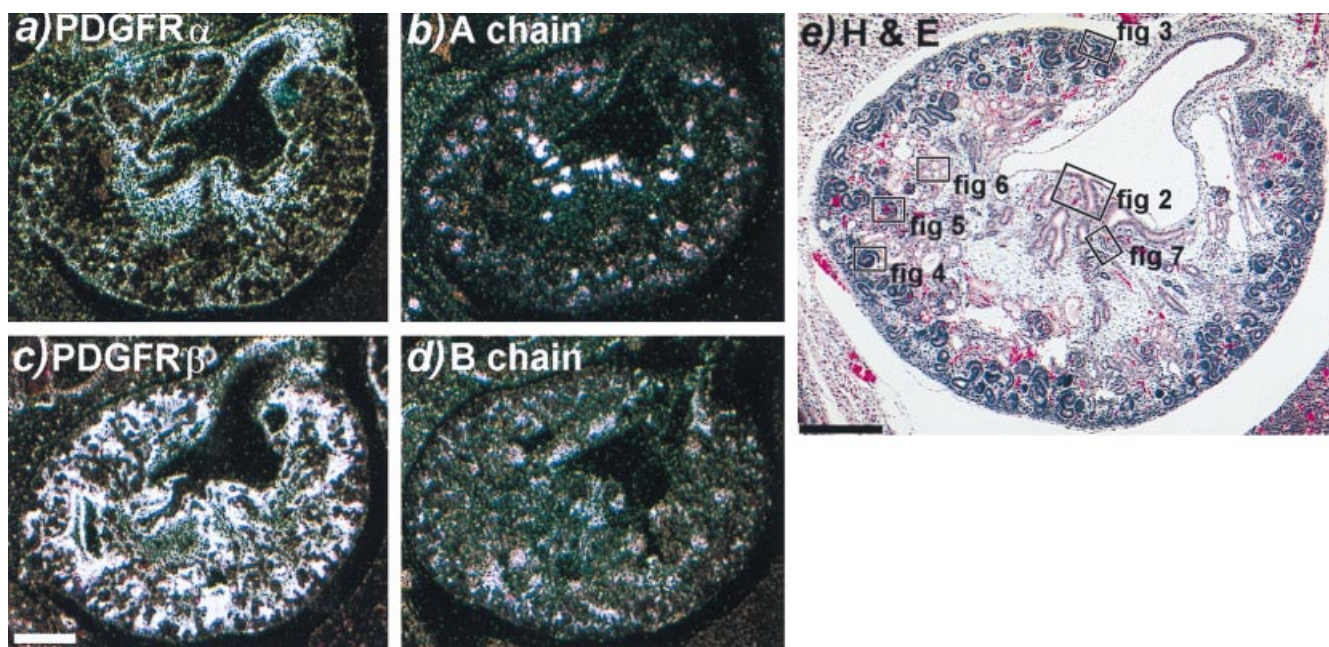


Fig. 1. PDGF/PDGFR expression in embryonic day 17.5 (E17.5) mouse kidney. These micrographs of kidney were obtained from parasagittal sections through whole E17.5 embryos and include the renal pelvis. Low magnification darkfield micrographs illustrate the pattern of expression of PDGFR α (a), PDGF A-chain (b), PDGFR β (c), and PDGF B-chain (d). Panel (e) is a brightfield micrograph of an adjacent H&E stained section that shows the morphology of the embryonic kidney and indicates the locations at which the higher magnification micrographs in Figures 2 to 7 were obtained. The white bar in panel c indicates 250 microns and the black bar in panel e indicates 200 microns.

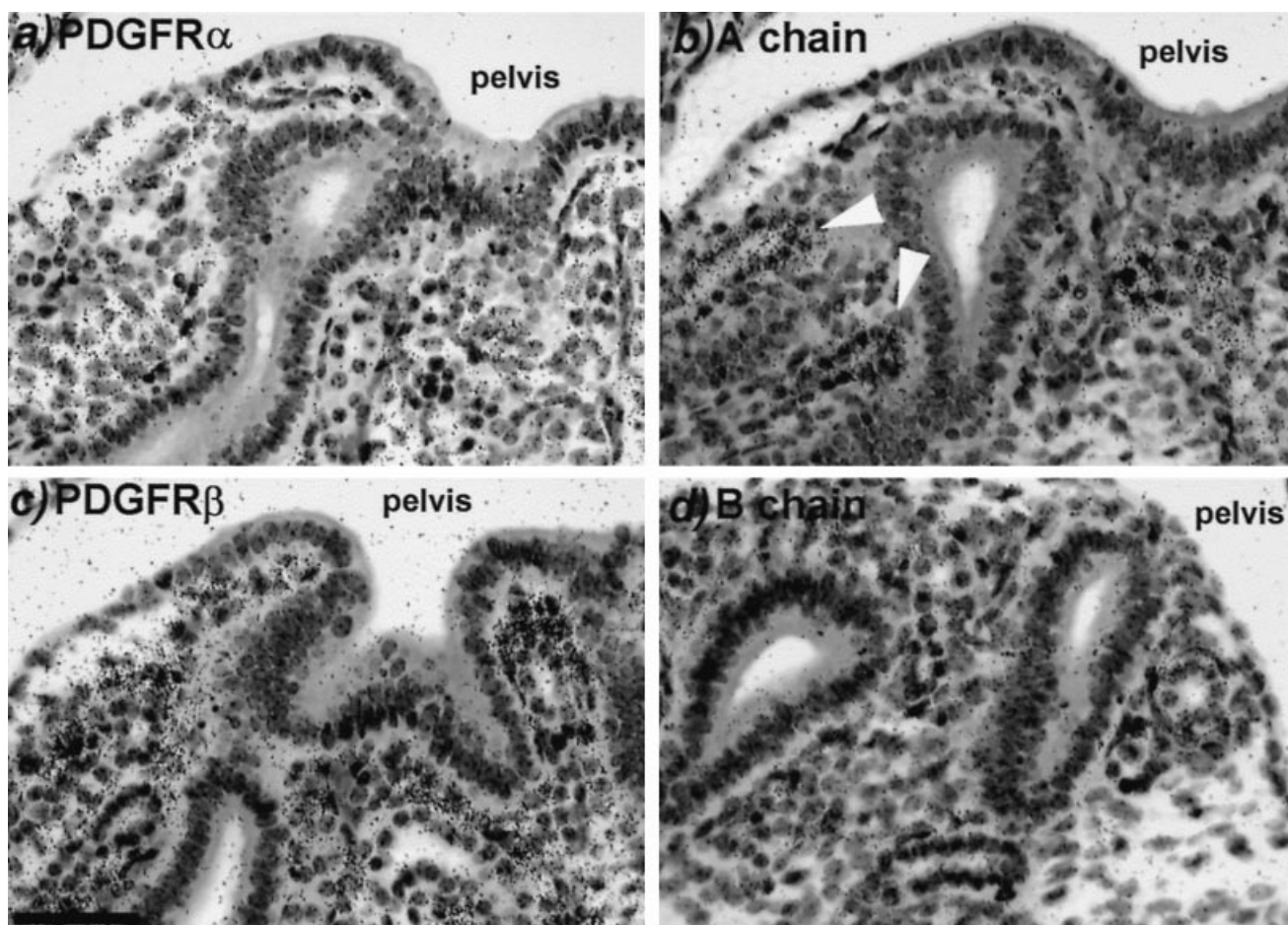


Fig. 2. PDGF/PDGFR expression near the renal pelvis of an E17.5 mouse. The white arrowheads in panel b indicate the tubular epithelia that express very high levels of PDGF A-chain. The bar in panel c indicates 50 microns.

