Drugs of the Future 2004, 29(2): 179-184

CCC: 0377-8282/2004

**Review Article**

# PDGF-D: a novel mediator of mesangioproliferative glomerulonephritis

***Jürgen Floege*, Claudia van Roeyen and Tammo Ostendorf***

*Division of Nephrology and Immunology, University of Aachen, Germany. *Correspondence: Jürgen Floege, M.D., Medizinische Klinik II, Klinikum der RWTH, Pauwelsstr. 30, D-52074 Aachen, Germany.*
*e-mail: Juergen.Floege@rwth-aachen.de.*

**CONTENTS**



**Abstract**

In view of the increasing number of patients with end-stage renal disease (ESRD), new approaches to common underlying diseases such as mesangioproliferative glomerulonephritis, including IgA nephropathy, are urgently needed. Whereas the role of platelet-derived growth factor (PDGF) B-chain (PDGF-B) in mediating mesangioproliferative changes is well established, the role of PDGF D-chain (PDGF-D) has only recently been elucidated. Like PDGF-B, PDGF-D signals through the PDGF β-receptor and therefore shares a number of biological activities with PDGF-B. Recent studies have shown that PDGF-D induces mesangial cell proliferation *in vitro* and is overexpressed in mesangioproliferative glomerulonephritis *in vivo*. In addition, hepatic transfection with a PDGF-D expression plasmid induced prominent mesangioproliferative nephritis in mice, whereas antagonism of PDGF-D in a rat model of mesangioproliferative disease ameliorated the renal changes. These observations establish PDGF-D, along with PDGF-B, as an important mediator of mesangioproliferative nephritis *in vivo* and suggest that it may be an attractive therapeutic target. In addition, preliminary observations suggest that PDGF-D may also contribute to secondary renal changes that characterize progressive renal failure, *i.e.*, tubulointerstitial fibrosis.

## The clinical problem

The number of patients with end-stage renal disease (ESRD) is growing worldwide. In the United States, for example, it is predicted that between the years 2000 and 2010, a 50% increase in the ESRD population will occur (1). Apart from the serious personal consequences and suffering in the case of ESRD, this predicted growth of the patient population will have dramatic consequences for health insurance and national health systems given the cost of dialysis therapy. In Germany, it can be estimated that a 5% annual net increase in the ESRD population will add about 100-150 million euros to healthcare expenditures per year.

Whereas diabetic nephropathy, in particular that associated with type 2 diabetes, is now the major cause of newly discovered ESRD and largely responsible for the continuing growth[1], the absolute number of glomerulonephritis cases resulting in ESRD has not changed over the last years. These latter patients are a particular economic problem, since they often exhibit only renal disease, are often relatively young, and thus have a much lower mortality compared to older ESRD patients with underlying type 2 diabetes. Consequently, the prevention of ESRD in young patients with glomerulonephritis is a particularly important task.

The most common type of glomerulonephritis in the Western world is IgA nephropathy. About 20-30% of the patients with IgA nephropathy will develop progressive renal failure within 10-20 years after the onset of disease (2, 3). IgA nephropathy is a mesangioproliferative glomerulonephritis characterized by extensive deposition of IgA in the glomerular mesangium. Other diseases characterized by mesangioproliferative changes include membranoproliferative glomerulonephritis, variants of idiopathic focal sclerosis and lupus nephritis (4, 5).

The pathogenesis of IgA nephropathy is only partially understood (2, 3). Patients with IgA nephropathy suffer

[1]See for example, the ERA-EDTA registry at http://www.era-edta.org.

from an excessive response of their IgA system following antigenic stimulation (*e.g.*, infection), resulting in increased and prolonged levels of circulating IgA immune complexes after a challenge. The site of the increased IgA production appears to be the bone marrow. One of the most exciting findings of recent years is a relative deficiency of galactose residues in the hinge region of the IgA molecule in patients with IgA nephropathy. Apparently, this overproduced and undergalactosylated IgA is preferentially deposited in the glomerular mesangium (6), where it can then activate complement (7) and induce a proinflammatory response of the resident mesangial cells, including enhanced proliferation and matrix synthesis.

