

Paracrine PDGF-B/PDGF-R β signaling controls mesangial cell development in kidney glomeruli

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SUMMARY

Kidney glomerulus mesangial cells fail to develop in mice carrying targeted null mutations in the platelet-derived growth factor (PDGF)-B or PDGF-R β genes. We have examined the pattern of expression of these genes and smooth muscle markers during kidney development, to address the possible mechanisms underlying the mutant phenotypes. In wild-type embryos, PDGF-B was expressed in vascular endothelial cells, particularly in capillary endothelial cells in the developing glomeruli, whereas PDGF-R β was found in perivascular mesenchymal cells in the developing renal cortex. In the course of glomerular development, small groups of PDGF-R β and desmin-expressing cells collected in the 'S'-shaped and early cup-shaped vesicles, and at later stages such cells were found in the glomerular mesangium. In PDGF-B or -R β null embryos, some PDGF-R β /desmin or desmin-positive cells,

respectively, were seen in early cup-shaped vesicles, but fewer than in the wild type, and further development of the mesangium failed. In mouse chimeras composed of PDGF-R β $+/+$ and $-/-$ cells, the R β - $-/-$ cells failed to populate the glomerular mesangium. Our results show that while the mesangial cell lineage is specified independently of PDGF-B/R β , these molecules provide critical permissive signals in mesangial cell development. We propose a model in which mesangial cells originate from PDGF-R β -positive progenitors surrounding the developing glomerular afferent and efferent arterioles, and are co-recruited in response to PDGF-B during angiogenic formation of the glomerular capillary tuft.

Key words: PDGF, Kidney, Mouse, Development, Mesangial cell

INTRODUCTION

The metanephric kidney develops as a result of reciprocal signaling between the nephric duct and the metanephrogenic mesenchyme (Grobstein, 1955). This leads to the formation of a branch from the nephric duct, the ureteric bud, which grows into and branches within the metanephrogenic mesenchyme. The ureteric bud and its branches eventually forms the ureter, the renal pelvis and the collecting ducts. The functional units of the metanephric kidney, the nephrons, consist of the glomeruli, the proximal and distal convoluted tubules and Henle's loop. All epithelial cell types of the nephron originate from small groups of mesenchymal cells that aggregate at the branching ureter buds and undergo epithelial conversion. These aggregates go through a series of well-characterized morphological stages during their development, referred to as 'comma'-, 'S'- and 'cup'-shaped. In addition to the nephric duct- and metanephrogenic mesenchyme-derived epithelial cell types, a third important component of the metanephrogenic kidney is the vasculature. In the glomerulus, this consists of a complex of capillary loops and associated mesangial cells. The

formation of the glomerulus is initiated when a capillary loop enters into the epithelial cleft formed in the S-shaped stage of the developing nephron. In the cup-shaped stage, and later in the mature glomerulus, this loop branches to produce the complex capillary tuft. In this process, mesangial cells populate the core of the tuft and connect the capillary loops through the deposition of mesangial matrix; this becomes focally attached to the glomerular basement membrane (GBM), which is produced by the podocytes and endothelial cells (Ekblom, 1981; Sariola et al., 1984). The GBM constitutes the glomerular filter together with its cellular lining: fenestrated capillary endothelial cells on the inside and epithelial podocytes on the outside. The development of the glomerular mesangium takes place late in the formation of the nephron and coincides with capillary tuft maturation. The origin of mesangial cells has been unclear, but sometimes these cells are referred to as specialized microvascular pericytes (Martinez-Hernandez and Amenta, 1983; Michael et al., 1980; Sariola et al., 1984).

During recent years a number of growth and differentiation factors and their receptors have been identified as key signaling

molecules in the development of the metanephric kidney. Glial-derived neurotrophic factor (GDNF) is produced by the metanephrogenic mesenchyme and triggers signaling through the Ret protein expressed on the ureteric bud. GDNF or Ret null mice fail to develop kidneys (Moore et al., 1996; Pichel et al., 1996; Sánchez et al., 1996; Schuchardt et al., 1996). Fibroblast growth factor-2 (FGF-2 or basic FGF) expressed by the ureteric bud appears to provide a survival signal to mesenchymal cells and to stimulate their aggregation as a prelude to the epithelial conversion (Perantoni et al., 1995). Bone morphogenic protein-7 (BMP-7), also expressed by the ureteric bud, appears to be important for epithelial differentiation and to inhibit apoptosis of the renal mesenchyme, and its absence in mice leads to severe renal hypoplasia (Dudley et al., 1995; Luo et al., 1995). Epithelial conversion of the mesenchymal aggregates has been suggested to depend on their autocrine production of Wnt-4, and Wnt-4 null mice fail to develop pretubular epithelial aggregates (Stark et al., 1994). Hepatocyte growth factor/scatter factor (HGF) is expressed by metanephrogenic mesenchyme and its receptor Met is expressed by the ureteric bud (Santos et al., 1994; Woolf et al., 1995). Their roles in kidney development remain to be established, as does the role for Wnt-11, which is expressed by the ureteric bud (Kispert et al., 1996).

The in-growth of the capillary loop into the S-shaped glomerular vesicle and the further growth and branching of glomerular capillaries may be driven by VEGF, since this factor is produced by the developing glomerular epithelium (Breier et al., 1992). The emergence of mesangial cells in the glomerulus is dependent on platelet-derived growth factor-B (PDGF-B) and its receptor PDGF receptor- β (PDGF-R β), since mice carrying null mutations in the PDGF-B or PDGF-R β genes lack mesangial cells in their glomeruli (Levéen et al., 1994; Soriano, 1994). The complex capillary tufts were also lost in these mutants and replaced by a single, or a few, dilated capillary loops. The reason for mesangial cell loss in PDGF-B and -R β null mice remains unclear. Mesangial cells have been demonstrated to carry PDGF-R β in the developing human kidney (Alpers et al., 1992, 1993) and in conjunction with pathological mesangial cell proliferation associated with glomerular disease (Fellström et al., 1989; Floege et al., 1992; Iida et al., 1991). These cells might be direct targets of PDGF-B, but the local source of PDGF-B and the cellular mechanisms controlled by PDGF-B have not been clarified. In vitro, PDGFs have been shown to stimulate mesenchymal cell proliferation, migration, production of extracellular matrix and certain functions such as contraction (reviewed in Betsholtz and Raines, 1997). For mesangial cells specifically, PDGF-B dimers have been shown to be mitogenic (Shultz et al., 1988; Silver et al., 1989; Floege et al., 1991; Madri and Marx, 1992; Abboud et al., 1994) and chemotactic (Barnes and Hevey, 1990; H. Abboud, personal communication) and to stimulate production of extracellular matrix (Doi et al., 1992) and contraction (Mene et al., 1987). The present analysis of glomerular development in PDGF-B and -R β null mice, and in mouse chimeras composed of R β ^{-/-} and R β ^{+/+} cells, was undertaken to provide insight into the ontogeny of mesangial cells, to pinpoint the stage at which mesangial cell development fails in the absence of PDGF-B/R β signals and, potentially, to reveal the cellular mechanism(s) controlled by PDGF-B in the development of the glomerular mesangium.

