PDGF-C Expression in the Developing and Normal Adult Human Kidney and in Glomerular Diseases

FRANK EITNER,* TAMMO OSTENDORF,* MATTHIAS KRETZLER,†
CLEMENS D. COHEN† for the ERCB-Consortium, ULF ERIKSSON,‡
HERMANN-JOSEF GRÖNE,§ and JÜRGEN FLOEGE*  
*Division of Nephrology and Immunology, Aachen University, Aachen, Germany; †Medical Policlinic, Ludwig Maximilians University of Munich, Munich, Germany; ‡Ludwig Institute for Cancer Research, Stockholm, Sweden; §Department of Cellular and Molecular Pathology, German Cancer Research Center Heidelberg, Germany.

Abstract. PDGF-C is a new member of the PDGF-family and has recently been identified as a rat mesangial cell mitogen. Its expression and function in human kidneys is unknown. Localization of PDGF-C protein was analyzed by immunohistochemistry using a rabbit polyclonal antibody directed against the core-domain of PDGF-C in human fetal kidneys (n = 8), normal adult human kidneys (n = 9), and in renal biopsies of patients with IgA nephropathy (IgAN, n = 31), membranous nephropathy (MGN, n = 8), minimal change disease (MC, n = 7), and transplant glomerulopathy (TxG, n = 12). Additionally, PDGF-C mRNA was detected in microdissected glomeruli by real-time RT-PCR in cases of normal adult kidneys (n = 7), IgAN (n = 27), MGN (n = 11), and MC (n = 13). In the fetal kidney, PDGF-C localized to the developing mesangium, ureteric bud epithelium, and the undifferentiated mesenchyme. In the adult kidney, PDGF-C was constitutively expressed in parietal epithelial cells of Bowman’s capsule, tubular epithelial cells (loops of Henle, distal tubules, collecting ducts), and in arterial endothelial cells. A marked upregulation of glomerular PDGF-C protein was seen in MGN and TxG with a prominent positivity of virtually all podocytes. In MC, PDGF-C localized to podocytes in a more focal distribution. In MGN, increased glomerular PDGF-C protein expression was due to increased mRNA synthesis as a 4.3-fold increase in PDGF-C mRNA was detected in microdissected glomeruli from MGN compared with normal. PDGF-C protein was additionally expressed in individual mesangial cells in TxG. Finally, upregulated PDGF-C protein expression was detected within sclerosing glomerular and fibrosing tubulointerstitial lesions in individual cases from all analyzed groups. We conclude that PDGF-C is constitutively expressed in the human kidney and is upregulated in podocytes and interstitial cells after injury/activation of these cells.

Members of the platelet-derived growth factor (PDGF) family of cytokines are involved in different aspects of renal disease, particularly the mediation of glomerular mesangial cell proliferation and the induction of renal interstitial fibrosis (1,2). Until recently, the PDGF family comprised three dimers composed of a PDGF A-chain and B-chain, i.e., PDGF-AA, PDGR-AB, and PDGF-BB, that act through the α-subunit and β-subunit of the PDGF receptor (PDGFR) (3–5). In 2000, a new member of the PDGF family was identified and subsequently termed PDGF-C (6). PDGF-C, like PDGF-A and PDGF-B, forms a disulphide-bonded dimer, PDGF-CC. Li et al. (6) identified PDGF-CC as a PDGFR-α-specific ligand. Whereas the α-subunit of the PDGF-receptor is present in vascular smooth muscle cells and the renal interstitium, the β-subunit is constitutively expressed in mesangial and parietal glomerular epithelial cells, in vascular smooth muscle cells, as well as in renal interstitial cells (5). Increased expression of PDGF receptors at sites of renal injury has been documented in a large variety of diseases (5).

We have previously identified PDGF-C as a potent mitogenic stimulus for cultured rat mesangial cells in vitro (7). Immunohistochemical studies in rat tissues detected a constitutive expression of PDGF-C within arterial smooth muscle cells and collecting duct epithelial cells. However, a marked upregulation of PDGF-C was identified within the mesangium in rat experimental mesangio proliferative glomerulonephritis (7). Other rat glomerular disease models with predominant sclerotic lesions or predominant injury to podocytes were almost completely negative for glomerular PDGF-C. In a single rat model of spontaneous glomerulosclerosis (Milan normoten sive glomerulosclerosis), PDGF-C localized to podocytes at sites of focal glomerular injury (7). The expression and potential role of PDGF-C in human kidneys and in human renal diseases is completely unknown. In the current study, we analyzed the tissue localization of PDGF-C by immunohistochemistry in developing fetal kidneys, in normal adult kidneys,
and in renal biopsy tissues with different glomerular diseases. Furthermore, we analyzed PDGF-C mRNA expression by real-time RT-PCR in microdissected glomeruli from patients with different glomerular diseases. The present study is the first to identify a significant increase of podocytic PDGF-C in human glomerular diseases primarily associated with injury to podocytes, i.e., membranous nephropathy (MGN), transplant glomerulopathy (TxG), and to a lesser degree minimal change disease (MC). Furthermore, mesangial PDGF-C expression was detected in TxG, a glomerular disease characteristically preceded by mesangiolysis. However, in human mesangio-proliferative IgA nephropathy (IgAN), no upregulation of either glomerular PDGF-C protein or mRNA was detectable.