Given the fragmentary knowledge of the pathogenesis of IgA nephropathy, causal therapy is usually not possible. Apart from nonspecific measures such as antihypertensive therapy, some patients with progressive renal function loss or at risk for it appear to benefit from immunosuppression with corticosteroids and/or cytotoxic agents (8).

Insights gained from animal studies, in particular the rat mesangioproliferative anti-Thy 1.1 glomerulonephritis model, have led to a better understanding of the processes that may follow immune complex deposition (and/or formation) and subsequent complement activation in the mesangium. While a large variety of growth factors and cytokines are likely to be involved in mediating mesangioproliferative glomerulonephritis, one of the most extensely characterized is platelet-derived growth factor (PDGF). The single, best-established role for PDGF in the kidney is the mediation of glomerular mesangial cell proliferation, migration and matrix production.

## The PDGF system

Until recently, the PDGF system was thought to consist of two PDGF chains, PDGF-A and PDGF-B, which are secreted as homo- or heterodimers and bind to dimeric PDGF receptors composed of α- and/or β-chains. Whereas PDGF-A binds to the α-chain only, PDGF-B is a ligand for all receptor types (9). Recently, using an EST database homology search, two novel PDGF isoforms, designated PDGF-C and PDGF-D, were identified, which are released as the respective homodimers PDGF-CC and PDGF-DD (10-12). These two new isoforms differ from the classical PDGFs since they form homodimers only and are produced as latent factors. Proteolytic cleavage of a CUB domain from each chain is then required for activation (13). The proteases involved in this process *in vivo* remain to be identified. To add to the complexity, it has been suggested that latent PDGF-CC or PDGF-DD homodimers become antagonists after removal of only one CUB domain, whereas agonist activity only results if both CUB domains are removed (13). Following this proteolytic processing, the core domain of PDGF-CC appears to be largely a ligand for the PDGF αα-receptor, while PDGF-DD binds predominantly to the PDGF ββ-receptor (13) (Fig. 1).

The PDGF receptor possesses tyrosine kinase activity and is autophosphorylated upon ligand binding (14). The receptor then interacts with several other cytoplasmic proteins containing SH2 domains, including phospholipase C (PLC-α), *ras* GTPase-activating protein, phosphatidylinositol 3-kinase, members of the pp60$^{src}$ family of protein tyrosine kinase, the protein-tyrosine-phosphatase SHP-2 and members of the signal transducers and

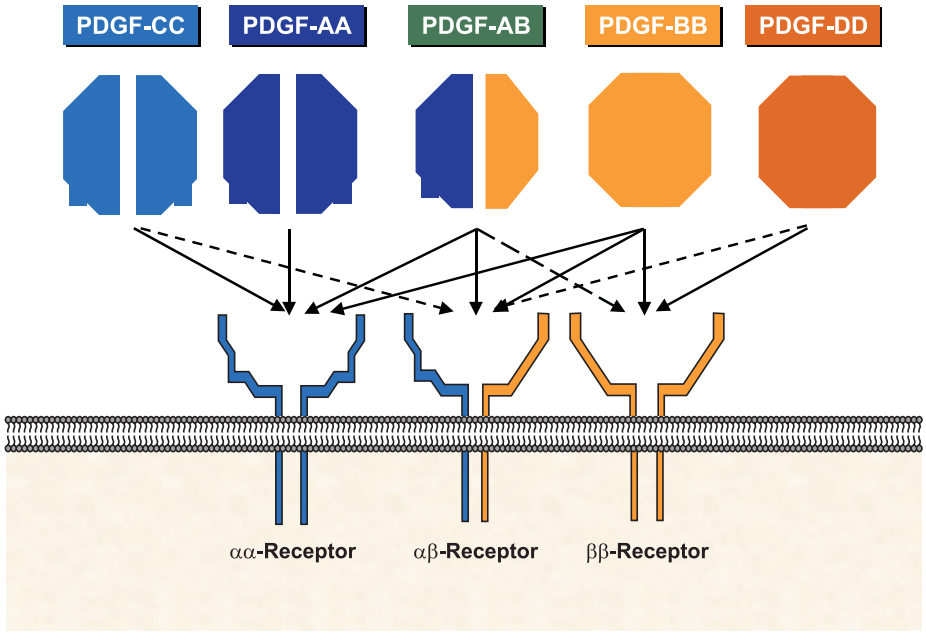


Fig. 1. PDGF isoforms and receptor binding characteristics.

