Developmental roles of platelet-derived growth factors

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Summary
Platelet-derived growth factor (PDGF) was originally identified in platelets and in serum as a mitogen for fibroblasts, smooth muscle cells (SMC) and glia cells in culture. PDGF has since expanded to a family of dimers of at least four gene products, whose biological actions are mediated through two receptor tyrosine kinases, PDGFRs. The present review summarizes and discusses the biological functions of PDGFs and PDGFRs in developmental processes, mainly as revealed through genetic analysis in mice. Such studies have demonstrated multiple critical roles of PDGFs and PDGFRs in embryonic and postnatal development. PDGFs seem to act upon specific populations of progenitor cells that give rise to several different cell types with distinct functions in a variety of developmental processes. Analogies are seen between the cell functions and the developmental processes controlled by PDGFs. This suggests that ancestral PDGF and PDGFR expression patterns and functions may have been iterated in related sets of morphogenetic processes in the course of evolution. ***BioEssays* 23:494–507, 2001. © 2001 John Wiley & Sons, Inc.**

Introduction

The PDGFs

The constituent polypeptide chains of the PDGFs and the vascular endothelial growth factors (VEGFs) share a core motif consisting of eight cysteine residues with a characteristic spacing in the polypeptide chains.[1] For almost two decades, only two PDGF polypeptides, PDGF-A and PDGF-B, were known and hence seemed to comprise the complete mammalian PDGF family (for a recent comprehensive review on PDGF, see Ref. 2). This was in contrast to the VEGF family, which expanded over the past few years to include five members, VEGF-A, VEGF-B, VEGF-C, VEGF-D and placenta growth factor (PlGF) (reviewed in Refs. 1,3). Recently, however, PDGF-C and PDGF-D, two new PDGFs were discovered, which form a separate PDGF subfamily.[4–6]

The biologically active PDGF and VEGF molecules are homodimers or heterodimers. In the PDGF family, the four homodimers PDGF-AA, PDGF-BB, PDGF-CC and PDGF-DD and the heterodimer PDGF-AB have all been demonstrated as endogenous cell products. The PDGF dimers are assembled intracellularly and, in the case of PDGF-A and PDGF-B, without apparent bias towards a specific dimer configuration. A cell may therefore produce either PDGF-AA or PDGF-BB if either of these PDGF genes is solely transcribed. If both genes are transcribed simultaneously, a mixture of PDGF-AA, PDGF-BB and PDGF-AB will be produced. PDGF-C does not appear to heterodimerize with PDGF-A or PDGF-B.[4] The reason for this may be that PDGF-C is rather distantly related to PDGF-A and -B in its core domain. In contrast, PDGF-C and PDGF-D are closely structurally related, but it remains to be established if they can heterodimerize with each other. Four research groups independently cloned the cDNA sequence for PDGF-C,[4,9] which has also been named fallotein[7] and spinal-cord-derived growth factor (SCDGF).[8] Based on primary sequence, PDGF-C and PDGF-D are as closely related to the VEGFs as to PDGF-A and PDGF-B. The basis for their characterization as novel PDGFs is, however, their PDGF-receptor-binding specificity (see below). PDGF-C and PDGF-D possess a novel N-terminal domain among the PDGF/VEGFs, referred to as a CUB domain. CUB domains are found in many proteins, but their function(s) is unknown.[10] In contrast to the N-terminal pro-peptides of PDGF-A and PDGF-B, the CUB domains of PDGF-C and PDGF-D appear not to be obligatorily removed by intracellular proteolytic processing prior to secretion, but remain on the secreted PDGF-CC and PDGF-DD molecules .[4,6] Proteolytic removal of the CUB domain is a prerequisite for binding of PDGF-CC and PDGF-DD to PDGF receptors. Proteases involved in the assumed endogenous extracellular processing of PDGF-CC and PDGF-DD remain to be identified. It is attractive to postulate that PDGF-CC and PDGF-DD are secreted as latent, conditionally inactive, growth factors similar to the situation for transforming growth factor β (TGF β) (reviewed in Ref. 11).

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Abbreviations: PDGF, platelet-derived growth factor; PDGFR, platelet-derived growth factor receptor; SMC, smooth muscle cell(s); vSMC, vascular smooth muscle cell(s); VEGF, vascular endothelial growth factor; CUB, domain found in complement subcomponents C1r/C1s, urchin EGF-like protein and bone morphogenetic protein-1; PLC, phospholipase C; PI3K, phosphatidylinositol 3′ kinase; GAP, GTPase activating protein; TGFβ, transforming growth factor β; ALK, activin receptor like kinase.

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Through the identification of PDGF-C and PDGF-D, it has become clear that the genetic complexity of the PDGF family of ligands is larger than previously thought. In addition to transcriptional and post-transcriptional control of PDGF biosynthesis and subsequent proteolytic processing, PDGF bioavailability in vivo is probably also dependent on association of the secreted growth factor to proteoglycans and other extracellular matrix molecules. For PDGF-A and PDGF-B, this seems to be mediated by a C-terminal basic motif in the PDGF molecule, the presence or absence of which is determined by alternative splicing in PDGF-A and alternative proteolytic processing in PDGF-B (reviewed in Ref. 12).

PDGF receptors

PDGFs bind to and signal through two cell-surface receptor tyrosine kinases, PDGFR-α and PDGFR-β. The PDGF molecules are bivalent and PDGF-dependent receptor dimerization is a prerequisite for receptor autophosphorylation and subsequent signal propagation (reviewed in Refs. 2,13). The two PDGF receptors have different ligand-binding capacities. PDGFR-β binds PDGF-B and PDGF-D with high affinity whereas PDGFR-α has a broader specificity and binds PDGF-A and PDGF-C, but also PDGF-B (Fig. 1). PDGFR-α binds PDGF-A with higher affinity than PDGF-B, which may be of relevance in vivo (see below). The two receptor genes may be expressed individually or together in cells. The structure of the signaling PDGF receptor unit in any given situation (PDGFR-$\alpha\alpha$ PDGFR-$\alpha\beta$, or PDGFR-$\beta\beta$) is both dependent on the pattern of PDGF receptor gene expression by the responding cell (α, β or both) and on the bioavailability of various PDGF dimers (AA, AB, BB, CC or DD). It is presently unclear if the receptor heterodimer is endowed with unique signaling activities, although it appears to mediate a slightly stronger mitogenic signal than any of the two receptor homodimers.

PDGF receptors have been extensively studied regarding their signaling mechanism, in particular PDGFR-β. Through intracellular phosphotyrosine residues, the receptor dimers contact a number of SH2 domain-containing molecules that connect to different intracellular signaling pathways (reviewed in Ref. 14). Cytosolic proteins connecting to PDGFR-β include phospholipase C-γ (PLCγ), phosphatidylinositol 3$'$ kinase p85 subunit (PI3K), Ras GTPase-activating protein (Ras-GAP), SHP2 phosphatase and Src family kinases. These pathways appear to converge on a set of immediate early genes that become transcriptionally activated. Analysis of the importance of distinct phosphotyrosine residues in the PDGFR-β molecule has demonstrated a high degree of specificity in their binding to and activation of various signaling pathways, but surprising redundancy in the activation of immediate early genes[15] (reviewed in Ref. 16).

The two PDGF receptors have partially overlapping and partially distinct signaling capabilities in vitro (reviewed in Refs. 14,17). Differences include Ras-GAP, which binds PDGFR-β, but not PDGFR-α, and the Crk family of adaptor proteins, which bind only to PDGFR-α. Knockout studies have

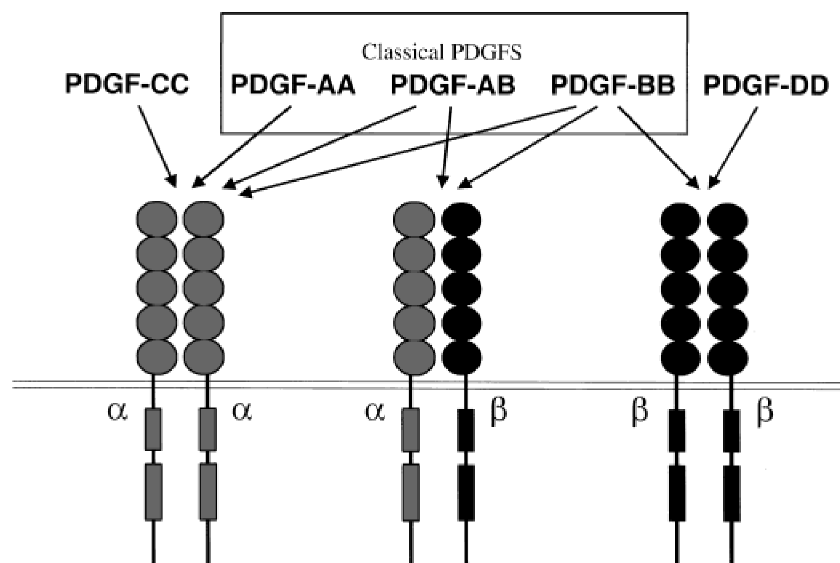

Figure 1. PDGF ligand-receptor specificities. PDGF-A, PDGF-B, PDGF-C and PDGF-D polypeptides may assemble into at least five different dimers with different receptor-binding specificities. Ligand-binding promotes dimerization of the receptors PDGFR-α and PDGFR-β into the configurations shown.

clearly demonstrated that the two receptors have different developmental roles in vivo (see below), which may primarily relate to differences in their expression patterns and PDGF ligand binding. Intracellular domain replacement experiments demonstrate that the signaling downstream of the two PDGF receptors can largely compensate for each other in the support of embryonic development.[18] PDGFR-β cannot be fully compensated by that of PDGFR-α in vascular development and function, however, suggesting that the biochemical differences in signaling have specific biological consequences[18] (see also below).

