This Review is part of a thematic series on **Vascular Cell Diversity**, which includes the following articles:
Heart Valve Development: Endothelial Cell Signaling and Differentiation
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Endothelial/Pericyte Interactions

Endothelial-ECM: Biosynthesis, Remodeling, and Functions During Vascular Morphogenesis and Neovessel Stabilization ***Joyce Bischoff, Guest Editor***

Endothelial/Pericyte Interactions

Annika Armulik, Alexandra Abramsson, Christer Betsholtz

Abstract—Interactions between endothelial cells and mural cells (pericytes and vascular smooth muscle cells) in the blood vessel wall have recently come into focus as central processes in the regulation of vascular formation, stabilization, remodeling, and function. Failure of the interactions between the 2 cell types, as seen in numerous genetic mouse models, results in severe and often lethal cardiovascular defects. Abnormal interactions between the 2 cell types are also implicated in a number of human pathological conditions, including tumor angiogenesis, diabetic microangiopathy, ectopic tissue calcification, and stroke and dementia syndrome CADASIL. In the present review, we summarize current knowledge concerning the identity, characteristics, diversity, ontogeny, and plasticity of pericytes. We focus on the advancement in recent years of the understanding of intercellular communication between endothelial and mural cells with a focus on transforming growth factor β, angiopoietins, platelet-derived growth factor, spingosine-1-phosphate, and Notch ligands and their respective receptors. We finally highlight recent important data contributing to the understanding of the role of pericytes in tumor angiogenesis, diabetic retinopathy, and hereditary lymphedema. **(*Circ Res*. 2005;97:512-523.)**

Key Words: pericyte ■ transforming growth factor β ■ platelet-derived growth factor ■ angiopoietin ■ sphingosine-1-phosphate

Pericytes is the term for vascular mural cells embedded within the vascular basement membrane of blood microvessels, where they make specific focal contacts with the endothelium. For a long time, the existence and role of pericytes were neglected, but during recent years these, these cells have gained increasing attention as obligatory constituents of blood microvessels and important regulators of vascular development, stabilization, maturation, and remodeling. Pericytes may also mediate physiological and pathological repair processes, therefore constituting potential future targets of therapy. Much of the recently gained insight into pericyte biology stems from the analysis of genetic mouse mutants. In some cases, the phenotypes of these mutants resemble human diseases, suggesting that pericytes may play a role in the pathomechanisms of such diseases. This work has shown the absolute requirement for pericytes in the microvessel wall and pinpointed specific signaling pathways and enzymatic processes directly or indirectly involved in pericyte formation and regulation. Recent studies also highlight the intimate interactions between endothelial cells and pericytes, leaving us with the notion that impairments of 1 vessel wall cell type will inevitably affect the other. The present review focuses on endothelial/pericyte interactions, with an emphasis on the signaling molecules involved. We also summarize advancements in recent years concerning pericyte identification, diversity, development, and function. Although the progress of the past years has been rapid, we are clearly only beginning to understand pericyte biology.

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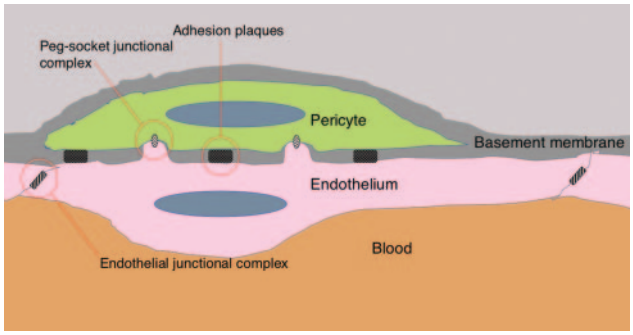

Figure 1. Endothelial–pericyte interactions in microvessels. Pericytes surrounding endothelial cells share the basement membrane with endothelial cells. A direct pericyte–endothelial contact is established via junctional complexes located to peg–socket contacts at sites were the basement membrane is absent.