activators of transcription (STAT family) (14). Second messengers include inositol-(1,4,5)-triphosphate and diacylglycerol, intracellular calcium release and protein kinase C (PKC) activation (14). In the nucleus, PDGF signaling activates various proto-oncogenes and immediate early response genes, including c-*fos*, c-*myc* and *egr*-1 (15). Homodimeric αα- and ββ-receptor complexes induce overlapping but distinctly different effects on target cells, which might be explained by differential interactions with various SH2 domain proteins. Autophosphorylation on different tyrosine residues might explain the unique properties of the heterodimeric αβ-receptor complex in comparison to homodimeric receptors. Finally, PDGF signaling can also be modulated in the extracellular milieu by matrix molecules.

All four PDGF isoforms, as well as both receptor chains, are expressed in the kidney, albeit in distinct spatial arrangements (9, 16-19). Of relevance in the context of PDGF-D is the observation that the PDGF receptor β-subunit is constitutively expressed in mesangial and parietal glomerular epithelial cells, vascular smooth muscle cells, as well as renal interstitial cells (20-25).

## The PDGF system in renal disease

Increased expression of PDGF in glomerular and/or interstitial locations has been documented in a large variety of renal diseases. In addition, increased expression of PDGF receptors occurs in experimental and human renal diseases (18-30).

Functional *in vivo* studies demonstrated that infusion of recombinant PDGF-BB into rats or transfection of glomeruli *in vivo* with PDGF B-chain cDNA leads to a selective increase in glomerular mesangial cell proliferation (31, 32). Platelet-derived growth factor-induced mesangial cell proliferation was markedly augmented if the mesangial cells had suffered a minor (subclinical) injury prior to the PDGF infusion (31). Finally, in view of the similarity of events occurring during ontogenesis and renal pathology, the observation that both the PDGF-B chain and the PDGF receptor β-subunit play a critical role in mesangial development is of importance (33-35).

Experiments with a neutralizing antibody against PDGF-B demonstrated that it can decrease mesangial cell proliferation and matrix accumulation in the anti-Thy 1.1 mesangioproliferative nephritis model in rats (36). A more powerful PDGF-B antagonist, a specific DNA aptamer (37), was recently demonstrated to be highly effective in reducing mesangial proliferation and matrix accumulation in the same model (38). More importantly, transient treatment with this antagonist during the mesangioproliferative phase of a chronic anti-Thy 1.1 model completely prevented the development of renal failure and glomerular as well as tubulointerstitial scarring (39). Other therapeutic approaches by which PDGF-B bioactivity was inhibited, *e.g.,* gene transfer of soluble PDGF β-receptor (40) or PDGF β-receptor blockers (41), have also substantiated the notion that PDGF-B is a potentially very important novel target in glomerular disease. Given that PDGF-D, like PDGF-B, signals through the PDGF β-receptor, overlapping biological activity was to be expected.

## PDGF-D and mesangioproliferative glomerulonephritis

In order to ascribe a specific biological effect to the action of an individual cytokine or growth factor, several requirements have to be fulfilled. In this section, we will examine whether these requirements are fulfilled in the case of PDGF-D and mesangioproliferative disease.

### *The factor exerts the effect in vitro*

Incubation of growth-arrested cultured rat and human mesangial cells with PDGF-DD led to a dose-dependent increase in proliferation (42). The mitogenic activity of PDGF-DD was independent from that of PDGF-BB, as demonstrated by incubating the cells with antagonistic PDGF-BB aptamers simultaneously with PDGF-DD. While the aptamers blocked PDGF-BB-induced proliferation, they had no effect on the mitogenic effects of PDGF-DD.