Biological roles of PDGFs

The in vivo functions of the PDGFs and its receptors have been studied extensively by gene targeting, but several antagonists have also been described recently that may prove useful in pharmacological interference with PDGF signaling (see Ref. 2 and references therein). The interest in PDGF/PDGF receptor antagonists is primarily based on assumed pathogenic functions of PDGFs in diseases associated with connective tissue overgrowth, such as atherosclerosis, different types of fibrotic responses, rheumatoid diseases and cancer. For the latter, both transforming and stroma-modulating activities have been suggested (reviewed in Refs. 2,19).

As will be discussed in detail below, paracrine signaling appears to be the common route of signaling by PDGFs in embryogenesis. Autocrine PDGF signaling plays a role in some cases of carcinogenesis, for example, in simian sarcoma virus (SSV)-transformed cells and in certain human tumor cell lines, particularly gliomas. Human dermal fibrosarcomas show a very high frequency of translocations in which PDGF-B coding sequences are placed under the transcriptional control of collagen 1A promoter sequences.[20] Collagen 1A producing skin fibroblasts carry PDGF receptors, and hence, autocrine growth stimulation likely occurs as a result of the translocation. Readers interested in the proposed pathological effects of PDGF (over)-expression in adult mammals should consult other review articles on this subject.[2,19,21] Here onwards, this review will focus primarily on developmental roles of PDGFs and PDGF receptors as revealed through genetic analysis in mice.

Developmental role of PDGF-B and PDGFR-β

PDGF-B and PDGFR-β knockouts

PDGF-B- or PDGFR-β-negative mice[22,23] die during late gestation from cardiovascular complications. Clinically, mutants appear healthy and normal until E16-19, at which time there is a sudden onset of edema formation, dilation of the heart and large blood vessels, and dilatation and rupture of capillaries. Mutants delivered by Cesarean section immediately prior to birth fail to breathe and die quickly. Histological findings in PDGF-B and PDGFR-β -negative mice include abnormal kidney glomeruli, capillary microaneurysms, arterial SMC hypoplasia, cardiac muscle hypotrophy, placenta defects and widespread edema and hemorrhage.[22–27] The PDGF-B and PDGFR-β null phenotypes have so far not revealed any differences and appear to be identical. This raises the question of the biological significance in vivo of PDGF-B signaling via PDGFR-α and of PDGF-D signaling via PDGFR-β. For PDGF-D, we presently lack information about its embryonic expression pattern. It is possible that its major function(s) occurs in the adult. As discussed in more detail below, the PDGF-B, PDGF-A, PDGF-R-α and PDGF-R-β expression patterns are distinct in the mouse embryo and it is possible that PDGFR-α-expressing cells are not exposed to PDGF-B during embryonic development.

PDGF-B and PDGFRβ expression patterns

In the mouse embryo, PDGF-B expression appears restricted to vascular endothelium and megakaryocytes.[24] Neither cell type expresses detectable levels of PDGF-A during mouse embryogenesis, and hence it can be assumed that the PDGF isoform produced by these cells is PDGF-BB. PDGFR-β-positive mesenchymal cells were found in cells distinct from but in tight contact with endothelial cells, and it is assumed that these cells constitute vascular SMC (vSMC) or pericyte progenitors.[24,26] Detailed analysis of the vascular PDGF-B/R-β expression patterns in wild-type embryos and young pups has revealed that vascular PDGF-B expression is highest in sprouting, immature, capillary endothelium and in the endothelium of growing arteries.[26]

Vascular SMC- and pericyte recruitment to new blood vessels depends on PDGF-B/PDGFR-β signaling

The spatial and temporal expression patterns of PDGF-B and PDGFR-β suggest that PDGF-BB released from endothelial cells normally promotes proliferation in adjacent vSMC/pericyte progenitors (Fig. 2A). This hypothesis is confirmed by the PDGF-B and PDGFR-β null phenotypes. Both PDGF-B and PDGFR-β knockout embryos show a reduced BrdU labeling index (indicative of reduced proliferation) and a pronounced reduction in the number of vSMC/pericyte progenitors, as demonstrated by the use of several different markers for such cells. The blood vessel dilation and microaneurysms seen in mutant embryos affect vessels in which the reduction in vSMC/pericyte abundance is particularly dramatic. Pericyte deficiency was most apparent in brain, heart and brown adipose tissue, but was less pronounced or undetectable at other sites, such as the gastrointestinal tract, adrenal, pancreas, liver and skin.[26] The porta circulation of the liver was atypical in that these vessels did not express PDGF-B or PDGFR-β, and, as expected, therefore, the sinusoidal pericytes (Ito cells)

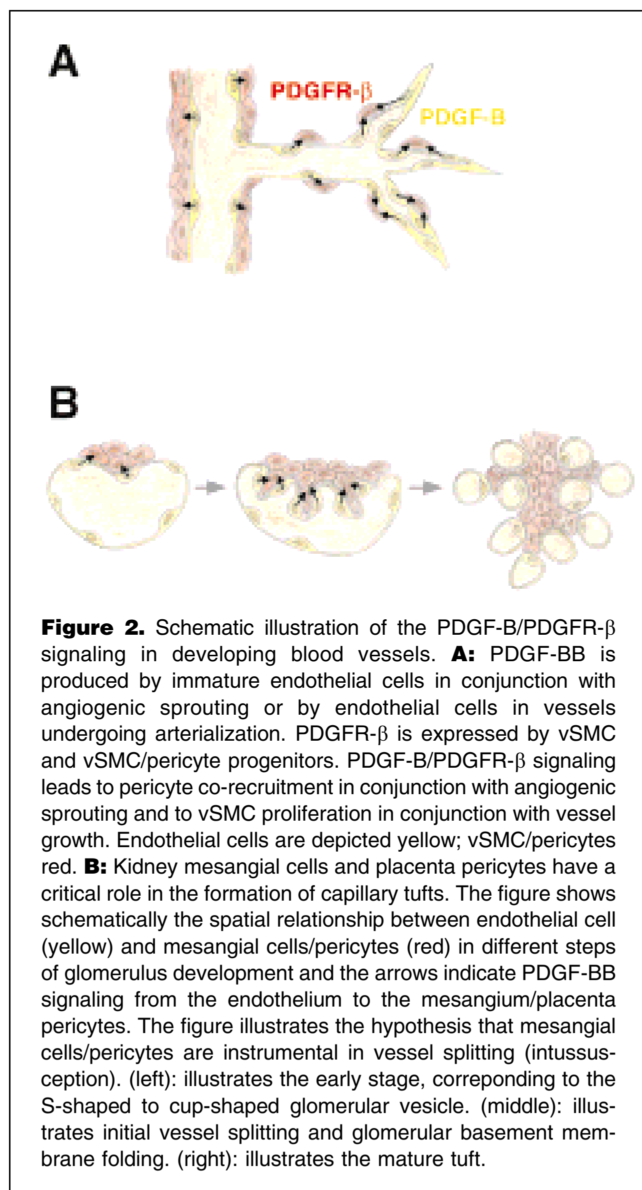

Figure 2. Schematic illustration of the PDGF-B/PDGFR-β signaling in developing blood vessels. **A:** PDGF-BB is produced by immature endothelial cells in conjunction with angiogenic sprouting or by endothelial cells in vessels undergoing arterialization. PDGFR-β is expressed by vSMC and vSMC/pericyte progenitors. PDGF-B/PDGFR-β signaling leads to pericyte co-recruitment in conjunction with angiogenic sprouting and to vSMC proliferation in conjunction with vessel growth. Endothelial cells are depicted yellow; vSMC/pericytes red. **B:** Kidney mesangial cells and placenta pericytes have a critical role in the formation of capillary tufts. The figure shows schematically the spatial relationship between endothelial cell (yellow) and mesangial cells/pericytes (red) in different steps of glomerulus development and the arrows indicate PDGF-BB signaling from the endothelium to the mesangium/placenta pericytes. The figure illustrates the hypothesis that mesangial cells/pericytes are instrumental in vessel splitting (intussusception). (left): illustrates the early stage, correponding to the S-shaped to cup-shaped glomerular vesicle. (middle): illustrates initial vessel splitting and glomerular basement membrane folding. (right): illustrates the mature tuft.