Identity and Characteristics of Pericytes

Pericytes and Vascular Smooth Muscle Cells

Different cell types contact the microvascular basement membrane (BM) in different microvascular beds to communicate with the endothelial cells and help to determine and maintain local microvessel characteristics. Among these cells, pericytes are unique by their distribution and relationship with the BM and by the type of contacts formed with the endothelial cells.

Pericytes are found around blood capillaries, precapillary arterioles, postcapillary venules, and collecting venules.[1,2] Although they are related to vascular smooth muscle cells (VSMC) and are generally assumed to belong to the same cell lineage, they distinguish from the VSMC by their location relative to the endothelium, their morphology, and, to some extent, by their marker expression. However, it is important to remember that the distinction between pericyte and VSMC morphology and location is not absolute. Rather, there appears to exist a continuum of phenotypes ranging from the classical VSMC to the typical pericyte, distributed along intermediate size to small vessels, ie, between arteriole, capillary, and venule. It has also been suggested that pericytes may reside subjacent to the endothelium of large vessels.[3]

The classical VSMC of a large artery or vein distinguish from pericytes through their separation from the vascular BM by a layer of mesenchymal cells and extracellular matrix, the intima. In these vessels, the VSMC compose a separate layer in the vascular wall, the media. Pericytes, on the other hand, are embedded within the endothelial BM, to which they also are thought to contribute products (Figure 1). In areas where the BM is absent between the endothelial cells and pericytes, different types of endothelial/pericyte cell contacts have been described.[4] In adhesion plaques, the intercellular space between the 2 cell types is maintained and contains fibronectin deposits.[5] Areas called *peg–socket contacts* represent membrane invaginations extending from either cell type, which contain tight-, gap-, and adherence junctions[6–9] (Figure 1). Thus, whereas VSMC are arranged to mediate vascular tone and contraction, pericytes appear, rather, to be arranged to facilitate and integrate cell communication. Because a single pericyte often contacts several endothelial cells through these specialized contacts, they may integrate and coordinate neighboring endothelial cell responses.

Pericyte Abundance, Distribution, and Diversity

The pericyte coverage of the abluminal vessel area of the endothelium is partial, ranging from around 10% to 50%, depending on the vascular bed. This difference reflects a variation in the morphology and relative frequency of pericytes to endothelial cells, the latter varying between 1:100 (in skeletal muscle) to 1:1 (in the retina).[10] The highest pericyte coverage around microvessels is found in the central nervous system (CNS). It is not clear why the CNS needs higher vascular pericyte coverage than other organs, but 1 of the possibilities is that pericytes contribute to the formation of the blood–brain barrier. In vitro coculture experiments suggest that pericytes may increase the barrier function established by endothelial cells,[11] a process to which transforming growth factor (TGF)-β and angiopoietin 1 (Ang1) contribute.[12,13] In addition, pericytes could function as sensors of hypoxia and hypoglycemia and mediate adaptive responses required to protect the vulnerable neurons of the CNS. In response to hypoxia, brain pericytes in primary culture release prostaglandins D2 and J2, which, in turn, promote the upregulation of glucose transporter glut-1 in endothelial cells.[14] The highest pericyte coverage is observed in the retina, a tissue with meticulous metabolic demands. As discussed further below, the retina appears to be the most sensitive site for partial pericyte loss.

The anatomy of the pericyte/endothelial interface reflects the vessel function. In the choroid and in the skin, pericytes are located such that the exchange of gas and metabolites between blood vessels and the surrounding tissue is minimally hindered. Similar avoidance of pericytes is seen on surfaces engaged in gas exchange, nutrient transport, filtration, and secretion in organs such as the lung, placenta, kidney, and plexus choroideus.

Pericytes are morphologically distinct in different organs. Their morphology may range from that of the typical CNS pericyte, a flattened, or elongated, stellate-shaped solitary cell with multiple cytoplasmic processes encircling the capillary endothelium and contacting a large abluminal vessel area, to that of a mesangial cell of the kidney glomerulus, rounded, compact and contacting a minimal abluminal vessel area, making only focal attachments to the BM. Mesangial cells form a branched multicellular core around which the glomerular capillaries wind.