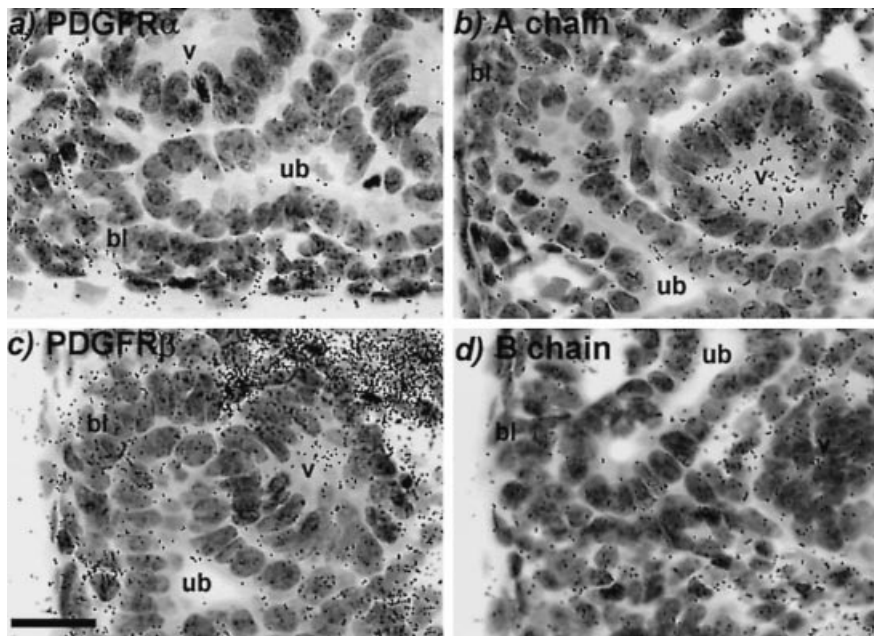


Fig. 3. PDGF/PDGFR expression in blastema and ureteric bud of an E17.5 mouse. Abbreviations are: ub, ureteric bud; v, epithelial-lined vesicle; bl, blastema. The bar in panel c represents 20 microns.

Vascular structures

PDGFR β , and to a lesser extent PDGFR α , transcripts are highly expressed by connective tissue cells surrounding both small arteries and arterioles (Figs. 13 and 14), but are not detectably expressed in the vascular SMCs themselves. In contrast, PDGF B-chain is expressed by endothelial cells of arterioles and small arteries (Figs. 13 and 14). PDGF A-chain expression is difficult to detect in vessel walls but occasionally appears to be expressed by vascular smooth muscle cells (for example, Fig. 13).

DISCUSSION

Comparison of PDGF and PDGFR expression in mouse to expression in other species

Some of the patterns of PDGF/PDGFR expression that we observed in the mouse kidney are similar to the pattern of expression observed in other species, but there are also notable differences between species.

PDGF A-chain

In both fetal human [22] and developing mouse kidney, PDGF A-chain is expressed by epithelial cells lining the renal pelvis and by the early blastemally-derived vesicular cells that will form glomerular and tubular epithelia. In the mouse, PDGF A-chain transcripts are most highly expressed in tubules with a tightly packed cuboidal epithelium that are found near the renal pelvis (Fig. 7). In humans, expression by the epithelia lining the renal pelvis remains very high in the adult kidney [22], whereas in the mouse it declines below the detection limit. In immature human glomeruli, PDGF A-chain is highly expressed by podocytes and continues to be expressed by podocytes and visceral epithelia in adult glomeruli [22]. By contrast, mouse podocytes

do not detectably express PDGF A-chain either during development nor in the adult glomerulus.

PDGF A-chain expression is readily detected in vascular smooth muscle cells of both fetal and adult human kidney [18, 22] and in larger muscular arteries elsewhere in the body [23, 24]. The E17.5 mouse kidney contains few vessels large enough to include obvious vascular smooth muscle cells. However, we did observe that PDGF A-chain is expressed by vascular smooth muscle cells in arteries elsewhere in the mouse embryo (unpublished observations). In the mouse, however, PDGF A-chain expression by vascular smooth muscle cells declines to undetectable levels in the adult kidney (Figs. 13 and 14).

PDGFR α

PDGFR α is broadly expressed by interstitial cells in developing mouse and human [25] kidneys especially by adventitial fibroblasts (Fig. 10 [25]). PDGFR α is expressed in vascular arcades in the developing human but not mouse kidney. Conversely, PDGFR α is expressed at significant levels by blastemal cells in mouse but not human. We did not detect PDGFR α expression in adult mouse glomeruli (Fig. 9) and we rarely detected PDGFR α expression in adult human glomeruli [25], although Gesuldo et al reported that PDGFR α expression is readily detectable in normal adult glomeruli [10].

PDGF B-chain

In both the mouse and human kidney, PDGF B-chain is expressed by epithelial cells of the developing glomerulus. In humans, PDGF B-chain expression is already apparent in S- and comma-shaped vesicles, whereas in mice expression is not detected until after the glomerulus has become