were of normal abundance in the PDGF-B and PDGFR-β mutants.[26]

Conceivably, the PDGFR-β-positive vSMC/PC progenitors are first induced from competent mesenchyme in limited numbers at perivascular sites. Subsequently, this population is selected when it expands and spreads to new blood vessels under the influence of PDGF-BB. This idea, introducing two distinct steps in vSMC/pericyte development, would offer a possible explanation for the tissue-specific differences seen in the degree of vSMC/pericyte loss in PDGF-B and PDGFR-β mutants.[26] Sites where abundant vSMC/pericyte progenitors remain in mutants would reflect sites where induction is particularly active. Alternatively, such sites harbor putative vSMC/pericyte growth factors other than PDGF-BB. Sites devoid of competent mesenchyme (e.g. the brain) would rely on PDGF-BB-dependent vSMC/pericyte selection. The concept of vSMC induction is supported by in vitro studies suggesting that endothelial cells promote SMC differentiation when contacting co-cultured undifferentiated mesenchymal cells.[28] Antibody neutralization experiments identified TGFβ as a SMC-inducing factor.[28] Gene knockout studies confirm that TGFβ is important for proper vessel formation and that it might use the TGFβ-binding protein endoglin, the TGFβ receptors ALK-1 and ALK-5, and the signal transducer Smad5 to mediate this function.[29–33] Interestingly, the endoglin and ALK-1 knockouts were both shown to have defective vSMC development.[31,32] A current working model of vSMC formation is therefore that TGFβ mediates vSMC/pericyte induction followed by PDGF-BB-mediated expansion of the vSMC/pericyte population.

Similar roles of PDGF-B in kidney and placenta development

In kidney glomeruli and in the labyrinthine layer of the mouse placenta, failure of recruitment of pericytes to the newly formed blood vessels, as seen in PDGF-B and PDGFR-β knockout embryos, appears to generate specific and highly similar vascular defects. Normally, both organs develop complex networks, or tufts, of capillaries, which create large active surfaces to allow for efficient filtration/excretion in the kidney and excretion/absorption in the placenta. In PDGF-B and PDGFR-β knockout kidneys, mesangial cells, the specialized glomerular capillary pericytes, are not properly recruited to the developing glomerular tuft. Capillary branching fails and, as a consequence, a single (or a few) dilated capillary loop forms that occupy the entire Bowman's space.[22,23,25] The mesangial cells express PDGFR-β and should constitute the primary target for endothelium-derived PDGF-BB. In addition, the effect of PDGF-B or PDGFR-β deficiency on the capillary tuft is similar to the effect of experimental mesangiolysis, as produced in rats by Thy-1 antibodies,[34] or by certain snake venomes.[35] The PDGF-B and PDGFR-β knockout data thus suggest that mesangial cells play an important morphogenetic role in glomerulus formation, in that they induce the generation of correct size and density of capillaries in the kidney glomerulus.

The labyrinthine layer in the mouse placenta is where the fetal and maternal vessel networks meet (note: labyrinthine layers do not exist in all mammals; mice have labyrinthine placentas, but humans, for example, do not). The fetal vessels in the placenta are composed of endothelial tubes with associated pericytes, whereas the vessels channeling the maternal blood are instead lined by trophoblasts. The fetal vessels are arranged around cords of pericytes that, as for the mesangial cells in the glomeruli, appear to organize the vessel network. The pericyte loss in PDGF-B and PDGF-R-β knockouts was less profound in

the placenta (approx. 50% reduction) than in the kidney glomeruli (90–100% reduction), but nevertheless had dramatic effects on the shape of the blood vessels. In knockout placentas, the fetal capillaries were disorganized and extensively dilated and appeared fused into fewer but wider structures.[27] This phenotype was evident from E13.5. Morphometric analysis demonstrated a reduction in the surface area for nutrient exchange.[27] In spite of this, embryonic development of the PDGF-B and PDGF-R-β knockout embryos proceeds overtly normally until E16, with only a slight (10–20%) weight reduction as a putative sign of placenta dysfunction.

The spatial relationship between the endothelial tubes and associated pericytes appears strikingly analogous in kidney glomeruli and mouse placenta. At both sites, lack of PDGF-B or PDGFR-β have similar effects. In contrast to other sites (e.g. the brain) where pericyte loss does not appear to affect the sprouting or branching of capillaries, the loss of pericytes in the kidney glomeruli and placenta leads to a dramatic reduction in the number of capillary loops formed in each tuft. This suggests that mesangial cells and placenta pericytes may control capillary branching, possibly by a process of vessel splitting, referred to as intussusception (Fig. 2B).

Primary or secondary phenotypes in PDGF-B and PDGFR-β mutants?

In interpreting the consequences of gene disruption, it is important to discriminate between primary and secondary defects. Cell autonomous defects often occur if the targeted gene encodes a membrane-bound or intracellular protein and, in such cases, the normal expression patterns of the gene is often informative. It is more difficult to deduce the primary effect of loss of a secreted ligand. Expression patterns do not reveal the action range of the ligand, which may be autocrine, paracrine or endocrine. It may also be difficult to distinguish primary from secondary defects. In mouse mutants suffering from vascular defects, this is a particularly relevant consideration, since vascular defects may have a plethora of secondary consequences. A primary defect in one part of the vascular system (e.g. the placenta) may also have secondary consequences in other parts of the vascular system (e.g. the heart). In addition to the vascular defects described above, both PDGF-B and PDGFR-β mutants show cardiac dilation, myocardial hypotrophy and an increased frequency of ventricular septum defects.[22,26] There are also skeletal muscle abnormalities that have not been well characterized (M. Hellström and C. B., unpublished observations). The placenta and heart defects seen in PDGF-B and PDGFR-β knockouts may have systemic consequences, although it seems unlikely that the specific blood vessel defects seen would be caused by these defects, rather than by failure of paracrine PDGF/PDGF receptor interaction within the developing blood vessels. Conversely, the blood vessel abnormalities may lead to problems of organ nutrition and growth. The heart and skeletal muscle abnormalities in PDGF-B and PDGF-R-β-deficient mice may therefore develop secondary to poor blood flow in these organs. Indeed, both heart and skeletal muscle show severe pericyte deficiency, and, in the heart, the developing intra-myocardial branches of the coronary arteries fail almost completely to recruit a vSMC coat. Although this may offer a plausible explanation for the cardiac phenotype, other possibilities have to be considered, as shown by chimeric analysis.

Chimeras reveal additional roles for PDGFR-β in muscle development

To aid in the discrimination between primary and secondary phenotypes, chimeric analysis presents an alternative to the classical knockout and transgenic approaches. This type of analysis may reveal the cellular function of a gene in a situation of "competition" between wild-type cells and mutant cells in a chimeric individual. In chimeras consisting of PDGFR-β null and wild-type cells, the null cells were excluded from the vSMC/pericyte/mesangial lineage, as expected, but also from all other muscle lineages, including cardiac muscle, skeletal muscle and visceral smooth muscle.[36] This demonstrates a cell autonomous dependence on PDGFR-β in cells at some level(s) of the entire muscle lineage. In view of this, it is somewhat mysterious that PDGFR-β expression is undetectable in cardiac and skeletal muscle cells, at least during the later part of mouse embryonic development. An independent chimeric analysis has yielded contradictory results on the cell autonomous role for PDGFR-β in cardiomyocytes.[37] In confirming the suggested roles for PDGFR-β in skeletal and cardiac muscle development, several possibilities need to be considered. Firstly, muscle cells may express low, seemingly undetectable but biologically significant, levels of PDGFR-β, or expression may be transient during a narrow window of early embryogenesis. Secondly, PDGFR-β-positive perivascular cells, which are clearly competent to differentiate into vSMC and pericytes, may represent a multipotent muscle progenitor that contributes also to cardiac and skeletal myogenesis. In this context, it is noteworthy that the ontogeny of satellite cells is unclear. Such cells contribute to skeletal muscle formation by fusing with preexisting myofibres.