MATERIALS AND METHODS

Animals

PDGF-B ^{+/−} mice (Levéen et al., 1994) and PDGF-R β ^{+/−} mice (Soriano, 1994) were bred as 129Ola/C57BL6J and 129Sv/C57BL6J hybrids, respectively. Heterozygotes were intercrossed and E15.5–17.5 embryos were isolated and fixed for immunohistochemistry or in situ hybridization. Tie^{lcz} mice (Puri et al., 1995) (kindly donated by Dr A. Bernstein) represent heterozygous carriers of a lacZ coding sequence integrated in the Tie1 locus and, hence, under the transcriptional control of Tie1 regulatory elements. Tie^{lcz} mice (129Sv/C57BL6J background) were propagated by heterozygous breeding and *tie*^{lcz} carriers identified by lacZ staining of tail tissue.

Histological analysis

Immunohistochemistry was performed using antibodies directed against smooth muscle α -actin/ASMA (clone 1A4; Dako, Denmark) and desmin (clone D33; Dako) according to protocols supplied by the manufacturers. For detection of renin we used a polyclonal rabbit anti-mouse renin antibody (kindly supplied by Dr Tadashi Inagami, Vanderbilt University; Michelakis et al., 1974) and horseradish peroxidase-conjugated antibodies against rabbit immunoglobulins (Dako).

In situ hybridization

We applied a non-radioactive protocol for in situ hybridization, which involves the use of digoxigenin-labeled RNA probes (Boehringer-Mannheim), and their detection on sections using alkaline phosphatase-conjugated antibodies (Boström et al., 1996). PDGF-B and -R β sense and antisense probes were generated as described (Lindahl et al., 1997b). The results shown were obtained on sections 14 μ m thick. Unstained sections in combination with Nomarski optics were used to allow for good sensitivity and resolution. As negative controls, the corresponding sense probes were used. No hybridization signal was obtained with any of these probes.

Chimera analysis

PDGF-R β ^{-/-} ES cells were isolated from β R2 ES cells, in which one allele of the PDGF-R β gene was disrupted with a neo expression cassette (Soriano, 1994). Three homozygous mutant ES cells were isolated from a total of 52 clones selected at 3 mg/ml G418 (1.5 mg/ml active powder), using the 'step-up' procedure (Mortensen et al., 1992). 5–15 ES cells were injected in blastocysts derived from matings of ROSA26 mice backcrossed seven generations to C57BL6/J, which express lacZ ubiquitously in the embryo (Zambrowicz et al., 1997). Tissues isolated from E14–E18 embryos and from adults were cut to small sizes to allow dye penetration, then fixed and stained with X-Gal as described previously (MacGregor et al., 1995).

BrdU labeling

Cells replicating their DNA were labeled at E15.5 by intraperitoneal injection of BrdU (Sigma, 100 μ g/g body mass) in the pregnant mother. The embryos were delivered 2 hours later by caesarean section, fixed in 4% PFA, embedded in paraffin and sectioned (4 μ m). The sections were incubated with an anti-BrdU antibody (Becton Dickinson, Catalog number 347580), a rabbit anti-mouse biotin-conjugated secondary antibody (Dako) and HRP-conjugated streptavidin (Dako), according to standard immunohistochemical protocols.

RESULTS

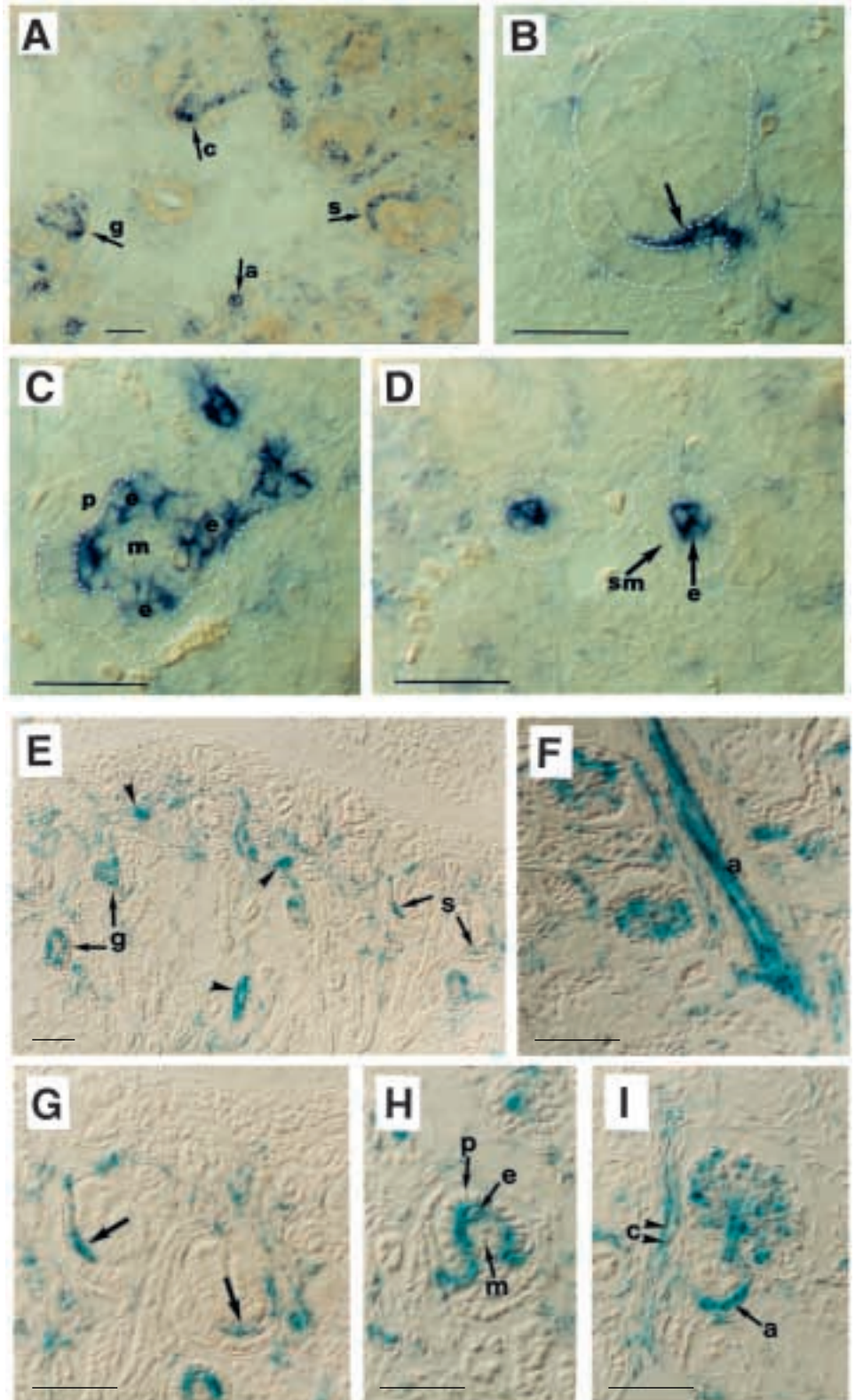
PDGF-B and -R β expression patterns in the developing kidney