### *The effect in vivo is associated with overproduction or release of the factor*

By immunohistochemistry, the localization of PDGF-DD in normal rat kidney was confined to arterial and arteriolar vascular smooth muscle cells, whereas no immunoreactivity was noted in glomeruli (42). Following the induction of mesangioproliferative anti-Thy 1.1 nephritis in rats, prominent glomerular localization of PDGF-DD in the expanded mesangium was present. In addition, glomerular PDGF-D mRNA expression increased 2.4- and 2.9-fold at days 7 and 9, respectively, in comparison to non-nephritic rats (42). Of particular interest in this latter study was the observation that PDGF-D, unlike PDGF-B, apparently also acts as an endocrine growth factor, since plasma levels increased about 1,000-fold in nephritis (42).

### *The effect is reproduced in vivo by administration or overexpression of the factor*

In mice, hepatic viral overexpression of a human PDGF-D construct has been demonstrated to potently induce mesangioproliferative changes, characterized by enlarged glomeruli, as well as marked increases in glomerular cellularity, cell proliferation, extracellular matrix accumulation and macrophage counts (43). Similar changes were obtained with a PDGF-B construct (43).

*The effect can be abolished or diminished in vivo by specific antagonism of the factor*

Following the induction of mesangioproliferative anti-Thy 1.1 nephritis, glomerular cell proliferation, as assessed by counting the number of glomerular mitoses or BrdU-positive nuclei, was significantly reduced in a dose-dependent manner in rats receiving a specific neutralizing anti-PDGF-D monoclonal antibody (42). Specific identification of proliferating mesangial cells on day 8 after disease induction showed that the anti-PDGF-D antibody, but not a control antibody, led to a 57% reduction in mesangial cell proliferation. In addition, treatment with the antibody reduced the *de novo* glomerular expression of smooth muscle α-actin, which is only expressed by activated mesangial cells (44). Other effects of the anti-PDGF-D antibody included a reduction of glomerular fibronectin accumulation and a marked reduction of glomerular monocyte/macrophage influx (42).

The above observations establish PDGF-D, like PDGF-B, as a mediator of mesangioproliferative disease *in vivo*. If PDGF-D and PDGF-B are so similar in terms of biological activities in the kidney, it is at first glance puzzling that specific inhibition of either isoform can dramatically affect renal disease even in instances where both are overexpressed, such as in mesangioproliferative nephritis (38, 42). Three explanations may account for these observations: 1) overexpression of PDGF-B and PDGF-D is temporally separated, a possibility not supported by studies in glomerular or tubulointerstitial disease (19, 42); 2) overexpression of PDGF-B and PDGF-D is spatially separated, which may be the case to some degree in the tubulointerstitium (19), but not the glomerulus (42); and 3) PDGF-B and PDGF-D interact with each other, which remains to be clarified.

Is chronic or repeated inhibition of PDGF-D safe *in vivo*? Whereas, as noted above, the PDGF-B chain and PDGF receptor β-subunit are critically important for the development of the glomerular mesangium, PDGF-B signalling apparently is not required in normal adult life. Thus, mice that transgenically overexpress a soluble PDGF receptor β-subunit only in adult life do not exhibit any phenotype and have a normal life span (45). Since the soluble β-receptor can be expected to bind both PDGF-B and PDGF-D, this provides evidence for the safety of PDGF-D blockade in adult life. In addition, experimental and clinical studies with a PDGF β-receptor tyrosine kinase blocker lend further support to the notion that PDGF-B and PDGF-D can be safely blocked in patients.

## A role for PDGF-D in secondary renal interstitial fibrosis?

In addition to mediating mesangioproliferative disease *in vivo*, PDGF-D, like PDGF-B, might also be of relevance for additional pathological events in progressive renal disease: Nearly all progressive renal diseases that can lead to ESRD are characterized by a progressive renal tubulointerstitial fibrosis, which occurs secondary to glomerular damage.