Whereas fibroblasts and endothelial cells appear unaffected (in a direct, cell-autonomous sense) by PDGFR-β deficiency in the embryo, the same cells types in the adult mouse were shown by chimeric analysis to depend on PDGFR-β expression in granulation tissue formation.[38] Adult fibroblasts are known to express PDGFR-β but available data are less clear for endothelial cells. Chimeric analysis reveals dependence, but not necessarily in the latest step in a cell lineage. The apparent PDGFR-β dependency of endothelial cells may therefore reflect an earlier role, perhaps of a rather distant progenitor. For example, granulation tissue endothe-

lium may be recruited from surrounding tissue, or from the bone marrow.[39]

Mutations in the PDGFR-β gene affecting selective signaling pathways in vivo

PDGFR-β autophosphorylation in response to ligand binding leads to the creation of docking sites for a number of signaling molecules, including enzymes, adapters and transcription factors.[14] At the biochemical level, these molecules couple to distinct signaling pathways, but it is less clear how (and if) they trigger different cellular responses in vitro or in vivo. One way of addressing this is to change single phosphotyrosine residues in the endogenous PDGFR-β gene by targeted mutagenesis and to analyze the effect of the homozygous mutation in mice and cell cultures. One of the most extensively studied targets of PDGFR-β is PI3K, which binds to two phosphorylated tyrosine residues in the receptor cytoplasmic domain.[40,41] A change of these residues to phenylalanine in PDGFR-β had surprisingly little effect on development, in spite of a reduced chemotactic and contractile effect of PDGF-BB on mutant cells in vitro.[42] However, mutant mice were impaired in resolving experimental edema in response to PDGF. It is possible therefore that PDGFR-β signaling via PI3K has a role in increasing interstitial fluid pressure by stimulating tissue contraction.[42]

A PDGFR-β molecule containing a mutated PLCγ-binding tyrosine in addition to the two mutated PI3K-binding tyrosines failed to mediate cell migration in response to PDGF-BB in vitro.[37] Surprisingly, mice carrying this mutation developed normally and survived until adulthood, but showed an increased pathological response to experimentally induced glomerulonephritis, probably because of inhibited function of mesangial cells.[37]

Exchange of the entire intracellular domain of PDGFR-β for the corresponding region of PDGFR-α led to a more dramatic effect in vivo, and spontaneous pathology appeared in the eye, heart and kidneys.[18] In the eye and kidney of these mice, pericytes and mesangial cells, respectively, failed to be recruited properly, i.e. cells known to depend on PDGF-B/R-β signaling for their recruitment to capillaries were particularly affected. This demonstrates that PDGFR-β but not PDGFR-α can activate a pattern of intracellular signaling (or couple to distinct pathways) of specific importance for the development of the mural cells of small blood vessels. The identity of the critical PDGFR-β-mediated signal(s) remains to be established.

Developmental role of PDGF-A / PDGFR-α signaling

PDGF-A and PDGFR-α knockouts

PDGF-A knockouts[43] and PDGFR-α knockouts[44] are both lethal, but show substantial phenotypic differences. PDGF-A knockouts either die before E10 or survive until birth. The early lethality is not well understood, and mutant embryos at E10 are either grossly retarded, displaying a severe reduction in paraxial mesoderm, or of normal size and phenotypically indistinguishable from wild-type and heterozygote littermates (M. Hellström, C. B. and P. Soriano, unpublished observations). Mutants that survive E10 develop further until birth. Most postnatal PDGF-A$^{-/-}$ mice die within the first couple of days, however an appreciable number live for up to 6 weeks, allowing for analysis of postnatal development. PDGF-A knockouts are slightly smaller than littermates at birth, but this size difference increases with age, and by 3–4 weeks knockouts are half the weight or less compared to wild-type or heterozygote littermates. PDGF-A knockouts develop a broad range of defects in several different tissues (Fig. 3). These are discussed in more detail below. The phenotype of PDGFR-α knockouts is more severe, and includes defects that are not seen in PDGF-A knockouts, such as cleft face, subepidermal blistering, spina bifida, and skeletal and vascular defects, leading to death between E8 and E16.[44] A natural mouse mutation encompassing the PDGFR-α locus, *patch*, shows a genetic background-dependent cleft face phenotype, malformations in the cardiac outflow tract and severe general mesenchymal loss. The overall phenotypes of PDGFR-α null and *patch* homozygotes appear similar, however, the patch deletion probably affect additional genes, such as *kit*, which may accounts for the pigmentation defect seen in *patch* heterozygotes. PDGFR-α null embryos show increased apoptosis in neural crest-derived mesenchyme.[44] The cleft face, spina bifida and cardiac outflow tract abnormalities in PDGFR-α and *patch* mutants also point to a problem in neural-crest-derived mesenchyme.

PDGF-A, PDGF-C and PDGFR-α expression patterns and functions during early embryogenesis

A large number of cell types have been reported to express PDGF-A and PDGFR-α in vitro and in vivo (reviewed in Ref. 2). In the developing mouse embryo, PDGF-A expression onsets early[45] and is seen already in the preimplantation embryo.[46] At this stage, it is coexpressed with PDGFR-α, for instance in the blastocyst inner cell mass. Following implantation, expression becomes paracrine,[46] and, as development proceeds, PDGF-A becomes widely expressed in epithelia, muscle and nervous tissue[47–51] (additional citations below). PDGFR-α, in contrast, is expressed by most mesenchyme and in a few cases epithelium, such as the lens epithelium and the limb apical ectodermal ridge (Ref. 51,52 and L. Karlsson and C. B., unpublished results). The general picture is that the expression patterns of ligand and receptor are non-overlapping. Often PDGF-A-positive epithelium is closely associated with PDGFR-α-positive mesenchyme, suggesting a paracrine mode of signaling across epithelial