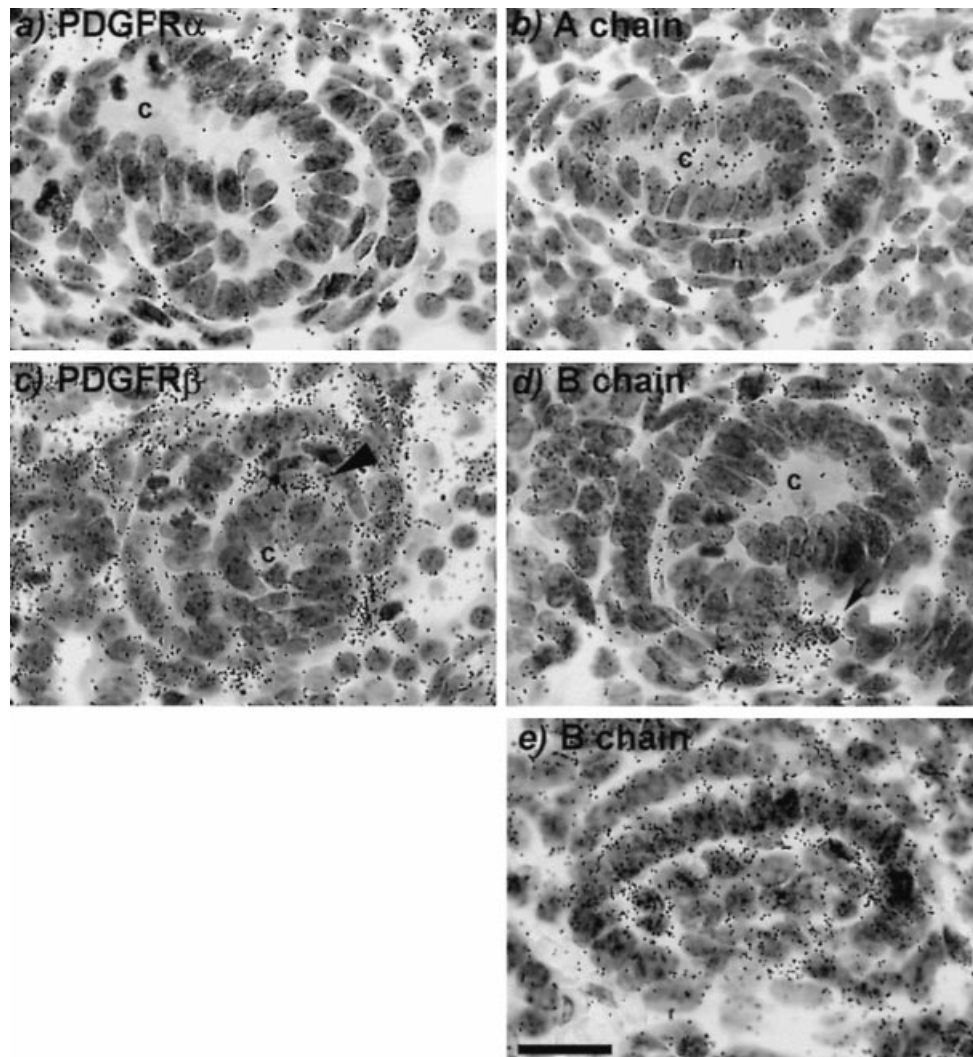


Fig. 4. PDGF/PDGFR expression during early glomerular differentiation of E17.5 mouse. The abbreviation c is the “comma-shaped” early stage differentiating glomeruli. The black arrowhead points to PDGFR β expressing cells entering the glomerular structure (c), and the black arrow points to PDGF B-chain expressing cells entering the glomerular structure (d). Epithelial expression of PDGF B-chain expression in early glomerulus is shown in panel e. The bar in panel e represents 20 microns.

vascularized. In humans PDGF B-chain is expressed in more differentiated glomeruli in a pattern suggesting that it is expressed by mesangial cells [26], whereas in the mouse PDGF B-chain expression in later stage glomeruli is more diffuse and the resolution of the ISH does not allow us to determine which cell types make PDGF B-chain. For the most part PDGF B-chain expression in the developing mouse kidney is associated with blood vessels and is probably due to endothelial cell expression. B-chain-expressing cells are seen being recruited into the forming mouse glomeruli and B-chain is relatively highly expressed in vascularized glomeruli. In the adult mouse kidney, expression of PDGF B-chain remains restricted to endothelial cells in glomeruli and blood vessels. Low but detectable expression of PDGF B-chain transcripts are also found in adult rat glomeruli [11] [27]. We did not observe PDGF

B-chain expression in distal and collecting tubules as is seen in normal rat kidney [28, 29].

PDGFR β

PDGFR β is expressed by blastemal cells in both developing mouse and human [26] kidneys, but at different levels. In fetal human kidney, PDGFR β expression in blastema is at least as strong as expression in kidney mesenchyme. In embryonic mouse kidney, PDGFR β expression in blastema is much lower than in kidney mesenchyme. Strong expression of PDGFR β is observed in newly formed glomeruli in both mouse (Fig. 5) and human [26] and PDGFR β expression by mesangial cells persists in adult kidneys from mouse, rat and human. Studies in rats have suggested that PDGFR β expressed by mesangial cells in adult kidneys can be engaged by PDGF released locally

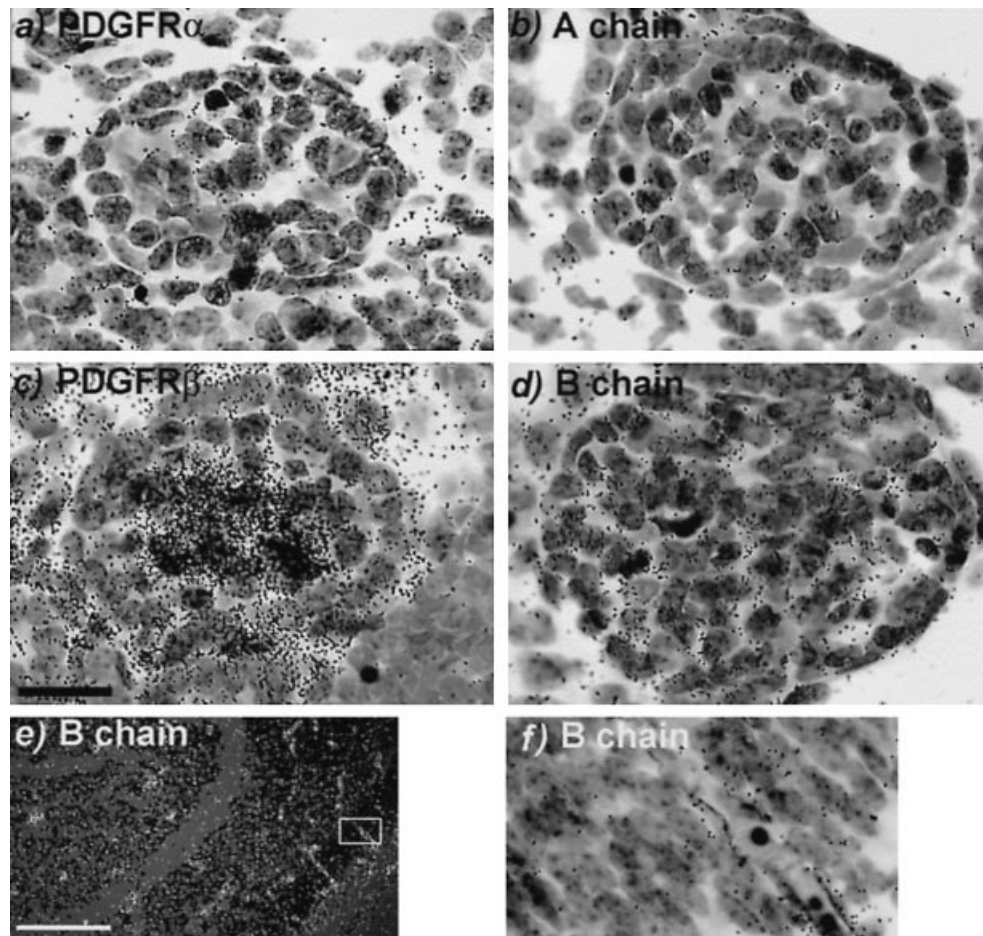


Fig. 5. PDGF/PDGFR expression in vascularized glomeruli of E17.5 mouse. Early stage glomerular structures containing capillaries are shown in panels a-d. The low power darkfield view of brain from a 15-day-old mouse embryo shows PDGF B-chain transcript expression (e). The area encompassed by the white box is shown at higher magnification in panel f. The black bar in panel c represents 20 microns and applies to panels a-d and f; the white bar in panel e represents 200 microns.

by infiltrating macrophages or platelets or by the mesangial cells themselves in injury states [11]. The second class of PDGFR β -expressing cells are connective tissue cells, including adventitial fibroblasts surrounding blood vessels, and interstitial cells (Figs. 9, 10, 13 and 14) [11, 20, 26, 30, 31]. The interstitial cells expressing PDGFR β are probably fibroblasts, not endothelial cells [20]. We did not observe PDGFR β expression in parietal epithelial cells as has been reported in adult human kidneys [20], nor in distal or collecting epithelial cells as observed in rat kidney [29].

Significance and correlates of the pattern of PDGF/PDGFR genes in mouse kidney

Orr-Urtreger and Lonai compared expression of PDGF A-chain and PDGFR α in mouse embryos (but not in the kidney) and observed that PDGF A-chain is generally expressed in epithelial cells, whereas PDGFR α is expressed by adjacent mesenchymally-derived stromal cells [32]. In their review of PDGF/PDGFR expression during develop-

ment, Ataliotis and Mercola support this relationship and suggest that this reciprocal expression pattern indicates a role for PDGF/PDGFR in inductive interactions during development [33]. We also observe pair-wise expression of PDGF ligand and PDGFR in both the developing and adult kidney in the current study. PDGF A-chain expression in epithelial cells is associated with PDGFR α expression in mesenchymal cells and PDGF B-chain expression in endothelial cells is associated with PDGFR β expression in vascular smooth muscle cells.