To provide insight into the mechanism of mesangial cell failure in PDGF-B or -R β null embryos, we mapped the expression patterns of PDGF-B and -R β in the developing mouse kidney

using non-radioactive in situ hybridization. PDGF-B was expressed in the endothelium of developing glomerular capillaries (Fig. 1A-C) and interlobular arteries (Fig. 1A,D). In the course of glomerulogenesis, the PDGF-B probe labeled the capillary sprout entering into the epithelial cleft formed by the

S-shaped glomerulus (Fig. 1A,B). In cup-shaped vesicles PDGF-B expression fell in the endothelial layer, intermediate to the outermost layer of PDGF-B-negative epithelial cells and the likewise PDGF-B-negative central core of mesenchymal cells (Fig. 1C).

Fig. 1. PDGF-B and *Tie-lacZ* expression in the developing mouse kidney. Non-radioactive in situ hybridization showing the expression of PDGF-B (A-D) and *tie-lacZ* (E-I) in the wild-type embryonic kidney. Expression is seen as blue staining in unstained sections viewed by interference contrast. (A) PDGF-B expression is seen in vascular endothelial cells in developing S-shaped (s) and cup-shaped glomeruli (c) and in mature glomeruli (g). The endothelium of a cross-sectioned arteriole (a) is also labeled. (B) A higher magnification of an S-shaped glomerulus. Note the PDGF-B expression in the endothelial sprout between the epithelial cell layers. (C) PDGF-B expression in a late cup-shaped vesicle shown at high magnification. PDGF-B mRNA is evident in the cytoplasm of endothelial cells (e) situated beneath the developing podocyte layer (p). Note the lack of labeling in the mesangial cells (m) situated at the center of the developing glomerulus. (D) PDGF-B expression in arteriolar endothelium (e). The lumen of these vessels is commonly very narrow and, hence, cross-sections appear as illustrated with the labeled endothelial cells tightly clustered. The surrounding developing vascular wall SMC (sm) do not express PDGF-B, but are strongly positive for PDGF-R β . Dashed lines indicate developing glomerular epithelium in B, C and vascular wall in D. (E) Overview of *tie-lacZ* expression in the developing E16.5 kidney. Arrowheads point at arterial and arteriolar endothelium. Two S-shaped glomeruli (s) and two late cup-shaped glomeruli (g) are indicated. (F) A longitudinally sectioned interlobular artery (a) flanked on the left by two glomeruli. (G) Magnification of section in E showing *tie-lacZ* labeling of the capillary sprout (arrows) entering into the cleft of the S-shaped stage. (H) A cup-shaped glomerulus in which the *tie-lacZ* negative podocytes (p), the positive endothelium (e) and the negative mesangium (m) are indicated. (I) Mature glomerulus with folded capillary tuft. Arrow points at an afferent or efferent arteriole (a) and arrowheads point at capillaries (c). Bars, 50 μ m.



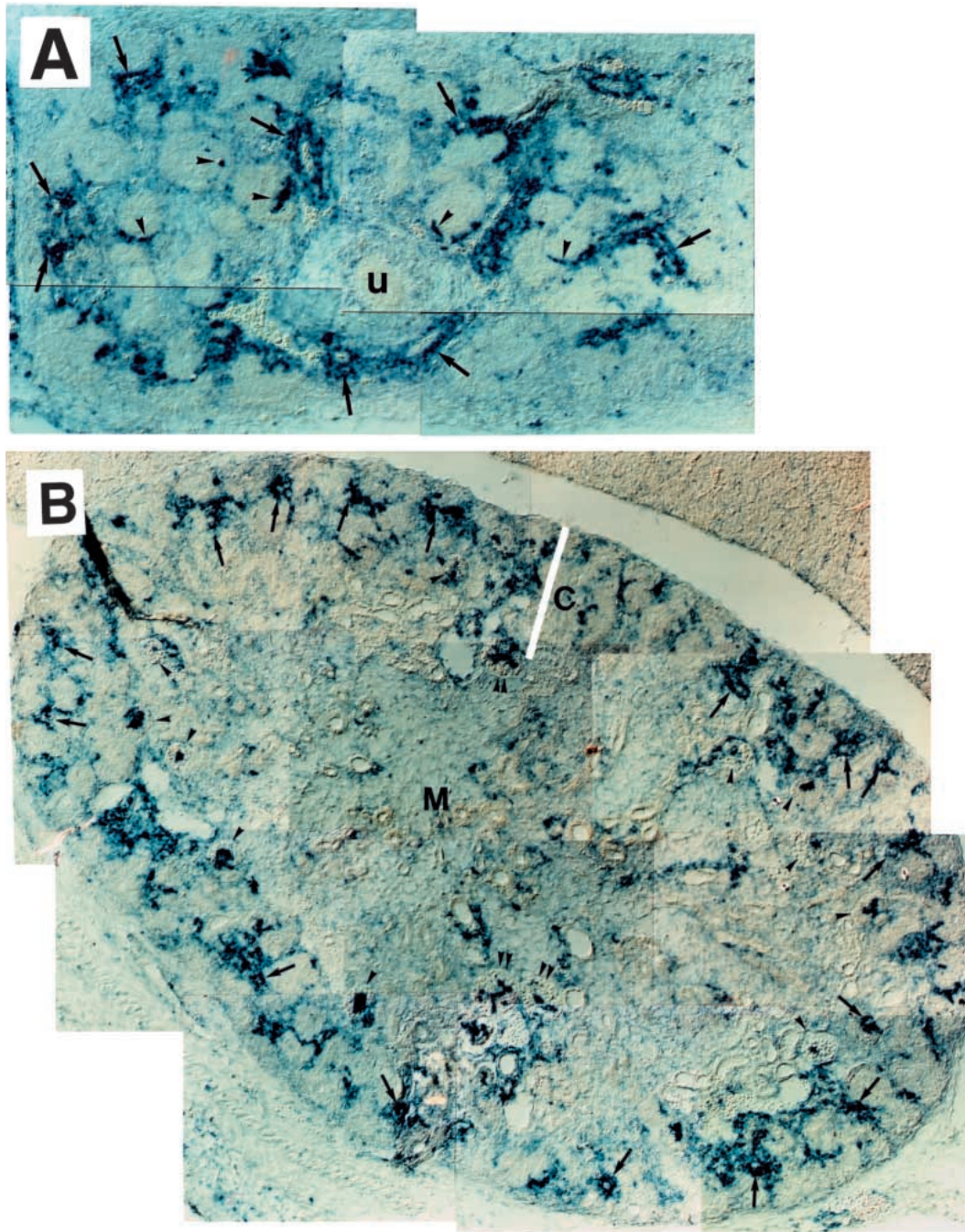
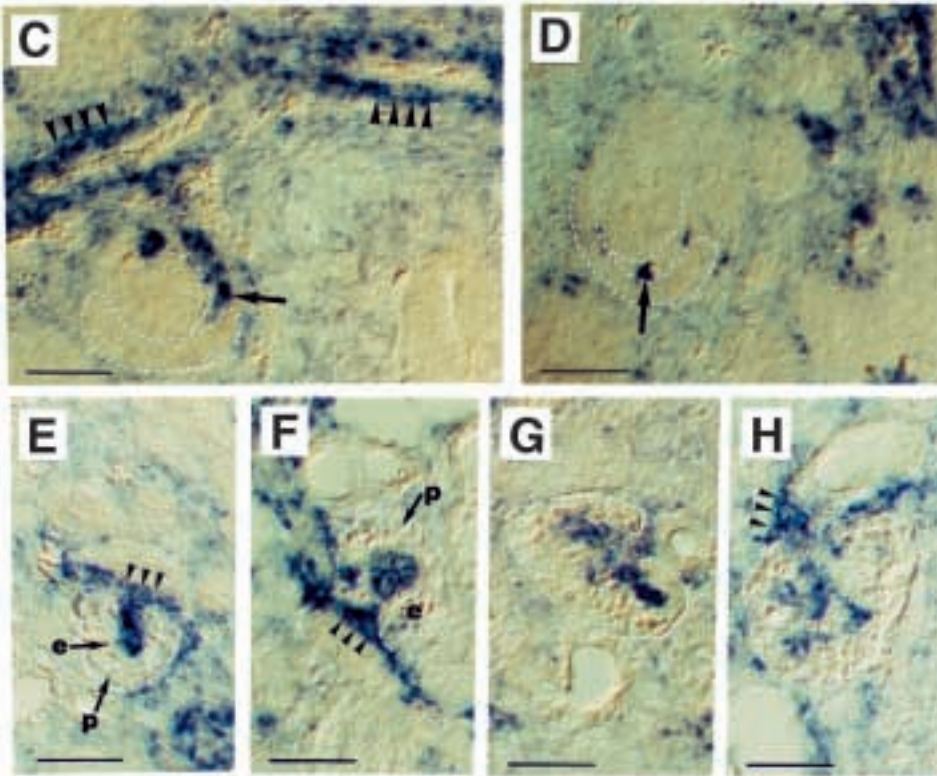