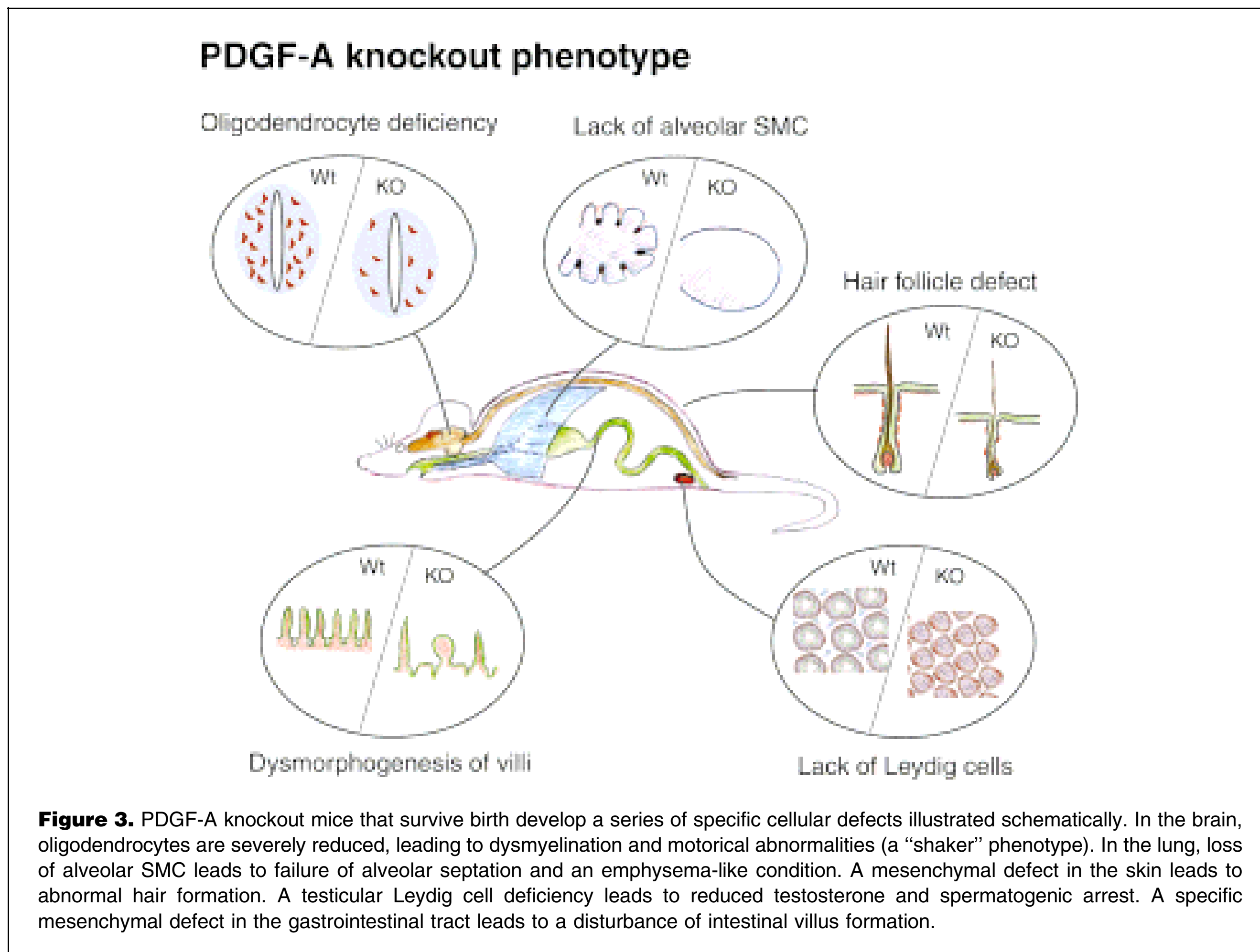

Figure 3. PDGF-A knockout mice that survive birth develop a series of specific cellular defects illustrated schematically. In the brain, oligodendrocytes are severely reduced, leading to dysmyelination and motorical abnormalities (a "shaker" phenotype). In the lung, loss of alveolar SMC leads to failure of alveolar septation and an emphysema-like condition. A mesenchymal defect in the skin leads to abnormal hair formation. A testicular Leydig cell deficiency leads to reduced testosterone and spermatogenic arrest. A specific mesenchymal defect in the gastrointestinal tract leads to a disturbance of intestinal villus formation.

basement membranes. The PDGF-A and PDGFR-α expression patterns suggest roles early in development as well as in organogenesis. A role for PDGF-A has been implicated in frog gastrulation,[53] and a PDGF-like molecule has also been functionally implicated in sea urchin gastrulation.[54] The expression of PDGF-A and PDGFR-α during frog gastrulation suggests a role in promoting spreading of mesoderm to anterior positions.[53] It is presently unclear what role (if any) PDGF-A and PDGFR-α may have in early mouse embryogenesis including gastrulation. Clearly a proportion, but not all, PDGF-A and PDGFR-α null embryos die at a very early age, which might be consistent with a gastrulation defect. Further phenotypic analysis of these early lethal mutants is required together with experiments addressing why such phenotype is not fully penetrant.

PDGF-A and PDGFR-α are implicated in the patterning of somites. During somite development, the myotome expresses PDGF-A and the sclerotome expresses PDGFRα.[44,48,51] Both PDGFR-α and PDGF-A knockouts show abnormal expression of myotomal markers (Ref. 44 and L. Hellström, C. B. and P. Soriano, unpublished) indicating that a primary defect in the sclerotome feeds back into the myotome. PDGFR-α knockouts develop rib truncations and fusion, probably as a consequence of the somite patterning defect. Myotomal PDGF-A expression is dependent on the transcription factor Myf5, and seems to mediate part of the Myf5 function in the myotome, as was indicated by a knock-in experiment placing a PDGF-A coding sequence in the Myf5 locus.[55] This led to a partial rescue of the severe rib truncation typical of the Myf5 knockout.[55]

At E9.5-12.5 the pattern of expression of PDGF-C largely overlaps with that of PDGF-A.[9,55] PDGF-C is strongly expressed in surface ectoderm in the developing facial region, hair follicles, somitic myotome and skeletal muscle, gut, lung and kidney (Refs. 4,9 and K. Aase, C. B. et al, unpublished data). As development proceeds both PDGF-C and PDGF-A expression are also seen in visceral smooth muscle cells. PDGF-A is also expressed by vascular smooth muscle. That subtle differences may exist between the expression patterns of PDGF-C and PDGF-A is illustrated in the developing kidney (discussed in more detail below).

Is PDGF-C the missing link between the PDGFR-α and PDGF-A null phenotypes?

As discussed above, PDGFR-α null mice display a series of defects in the somites, neural crest derivatives and skeleton that are not seen in PDGF-A or PDGF-B knockouts. Also PDGF-A/B double knockouts also fail to reproduce the typical PDGFR-α knockout defects. In addition, at sites where PDGF-A null mice display defects, such as skin, gut and lung, the PDGFR-α knockouts show more severe defects than PDGF-A knockouts.[43,44,56–58] The PDGF-C and PDGF-A expression patterns are strikingly similar in E9.5- 12.5 mouse embryos and it is likely that the two factors have overlapping function at sites where they are coexpressed, since they are both selective agonists of PDGFR-α. The particularly striking expression of PDGF-C[9] and PDGF-A[55] in the epithelium of facial processes and branchial arches may suggest a role in attracting PDGFR-α-positive neural-crest-derived mesenchyme into these structures, a process that apparently is abnormal in PDGFR-α knockouts. In addition, Both PDGF-C and PDGF-A are expressed in the myotome of the developing somites, and may have critical functions for the sclerotome. Sclerotome gives rise to vertebrae and ribs, which are defective in PDGFR-α knockouts. In summary, the expression patterns of PDGF-C and PDGF-A during early development is highly suggestive of overlapping functions. Comparing PDGF-C- and double PDGFA/C- with PDGFR-α knockout mice will ultimately test this hypothesis.

Lung alveogenesis

PDGF-A$^{-/-}$ mice surviving birth invariably develop an advanced lung emphysema-like condition with a complete lack of alveolar septa.[43] Alveogenesis is a postnatal process in the mouse. Prenatally, lung development proceeds through glandular, canalicular and saccular stages. The respiratory unit of the newborn mouse consists of alveolar sacs, which have not yet been further subdivided into alveoli. Alveogenesis starts around P5 and is essentially completed by three weeks of age. In PDGF-A$^{-/-}$ mice, an emphysema-like condition results from the failure of alveolar septum formation.[43,59] The process of alveogenesis appears to be driven by a cell type, alveolar SMC, that is missing in PDGF-A$^{-/-}$ lungs. These cells have been suggested to originate from clusters of PDGFR-α-positive mesenchymal cells that form at the epithelial buds during the pseudoglandular stage of lung development. During the canalicular and saccular stages, these cells detach from the cluster and spread distally to the walls of the alveolar sacs. They remain at this site until the onset of alveogenesis, at which time they downregulate PDGFR-α and strongly upregulate tropoelastin expression. In PDGF-A$^{-/-}$ lungs, PDGFR-α-positive clusters of mesenchymal cells are formed during the pseudoglandular stage, but the proliferation and distal spreading of PDGFR-α-positive mesenchymal cells during later stages fails. Consequently, the cells do not reach the alveolar saccules.[59] In the developing lung, PDGF-A (but not PDGF-B) is expressed by the lung epithelium. This suggests that PDGF-AA produced by the lung epithelium acts in a paracrine mode to regulate the spreading of the PDGFR-α-positive alveolar SMC progenitors. The spreading process appears to include both proliferation and migration.

Tropoelastin expression by the alveolar SMC occurs only during alveogenesis. Since alveolar septa contain large extracellular elastin deposits and, since the amount of lung elastin is severely reduced in PDGF-A$^{-/-}$ lungs, its appears likely that the alveolar SMC is the major source of lung elastin.[59] It is possible that the deposition of extracellular elastin provides a major mechanical force in alveolar septation. Once formed, septal elastin is not turned over significantly during the lifetime of the mammal. The elastin deposits are probably critical to the integrity of the septum, since an imbalance between elastase and anti-elastase activities of genetic (1-antitrypsin deficiency) or environmental (smoking) origin is associated with the development of alveolar septum destruction and emphysema in humans.