Pericyte Identification

The morphological diversity of pericytes mirrors diversity also at the molecular level. Several markers have been used to identify pericytes, including smooth muscle α-actin (SMA), desmin, NG-2, platelet-derived growth factor receptor (PDGFR)-β, aminopeptidase A and N, RGS5, and the promoter trap transgene *XlacZ4*.[9] However, none of these markers is absolutely specific for pericytes, and none of the markers recognizes all pericytes; their expression is dynamic and varies between organs and developmental stages.[9,15–17] For example, SMA is not appreciably expressed by skin or CNS pericytes under normal circumstances but becomes

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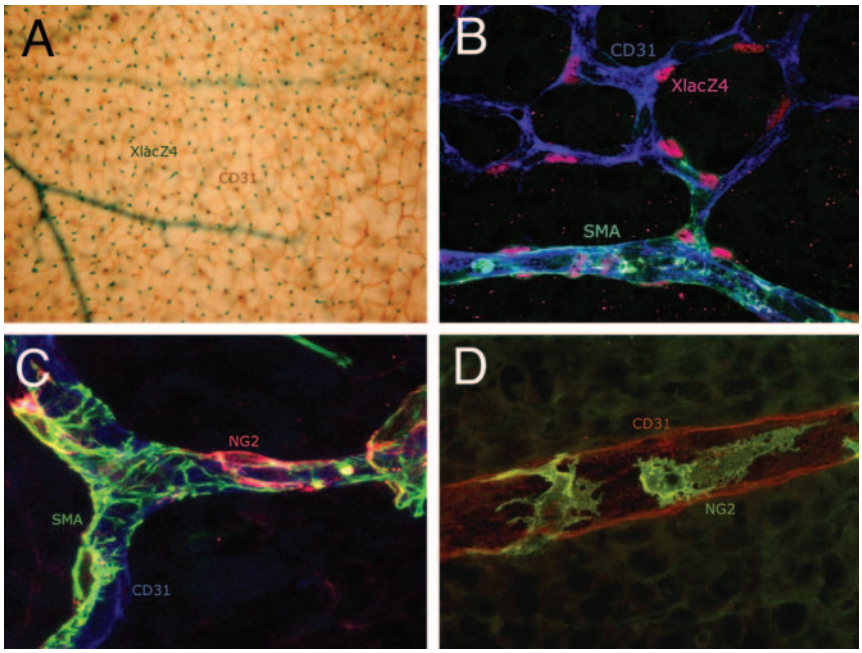

Figure 2. Marker identification of pericytes; examples of pericyte identification and pericyte heterogeneity in microvessels using different markers. A, In the XlacZ4 promoter trap transgenic mouse, pericyte nuclei are identified by lacZ staining. Image shows pericytes (nuclei, stained blue/green) in the deeper retinal plexus of a mouse, where the capillary endothelium is stained with CD31 (brown). B, Triple staining of endothelium (CD31, blue) and mural cells (SMA, green; XlacZ4, red) in the mouse retina demonstrates XlacZ4 labeling in the nuclei of mural cells in both arteries (bottom) and capillaries, whereas SMA labels the cytoskeleton of the mural cells in the artery and precapillary arteriole only. C, Pericyte heterogeneity in a tumor capillary segment revealed by staining for SMA (green), which decorates the cytoskeleton of 2 pericytes to the left, and NG2 labeling (red), which is observed in the same 2 pericytes but, in addition, also in a third, SMA-negative pericyte. D, NG2 staining (green) reveals morphological heterogeneity among tumor vessel pericytes.

upregulated during retinopathy and in subcutaneously transplanted tumors.[15,18–21] Likewise, the expression of RGS5 was found to be upregulated during tumor and physiological angiogenesis and to correlate with the degree of vascular remodeling.[22] In addition, there are species-specific differences in pericyte marker expression. In chicken embryos, expression of the transcription factor Slug, which is involved in epithelial-mesenchymal transition, is observed in pericytes and VSMC, whereas such expression has not been observed in mouse embryos.[23] Also, SMA readily detects brain pericytes in the chicken embryo but fails to identify brain pericytes in the mouse.[8,15]