In contrast to glomerular diseases, much less is known about the biological actions of PDGF in the renal tubulointerstitium. Whereas the α-receptor is expressed constitutively in interstitial cells (29), the β-receptor is only present in injured interstitium (21, 46). A role for PDGF-B and the β-receptor in renal interstitial disease is derived from a study by Tang *et al*. (47), in which pharmacological doses of recombinant PDGF-BB, but not PDGF-AA, induced tubulointerstitial myofibroblast transformation and fibrosis. Upregulation of both receptor chains in fibrotic renal interstitium was confirmed in a study by Taneda *et al.* (19). More importantly, this latter study also assessed the expression of PDGF-D. Like PDGF-B, expression of PDGF-D rapidly increased in renal interstitial cells following unilateral ureteral obstruction, whereas PDGF-A and PDGF-C remained unchanged in fibrotic areas. Areas of PDGF-D overexpression closely overlapped with regions of β-receptor upregulation, providing the basis for increased biological activity. Taneda *et al.* (19) then confirmed these mouse data using renal biopsies from patients with chronic obstructive nephropathy. This study thereby provides a first hint to the possibility that interference with PDGF-D may not only be an attractive goal in glomerular disease, but also in either the primary or the much more common secondary renal tubulointerstitial damage process. Specific intervention studies will be needed to clarify this assumption.

## References

1. Xue, J.L., Ma, J.Z., Louis, T.A., Collins, A.J. *Forecast of the number of patients with end-stage renal disease in the United States to the year 2010*. J Am Soc Nephrol 2001, 12: 2753-8.

2. Donadio, J.V., Grande, J.P. *IgA nephropathy*. New Engl J Med 2002, 347: 738-48.

3. Floege, J., Feehally, J. *IgA nephropathy: Recent developments*. J Am Soc Nephrol 2000, 11: 2395-403.

4. Klahr, S., Schreiner, G., Ichikawa, I. *The progression of renal disease*. New Engl J Med 1988, 318: 1657-66.

5. Striker, L.J., Peten, E.P., Elliot, S.J., Doi, T., Striker, G.E. *Mesangial cell turnover: Effect of heparin and peptide growth factors*. Lab Invest 1991, 64: 446-56.

6. Allen, A.C., Bailey, E.M., Brenchley, P.E., Buck, K.S., Barratt, J., Feehally, J. *Mesangial IgA1 in IgA nephropathy exhibits aberrant O-glycosylation: Observations in three patients*. Kidney Int 2001, 60: 969-73.

7. Stad, R.K., Bruijn, J.A., van Gijlswijk-Janssen, D.J., van Es, L.A., Daha, M.R. *An acute model for IgA-mediated glomerular inflammation in rats induced by monoclonal polymeric rat IgA antibodies*. Clin Exp Immunol 1993, 92: 514-21.

8. Floege, J. *Evidence-based recommendations for immunosuppression in IgA nephropathy: Handle with caution*. Nephrol Dial Transplant 2003, 18: 241-5.

9. Floege, J., Ostendorf, T., Wolf, G. *Growth factors and cytokines*. In: Immunologic Renal Diseases. Neilson, E.G. and Couser, W.G. (Eds.). Lippincott-Raven: Philadelphia 2001.

10. Li, X., Ponten, A., Aase, K. et al. *PDGF-C is a new protease-activated ligand for the PDGF alpha-receptor*. Nat Cell Biol 2000, 2: 302-9.

11. LaRochelle, W.J., Jeffers, M., McDonald, W.F. et al. *PDGF-D, a new protease-activated growth factor.* Nat Cell Biol 2001, 3: 517-21.

12. Bergsten, E., Uutela, M., Li, X., Pietras, K., Ostman, A., Heldin, C.H., Alitalo, K., Eriksson, U. *PDGF-D is a specific, protease-activated ligand for the PDGF β-receptor*. Nat Cell Biol 2001, 3: 512-6.

13. Li, X., Eriksson, U. *Novel PDGF family members: PDGF-C and PDGF-D*. Cytokine Growth Factor Rev 2003, 14: 91-8.

14. Heldin, C.H., Westermark, B. *Mechanism of action and in vivo role of platelet-derived growth factor*. Physiol Rev 1999, 79: 1283-316.