PDGF A-chain and PDGFR α

Of the four PDGF/PDGFR genes, PDGF A-chain is the gene primarily expressed by differentiated epithelial structures in the mouse kidney. PDGF A-chain is expressed at day E14.5 throughout the length of the ureteric tube (data not shown) and at later developmental times (E17.5) expression is still elevated at the tips of the ureteric bud in the cortex and in epithelial cells lining the renal pelvis (Figs. 1 and 3). The highest expression of PDGF A-chain in

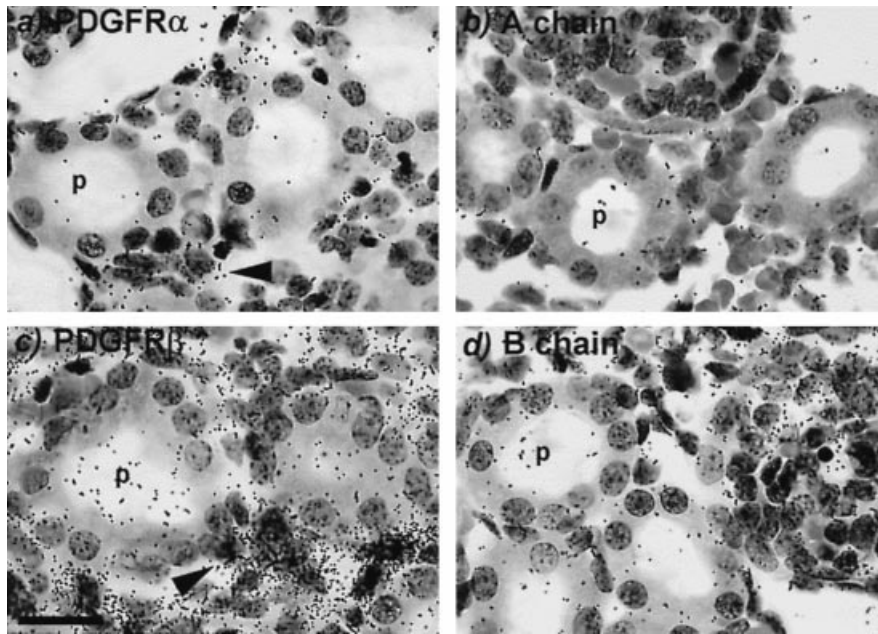


Fig. 6. PDGF/PDGFR expression around cortical tubules of an E17.5 mouse. Abbreviation P is proximal tubule. Black arrowheads point to interstitial cells expressing PDGFR α (a) or PDGFR β (c) transcripts. The bar in panel c represents 20 microns.

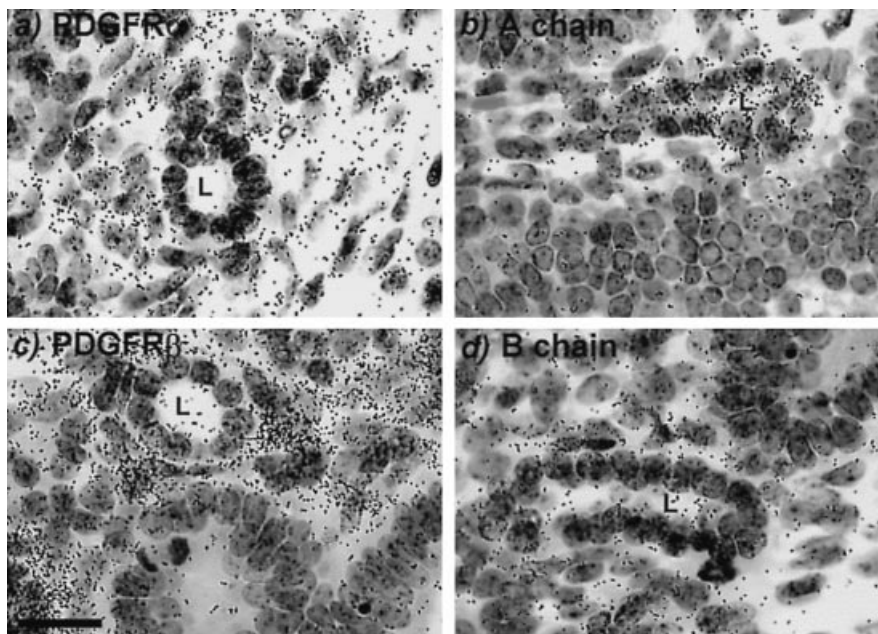


Fig. 7. PDGF/PDGFR expression in medulla of an E17.5 mouse. Abbreviation L is loop of Henle tubular epithelia. The bar in panel c represents 20 microns.

both embryonic and adult kidney is in tubular epithelial cells near the renal pelvis (Figs. 7 and 8). We suggest that these tubular structures in both the embryonic and the adult kidney are portions of the loop of Henle. We base this suggestion on the following observations. (a) Neither the proximal nor distal tubule epithelia in either the embryonic or the adult kidney cortex express PDGF A-chain. (b) PDGF A-chain labeled tubular epithelial cells are located near the fetal renal pelvis and in the adult renal papilla but yet are not seen emptying into the renal pelvis. Rather, they

appear to terminate or to loop back to the cortex, which are the expected location and characteristics of the tubular epithelia of the loop of Henle. Within this structure, A-chain expression appears to be restricted to the epithelia occupying the deepest position of the loop (Fig. 7) and, at least in the adult kidney, the ascending and descending arms of the loop of Henle do not appear to have PDGF A-chain expressing epithelial cells.

PDGFR α transcripts are broadly expressed by mesenchymal/interstitial cells, and are highest in cells nearest the