The heterogeneous morphology and marker expression make unambiguous identification of pericytes a challenge. Use of single markers in previous studies has clearly led to misinterpretations. Moreover, the lack of a distinct BM around immature vessels excludes the application of anatomical criteria in all situations. State-of-the-art identification of pericytes, therefore, relies on combinations of methods, including the use of multiple markers and high-resolution confocal imaging (Figure 2). By using such approaches, immature angiogenic sprouts and tumor vessels, which were often previously reported to lack or have few pericytes, have recently been shown to have abundant pericytes[9,16,22,24] (Figure 2).

Development of Vascular Mural Cells

Ontogeny

Similar to VSMC, pericytes may have multiple origins. A mesodermal origin has been suggested for the mural cells that develop around the developing trunk vessels in the axial and lateral plate mesenchyme,[25] whereas neural crest origin has been demonstrated for at least part of the brain pericytes.[26] Coronary vessel mural cells may derive from epicardial cells, which are derived from the splanchnic mesoderm.[27] Pericytes are generally thought to be of mesenchymal origin, but transdifferentiation from endothelial cells has also been suggested,[28] although this is not a major route of pericyte formation during normal development. Evidence for bone marrow origin of mural cells during adult angiogenesis was recently presented.[29]

The multiple origins of mural cells may reflect the finding that a rather small core set (a *gene battery*) of expressed genes dictates the general hallmarks of a SMC.[30] The turning on or off of this gene battery may not be a very complicated task for the cell, hence allowing cells originating from different germ layers to undertake a VSMC differentiation route. In this sense, the ontogeny of mural cells is quite distinct from that of other specialized cell types, for which the classically held view is that they undergo differentiation along a linear path, requiring successive specification of progenitor cells paralleled by progressive restrictions in the potency.

Plasticity

Pericytes are believed to be able to differentiate into VSMC and vice versa in conjunction with vessel growth and remodeling.[31] In addition to their ability to modulate their phenotype along the pericyte–VSMC axis, pericytes may also give rise to other types of mesenchymal cells, including fibroblasts, osteoblasts, chondrocytes, and adipocytes.[32] It is unclear whether this type of cellular plasticity should be regarded as transdifferentiation between completely different cellular phenotypes or whether a common mesenchymal cell type may function in different roles with only small and dynamic modulations of the phenotypic states. Pericytes have been suggested to escape from the capillary BM and differentiate into fibroblast-like cells that may contribute to the collagenous matrix of scars in would healing, to fibrosis in conjunction with chronic inflammation, and to the formation of fibrous tumor stroma in cancer.[33] However, fibroblasts preexist in most organs, and the relative importance of pericyte–fibroblast transdifferentiation versus activation of

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preexisting fibroblasts or immature mesenchymal progenitors to the fibrogenic cell population under these conditions is unclear.

Pericytes may also play a role in ectopic tissue calcification.[32] This pathological process occurs, for example, in arteries, cardiac valves, skeletal muscle, kidney, and skin and is reminiscent of endochondral bone formation. Ectopic calcification depends on the local formation of osteogenic cells and the production of a mineralized matrix. Several lines of evidence suggest that pericytes can be triggered to differentiate into chondrocytes and osteoblasts,[34,35] and contribution of pericytes to the osteoprogenitor cells has been suggested in the ectopic calcification of the vessel wall,[32] cardiac valves,[36] and skeletal muscle.[37]

Although the view of the pericyte as a multipotent progenitor cell of pathophysiological importance is gaining increasing attention, it is important to remember that the lack of definitive markers for these cells calls for care in the interpretation of results and in the review of available literature. Reliable fate mapping of pericytes in vivo is currently not possible, and in vitro experiments are generally confounded by the uncertain origin and identity of the so-called pericyte cultures.