Materials and Methods

Analyzed Kidney Tissues

Formalin-fixed, paraffin-embedded renal tissue specimens obtained between 1999 and 2002 at the German Cancer Research Center, DKFZ Heidelberg, Germany, were included in this study. Human tissue was used after approval by, and following the guidelines of, the local Ethics Committee. Renal biopsy cases with sufficient tissue for immunohistochemical evaluation after completion of diagnostic workup were included. Normal adult human kidney tissue was obtained from kidneys surgically excised because of the presence of a localized neoplasm. Tissues utilized for this study were obtained from macroscopically normal portions of kidney located at some distance from the neoplastic process. Eight human fetal kidneys (estimated gestational age ranging from 14 to 22 wk) were obtained fresh from tissue examined after therapeutic abortion. Both adult and fetal kidney tissues were fixed in formalin and embedded in paraffin. Table 1 summarizes the details of the analyzed materials.

Antibodies

Rabbit antiserum directed against PDGF-C was generated as described previously in detail (6). An antiserum generated against human PDGF-CC, affinity-purified against the core domain of PDGF-CC, was utilized in the present study. Specificity of this antiserum for human PDGF-CC, affinity-purified against the core domain of PDGF-CC, was confirmed by detection of the glomerulus-specific cDNA for PDGF-C. Immunohistochemistry was performed according to previously published protocols (7,8). Briefly, formalin-fixed, paraffin-embedded tissues were sectioned at 4 μm. Sections were deparaffinized in xylene and rehydrated in graded ethanols. Endogenous peroxidase was blocked by incubation in 3% hydrogen peroxide. The sections were saturated with 4% skim milk in PBS (pH 7.6) at 22°C for 1 h. The primary antibody was added to the sections for 18 h at 4°C. Then, with the application of avidin followed by biotin (avidin/biotin blocking kit; Vector), endogenous biotin, biotin receptors, or avidin binding was labeled. This was followed by application of a biotinylated pan-specific secondary antibody (BA-1300; Vector) in 1% BSA/PBS at 22°C for 1 h. A horseradish peroxidase-streptavidin complex (SA 5004, diluted 1:200; Vector) was then applied at 22°C for 1 h. 3-Amino-9-ethylcarbazole or 3,3′-diaminobenzidine substrate kits (SK-4200 or SK 4100, respectively; Vector) were used for specific staining. Counterstaining was performed with hematoxylin at 22°C for 4 min. Negative controls consisted of replacement of the primary antisemur with non-immune rabbit serum.

Microdissection, RNA Isolation, Real-Time RT-PCR

Human kidney biopsies were obtained in a multicenter study for gene expression analysis in renal biopsies (see Appendix for participating centers). Clinical data including gender, age, serum-creatinine, and quantitative proteinuria were obtained from each patient at the time of biopsy. Informed consent of the patients and acknowledgment of the respective local ethical committees were obtained. For a detailed description of the protocol used see reference 11. In brief, directlycur after biopsy a cortical tissue segment was transferred into a commercially available RNase inhibitor (RNAlater; Ambion), stored at −20°C, and then manually microdissected in glomeruli and tubulointerstitial compartments in RNAlater. Glomerular microdissection was confirmed by detection of the glomerulus-specific cDNA for Wilms tumor antigen 1. Total RNA was isolated using a commercially available silica-gel-based isolation protocol (RNeasy-Mini, Qiagen, Germany). Reverse transcription was performed in a 45-μl volume, containing 9 μl of buffer, 2 μl of DTT (both Life Technologies), 0.9 μl of 25 mM dNTP (Amersham Pharmacia, Freiburg, Germany), 1 μl of RNase inhibitor (Rnasin; Promega, Mannheim, Germany), and 0.5 μl of buffer, 2 μl of DTT (both Life Technologies), 0.9 μl of 25 mM dNTP (Amersham Pharmacia, Freiburg, Germany), 1 μl of RNase inhibitor (Rnasin; Promega, Mannheim, Germany), and 0.5...