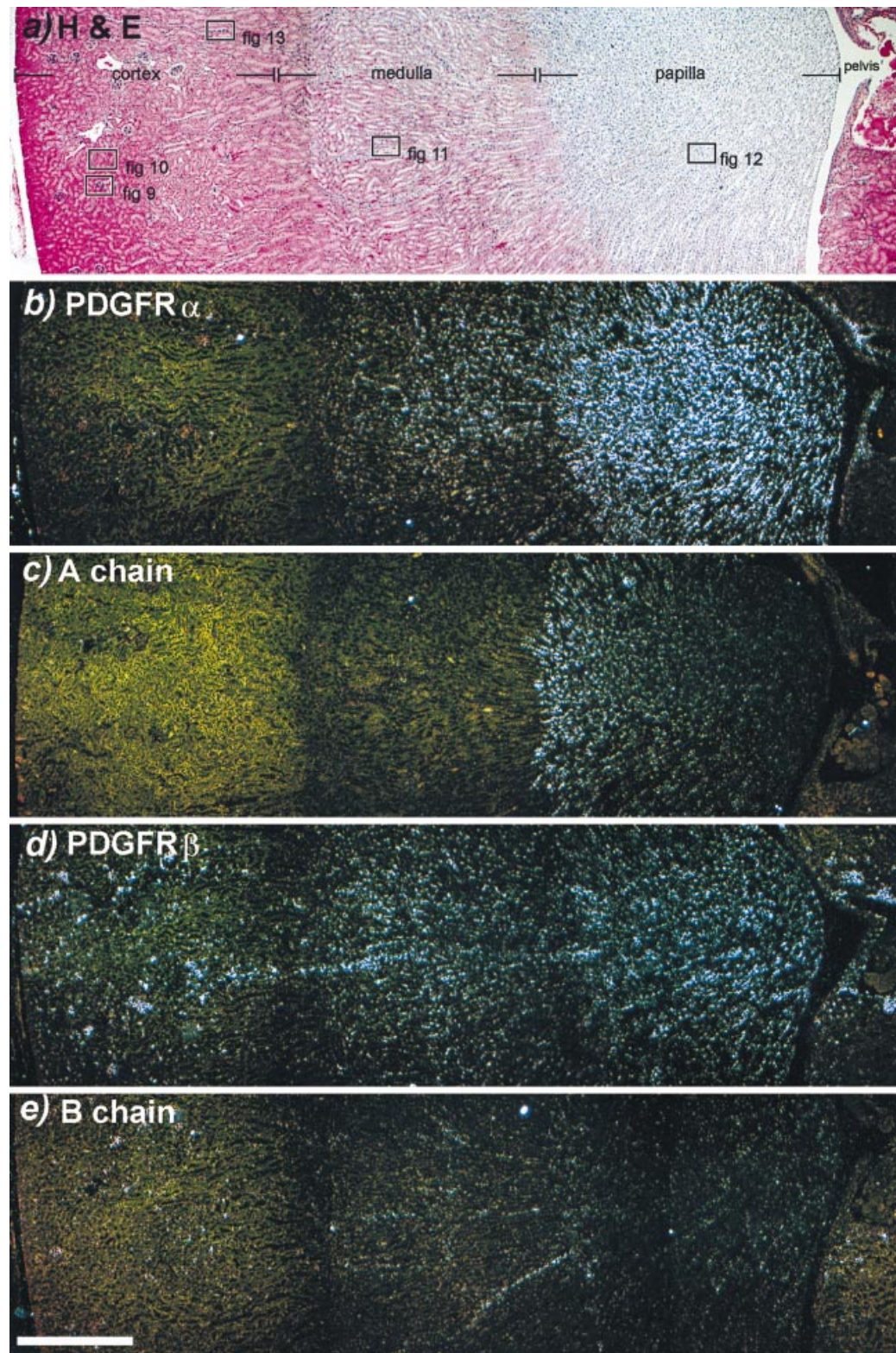


Fig. 8. PDGF/PDGFR expression in the adult mouse kidney. (a) Brightfield micrograph of an H & E stained longitudinal section that shows the morphology of the adult kidney including the cortex, medulla and the papilla extending into the renal pelvis and indicates the approximate locations at which the higher magnification micrographs in Figures 9 to 13 were obtained. Darkfield micrographs of adjacent sections evaluated by *in situ* hybridization of expression of PDGFR α (b), PDGF A-chain (c), PDGFR β (d), and PDGF B-chain (e). The bar in panel e represents 500 microns.

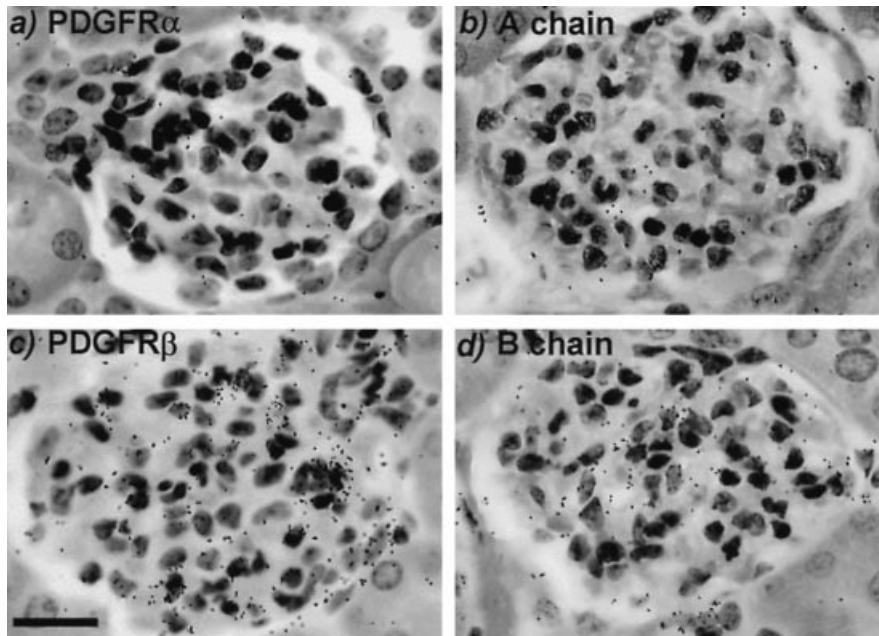


Fig. 9. PDGF/PDGFR expression in the glomerulus of an adult mouse. The bar in panel c represents 20 microns.

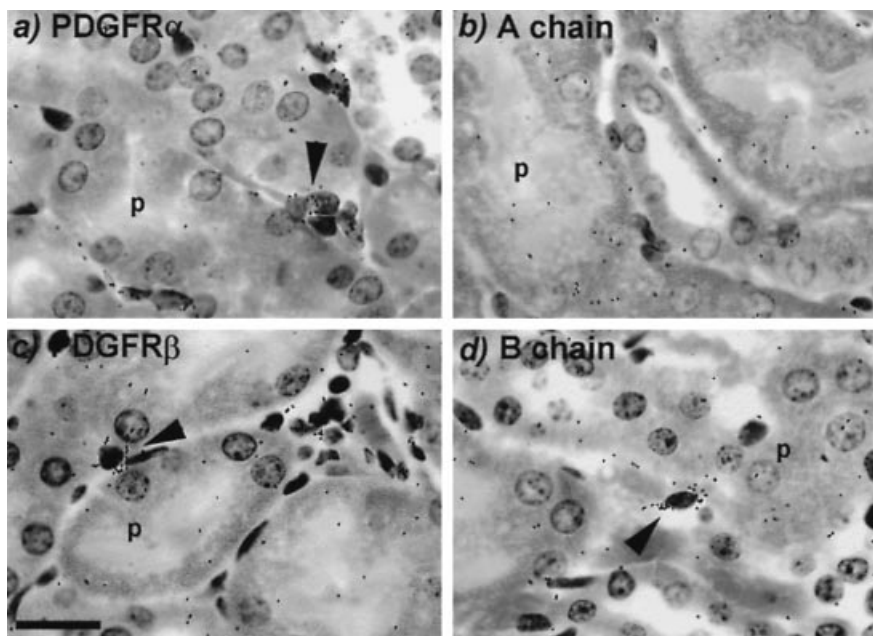


Fig. 10. PDGF/PDGFR expression around proximal tubules of an adult mouse kidney. Abbreviation P is proximal tubule. The black arrowheads indicate interstitial cells expressing PDGFR α (a), PDGFR β (c) or PDGF B-chain (d). The bar in panel c represents 20 microns.

sources of PDGF A-chain. Adventitial fibroblasts around developing blood vessels express PDGFR α and may receive trophic support via PDGF A-chain from the underlying vascular smooth muscle cells. The highest expression of both PDGF A-chain and PDGFR α is in the renal pyramid of both the embryonic and adult kidney (Figs. 1 and 8). In the embryonic kidney, PDGFR α is expressed by undifferentiated mesenchymal cells but in the adult, expression in the renal pyramid is largely limited to a distinctive interstitial cell type that we believe is the lipid-laden interstitial cell described by Lemley and Kriz [21].