Fig. 2. PDGF-R β expression in the developing mouse kidney. (A) E14.5 kidney. Note the predominant expression of PDGF-R β surrounding the developing renal arterioles (arrows). Some R β -positive cells can also be seen in the cleft formed by comma and S-stage glomeruli (arrowheads). (B) Expression of PDGF-R β is seen in E16.5 arterioles (arrows) and in the mesangium of mature glomeruli (arrowheads) which collect in the deeper part of the cortex. (C,D) Higher magnification of PDGF-R β expression in developing arteriolar walls (arrowheads) and in cells associated with the capillary sprout entering into the S-shaped glomerulus (arrows). (E) Early and (F) late cup-shaped glomeruli labeled for PDGF-R β . Note that the mesangial core is strongly labeled and that this labeling is continuous with afferent and efferent arteriolar walls (arrowheads). Note also that the podocytic (p) and endothelial (e) layers lack PDGF-R β expression. (G,H) PDGF-R β expression in mature glomeruli in which the GBM has folded. The R β probe typically labels the glomerular mesangium. In H the mesangial labeling is seen continuous with afferent and efferent arteriolar walls (arrowheads). U, ureter; M, medulla; C, cortex. The white bar in B indicates the approximate depth of the developing cortex. Dashed lines in C-H indicate developing glomerular epithelium. Bars, 50 μ m.

The expression of PDGF-B was similar to that of the *tie-1* gene. The Tie-1 receptor tyrosine kinase is expressed specifically in vascular endothelium and particularly in immature endothelial cells (Korhonen et al., 1995; Puri et al., 1995). We have previously observed co-expression of PDGF-B and *tie-1* in vascular endothelium at extrarenal sites (Lindahl et al., 1997a). In the kidney Tie-1 labeling was apparent in the capillary sprout entering the S-shaped stage, and at later stages expression was clearly confined to the glomerular endothelium, whereas mesangial cells were negative (Fig. 1E-I).

PDGF-R β expression was seen in a proportion of the mesenchymal cells in the developing renal cortex (Fig. 2A,B) as well as in a few cells in the medulla (Fig. 2B). The PDGF-

R β positive (PDGF-R β ⁺) cells in the cortex were located in the vicinity of developing interlobular arteries and afferent or efferent glomerular arterioles. The PDGF-R β ⁺ cells have a typical perivascular location and may represent precursors to vascular smooth muscle cells (SMC). Interlobular arteries were surrounded by several layers of PDGF-R β ⁺ cells (Fig. 2A, arrows; 2C, arrowheads). The perivascular expression was particularly clear when serial sections were analyzed and blood vessels could be followed over longer distances (not shown). Glomerular expression of PDGF-R β was first seen in singular cells associated with the ingrowing capillary loop in the S-shaped stage of glomerular development (Fig. 2C,D, arrows). Cell counting on serial sections revealed that groups of 10-15



PDGF-R β ⁺ cells collected at the base and in the mesenchymal core of cup-shaped vesicles (Fig. 2E,F and data not shown). In the further maturation of the glomerular tufts the PDGF-R β labeling was clearly confined to the mesangial cells of the tuft (Fig. 2G,H). PDGF-R β ⁺ cells in the extraglomerular blood vessel wall were in physical contact with the developing mesangium (see glomeruli in Fig. 2E-H). This is consistent with the view that later the mesangium is continuous with the vascular wall of afferent and efferent arterioles. In summary, the expression studies establish that PDGF-B is expressed by renal arteric and glomerular capillary endothelium and that PDGF-R β is a marker for developing vascular SMC and mesangial cells.

Mesangial cell failure in PDGF-B and -R β null mice occurs during formation of the cup-shaped stage of glomerular development

The fate of mesangial cells in wild-type and PDGF-B^{-/-} embryos was studied using three markers for developing SMC, pericytes and mesangial cells: PDGF-R β , desmin and smooth muscle α -actin (ASMA). In PDGF-B^{-/-} embryos PDGF-R β expression appeared normal in the perivascular mesenchymal cells surrounding interlobular arteries and glomerular afferent and efferent arterioles (Fig. 3A and data not shown). PDGF-R β ⁺ cells were also seen in association with the early stages of glomerulogenesis (Fig. 3A, arrows) but as the glomerulus developed further and the dilated capillary loops typical for mutant glomeruli became apparent, PDGF-R β ⁺ cells were located at the juxtaglomerular area (Fig. 3B, arrows).