Oligodendrocyte development and myelination

Oligodendrocytes are glia cells that form myelin sheaths around nerve processes in the CNS. In mammals, oligodendrocytes develop from PDGFR-α-positive progenitor cells that are first detected as bilateral rows of cells in the mouse spinal cord around E12 (reviewed in Ref. 60). From the site of origin, the cells multiply and spread laterally through the cord. PDGF-A is widely expressed by neurons and glia (presumably astrocytes) in the CNS. In mice, myelination onsets after birth and is essentially completed by weaning (3–4 weeks). The origin of brain oligodendrocytes is not mapped in detail, but most likely they have a peri-ventricular origin similar to cord oligodendrocytes. In vitro, oligodendrocytes derive from a bi-potent precursor, the O2A progenitor, which may also differentiate into astrocytes. In vitro as well as in vivo, PDGF-AA is a major mitogen for oligodendrocyte progenitors (reviewed in Ref. 61). PDGF-A$^{-/-}$ embryos are severely deprived of PDGFR-α-positive oligodendrocyte progenitors,[62] and postnatally surviving mutants become severely hypo-myelinated.[63] The progenitor loss and the subsequent myelination deficiency were both more severe in the periphery of the brain, i.e. at sites distant from the assumed peri-ventricular site of origin. Whereas the density of myelinated fibers were reduced to approximately 50% of the wild-type density in the striatum (central location), it was further reduced to less than 10% of normal in the cerebellum and optic chiasm (peripheral locations). In the optic nerve, PDGF-A$^{-/-}$ mice had few fibers myelinated close to the chiasm but a complete lack of myelin near the retina.[63] Thus, the most peripheral extension of the CNS, the optic nerve, showed the most extensive loss of oligodendrocytes and

myelin. This implies that PDGF-AA is particularly important for the long-distance migration of the oligodendrocyte progenitors. The role of PDGFs in migrating populations of cells will be further discussed below.

Oligodendrocyte numbers are balanced through a combination of progenitor division, and dropout of progenitors from the cell cycle followed by differentiation or death by apoptosis.[62] Heterozygote PDGF-A mutants show half the density of oligodendrocyte progenitors before birth, but are fully myelinated postnatally. Transgenic mice overexpressing PDGF-A from neurons develop several-fold higher numbers of oligodendrocyte progenitors before birth, but the situation normalizes after birth, since superfluous progenitors are eliminated through apoptosis.[62] Although PDGF-B, like PDGF-A, can bind to PDGFR-α, PDGF-BB, which is expressed by developing brain vascular endothelium, does not appear to contribute to oligodendrocyte progenitor proliferation since PDGF-B$^{-/-}$ embryos showed normal oligodendrocyte progenitor density.

Skin and hair development

Both PDGF-A and PDGFR-α knockouts show mesenchymal defects in the dermis. PDGFR-α null mice show severe dermal mesenchymal hypoplasia and regional detachment of the epidermis. PDGF-A null mice do not show significant dermal defects before birth, but show progressive loss of dermal mesenchyme in conjunction with increased age. Expression studies demonstrate PDGF-A mRNA in epidermis and hair follicle epithelium and PDGFR-α in subjacent mesenchyme. Paracrine signaling of PDGF-A in the skin appears to stimulate proliferation in the dermal mesenchyme. BrdU labeling experiments showed extensive reduction of DNA synthesis in the dermal mesenchyme in PDGF-A$^{-/-}$ compared with wild-type mice.[56]

Postnatal PDGF-A$^{-/-}$ mice show abnormal development of the hair. Mutant hair follicles are smaller, regionally crowded, and misshapen. This defect appears skin-autonomous, since it is reproduced in skin grafts to nude mice.[56] There are specific cell deficits in the mesenchymal components of the hair follicles. A continuous mesenchymal socket, called the dermal sheath, normally surrounds the hair follicle epithelium. This sheath is incompletely developed in PDGF-A null mice. During development of the follicles of the first hair cycle, PDGF-A is particularly strongly expressed by the upper portion of the hair follicle, and PDGFR-α is abundant in surrounding mesenchyme. At these early stages of hair development there is a significant reduction PDGFR-α-positive cells surrounding the follicular epithelium in PDGF-A$^{-/-}$ embryos, suggesting that PDGF-A, presumably derived from the hair follicle epithelium, stimulates the proliferation of surrounding cells, predestined to become dermal sheath cells. PDGF-A$^{-/-}$ follicles also show smaller dermal papillae, the mesenchymal cell cluster enclosed in an epithelial pocket at the follicle base. Dermal papillae form during follicle induction by the clustering of PDGFR-α-positive cells.[56] Once the dermal papilla is formed, its constituent cells become post-mitotic. It is therefore uncertain if PDGF-A has a direct role in the dermal papilla. It seems more likely that the role of PDGF-A in the developing skin is to secure the renewal of PDGFR-α-positive mesenchymal stem or progenitor cells that are consumed as they are recruited to hair follicles to become dermal papillae and dermal sheaths.

Testis development

Postnatal PDGF-A$^{-/-}$ mice develop a progressive reduction in testicular size and show loss of adult Leydig cells, reduced circulating testosterone and disrupted spermatogenesis.[64] During embryonic testicular development, tubular epithelial cells (presumably Sertoli cells but possibly also spermatocytes) express PDGF-A, whereas interstitial mesenchymal cells from which the Leydig cells develop express PDGFR-α. Prenatal testicular development was found to be normal in PDGF-A$^{-/-}$ mice, and the fetal population of Leydig cells is present and functional as evidenced by normal androgenization of male PDGF-A$^{-/-}$ embryos. It is assumed also that the adult population of Leydig cells develops from mesenchymal stem cells in the testis, rather than from preexisting fetal Leydig cells. Thus, the PDGFR-α-positive interstitial mesenchymal cells are putative adult Leydig cell precursors. Lack of PDGF-A could lead to adult Leydig cell deficiency through proliferative arrest and progressive depletion of the Leydig cell precursors, i.e. a situation similar to the skin, discussed above. Alternatively, PDGF-A controls directly Leydig cell differentiation. Further studies are required to discriminate between these possibilities.

Intestinal development

Both PDGF-A$^{-/-}$ and PDGFR-α$^{-/-}$ mice show intestinal villus dysmorphogenesis.[57] The expression patterns of PDGF-A and PDGFR-α in the gastrointestinal tract follow the overall consensus, with ligand expressed in the epithelium and receptor in the mesenchyme. Villus morphogenesis was found to correlate with the formation of discrete clusters of PDGFR-α-positive cells, villus clusters[57] that localized to the tip of the mesenchymal core of the growing villus. As int he dermal papillae of hair follicles, these clusters were postmitotic, whereas non-clustered PDGFR-α-positive cells proliferated actively. The spatial and temporal relationship between villus clusters and villus morphogenesis, and the fact that certain signaling molecules are expressed by the clusters, suggest that the clusters play a critical role in villus formation. In the PDGF-A$^{-/-}$ intestine, a reduced number villus clusters and villi are formed but, importantly, the non-clustered PDGFR-α-positive cells showed reduced

proliferation and were progressively depleted in the intestinal mesenchyme. Based on these observations, we proposed that PDGF-AA, produced by the intestinal epithelium, drives the proliferation of a subset of mesenchymal cells that are strongly positive for PDGFR-α which are subsequently recruited to villus clusters to play an important role in villus formation.[57]

Knockout phenotypes and distinct expression patterns suggest specific roles for three PDGFs in kidney development

All three PDGFs and both PDGF receptors are expressed in the developing kidney. As discussed above, PDGF-B and PDGFR-β are expressed in developing vascular endothelium and smooth muscle/mesangial cells, respectively, and play a critical role in glomerular morphogenesis. PDGF-C is expressed in the metanephric mesenchyme,[4] which undergoes aggregation and epithelial conversion as a result of interaction with the ureteric bud, and further develops into the epithelial tubuli of the nephron. PDGF-A is expressed in the developing tubular system, in particular in the descending Henle's loop. PDGFR-α is expressed throughout the mesenchyme of the developing mouse kidney. The significance of PDGFR-α expression in the developing kidney was apparent from the knockout, which showed a pronounced loss of mesenchymal cells in the kidney cortex.[4] The metanephric blastema was not affected, however, and tubulogenesis was seemingly normal. Neither PDGF-A knockouts nor PDGF-A/B