Signaling Pathways in Mural Cell Development and Endothelial/Mural Cell Interactions

Much of the insight into signaling between endothelial cells and pericytes comes from the analysis of genetic mouse models. One important lesson learned from these studies is that the 2 vascular cell types are interdependent; primary defects in 1 cell type have obligated consequences for the other. Proper identification of cell-type–restricted gene expression patterns and cell-type–specific mutagenesis has, in many cases, helped to distinguish primary events from secondary. In some cases, a single factor (eg, TGF-β) may affect both cell types in intricate ways. In addition to the signaling pathways discussed in more detail below, several additional genes and proteins have been implicated in mural cell development, as demonstrated by vascular wall defects in gene-targeted mice. These include the enzymes retinal aldehyde dehydrogenase 2,[38] membrane-type 1 matrix metalloproteinase,[39] and T-synthase,[40] as well as integrin $\alpha_4\beta_1$[41] and the transcription factors Fli1,[42] myocyte enhancer factor 2c,[43] L-Krüppel-like factor,[44] HAND1,[45] and HAND2.[46]

TGF-β in Endothelial/Mural Cell Interactions

The de novo induction of VSMC around the first blood vessels depends on TGF-β and, perhaps, on additional factors that remain to be characterized. A number of studies demonstrate the critical importance of various components of the TGF-β–signaling machinery for vascular development and function. Genetic inactivation in mice of *tgfb1*[47] and genes encoding its receptors, activin-receptor-like kinase 1 (*alk1*),[48,49] *alk5*,[50] TGF-β receptor II (*tβrII*),[51] and *endoglin* (type III TGF-β receptor),[52–54] as well as its downstream effector Smad5,[55,56] all lead to comparable cardiovascular defects and embryonic lethality. In humans, mutations in *ENDOGLIN* and *ALK1*, which are both expressed by endothelial cells, cause hereditary hemorrhagic telangiectasia (HHT) type 1 and 2, respectively, diseases characterized by bleeding caused by vascular malformations.[57,58]

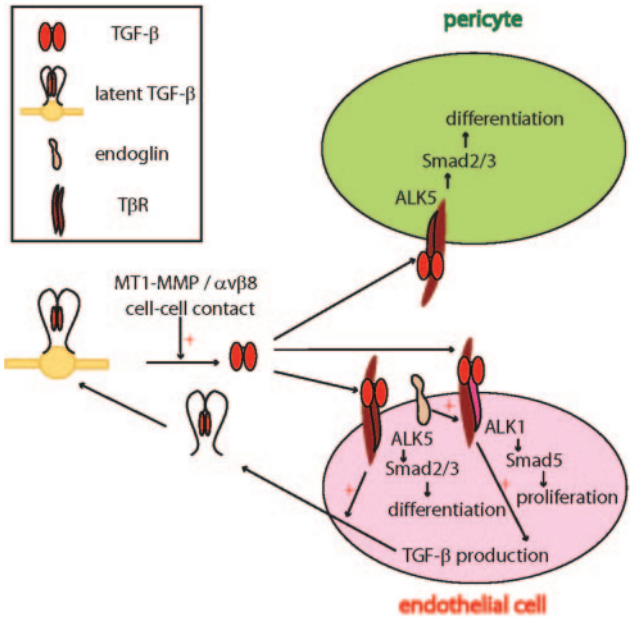

Figure 3. Endothelium-derived TGF-β signaling is important for pericyte differentiation. The outcome of TGF-β signaling in endothelial cells is dependent on the balance between 2 pathways originating from TβR/ALK-1 and TβR/ALK-5. TβR signaling has a positive autoregulatory effect on TGF-β synthesis in endothelial cells. Activation of latent TGF-β is dependent on endothelial–pericyte contact (and also in the brain on astrocyte–endothelial contacts); TGF-β signaling in mesenchymal cells is required for their differentiation into the mural cell lineage.