A similar pattern was seen when desmin was used as a marker for mesangial development. Desmin was expressed in juxtaglomerular blood vessel walls, in the mesenchymal core

of early to late cup-shaped vesicles (Fig. 3C,D) and in the mature mesangium (Fig. 3E,F). In PDGF-B^{-/-} embryos (Fig. 3) or in PDGF-R β ^{-/-} embryos (not shown), desmin-labeled cells were found in or in apposition to cup-shaped vesicles (Fig. 3G,H), but were fewer than in wild type and preferentially located at the juxtaglomerular region (compare Fig. 3G,H with C,D). In a few late-stage glomeruli, however, single or small groups of desmin⁺ cells were seen between the expanded capillary loops (Fig. 3I).

ASMA antibodies labeled renal arteric and arteriolar walls strongly (Fig. 3J-M), and also mesangial cells (Fig. 3J). In contrast to the PDGF-R β and desmin expression, ASMA labeling of the developing glomerulus was seen first in the late cup-shaped vesicles as a weak staining (Fig. 3J,K), that was markedly intensified upon folding of the GBM (Fig. 3J). Thus, ASMA appears to be a marker for a more differentiated population of SMC and mesangial cells than PDGF-R β

and desmin. In the PDGF-B^{-/-} embryo, no ASMA expression was seen in the late cup-shaped vesicles or in mature glomerular structures (Fig. 3L,M), except for rare individual mesangial cells that apparently escape the PDGF-B/R β signaling block (Fig. 3N, arrows).

No obvious discrepancies in extraglomerular blood vessel expression of PDGF-R β , desmin or ASMA were revealed when PDGF-B^{-/-}, PDGF-R β ^{-/-} and wild-type mice were compared (Fig. 3A-N and data not shown). Also renin, which is selectively expressed in a subset of the juxtaglomerular blood vessel wall cells (Michelakis et al., 1974; Taugner et al., 1982), was found to be expressed in PDGF-B^{-/-} kidneys in a pattern which did not seemingly diverge from wild-type kidneys (Fig. 3O-R).

Cell proliferation in glomerular development

BrdU labeling was used to study cell proliferation in the developing mouse kidney. We focused our attention on the cup-shaped vesicles since glomerular development appears to fail at this stage in the PDGF-B^{-/-} and -R β ^{-/-} kidneys. In wild-type cup-shaped vesicles, BrdU labeling was evident both in the central part of the mesenchymal core presumed to contain mesangial progenitors and in the core periphery believed to contain developing endothelial cells (compare BrdU-labeling pattern in Fig. 4C,D with PDGF-R β expression pattern in Fig. 4A,B, and PDGF-B/tie-1 expression patterns in Fig. 1C,H). In contrast, the developing podocytes appeared to be post-mitotic or cycled at a very slow rate (a rare BrdU-labeled podocyte is seen in Fig. 4D). In PDGF-B^{-/-} kidneys, some cells were labeled in the mesenchymal core of the cups. Following ballooning of the capillaries during glomerular maturation (Fig. 4E,F) it was clear that endothelial cells were proliferating.