The paired expression pattern for PDGF A-chain and PDGFR α suggests that PDGF A-chain acting via PDGFR α may drive stroma formation in these regions. This is supported by the phenotype of the *Patch* mutation in mouse, in which inactivation of the PDGFR α gene results in a dramatic deficit of fibroblasts in every tissue [7]. The inactivation of PDGF A-chain by homologous recombination [6] did not have this general and severe effect on connective tissue development. Since both PDGF A-chain and PDGF B-chain can bind to PDGFR α , it is likely that both ligand genes contribute to activation of PDGFR α in

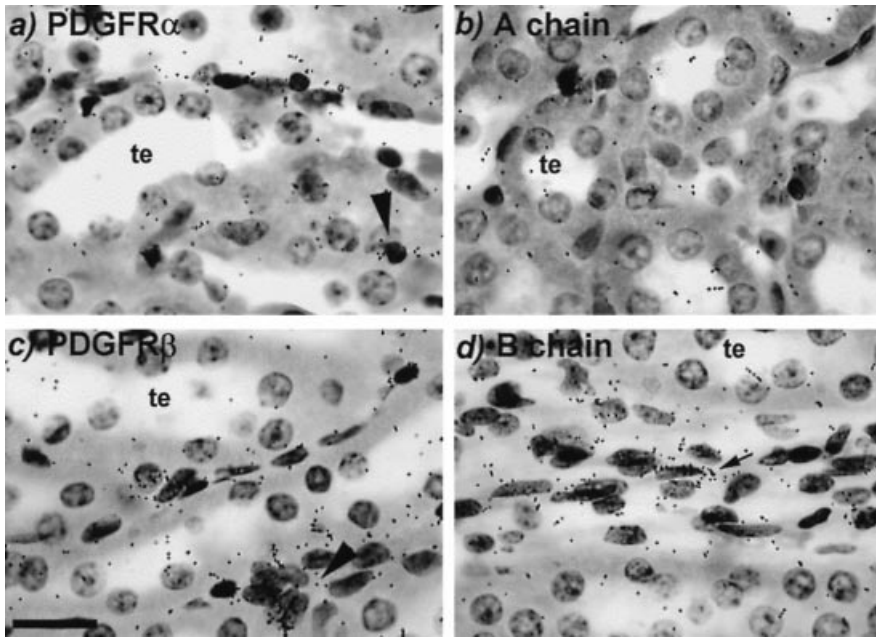


Fig. 11. PDGF/PDGFR expression in medullary region of an adult mouse kidney. Abbreviation te is tubular epithelia. The black arrowheads indicate interstitial cells expressing PDGFR α (a) or PDGFR β (c), and the black arrow in (d) indicates PDGF B-chain expressing cells in a longitudinally sectioned blood vessel in the medullary region. The bar in panel c represents 20 microns.

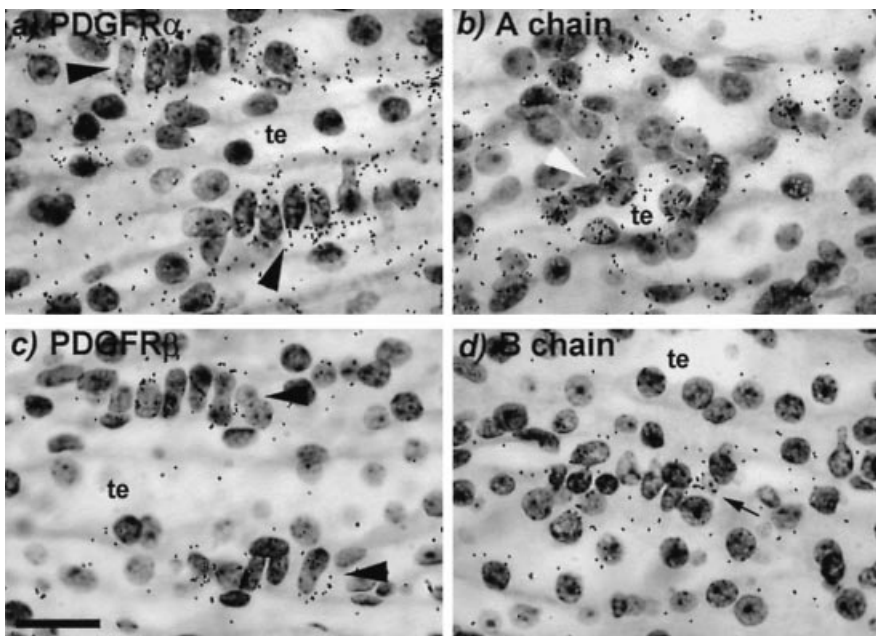


Fig. 12. PDGF/PDGFR expression in papillary region of an adult mouse kidney. The abbreviation te is tubular epithelia. The black arrowheads indicate lipid-laden interstitial cells expressing PDGFR α (a) or PDGFR β (c), the white arrowhead (b) indicates PDGF A-chain expression by tubular epithelial cells, and the black arrow (d) indicates interstitial cells expressing PDGF B-chain. The bar in panel c represents 20 microns.

connective tissue so that disruption of PDGFR α produces a more severe phenotype than does disruption of either ligand gene alone. The continued expression of PDGFR α in adult stroma, especially in the papilla, is consistent with a role for PDGF in the interstitial fibrosis that occurs in some pathological conditions. In humans, these cells have been considered the cell of origin of medullary fibrosis, a common finding at autopsy but one of unknown clinical significance [34]. These cells also have been implicated in the production of vasoactive hormones (possibly prostaglandins) that exert an anti-hypertensive effect in exper-

imental disease models [35]. Localization of PDGFR α and PDGFR β to these cells offers the first clue as to what signaling molecules may be involved in their regulation.

PDGF B-chain and PDGFR β

PDGF B-chain and PDGFR β expression in the embryonic kidney is greatest in glomeruli and in developing vascular structures. As the blastemally-derived vesicle elongates and folds, it encapsulates a portion of surrounding mesenchyme and associated vasculature. The mesenchyme

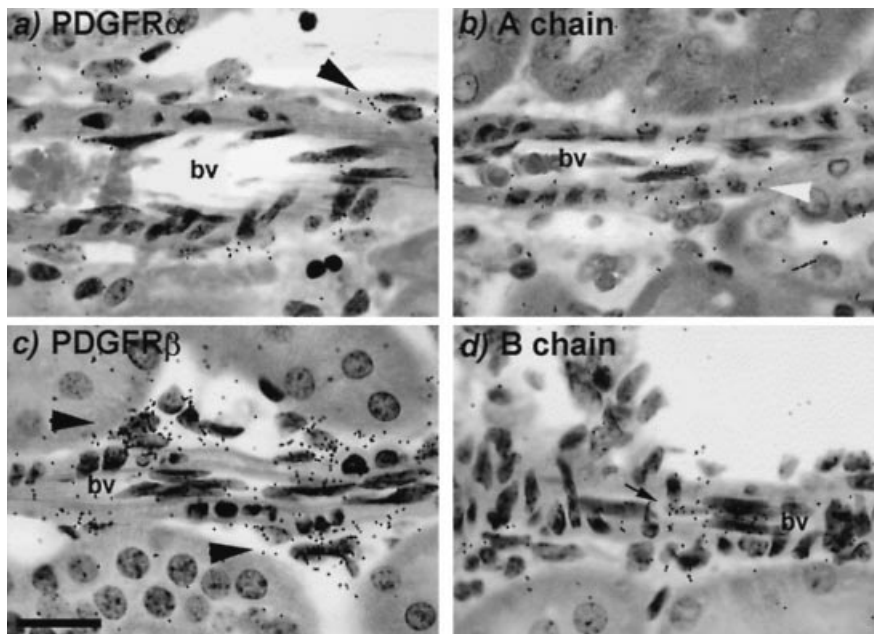


Fig. 13. PDGF/PDGFR expression in small blood vessels of an adult mouse. The abbreviation bv is blood vessel. The black arrowheads indicate adventitial cells expressing PDGFR α (a) or PDGFR β (c). The white arrowhead (b) indicates low level PDGF A-chain expression by vascular smooth muscle cells, and the black arrow indicates vascular endothelial cells expressing PDGF B-chain (d). The bar in panel c represents 20 microns.