Table 1. Tissues analyzed

| Diagnosis                        | n   | Analyzed Glomeruli | Mean Glomeruli/Case |
|----------------------------------|-----|--------------------|---------------------|
| Normal adult human kidney        | 9   | >450               | >50                 |
| Human fetal kidney               | 8   | nd                 | nd                  |
| IgA nephropathy (IgAN)           | 31  | 374                | 12                  |
| Membranous nephropathy (MGN)     | 8   | 98                 | 12                  |
| Minimal change disease (MC)      | 7   | 69                 | 10                  |
| Transplant glomerulopathy/Glomerulitis (TxG) | 12  | 157                | 13                  |

* nd, not defined.


µl of Microcarrier (Molecular Research Center, Cincinnati, OH), 1 µg of random hexamers (2 mg/ml stock; Roche, Mannheim, Germany), and 200 U of reverse transcriptase (Superscript; Life Technologies) for 1 h at 42°C. Real time RT-PCR was performed on a TaqMan ABI 7700 Sequence Detection System (PE Biosystems, Weiterstadt, Germany) using heat-activated TaqDNA polymerase (Amplitaq Gold; PE Biosystems, Weiterstadt, Germany). After an initial hold of 2 min at 50°C and 10 min at 95°C, the samples were cycled 40 times at 95°C for 15 s and 60°C for 60 s. PDGF-C and housekeeper cDNA templates were quantified by standard curves of diluted standard cDNA. Here, the threshold cycle of each sample corresponds with a dilution step of the standard cDNA (arbitrary units). For quantitative analysis, cDNA content of each sample was compared with another sample by PDGF-C/housekeeping gene. GAPDH and 18S ribosomal RNA served as housekeeping genes to assess the overall cDNA content, yielding comparable results. Data are given for ratios to 18S rRNA.

The following oligonucleotide primers (300 nM) and probes (100 nM) were used: Human PDGF-C (gb AF244813; bp 193 to 323): sense primer 5'-GCTTTCGGGCTTCTCC-3'; antisense primer 5'-TGAGATCTTGACTCCGGTCTGTT-3'; fluorescence-labeled probe (FAM) 5'-CCGCCCAGAGACGAGGACTCA-3'; Human Wilms Tumor WT1 (gb X51630; bp1155 to 1221): sense primer 5'-AAATGGACAGAAGGGCAGAGC-3', antisense primer 5'-GGATGGGCGTTGTGTGGT-3', fluorescence-labeled probe (FAM) 5'-ACCACACGACAGGATCGAGGCA-3'; commercially available predeveloped TaqMan reagents were used for human GAPDH and 18S RNA. Similar amplification efficiencies for all targets were demonstrated by analyzing serial cDNA dilutions showing a slope value of log input cDNA amount versus (Ct taget A – Ct target B) of < 0.1. The primers for PDGF-C and WT-1 were cDNA-specific, not amplifying genomic DNA. All primers and probes were obtained from Applied Biosystems, Weiterstadt, Germany.

Results

PDGF-C Expression in Normal Adult Human Kidney

The immunohistochemical staining pattern was very consistent in the nine analyzed adult human kidneys (summarized in Table 2). Within the glomerular compartment, parietal epithelial cells were consistently positive for PDGF-C in all cases (Figure 1A). The glomerular tuft was however negative for PDGF-C. Very few individual positive cells were rarely identified within the glomerular tuft (data not shown). In view of the infrequency, we were unable to identify the phenotype of these cells by morphologic criteria. Tubular epithelial cells frequently expressed PDGF-C (Figure 1B). PDGF-C expression localized to the loop of Henle, distal tubules, and collecting ducts, whereas proximal tubules (as identified by the presence of a brush border) did not show detectable PDGF-C expression. Few individual PDGF-C-expressing cells were localized throughout the interstitium in a scattered manner. There was a strong expression of PDGF-C within endothelial cells of renal arteries and arterioles (Figure 1C). Endothelial cells in the glomerular or peritubular microvasculature or in veins did not demonstrate detectable PDGF-C expression. In addition to the prominent endothelial PDGF-C expression, there was a weaker and more scattered positivity within the smooth muscle cell-rich media of arterial vessels. Identical results were obtained with two different staining methods used in this study. Negative control tissues that were incubated with equal amounts of non-immune rabbit serum did not demonstrate any staining signal in all analyzed normal and diseased cases.