15. Rupprecht, H.D., Sukhatme, V.P., Lacy, J., Sterzel, R.B., Coleman, D.L. *PDGF-induced Egr-1 expression in rat mesangial cells is mediated through upstream serum response elements*. Am J Physiol 1993, 265: F351-60.

16. Changsirikulchai, S., Hudkins, K.L., Goodpaster, T.A., Volpone, J., Topouzis, S., Gilbertson, D.G., Alpers, C.E. *Platelet-derived growth factor-D expression in developing and mature human kidneys*. Kidney Int 2002, 62: 2043-54.

17. Eitner, F., Ostendorf, T., Van Roeyen, C., Kitahara, M., Li, X., Aase, K., Grone, H.J., Eriksson, U., Floege, J. *Expression of a novel PDGF isoform, PDGF-C, in normal and diseased rat kidney.* J Am Soc Nephrol 2002, 13: 910-7.

18. Eitner, F., Ostendorf, T., Kretzler, M., Cohen, C.D., Eriksson, U., Gröne, H.J., Floege, J. *PDGF-C expression in the developing and normal adult human kidney and in glomerular diseases*. J Am Soc Nephrol 2003, 14: 1145-53.

19. Taneda, S., Hudkins, K.L., Topouzis, S. et al. *Obstructive uropathy in mice and humans: Potential role for PDGF-D in the progression of tubulointerstitial injury.* J Am Soc Nephrol 2003, 14: 2544-55.

20. Iida, H., Seifert, R., Alpers, C.E. et al. *Platelet-derived growth factor (PDGF) and PDGF receptor are induced in mesangial proliferative nephritis in the rat.* Proc Natl Acad Sci USA 1991, 88: 6560-4.

21. Alpers, C.E., Seifert, R.A., Hudkins, K.L., Johnson, R.J., Bowen-Pope, D.F. *PDGF-receptor localizes to mesangial, parietal epithelial, and interstitial cells in human and primate kidneys.* Kidney Int 1993, 43: 286-94.

22. Seifert, R.A., Alpers, C.E., Bowen-Pope, D.F. *Expression of platelet-derived growth factor and its receptors in the developing and adult mouse kidney.* Kidney Int 1998, 54: 731-46.

23. Gesualdo, L., Di Paolo, S., Milani, S. et al. *Expression of platelet-derived growth factor receptors in normal and diseased human kidney. An immunohistochemistry and in situ hybridization study*. J Clin Invest 1994, 94: 50-8.

24. Alpers, C.E., Seifert, R.A., Hudkins, K.L., Johnson, R.J., Bowen-Pope, D.F. *Developmental patterns of PDGF B-chain, PDGF-receptor, and α-actin expression in human glomerulogenesis*. Kidney Int 1992, 42: 390-9.

25. Fellstrom, B., Klareskog, L., Heldin, C.H. et al. *Platelet-derived growth factor receptors in the kidney – Upregulated expression in inflammation.* Kidney Int 1989, 36: 1099-102.

26. Gesualdo, L., Pinzani, M., Floriano, J.J. et al. *Platelet-derived growth factor expression in mesangial proliferative glomerulonephritis*. Lab Invest 1991, 65: 160-7.

27. Alpers, C.E., Hudkins, K.L., Ferguson, M., Johnson, R.J., Rutledge, J.C. *Platelet-derived growth factor A-chain expression in developing and mature human kidneys and in Wilms' tumor.* Kidney Int 1995, 48: 146-54.

28. Alpers, C.E., Davis, C.L., Barr, D., Marsh, C.L., Hudkins, K.L. *Identification of platelet-derived growth factor A and B chains in human renal vascular rejection.* Am J Pathol 1996, 148: 439-51.

29. Floege, J., Hudkins, K.L., Davis, C.L., Schwartz, S.M., Alpers, C.E. *Expression of PDGF α-receptor in renal arteriosclerosis and rejecting renal transplants.* J Am Soc Nephrol 1998, 9: 211-23.

30. Floege, J., Hudkins, K.L., Seifert, R.A., Francki, A., Bowen-Pope, D.F., Alpers, C.E. *Localization of PDGF α-receptor in the developing and mature human kidney.* Kidney Int 1997, 51: 1140-50.