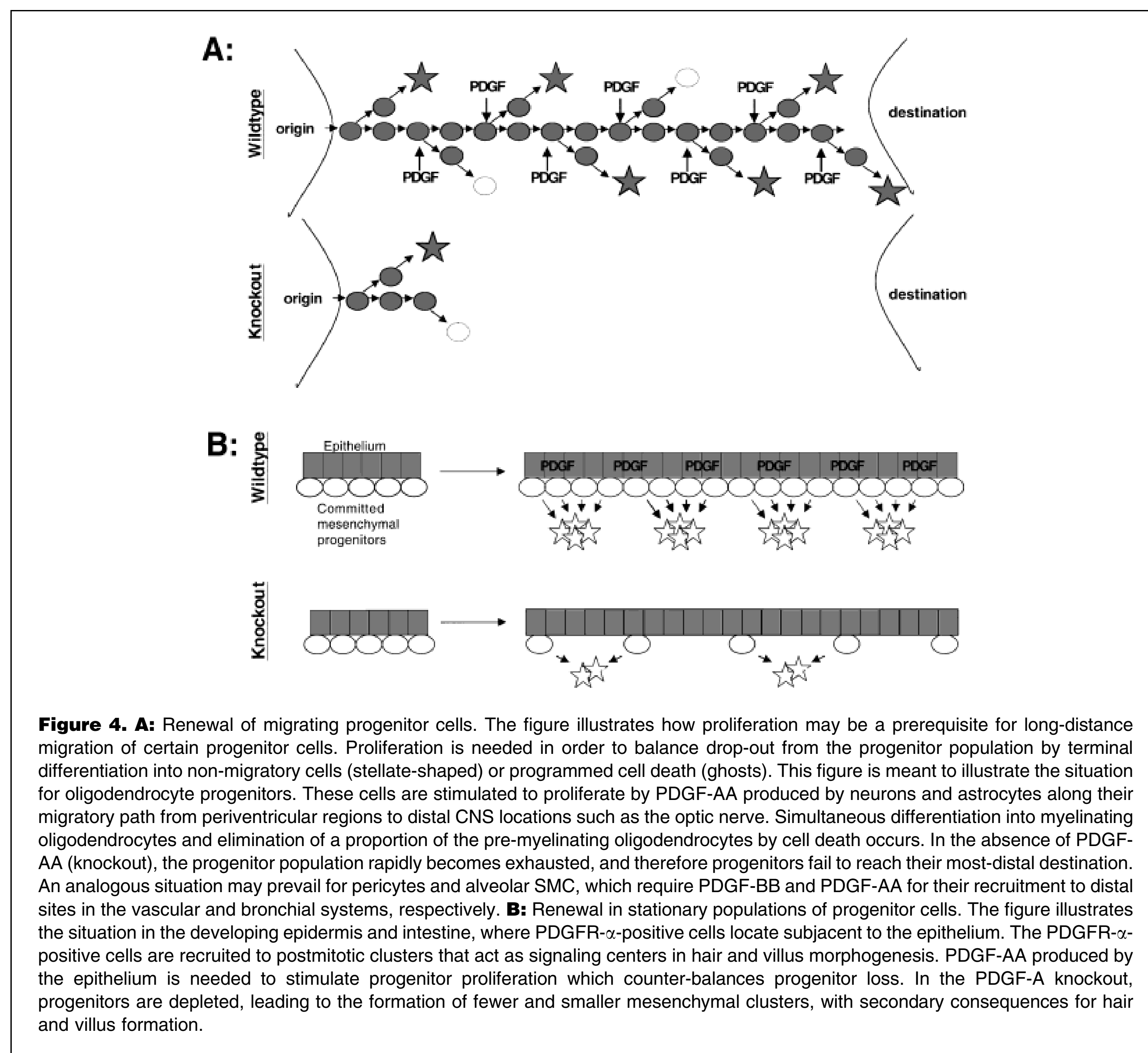

Figure 4. A: Renewal of migrating progenitor cells. The figure illustrates how proliferation may be a prerequisite for long-distance migration of certain progenitor cells. Proliferation is needed in order to balance drop-out from the progenitor population by terminal differentiation into non-migratory cells (stellate-shaped) or programmed cell death (ghosts). This figure is meant to illustrate the situation for oligodendrocyte progenitors. These cells are stimulated to proliferate by PDGF-AA produced by neurons and astrocytes along their migratory path from periventricular regions to distal CNS locations such as the optic nerve. Simultaneous differentiation into myelinating oligodendrocytes and elimination of a proportion of the pre-myelinating oligodendrocytes by cell death occurs. In the absence of PDGF-AA (knockout), the progenitor population rapidly becomes exhausted, and therefore progenitors fail to reach their most-distal destination. An analogous situation may prevail for pericytes and alveolar SMC, which require PDGF-BB and PDGF-AA for their recruitment to distal sites in the vascular and bronchial systems, respectively. **B:** Renewal in stationary populations of progenitor cells. The figure illustrates the situation in the developing epidermis and intestine, where PDGFR-α-positive cells locate subjacent to the epithelium. The PDGFR-α-positive cells are recruited to postmitotic clusters that act as signaling centers in hair and villus morphogenesis. PDGF-AA produced by the epithelium is needed to stimulate progenitor proliferation which counter-balances progenitor loss. In the PDGF-A knockout, progenitors are depleted, leading to the formation of fewer and smaller mesenchymal clusters, with secondary consequences for hair and villus formation.

double knockouts showed the same degree of mesenchymal loss in the kidney cortex as PDGFR-α knockouts.[4] Therefore, the PDGF-C expression in the metanephric blastema and the discrepancy between the phenotypes of PDGFR-α and PDGF-A/B knockouts in the kidney suggest a role for PDGF-C at this site.

Analogous developmental roles of the PDGFs

The different developmental functions of the PDGFs display some striking analogies, suggesting that a single ancestral function of PDGF may have been adapted to a number of separate but related developmental processes. Analogies are seen both at the level of PDGF function and at the level of cell function.

PDGFs are mitogens for specific stem- or progenitor cell types

The most well-documented functions of PDGFs in vitro are the control of cell proliferation, cell migration and actin reorganization and membrane ruffling. Additional suggested functions include extracellular matrix production, cell differentiation, cell contraction and cell survival (reviewed in Ref. 2). BrdU labeling in vivo has documented a role for PDGF-A in the proliferation of oligodendrocyte progenitors and subsets of dermal and intestinal mesenchymal cells as well as a role for PDGF-B in the proliferation of pericyte progenitors and vascular SMC. In the case of oligodendrocyte formation, PDGF-A deficiency leads to a reduced number of oligodendrocyte progenitors; however, in the spinal cord, these remaining progenitors are clearly able to migrate from their peri-ventricular site of origin and spread in the cord. Long distance spreading of the oligodendrocyte progenitors fails, however, and, consequently, the most peripheral extensions of the CNS remain completely devoid of myelin in PDGF-A$^{-/-}$ mice. Does this imply that PDGF-A controls migration of oligodendrocyte progenitors? Not necessarily. Published data suggest that many oligodendrocyte progenitors exit the cell cycle and enter a differentiation program prematurely. These