PDGF-C Expression in the Developing Fetal Kidney

In the developing human kidney, PDGF-C showed a distinct localization pattern (summarized in Table 3). Strong PDGF-C expression was frequently detected within ureteric buds (Figure 1D) and within the metanephric blastema (Figure 1E). Early glomerular stages, i.e., glomerular vesicles, comma-shape, or s-shape glomeruli, were almost completely negative for PDGF-C (Figure 1E). However, few s-shape glomeruli were expressed in the early stages of kidney development. In the developing glomeruli, PDGF-C expression was detectable within the mesangial cells, visceral epithelial cells, parietal epithelial cells, and capillary endothelial cells (Table 2). PDGF-C expression was also detectable within the arterial endothelial cells, venous endothelial cells, smooth muscle cells, and adventitial cells (Table 2). PDGF-C expression was not detectable within the mesangial cells, visceral epithelial cells, parietal epithelial cells, and capillary endothelial cells (Table 2). PDGF-C expression was most frequently detected in the tubulointerstitium, particularly in the proximal tubules, distal tubules, Loop of Henle, collecting ducts, interstitial cells, and mononuclear cells (Table 2).

Table 2. PDGF-C expression in normal adult kidneys and in glomerular diseases

|                      | Normal | IgAN | MGN | MC | TxG |
|----------------------|--------|------|-----|----|-----|
| Glomerulus           |        |      |     |    |     |
| mesangial cells      | -      | -    | -   | -  | +/- |
| visceral epithelial cells | - | +/−  | +   | +  | +   |
| parietal epithelial cells | + | +    | +   | +  | +   |
| capillary endothelial cells | - | -    | -   | -  | +/- |
| Vessel               |        |      |     |    |     |
| arterial endothelial cells | + | +    | +   | +  | +   |
| venous endothelial cells | - | -    | -   | -  | -   |
| smooth muscle cells  | +/−  | +/−  | +/− | +/−| +/− |
| adventitial cells    | +/-   | +/−  | +/− | +/−| +/− |
| Tubulointerstitium   |        |      |     |    |     |
| proximal tubules     | -      | -    | -   | -  | -   |
| distal tubules, Loop of Henle | + | +    | +   | +  | +   |
| collecting ducts     | +      | +    | +   | +  | +   |
| interstitial cells   | +/−   | +/−  | +/− | +/−| +/− |
| mononuclear cells    | -      | -    | -   | -  | +   |

* Normal, normal adult human kidneys; IgAN, IgA nephropathy; MGN, membranous nephropathy; MC, minimal change disease; TxG, Transplant glomerulopathy; −, no expression detectable; +/−, weak and/or variable expression; +, detectable expression.
weakly expressed PDGF-C in the mesangial stalk region (data not shown). In more differentiated glomeruli, PDGF-C was restricted to the developing mesangium while all other glomerular cell types, including developing endothelial cells as well as podocytes, remained uniformly negative (Figure 1F). Additionally, parietal epithelial cells expressed detectable PDGF-C in more differentiated glomeruli (Figure 1F). In the vasculature, PDGF-C regularly localized to smooth muscle cells in smaller arteries and arterioles. In these arterial vessels, PDGF-C was additionally expressed by endothelial cells.

**PDGF-C Expression in Human Glomerular Diseases**

Given the marked mesangial PDGF-C upregulation in rat mesangioproliferative glomerulonephritis and human renal development and its role as a mesangial cell mitogen *in vitro*, we next analyzed the expression of PDGF-C in human mesangioproliferative IgAN (summarized in Table 2). Thirty-one cases of IgAN were selected to represent a variety of pathologic lesions of IgAN. Cases included minimal lesions, classical cases with prominent mesangial hypercellularity and segmentally increased matrix deposition/sclerosis, and cases with se-
vere focal necrosis and crescents. Within the analyzed glomeruli, there was a consistent positivity of all parietal epithelial cells (Figure 2A). The glomerular tuft of the majority of all cases was completely negative for PDGF-C. There was no immunohistochemical signal localized to mesangial cells. Cases with prominent mesangial hypercellularity, as illustrated in Figures 2A and 2B, were regularly negative for PDGF-C in the mesangium. A weak granular staining signal for PDGF-C localized to occasional podocytes and glomerular endothelial cells. However, the weak and infrequent character of this staining pattern was considered with some caution as it was just above the level of nonspecific background signal. In contrast, IgAN biopsies with segmental necrosis/crescentic lesions typically demonstrated an increase in glomerular PDGF-C expression. Extracapillary proliferates and podocytes adjacent to segmental necrosis strongly expressed PDGF-C (Figure 2, C and D). In all IgAN biopsies, PDGF-C was expressed by the loop of Henle, distal tubules, and collecting ducts. Identical with the findings in normal adult kidneys, a strong expression of PDGF-C was identified in arterial endothelial cells. Additionally, individual arterial smooth muscle cells as well as individual cells within the adventitia of arteries labeled positively for PDGF-C.