31. Floege, J., Eng, E., Young, B.A., Alpers, C.E., Barrett, T.B., Bowen-Pope, D.F., Johnson, R.J. *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 1993, 92: 2952-62.

32. Isaka, Y., Fujiwara, Y., Ueda, N., Kaneda, Y., Kamada, T., Imai, E. *Glomerulosclerosis induced by in vivo transfection of transforming growth factor-β or platelet-derived growth factor gene into the rat kidney.* J Clin Invest 1993, 92: 2597-601.

33. Leveen, P., Pekny, M., Gebre-Medhin, S., Swolin, B., Larsson, E., Betsholtz, C. *Mice deficient for PDGF B show renal, cardiovascular, and hematological abnormalities*. Genes Dev 1994, 8: 1875-87.

34. Soriano, P. *Abnormal kidney development and hematological disorders in PDGF β-receptor mutant mice*. Genes Dev 1994, 8: 1888-96.

35. Lindahl, P., Hellstrom, M., Kalen, M. et al. *Paracrine PDGF-B/PDGF-Rβ signaling controls mesangial cell development in kidney glomeruli.* Development 1998, 125: 3313-22.

36. Johnson, R.J., Raines, E.W., 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 1992, 175: 1413-6.

37. Green, L.S., Jellinek, D., Jenison, R., Ostman, A., Heldin, C.H., Janjic, N. *Inhibitory DNA ligands to platelet-derived growth factor B-chain*. Biochemistry 1996, 35: 14413-24.

38. Floege, J., Ostendorf, T., Janssen, U. et al. *Novel approach to specific growth factor inhibition in vivo: Antagonism of platelet-derived growth factor in glomerulonephritis by aptamers.* Am J Pathol 1999, 154: 169-79.

39. Ostendorf, T., Kunter, U., Groene, H.J. et al. *Specific antagonism of PDGF prevents renal scarring in experimental glomerulonephritis*. J Am Soc Nephrol 2001, 12: 909-18.

40. Nakamura, H., Isaka, Y., Tsujie, M. et al. *Electroporation-mediated PDGF receptor-IgG chimera gene transfer ameliorates experimental glomerulonephritis*. Kidney Int 2001, 59: 2134-45.

41. Gilbert, R.E., Kelly, D.J., McKay, T. et al. *PDGF signal transduction inhibition ameliorates experimental mesangial proliferative glomerulonephritis*. Kidney Int 2001, 59: 1324-32.

42. Ostendorf, T., van Roeyen, C.R., Peterson, J.D. et al. *A fully human monoclonal antibody (CR002) identifies PDGF-D as a novel mediator of mesangioproliferative glomerulonephritis*. J Am Soc Nephrol 2003, 14: 2237-47.

43. Hudkins, K.L., Gilbertson, D.G., Carling, M. et al. *Exogenous PDGF-D is a potent mesangial cell mitogen and causes a severe mesangial proliferative glomerulopathy*. J Am Soc Nephrol 2004, 15: 286-98.

44. Johnson, R.J., Iida, H., Alpers, C.E. et al. *Expression of smooth muscle cell phenotype by rat mesangial cells in immune complex nephritis. α-Smooth muscle actin is a marker of mesangial cell proliferation*. J Clin Invest 1991, 87: 847-58.

45. Liao, X., Escobedo, J.A., Williams, L.T. *Viability of transgenic mice expressing a platelet derived growth factor (PDGF) antagonist in plasma.* J Invest Med 1996, 44: 139-43.

46. Kliem, V., Johnson, R.J., Alpers, C.E., Yoshimura, A., Couser, W.G., Koch, K.M., Floege, J. *Mechanisms involved in the pathogenesis of tubulointerstitial fibrosis in 5/6-nephrectomized rats*. Kidney Int 1996, 49: 666-78.

47. Tang, W.W., Ulich, T.R., Lacey, D.L. et al. *Platelet-derived growth factor-BB induces renal tubulointerstitial myofibroblast formation and tubulointerstitial fibrosis.* Am J Pathol 1996, 148: 1169-80.