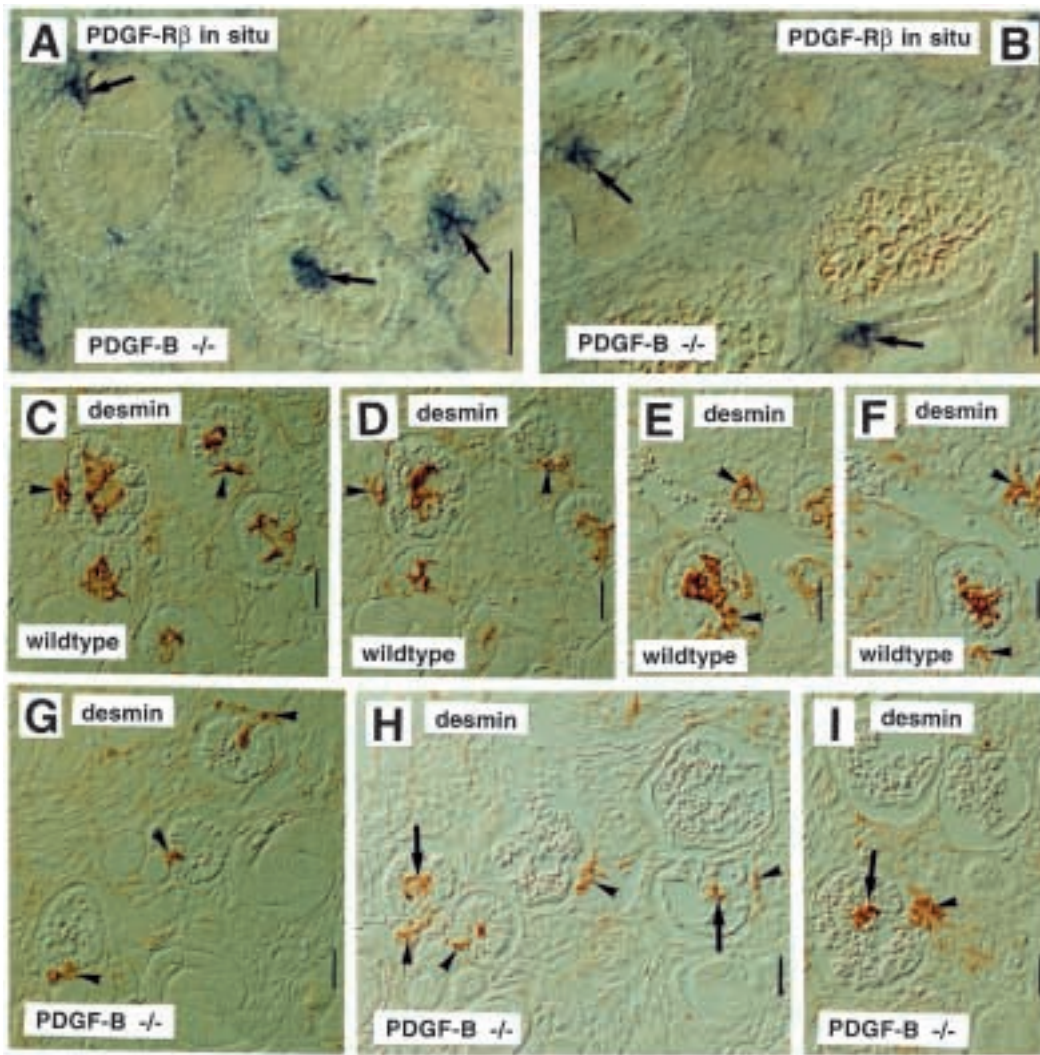


Fig. 3. Mesangial cell developmental block in PDGF-B $-/-$ kidneys. (A) Non-radioactive in situ hybridization demonstrates PDGF-R β expression (blue) in the developing S-shaped glomerulus (left arrow) and in early cup-shaped glomeruli (middle and right arrows). (B) In late-stage glomeruli in the PDGF-B $-/-$ kidney, the PDGF-R β positive cells are only seen in the juxtaglomerular region (arrows). Dashed lines in A and B indicate the developing glomerulus. (C-I) Desmin immunohistochemistry (brown staining). (C,D) Adjacent sections showing the desmin staining of various cup-shaped glomeruli in wild-type kidneys. Note that the glomerular mesangial core is strongly labeled, but that labeling also extends to the juxtaglomerular region (arrowheads). (E,F) Adjacent wild-type sections showing the desmin labeling of mesangial cells of mature glomeruli in which folding of the GBM has started. Juxtaglomerular blood vessel wall cells are also labeled (arrowheads). (G-I) Desmin labeling of PDGF-B $-/-$ kidneys. (G) shows three late cup-shaped vesicles.

The few cycling cells in the mesenchymal core in the cup-shaped vesicles could thus represent endothelial cells.

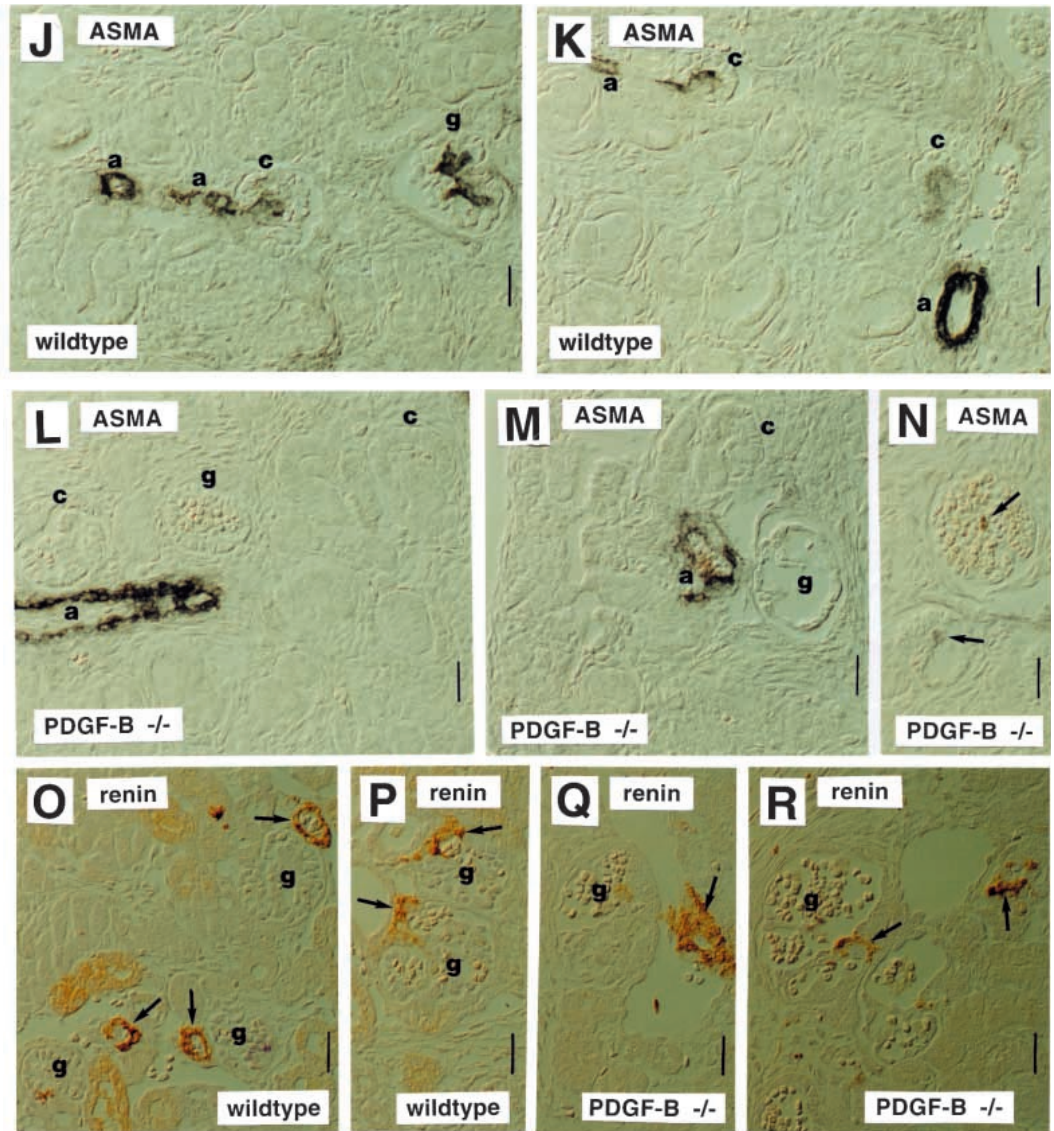
PDGF-R β $+/+$ cells are selected in the mesangium and vascular walls of PDGF-R β $+/+$ and $-/-$ chimeras

The perinatal death of PDGF-B and -R β null embryos limits the possibilities for analysis of the mechanism of mesangial failure to a short period immediately prior to birth. To further investigate the ability of PDGF-R β $-/-$ cells to populate the mesangium, chimeras were generated between wild-type and PDGF-R β $-/-$ cells by injecting PDGF-R β $-/-$ ES cells into PDGF-R β $+/+$ blastocysts. As PDGF-R β is a cell-associated molecule, its mutation might be expected to act cell-autonomously, and lead to reconstitution of the mutant phenotype in chimeras consisting to a high degree of PDGF-R β $-/-$ cells. No defect in kidney development was observed by hematoxylin and eosin staining in E14-E18 chimeras generated by injecting small (approx. 5) numbers of ES cells into blastocysts (not shown). Therefore, larger number of ES cells (approx. 15) were injected into recipient blastocysts taken from the ROSA26 mouse strain, which express beta-galactosidase (lacZ) ubiquitously (Zambrowicz et al., 1997), and the chimeras were allowed to develop as adults. In sections

from these chimeras, wild-type cells express lacZ whereas PDGF-R β $-/-$ cells do not.