In cases with a marked injury of podocytes, we identified strong expression of podocytic PDGF-C expression (Table 2). All cases of MGN expressed PDGF-C within the glomeruli. PDGF-C was localized to the cytoplasm of podocytes (Figure 3, A and B). Podocytes were identified by morphologic criteria as adjacent cells located at the outer aspect of the glomerular basement membrane. A very similar finding was obtained in the cases of TxG. Podocytes, both in a focal and segmental or a global distribution, labeled positively for PDGF-C (Figure 3C). Individual biopsies that had features of a transplant glomerulitis frequently had detectable PDGF-C in glomerular segments. In these cases, PDGF-C was located to focal glo-

| Site                      | PDGF-C Expression |
|---------------------------|-------------------|
| Metanephric blastema      | +                 |
| Glomerular vesicles       | −                 |
| Early glomeruli           | mesangial stalk +/−, otherwise negative |
| (comma, S-stage)          |                   |
| Differentiated glomeruli  | mesangium +       |
|                           | parietal epithelial cells + |
| Ureteric buds             | +                 |
| Vasculature               | arterial endothelial cells + |
|                           | smooth muscle cells + |

Table 3. PDGF-C expression in the developing human fetal kidney

Figure 2. PDGF-C expression in human IgA nephropathy (IgAN). Immunohistochemistry using a PDGF-C specific polyclonal antibody results in a black/brown color product. (A) In cases of IgAN, glomerular PDGF-C remains largely negative despite the presence of prominent focal mesangial hypercellularity. PDGF-C expression of parietal epithelial cells is unchanged compared with normal adult human tissues (compare with Figure 1A). (B) Higher power magnification illustration of Figure 2A indicating the absent mesangial PDGF-C. (C) In IgAN biopsies with segmental necrosis, strong PDGF-C expression is detected in podocytes adjacent to segmental necrosis. (D) Higher power magnification illustration of Figure 2C, indicating strong PDGF-C expression within podocytes (arrows). Original magnifications: ×400 in A and C; ×1000 in B and D.
merular endothelial cells as well as mesangial cells (Figure 3D). Within the tubulointerstitium and the vasculature, PDGF-C expression was unchanged compared with the normal adult and the IgAN cases. Immunohistochemical analysis of cases of MC detected PDGF-C expression in the cytoplasm of podocytes (data not shown). Compared with the prominent positivity in MGN, the staining pattern in MC was more focal.

We further addressed whether PDGF-C was locally produced within the diseased glomeruli in the course of the glomerulonephritis. We therefore microdissected individual glomeruli of normal adult kidneys and of biopsies with IgAN, MGN, or MC and analyzed PDGF-C mRNA expression by real-time RT-PCR. Consistent with our results obtained by immunohistochemistry at the protein level, we found no significant upregulation of PDGF-C mRNA expression in IgAN compared with normal adult kidneys (Figure 4). However, cases with the diagnosis of MGN demonstrated a significant increase in glomerular PDGF-C mRNA (Figure 4). Cases of MC reached an intermediate result. While some cases of MC demonstrated increased glomerular PDGF-C mRNA expression, the whole group did not differ significantly from the normal controls. These results indicate that the upregulation of podocytic PDGF-C protein that was detected by immunohistochemistry in MGN was related to an increase in local PDGF-C mRNA synthesis. Further analysis of clinical parameters revealed no significant correlation between glomerular PDGF-C mRNA expression and serum-creatinine, amount of proteinuria, age, or gender in any of the groups (data not shown).

PDGF-C Expression is Increased at Sites of Fibrosing Tubulointerstitial Injury

Individual cases of all analyzed groups had developed variable degrees of tubulointerstitial disease, including tubular atrophy, tubulointerstitial leukocyte infiltration, and tubulointerstitial fibrosis. The severity of the tubulointerstitial lesions correlated with the severity of the glomerular lesions in general. At sites of fibrosing lesions, tubulointerstitial PDGF-C expression was markedly upregulated in all cases analyzed (Figure 5, A and B). There was typically a finely granular immunohistochemical signal in fibrosing areas. Even at high magnifications (Figure 5B), we were unable to clearly identify whether PDGF-C was localized intracellularly within interstitial cells or within the extracellular matrix.