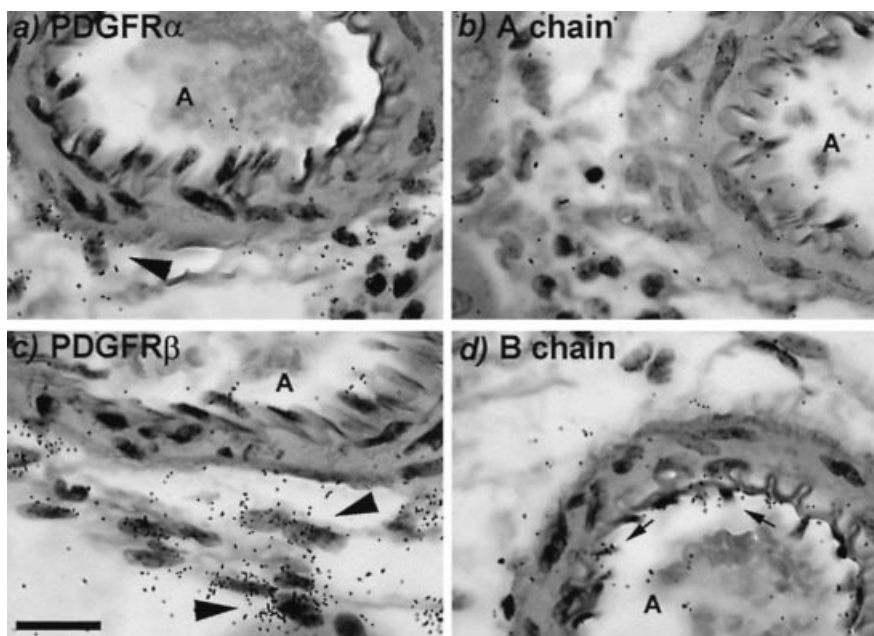


Fig. 14. PDGF/PDGFR expression in the renal arteries of an adult mouse. The abbreviation A is the artery in kidney. The black arrowheads indicate adventitial cells expressing PDGFR α (a) or PDGFR β (c). The black arrows indicate vascular endothelial cells expressing PDGF B-chain (d). The bar in panel c represents 20 microns.

enveloped by the folding vesicle expresses high levels of PDGFR β and PDGF B-chain transcripts (Fig. 4) associated with the recruited vasculature. PDGF B-chain and PDGFR β are both highly expressed in early stage glomeruli (Fig. 5), presumably by capillary endothelial cells and mesangial cell precursors, respectively, and PDGF B-chain expression remains detectable in glomerular capillaries in adult mouse kidneys (Fig. 9). The importance of PDGF B-chain and PDGFR β for formation of the glomerular vasculature is underscored by the characteristic malforma-

tion observed in the kidneys of mice in which expression of either of these genes has been eliminated by homologous recombination [4, 5]. The glomeruli in these mice do not contain mesangial cells and the endothelial cells form a single or bi-lobed aneurysmal bulb that fills the glomerulus. These results suggest that recruitment of capillary endothelial cells occurs independently of PDGF/PDGFR genes but that recruitment/proliferation of mesangial cells is highly dependent on PDGF B-chain expression by endothelial cells. This interaction has been proposed by Lindahl et al

based on similar observation of PDGF B-chain and PDGFR β expression patterns in the brain, kidney and other organs [36].

PDGF B-chain and PDGFR β are also highly expressed by developing vascular cells outside of the glomerulus. In adult kidneys, PDGF B-chain expression remains characteristic of endothelial cells in arteries and arterioles (Figs. 13 and 14), and probably interstitial capillary endothelial cells. (Figs. 10 to 12). PDGFR β is often expressed by cells adjacent to these PDGF B-chain expressing endothelial cells, for example, by pericytes and/or interstitial fibroblasts [20] in the cortex, medulla, and papilla (Figs. 10 to 12). An interesting exception is seen in larger blood vessels in adult kidneys (and in other adult organs). In these vessels, PDGFR β is highly expressed by adventitial cells but is not detectably expressed by the smooth muscle cells (Figs. 13 to 14). Since PDGFR β expression is high in smooth muscle cells of all of embryonic large arteries, even as late as E17 (our unpublished observations), PDGFR β expression by vascular smooth muscle cells must be markedly diminished at some time between late gestation and adulthood. This downregulation of receptor expression may be the mechanism through which the continued expression of PDGF B-chain by the endothelial cells is kept from driving continuous proliferation of adjacent smooth muscle cells in the mature vessel. Conversely, upregulation of PDGF receptors may be involved in enhancing signaling via the PDGF B-chain/PDGFR β system in pathological responses in the kidney [10, 11].

In summary, we provide the first complete map of transcript expression of the PDGF ligand/receptor system in the developing and mature mouse kidney, and have discussed how the pattern of expression compares with that in other species. An important and consistent observation between mice and humans is the widespread expression of PDGFR α receptor and PDGFR β receptor in the mesangial and interstitial cells of developing and mature kidneys. It has been shown in rats that these cells are responsive to infused PDGF B-chain *in vivo*, and that these cells mediate some of the sclerosing processes of chronic renal injury in rats and humans. Some of our findings, such as the predominant expression of PDGF A-chain in medullary regions, are without precedent from studies in other species. Knowledge of the similarities (and differences) between mouse and other species will be particularly useful as murine models of renal disease are developed. The mouse models will allow transgenic and knockout lines to be used to test the functions of specific genes that have been deduced from studies of rat models and human specimens. However, the significance of those findings for human pathologies will need to be considered in light of the similarities (and differences) between the ways that genes are expressed in different species.

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APPENDIX

Abbreviations used in this article are: PDGF, platelet-derived growth factor; PDGF/PDGFR, PDGF ligands and receptors; PDGFR α , PDGF receptor α -subunit; PDGFR β , PDGF receptor β -subunit.