Using this type of analysis with two independently derived mutant ES cell lines, we found that some of the chimeras exhibited extensive contribution of mutant cells. To quantify the extent of chimerism, we estimated the percentage of wild-type and mutant cells by Southern blots of tail biopsies. In 18 chimeras produced with two different PDGF-R β $-/-$ ES cell clones, the contribution of mutant cells in the tails varied from 26% to 68%, with a mean of 48.5% (± 12.5 s.d.). Four of the most extensive chimeras were analyzed in further detail. One part of the tissues was subjected to Southern blot analysis to quantify the percentage of mutant cells, and the other part was subjected to histological analysis. In these chimeras, the heart, spleen, lung, kidney, brain and testis were composed in an average of 76% mutant cells (80% in the two kidneys), and surprisingly these mice displayed no phenotypic abnormalities. Histological analysis revealed that among more than 50 glomeruli examined in each of two chimeras, the glomerular mesangium was invariably composed of PDGF-R β $+/+$ cells (Fig. 5B). Thus, the PDGF-R β mutant allele acts in a manner expected for a cell-autonomous mutation, i.e. the cell carrying the mutation gets eliminated in the particular process or tissue studied.

Labeling is seen in the juxtaglomerular area and in a small number of intraglomerular mesangial cells. (H) Labeling in an early cup-shaped vesicle (left arrow), in a late-stage glomerulus (right arrow) and in juxtaglomerular cells (arrowheads). (I) Desmin labeling of residual mesangial cells in a late-stage glomerulus (arrow) and in juxtaglomerular cells (arrowhead). (J-N) ASMA labeling (black) by immunohistochemistry. In wild-type kidneys (J,K) expression is seen in arteriolar walls (a) and in the mesangium of mature glomeruli (g), and as a weaker staining in cup-shaped glomeruli (c). In PDGF-B^{-/-} kidneys (L-N) staining was seen in arterioles (a), but not in cup-shaped glomeruli (c). Late-stage glomeruli occasionally contained single or small groups of ASMA-positive cells (N, arrows). (O-R) Renin immunohistochemistry demonstrates the presence of renin in juxtaglomerular blood vessel wall cells in both wild-type (O,P, arrows) and PDGF-B^{-/-} (Q,R, arrows) kidneys. g, glomeruli. Bars, 50 μ m.



In addition, strong selection for PDGF-R β ^{+/+} cells was also seen in renal interlobular arterial SMC (Fig. 5A). We show above that during prenatal development such arteries have endothelial expression of PDGF-B and perivascular expression of PDGF-R β , but PDGF-R β and other vascular SMC markers were still expressed in mutant embryos. This suggests that although the vascular wall of developing interlobular arteries is properly induced during embryogenesis, the initial selection of vascular SMC or further proliferation of these cells, which occurs in conjunction with postnatal vessel enlargement, is dependent on PDGF-B/R β signaling. This is consistent with a recent study in which chimera analysis demonstrated strong selection for PDGF-R β ^{+/+} cells over ^{-/-} cells in the vascular wall at many extrarenal sites (Crosby et al., 1998).

DISCUSSION

Paracrine PDGF-B/R β signaling controls mesangial cell development

Our previous work established that the development of

mesangial cells depends on PDGF-B and PDGF-R β (Levéen et al., 1994; Soriano, 1994), but did not distinguish between direct or indirect dependence. The present work supports a direct effect of PDGF-B on the mesangial cell lineage. The chimera analysis shows that the PDGF-R β mutation is cell-autonomous, arguing that the mesangial cells themselves, or their progenitors, as well as intrarenal arterial and arteriolar SMC are critical targets for the PDGF-B signal. This is in agreement with the expression pattern of PDGF-B and -R β in the developing glomerulus, where PDGF-R β is expressed by vascular SMC and developing mesangial cells and PDGF-B by vascular endothelium, including the developing glomerular capillary endothelial cells.

Ontogeny of mesangial cells

Mesangial cells are mesenchymal cells that connect the capillary loops in the glomerular tuft. Their role and origin have remained unclear. Contractile capacity and marker expression may suggest a relationship to SMC, but phagocytic activities and responsiveness to cytokines (Toyabe and

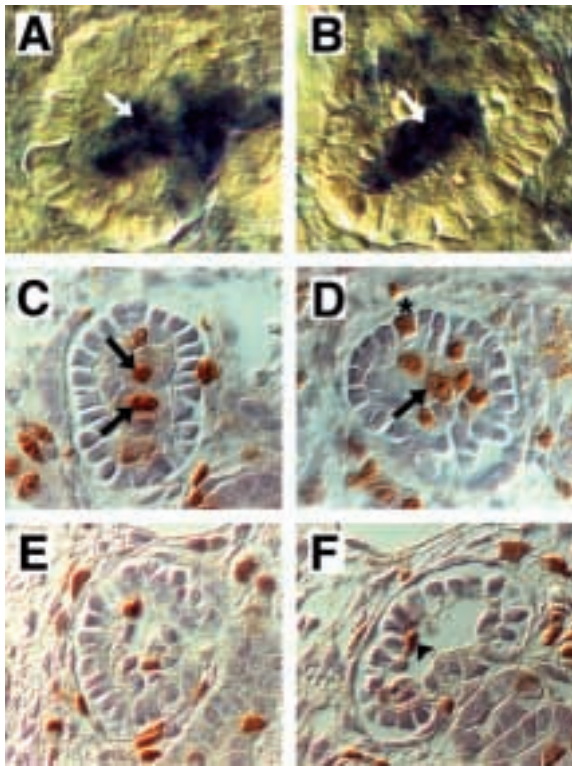


Fig. 4. Cell proliferation in developing glomeruli. BrdU was used to label nuclei of cells in the S-phase of the cell cycle. The BrdU-labeling pattern of wild-type cup-shaped glomeruli (C,D) is compared with the wild-type pattern of PDGF-R β expression (A-B). Note that the labeling index is high in general in the mesenchymal core of the vesicles and that abundant labeling is apparent in the central, PDGF-R β -positive, part of the core (compare C,D with A,B; presumptive mesangial progenitors indicated by arrows). E-F shows different stage PDGF-B $^{-/-}$ glomeruli labeled with BrdU. Note that while few podocytes are labeled (an exception is seen in D, asterisk), labeling of endothelial cells is apparent in the mutant glomeruli (arrowhead).

Iwanaga, 1992; Savill et al., 1992; reviewed in Schlondorff and Mori, 1990) show a resemblance to macrophages. It has also been suggested that mesangial and endothelial cells may be related or have a common origin, based on chimeric transplantation studies (Abrahamson, 1994; Hyink et al., 1996). Mesangial cells are sometimes referred to as specialized pericytes (Martinez-Hernandez and Amenta, 1983). Pericytes are contractile SMC-like cells embedded in capillary basement membranes. Similarly, mesangial cells are embedded in matrix that is focally contacting the inner leaflet of the GBM.