Discussion

PDGF cytokines have been identified as important mediators in the pathogenesis of glomerular diseases (1,2). The present study is the first to identify PDGF-C in human kidneys. PDGF-C is constitutively expressed in the adult human kidney and localizes to parietal epithelial cells, distal tubular epithelial cells, and arterial endothelial cells. After predominant injury to

![Figure 3](https://example.com/figure3.png)

**Figure 3.** PDGF-C expression in human membranous nephropathy and transplant glomerulopathy. Immunohistochemistry using a PDGF-C–specific polyclonal antibody results in a red color product. (A) All cases of membranous nephropathy express PDGF-C within the glomeruli. (B) Higher power magnification illustration of Figure 3A showing localization of PDGF-C within the cytoplasm of podocytes (arrows). (C) In the cases of transplant glomerulopathy, glomerular PDGF-C was detected in all cases. (D) Higher power magnification illustration of Figure 3C indicating strong expression of PDGF-C in individual podocytes (arrows). Original magnifications: ×400 in A and C; ×1000 in B and D.
podocytes, which is typically seen in membranous nephropathy and transplant glomerulopathy, there is a significant upregulation of glomerular PDGF-C. PDGF-C localizes immunohistochemically to podocytes. A significant upregulation of PDGF-C is additionally detected within the tubulointerstitium localizing to fibrosing lesions independent of the primary diagnosis of the different cases.

Our current understanding of the biology of the cytokine PDGF-C is still very limited. A first study by Li et al. (6) identified PDGF-CC as a PDGFRα-specific ligand, while Gilbertson et al. (12) additionally demonstrated that PDGF-CC could activate the beta-chain in the heterodimeric receptor complex but not in a homodimeric PDGFRββ receptor. Little is known about the potential functions of PDGF-C. Both, Li et al. (6) and Gilbertson et al. (12) have identified PDGF-C as a potent mitogenic stimulus for several mesenchymal cells in vitro. Furthermore, transgenic overexpression of PDGF-C in the mouse heart induced strong proliferation of cardiac fibroblasts and resulted in subsequent interstitial expansion with features of interstitial fibrosis (6). Further studies located PDGF-C expression in vascular smooth muscle cells as well as in endothelial cells (13). Additionally, PDGF-C was capable of inducing proliferation of human coronary artery and aortic smooth muscle cells in vitro, suggesting that PDGF-C participates in vascular development and pathology (13). Expression analysis of PDGF-C in adult and developing mouse tissues revealed a widespread and dynamic expression of PDGF-C in different organs (14). A strong expression was particularly prominent at sites of developing epidermal openings, indicating a function of PDGF-C in this developmental step (14).

The expression of the main PDGF-C receptor PDGFRα has previously been analyzed in human developing, normal adult, and in diseased kidneys. In the developing kidney, PDGFRα is present in interstitial cells, in vascular arcades, in the invaginating mesangium and the stalk region of early glomerular differentiation stages and strongly in the adventitia of the fetal arterial architecture (15). In normal adult kidneys, PDGFRα was extensively expressed by interstitial cells and only occasionally by mesangial cells (15). In cases of renal arteriosclerosis and/or renal vascular rejection, a widespread expression of PDGFRα was noted in renal cell types involved in fibrotic and sclerosing processes (16). Gesualdo et al. (17) detected only a slight increase of PDGFRα expression in diseased kidneys, mainly at the interstitial level, while a few cases of lupus nephritis also showed a moderate increase of PDGFRα at the glomerular level. In another study in biopsies from patients with glomerular diseases, Stein-Oakley et al. (18) identified increased PDGFRα in patients with IgAN and focal glomerulosclerosis, particularly in areas of mesangial hypercellularity. Taken together, these data show that PDGFRα is expressed in the normal renal interstitium and is upregulated at sites of renal fibrosis. One PDGFRα ligand, PDGF-A, is constitutively expressed in normal renal interstitium (19). PDGF-A was additionally detected in glomerular visceral epithelial cells, vascular arterial smooth muscle cells, and vascular endothelial cells in rejecting renal allograft (19, 20).

Correlation of the expression analyses of PDGF-C and its receptor might offer new insights into potential functional mechanisms of this cytokine. In the developing human kidney, increased PDGF-C expression occurs in close proximity to sites of previously reported PDGFRα expression. PDGF-C localizes to the metanephric blastema (PDGFRα to interstitial cells) and to arterial endothelial and smooth muscle cells (PDGFRα to adventitial cells), indicating a potentially paracrine action of PDGF-C in vascular and interstitial development. In the fetal glomeruli, both the ligand PDGF-C as well as the receptor PDGFRα localize to the developing mesangium, indicating a potentially paracrine or even autocrine role for PDGF-C in developing mesangial cells.