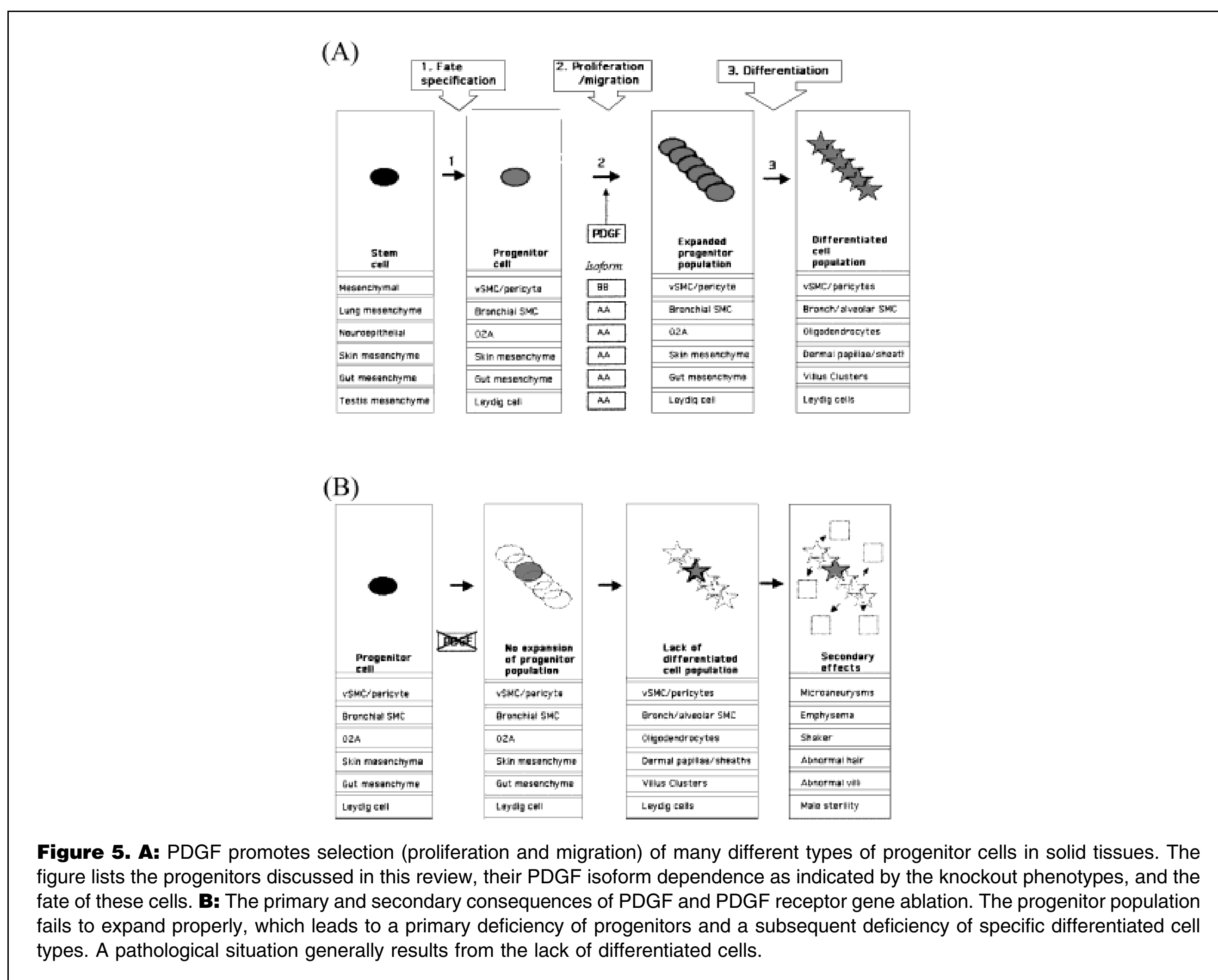

Figure 5. A: PDGF promotes selection (proliferation and migration) of many different types of progenitor cells in solid tissues. The figure lists the progenitors discussed in this review, their PDGF isoform dependence as indicated by the knockout phenotypes, and the fate of these cells. **B:** The primary and secondary consequences of PDGF and PDGF receptor gene ablation. The progenitor population fails to expand properly, which leads to a primary deficiency of progenitors and a subsequent deficiency of specific differentiated cell types. A pathological situation generally results from the lack of differentiated cells.

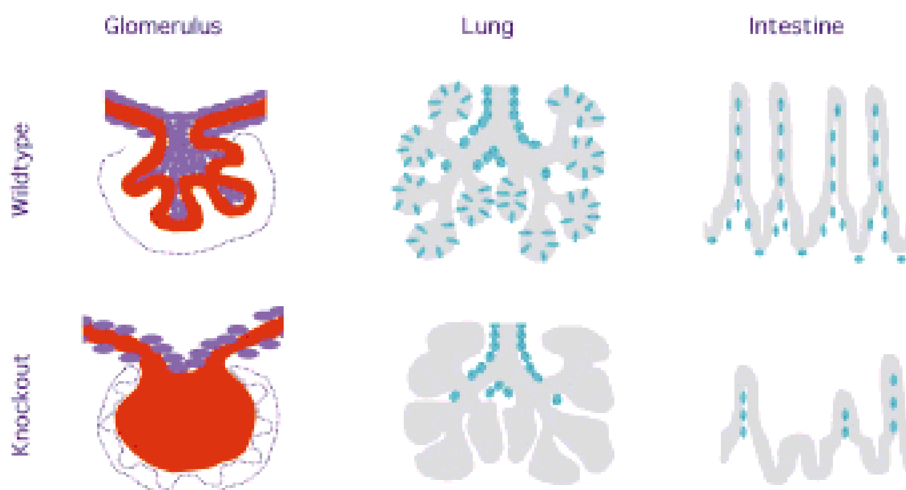

Figure 6. Analogous phenotypes affect the kidney, lung and intestine in PDGF and PDGF receptor knockouts. The figure illustrates the reduction in epithelial surface size, which results from gene knockout. In PDGF-B and PDGFR-β knockouts, the lack of formation of a capillary tuft in the kidney glomeruli and consequent folding of the glomerular basement membrane leads to a reduced surface for filtration. In the lung, lack of alveolar septation leads to an emphysema-like condition and a reduced surface for respiration. In the intestine, improper villus formation leads to a reduced surface for nutrient absorption. In all cases, PDGF-dependent mesenchymal cell types are involved in the morphogenesis of a folded epithelial sheath. See text for further explanations.

pre-differentiating oligodendrocytes become eliminated by apoptosis. Only when the relevant survival factors are present, is the differentiation program successfully completed. This points to the importance of renewal of the progenitor population, as dropout of cells by pre-differentiation and apoptosis would otherwise deplete the progenitor population (Fig. 4A). A similar reasoning may be applied to other populations of progenitors whose spreading is PDGF dependent, such as vSMC/pericyte progenitors that are spreading along vascular sprouts entering into certain tissues (e.g. brain), and alveolar SMC progenitors, which are spreading from proximal bronchiolar sites to the alveolar sacs. Although tempting, the conclusion from these observations that PDGFs control cell migration, should be made with some caution, as it remains theoretically possible that PDGF solely stimulates cell division, which becomes a prerequisite for spreading if there is simultaneous loss of progenitors through cell differentiation.

At sites where cells are not involved in directed migration, PDGFs may still be critical for progenitor renewal. The skin and intestine of a growing embryo and postnatal animal may represent such a situation, (Fig. 4B). The expansion of the surface epithelium is normally accompanied by a balanced proliferation of mesenchymal cells. PDGF-A secreted from the epithelium appears to promote proliferation of mesenchymal cells with specific functions in hair and villus morphogenesis. In the absence of PDGF-A, these cells become progressively depleted leading to abnormal hair and villus formation.

Taken together, available data imply that PDGFs regulate the renewal of several different populations of progenitor cells in solid tissues. The role of PDGFs in developing solid tissues thus appear similar to the role of colony stimulating factors, erythropoietin and other mitogens for hematopoietic progenitors and precursors in the bone marrow. In most cases, the PDGF targets are mesenchymal, but a noticeable exception is the oligodendrocytes, which are neuroectodermal. Figure 5 lists PDGF-dependent progenitor cells types, their progeny, and the phenotypic consequences of their depletion.

Functional analogies between PDGF-dependent cells

Lack of PDGFs affect cell types, which, in several cases, appear to have similar functions. The loss of pericytes leads to distension of capillaries, leading to the formation of rupturing microaneurysms at many sites. Certain specialized pericytes, such as the kidney mesangial cells and placenta pericytes form a core around which capillary tufts organize. In both organs the tuft arrangement provides a large surface for selective transport of fluid and macromolecules. Lack of PDGF-B or PDGFR-β, and the consequent loss of mesangial cells and placenta pericytes lead to formation of fewer and wider vessels. In the case of the kidney glomerulus, the complex capillary network resolve into a single microaneurysm. A general outcome of pericyte loss as a consequence of PDGF-B/-R-β deficiency is therefore capillary distension. Loss of mechanical stability in the micro-vessel wall is one potential explanation for the vessel distension, but other explanations need to be considered as well.

A more striking analogy appears from the comparison between the lung and intestinal phenotypes of PDGF-A knockouts and the kidney phenotypes of the PDGF-B and PDGFR-β knockouts. The failure of alveolar septation and the villus dysmorphogenesis in PDGF-A knockouts has resemblance to the distended glomerular capillary loops and the reduction in surface for filtration. In all cases, a normally highly convoluted and very large epithelial surface, composed of type 1 pneumocytes in the lung, enterocytes in the gut and podocytes in the kidney, is dramatically reduced in size due to a specific mesenchymal defect (Fig. 6). Thus, alveolar SMC, the intestinal villus cluster and the mesangial cells are all instrumental in a rather specific morphogenetic process — the folding of epithelial sheaths for the purpose of generating enormous surfaces.

Perspectives

Genetic analysis has revealed a number of analogous functions of the PDGF family of growth factors and the PDGF receptors in development. Since the PDGF receptors have been among the most intensely studied receptor protein tyrosine kinases (RTKs) with respect to signal transduction, a detailed knowledge of PDGF functions may provide an important basis for further investigations on the relative biological significance of the separate RTK-evoked signal transduction pathways. The specific loss of certain cell types in PDGF knockouts shed light on the functions of these cells and the developmental processes that they are involved in. Pathological conditions involving these cells, or the structures that they support, include cardiovascular and kidney diseases, lung emphysema, intestinal malabsorption, hair loss syndromes and male sterility. The study of PDGF null mice may shed light on causal links in the pathogenesis of these conditions, which, in turn, may lead to the development of new principles for intervention with the disease process, and the identification of new drug targets. The generation of conditionally mutated, or dysfunctional rather that non-functional PDGF and PDGF-receptor alleles should generate more specific developmental defects and, in certain cases, postnatal survival of the mutants. This will provide new possibilities to address the hypothetical pathogenic functions of PDGFs in diseases in which PDGF overactivity has been implicated, such as atherosclerosis, rheumatoid diseases, fibroses and cancer.

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