REFERENCES

- ROSS R, RAINES EW, BOWEN-POPE DF: The biology of platelet-derived growth factor. *Cell* 46:155–169, 1986
- SEIFERT RA, HART CE, PHILLIPS PE, FORSTROM JW, ROSS R, MURRAY MJ, BOWEN-POPE DF: Two different subunits associate to create isoform-specific platelet-derived growth factor receptors. *J Biol Chem* 264:8771–8778, 1989
- WILLIAMS LT: Signal transduction by the platelet-derived growth factor receptor. *Science* 243:1564–1570, 1989
- LEVEEN P, PEKNEY M, GEBRE-MEDHIN S, SWOLIN B, LARSSON E, BETSHOLTZ C: Mice deficient for PDGF B show renal, cardiovascular, and hematological abnormalities. *Genes Dev* 8:1875–1887, 1994
- SORIANO P: Abnormal kidney development and hematological disorders in PDGF beta-receptor mutant mice. *Genes Dev* 8:1888–1896, 1994
- BOSTROM H, WILLETTTS K, PEKNEY M, LEVEEN P, LINDAHL P, HEDSTRAND H, PEKNA M, HELLSTROM M, GEBRE-MEDHIN S, SCHALLING M, NILSSON M, KURLAND S, TORNELL J, HEATH JK, BETSHOLTZ C: PDGF-A signaling is a critical event in lung alveolar myofibroblast development and alveogenesis. *Cell* 85:863–873, 1996
- SCHATTEMAN GC, MORRISON-GRAHAM K, VAN KOPPEN A, WESTON JA, BOWEN-POPE DF: Regulation and role of PDGF receptor alpha-subunit expression during embryogenesis. *Development* 115:123–131, 1992
- SORIANO P: The PDGF alpha receptor is required for neural crest cell development and for normal patterning of the somites. *Development* 124:2691–2700, 1997
- FELLSTROM B, KLARESKOG L, HELDIN CH, LARSSON E, RONNSTRAND L, TERRACIO L, TUFVESON G, WAHLBERG J, RUBIN K: Platelet-derived growth factor receptors in the kidney—Upregulated expression in inflammation. *Kidney Int* 36:1099–1102, 1989
- 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
- IIDA H, SEIFERT R, ALPERS CE, GRONWALD RG, 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. *Proc Natl Acad Sci USA* 88:6560–6564, 1991
- SHULTZ PJ, DICORLETO PE, SILVER BJ, ABOUD HE: Mesangial cells express PDGF mRNAs and proliferate in response to PDGF. *Am J Physiol* 255:F674–F684, 1988
- BARNES JL, HEVEY KA: Glomerular mesangial cell migration in response to platelet-derived growth factor. *Lab Invest* 62:379–382, 1990
- GESUALDO L, PINZANI M, FLORIANO JJ, HASSAN MO, NAGY NU, SCHENA FP, EMANCIPATOR SN, ABOUD HE: Platelet-derived growth factor expression in mesangial proliferative glomerulonephritis. *Lab Invest* 65:160–167, 1991
- JOHNSON RJ, RAINES EW, FLOEGE J, YOSHIMURA A, PRITZL P, ALPERS C, ROSS R: Inhibition of mesangial cell proliferation and matrix expansion in glomerulonephritis in the rat by antibody to platelet-derived growth factor. *J Exp Med* 175:1413–1416, 1992

16. FLOEGE J, ENG E, YOUNG BA, ALPERS CE, BARRETT TB, BOWEN-POPE DF, JOHNSON RJ: Infusion of platelet-derived growth factor or basic fibroblast growth factor induces selective glomerular mesangial cell proliferation and matrix accumulation in rats. *J Clin Invest* 92:2952-2962, 1993
17. ISAKA Y, FUJIWARA Y, UEDA N, KANEDA Y, KAMADA T, IMAI E: Glomerulosclerosis induced by in vivo transfection of transforming growth factor-beta or platelet-derived growth factor gene into the rat kidney. *J Clin Invest* 92:2597-2601, 1993
18. ALPERS CE, DAVIS CL, BARR D, MARSH CL, HUDKINS KL: Identification of platelet-derived growth factor A and B chains in human renal vascular rejection. *Am J Pathol* 148:439-451, 1996
19. 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 platelet-derived growth factor B-chain. *Am J Pathol* 142:637-650, 1993
20. 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
21. LEMLEY KV, KRIZ W: Anatomy of the renal interstitium. *Kidney Int* 39:370-381, 1991
22. ALPERS CE, HUDKINS KL, FERGUSON M, JOHNSON RJ, RUTLEDGE JC: Platelet-derived growth factor A-chain expression in developing and mature human kidneys and in Wilms' tumor. *Kidney Int* 48:146-154, 1995
23. 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
24. MURRY CE, BARTOSEK T, GIACHELLI CM, ALPERS CE, SCHWARTZ SM: Platelet-derived growth factor-A mRNA expression in fetal, normal adult, and atherosclerotic human aortas. Analysis by competitive polymerase chain reaction. *Circulation* 93:1095-1106, 1996
25. FLOEGE J, HUDKINS KL, SEIFERT RA, FRANCKI A, BOWEN-POPE DF, ALPERS CE: Localization of PDGF alpha-receptor in the developing and mature human kidney. *Kidney Int* 51:1140-1150, 1997
26. ALPERS CE, SEIFERT RA, HUDKINS KL, JOHNSON RJ, BOWEN-POPE DF: Developmental patterns of PDGF B-chain, PDGF-receptor, and alpha-actin expression in human glomerulogenesis. *Kidney Int* 42:390-399, 1992
27. FLOEGE J, BURNS MW, ALPERS CE, YOSHIMURA A, PRITZL P, GORDON K, SEIFERT RA, BOWEN-POPE DF, COUSER WG, JOHNSON RJ: Glomerular cell proliferation and PDGF expression precede glomerulosclerosis in the remnant kidney model. *Kidney Int* 41:297-309, 1992
28. YOSHIMURA A, GORDON K, ALPERS CE, FLOEGE J, PRITZL P, ROSS R, COUSER WG, BOWEN-POPE DF, JOHNSON RJ: Demonstration of PDGF B-chain mRNA in glomeruli in mesangial proliferative nephritis by in situ hybridization. *Kidney Int* 40:470-476, 1991
29. KLIEM V, JOHNSON RJ, ALPERS CE, YOSHIMURA A, COUSER WG, KOCH KM, FLOEGE J: Mechanisms involved in the pathogenesis of tubulointerstitial fibrosis in 5/6-nephrectomized rats. *Kidney Int* 49:666-678, 1996
30. RUBIN K, TINGSTROM A, HANSSON GK, LARSSON E, RONNSTRAND L, KLARESKOG L, CLAESSON-WELSH L, HELDIN CH, FELLSTROM B, TERRACIO L: Induction of B-type receptors for platelet-derived growth factor in vascular inflammation: Possible implications for development of vascular proliferative lesions. *Lancet* 1:1353-1356, 1988
31. FRANKLIN WA, CHRISTISON WH, COLLEY M, MONTAG AG, STEPHENS JK, HART CE: In situ distribution of the beta-subunit of platelet-derived growth factor receptor in nonneoplastic tissue and in soft tissue tumors. *Cancer Res* 50:6344-6348, 1990
32. ORR-URTREGER A, LONAI P: Platelet-derived growth factor-A and its receptor are expressed in separate, but adjacent cell layers of the mouse embryo. *Development* 115:1045-1058, 1992
33. ATALIOTIS P, MERCOLA M: Distribution and functions of platelet-derived growth factors and their receptors during embryogenesis. *Int Rev Cytol* 172:95-127, 1997
34. HAGGITT RC, PITCOCK JA, MUIRHEAD EE: Renal medullary fibrosis in hypertension. *Hum Pathol* 2:587-597, 1971
35. MUIRHEAD EE, GERMAIN GS, ARMSTRONG FB, BROOKS B, LEACH B E, BYERS LW, PITCOCK JA, BROWN P: Endocrine-type antihypertensive function of renomedullary interstitial cells. *Kidney Int* 8(Suppl 5):S271-S282, 1975
36. LINDAHL P, JOHANSSON BR, LEVEEN P, BETSHOLTZ C: Pericyte loss and microaneurysm formation in PDGF-B-deficient mice. *Science* 277:242-245, 1997