The identification of increased PDGF-C at sites of fibrosing/sclerosing injury in diseased kidneys might indicate a functional role for PDGF-C in this process. The other known PDGFRα ligand, PDGF-A, is constitutively expressed in normal interstitium; therefore, a role of increased PDGFRα in fibrosing renal interstitium has remained speculative. Our current data suggest that upregulated expression and signaling of PDGF-C via upregulated PDGFRα might have a unique role in mediating renal fibrosis.

Another novel and important aspect of the present study is the identification of podocytic PDGF-C in human membranous nephropathy, transplant glomerulopathy, and minimal change disease. Our previous studies in rat experimental glomerulonephritis had revealed a focal expression of PDGF-C in some podocytes in a model of glomerulosclerosis (Milan glomerulosclerosis) (7). Glomerular PDGF-C was undetectable in a model of membranous nephropathy (passive Heymann nephritis) (7). In human membranous nephropathy, however, there was a prominent PDGF-C expression in all analyzed cases. Immunohistochemistry localized PDGF-C to the podocyte cytoplasm. Furthermore, our data indicate a local production of PDGF-C because glomerular PDGF-C mRNA was equally upregulated as PDGF-C protein in these cases. Transplant

Figure 4. PDGF-C mRNA expression in microdissected glomeruli. No significant difference of glomerular PDGF-C mRNA is observed in IgA nephropathy (IgAN) and minimal change disease (MC) as compared with normal adult kidney (normal). Glomerular PDGF-C mRNA is significantly increased in membranous nephropathy (MGN) as compared with normal adult kidneys or IgA nephropathy. Graphical points represent data obtained from individual patients.
glomerulopathy is another human disease that is thought to be associated with prominent injury to the podocytes. A strong podocytic PDGF-C expression mimicked the results of the membranous nephropathy cases. Interestingly, PDGF-C remained undetectable in podocytes during human renal development. From a more general perspective, these data might indicate that the activating injury (dedifferentiation) of podocytes in membranous and transplant nephropathy differs from the activation (differentiation) of podocytes in fetal glomerular development and results in distinct PDGF-C expression.

Finally, our data indicate a role for PDGF-C in modulating mesangial cell behavior in vivo. One central finding in our previous report analyzing PDGF-C expression in rat models of glomerular diseases was a marked and apparent de novo upregulation of mesangial PDGF-C in mesangioproliferative glomerulonephritis (7). Furthermore, PDGF-C acted as a potent rat mesangial cell mitogen in vitro (7). The present data obtained in the human organism are less consistent. Proliferating developing mesangial cells overexpressed PDGF-C in the human fetal glomerulus, indicating a role for PDGF-C in human mesangial cell proliferation in vivo. In adult human mesangioproliferative glomerulonephritis that was analyzed in IgA nephropathy and mesangioproliferative lupus nephritis (our own unpublished results), mesangial PDGF-C overexpression remained absent. Additionally, RT-PCR did not detect any significant upregulation of PDGF-C mRNA in IgAN. In contrast, in human transplant glomerulopathy, a disease typically preceded by mesangiolysis, PDGF-C expression was seen in individual mesangial cells. Thus, a different mesangial stimulus, i.e., mesangiolysis as it occurs in rat anti-Thy 1.1 nephritis or human TxG, apparently is needed for mesangial PDGF-C expression.

In conclusion, the present study is the first to identify distinct expression patterns of the novel cytokine PDGF-C in human adult and developing kidneys. An upregulated expression was seen in podocytes or interstitial cells and was associated with injury or activation of these specific cell types. Specific inhibition of PDGF-C in vivo will help to further define the roles of PDGF-C in renal development and disease.

Acknowledgments

The technical help of Kerstin Schenk, Gabi Dietzel, Gerti Minartz, Andrea Cosler, and Claudia Schmidt is gratefully acknowledged. This work was supported in part by grants SFB 542 C7 (FE, TO, JF) and SFB 405 B10 (HJG) from the Deutsche Forschungsgemeinschaft (DFG), by a grant from the Swedish Research Council (grant K2001–03P-12070–05B) and the Novo Nordisk Foundation (UE), and by grant DHG01K09922/2 and the Else-Kröner Fresenius Foundation (MK). FE is a recipient of a stipend of the German Kidney Foundation (Deutsche Nierenstiftung).