The presented analysis of marker expression supports a common origin of mesangial cells and SMC of glomerular afferent and efferent arterioles. These cells share the properties of PDGF-R β and desmin expression in their early development and ASMA expression later. The lining of the afferent and efferent arterioles with PDGF-R β -positive cells is continuous with the developing mesangium, and PDGF-R β $^{+/+}$ cells at both these locations are preferentially selected in chimeras between PDGF-R β $^{+/+}$ and $^{-/-}$ cells. The glomerular capillaries probably develop by angiogenic sprouting from the prospective afferent/efferent arteriole and it is conceivable that mesangial cells in this process are co-recruited from arteriolar SMC

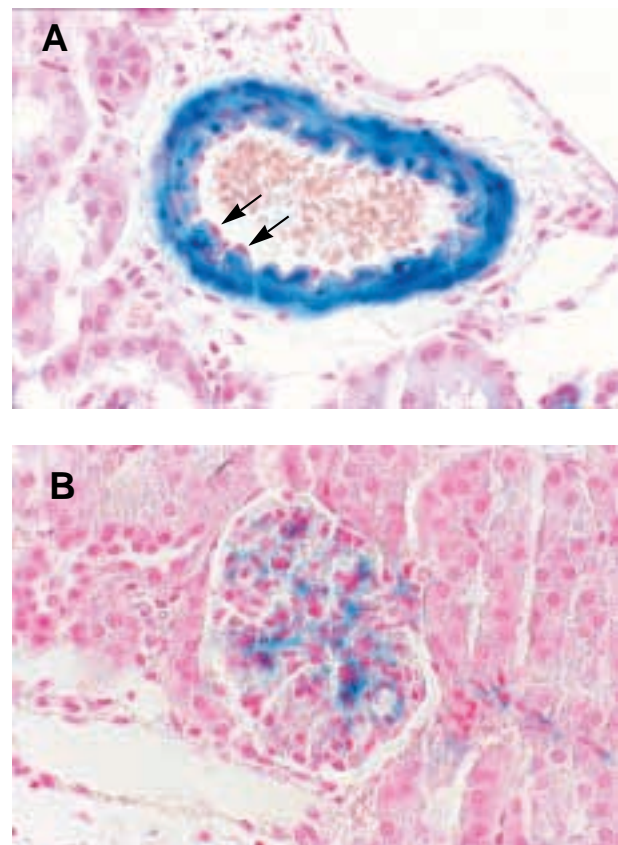


Fig. 5. Selection for PDGF-R β $^{+/+}$ cells in the adult kidney. Sections from adult chimeras between lacZ-positive PDGF-R β $^{+/+}$ and lacZ-negative PDGF-R β $^{-/-}$ cells. Note the preferential selection of R β $^{+/+}$ cells in the glomerular mesangium (B) and the interlobular arterial SMC (A). In A the majority of SMC are blue (R $\beta^{+/+}$) whereas the vascular endothelium (arrows) as well as surrounding tissue is R β $^{-/-}$ (unstained for lacZ).

progenitors, as schematically illustrated in Fig. 6. Our model is highly similar to a recently proposed scenario for repair of the mesangium following antibody-induced lysis of mesangial cells in rats (Hugo et al., 1997). In this study new mesangial cells were shown to be recruited from the juxtaglomerular area in a process involving proliferation and migration. A similar situation has also been proposed for the development of brain pericytes. These originate from PDGF-R β $^{+}$ vascular wall progenitors in the perineural vascular plexus, and are co-recruited in response to endothelium-derived PDGF-B in conjunction with angiogenic sprouting into the nervous tissue (Lindahl et al., 1997a).

Mechanism(s) of mesangial failure in PDGF-B and PDGF-R β null embryos

Studies of PDGF-induced cellular activities *in vitro* argue that PDGF-B chain-containing PDGF dimers can induce a spectrum of cellular activities, which may be relevant to the failure of a functional mesangium to form in the absence of PDGF-B or -R β , such as cell proliferation, survival, migration and extracellular matrix production. Although fewer PDGF-R β and desmin-positive cells were detected in PDGF-B and -R β mutants at juxtaglomerular perivascular sites and in early stages

Fig. 6. Schematic illustration of mesangium development in wild-type and PDGF-B/R β $-/-$ mouse embryos. Glomerular development is introduced and discussed in the text. Epithelial structures are depicted in blue. The diagram shows the entry of a capillary sprout, the prospective afferent/efferent arterioles (red), into the S-shaped vesicle, and its associated vascular wall SMC progenitor cells (black). The diagram illustrates the failure of glomerular capillary tuft development in $-/-$ embryos at the transition between the cup-shaped stage and the mature glomerulus as a result of either lack of PDGF-B in the endothelium or PDGF-R β in the mesangial progenitors.



of glomerular development, their presence nonetheless suggests that the defect in the mutant embryos is not solely related to a failure in cell-fate determination. The loss of mesangial cells in PDGF-B and -R β mutant glomeruli is therefore also likely coupled to impaired selection for mesangial progenitors in the course of glomerular maturation. Such selection by PDGF-B may involve an inability for the cells to migrate into the vesicle or a defect in the ability of these cells to proliferate or survive once they have entered the vesicle. TUNEL staining and routine histological examination (not shown) failed to detect increased apoptosis or pyknotic cells in the developing mesangium of mutant glomeruli, indicating that the loss of mesangial cells is unlikely to be attributed to increased cell death. In wild-type embryos, BrdU labeling indicates that proliferation is important for mesangial cell development, since these are actively proliferating in cup-shaped glomeruli. In mutant glomeruli, BrdU labeling indicated that fewer cells were proliferating, but this might simply reflect the smaller number of cells in these structures as the mitotic index did not change (data not shown). Likewise, marker analysis suggests that migration of mesangial cell progenitors from the afferent/efferent arterial wall into the glomeruli is necessary. Whether PDGF explicitly promotes one or both of these activities could not be resolved in this study. The sudden ballooning of the glomerular capillary loops at the late-cup stage may also suggest a failure of mesangial cells to make focal attachments to the GBM, which probably reflects lack of cells but possibly also improper cell differentiation or function, such as extracellular matrix synthesis.

Analogous PDGF functions in organogenesis

The paracrine function of PDGF-B suggested here to promote development of mesangial cells is largely analogous to the function suggested for PDGF-A in the development of lung alveoli (Boström et al., 1996; Lindahl et al., 1997b). In the developing lung, PDGF-A promotes the proliferation and distal spreading of progenitors of the alveolar SMC along the branches of the respiratory epithelium in much the same way as PDGF-B is proposed to promote the proliferation and/or migration of mesangial cells along the capillary endothelial tubes within the developing glomerular tuft. The two mature cell types, alveolar

SMC and mesangial cells, also appear to perform analogous functions in that they allow for the folding of epithelial surfaces and associated basement membranes, leading to the formation of large surfaces for gas exchange and blood filtration, respectively. As pointed out above, pericytes also depend on PDGF-B secreted from sprouting capillary endothelium for their spreading on new capillaries, at least in the brain. Pericyte absence, as studied in PDGF-B-negative embryos (Lindahl et al., 1997a), leads to microaneurysms that are similar to the capillary ballooning seen in the glomeruli of the PDGF-B- or PDGF-R β -negative embryos. PDGFs therefore seem to have multiple functions in the development of SMCs, and the specific subsets of SMCs affected in PDGF and PDGF-receptor mouse mutants may support similar morphogenetic events.

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