Members of the ERCB

C Cohen, M Kretzler, and D Schlöndorff, Munich; F Delarue and JD Sraer, Paris; MP Rastaldi and G D’Amico, Milano; P Doran and HR Brady, Dublin; D Mönks and C Wanner, Würzburg; AJ Rees, Aberdeen; P Brown, Aberdeen; F Strutz and G Müller, Göttingen; P Mertens and J Floege, Aachen; N Braun and T Risler, Tübingen; L Gesualdo and FP Schena, Wien; J Gerth and G Stein, Jena; R Oberbauer and D Kerjaschki, Vienna; M Fischereder and B Krämer, Regensburg; W Samtleben and W Land, Munich; H Peters and HH Neumayer, Berlin; K Ivens and B Grabensee, Düsseldorf.

References

1. Floege J, Johnson RJ: Multiple roles for platelet-derived growth factor in renal disease. Miner Electrolyte Metab 21: 271–282, 1995
2. Abboud HE: Role of platelet-derived growth factor in renal injury. Am J Physiol 247: F283–F287, 1984
3. Boekhoff F, Buenger C: Platelet-derived growth factor and renal disease. Annu Rev Physiol 44: 259–272, 1982
4. Heldin CH, Westermark B: Mechanism of action and in vivo role of platelet-derived growth factor. Trends Pharmacol Sci 10: 118–123, 1989
5. Floege J, Ostendorf T, Wolf G: Growth factors and cytokines. In: Immunologic Renal Diseases, edited by Neilson E, Couser WG, Philadelphia, Lippincott Williams and Wilkins, 2001, pp 415–463
6. Li X, Ponten A, Aase K, Karlsson L, Abramsson A, Utela M, Backstrom G, Hellstrom M, Bostrom H, Li H, Sorian P, Bet-
sholtz C, Heldin CH, Alitalo K, Ostman A, Eriksson U: PDGF-C is a new protease-activated ligand for the PDGF alpha-receptor. Nat Cell Biol 2: 302–309, 2000
7. Eitner F, Ostendorf T, Van Roeyen C, Kitahara M, Li X, Aase K, Grone HJ, Eriksson U, Floege J: Expression of a novel PDGF isoform, PDGF-C, in normal and diseased rat kidney. J Am Soc Nephrol 13: 910–917, 2002
8. Eitner F, Cui Y, Hudkins KL, Stokes MB, Segerer S, Mack M, Lewis PL, Abraham AA, Schlondorff D, Gallo G, Kimmel PL, Alpers CE: Chemokine receptor CCR5 and CXCR4 expression in HIV-associated kidney disease. J Am Soc Nephrol 11: 856–867, 2000
9. Grone HJ, Weber C, Weber KS, Grone EF, Rabelink T, Klier CM, Wells TN, Proudfoot AE, Schlondorff D, Nelson PJ: Met-RANTES reduces vascular and tubular damage during acute renal transplant rejection: Blocking monocyte arrest and recruitment. Faseb J 13: 1371–1383, 1999
10. Grone HJ, Cohen CD, Grone E, Schmidt C, Kretzler M, Schlondorff D, Nelson PJ: Spatial and temporally restricted expression of chemokines and chemokine receptors in the developing human kidney. J Am Soc Nephrol 13: 957–967, 2002
11. Cohen CD, Frach K, Schlondorff D, Kretzler M: Quantitative gene expression analysis in renal biopsies: A novel protocol for a high-throughput multicenter application. Kidney Int 61: 133–140, 2002
12. Gilbertson DG, Duff ME, West JB, Kelly JD, Sheppard PO, Hofstrand PD, Gao Z, Shoemaker K, Bukowski TR, Moore M, Feldhaus AL, Humes JM, Palmer TE, Hart CE: Platelet-derived growth factor C (PDGF-C), a novel growth factor that binds to PDGF alpha and beta receptor. J Biol Chem 276: 27406–27414, 2001
13. Uutela M, Lauren J, Bergsten E, Li X, Horellu-Kuitunen N, Eriksson U, Alitalo K: Chromosomal location, exon structure, and vascular expression patterns of the human PDGFC and PDGF genes. Circulation 103: 2242–2247, 2001
14. Aase K, Abramsson A, Karlsson L, Betsholtz C, Eriksson U: Expression analysis of PDGF-C in adult and developing mouse tissues. Mech Dev 110: 187–191, 2002
15. 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
16. Floege J, Hudkins KL, Davis CL, Schwartz SM, Alpers CE: Expression of PDGF alpha-receptor in renal arteriosclerosis and rejecting renal transplants. J Am Soc Nephrol 9: 211–223, 1998
17. 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
18. Stein-Oakley AN, Maguire JA, Dowling J, Perry G, Thomson NM: Altered expression of fibrogenic growth factors in IgA nephropathy and focal and segmental glomerulosclerosis. Kidney Int 51: 195–204, 1997
20. 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
20. 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