Mural cell defects have been reported in several of the abovementioned mouse mutants and also in HHT.[59] However, the primary defect likely occurs in the endothelium. TGF-β has context-dependent effects on endothelial cells, and different endothelial responses are mediated by signaling through ALK1/Smad1/5 (proliferation) and ALK5/Smad2/3 (differentiation).[60] Endoglin promotes ALK1 signaling, thereby shifting the TGF-β response toward proliferation.[61] Secondary changes in the mural cell compartment may relate to defects in the vascular basement membrane,[62] possibly caused by the defective balance between endothelial cell proliferation and differentiation. However, defective secondary signaling from endothelial to mural cells may also contribute to the mural cell defects seen in mouse models of HHT. Intriguingly, TGF-β itself might constitute such a second signal. In an elegant study, Carvalho et al analyzed TGF-β signaling in yolk sacs from *endoglin* knockouts and endothelium-specific knockouts of *tβrII* and *alk5* and found that the disrupted TGF-β signaling in endothelial cells also impaired the TGF-β/ALK5 signaling in adjacent mesenchymal cells, inhibiting their differentiation into VSMC and association with the endothelial tubes.[63] Therefore, TGF-β signaling in endothelial cells promotes TGF-β expression, synthesis, and release by these cells, which, in turn, induces differentiation of VSMC from surrounding mesenchymal cells but also reinforces TGF-β expression in the endothelial cells themselves in an autoregulatory loop[63] (Figure 3).

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Several studies have highlighted the critical importance of TGF-β for VSMC differentiation in vitro. Hirschi et al[64] demonstrated that TGF-β was required for differentiation of 10T1/2 cells to a SMC-like phenotype. Using soluble TGF-β trapping–receptor bodies, antibodies, and short-interfering RNA, Sinha et al showed that TGF-β signaling through Smad2/3 plays an important role in the development of SMCs in embryonic stem cell–derived embryoid bodies.[65] Elegant in vivo evidence for a direct role of TGF-β signaling in VSMC development comes from studies of mice with neural crest–specific ablation of *tβrII*. These animals develop a DiGeorge syndrome–like phenotype, including failure of neural crest derivatives to differentiate into VSMC in the cardiac outflow tract.[66] TGF-β signaling in neural crest cells involves the adaptor protein CrkL, which is also implicated in DiGeorge syndrome.

Although evidence is gathering for central role of TGF-β signaling in both endothelial and mural cells, additional studies highlight the importance of juxtaposition and collaboration of the 2 cell types for activation of latent TGF-β.[67,68] Interestingly, gap junctions between endothelial cells and pericytes appear to be involved in this process, and gap junctions are also required for endothelium-induced mural differentiation, as demonstrated by studies of connexin 43 (*cx43*)-knockout mice.[69] Other gene deletions/mutations may also result in vascular phenotypes because of interactions with TGF-β activation or signaling. One example is tissue factor (TF), a procoagulant receptor stimulated by TGF-β1. *Tf* knockouts develop a lethal vascular phenotype involving mural cell defects.[70] Another example is integrin $\alpha_v\beta_8$. Knockouts of α_v or β_8 genes result in brain hemorrhage and mid- to late-embryonic lethality.[71,72] Integrin $\alpha_v\beta_8$ is expressed by astrocytes, and its effect on brain blood vessels appears to be mediated by activating latent TGF-β.[73]

Angiopoietin–Tie2 Signaling in the Vascular Wall

Numerous studies suggest that the angiopoietin–Tie2 signaling pathway is also involved in the reciprocal communication between endothelial cells and pericytes. Activating mutations in *TIE2* causes human venous malformations associated with abnormal VSMC.[74] The Tie2 receptor is generally held as being endothelial specific,[75,76] whereas its agonistic ligand, Ang1, appears to be expressed mainly by perivascular and mural cells[77,78] (Figure 4). These expression patterns, thus, suggest that Ang1 is a pericyte-derived paracrine signal for the endothelium. Genetic loss- and gain-of-function studies in mice show that the Ang1–Tie2 signaling loop is essential for vessel maturation and stabilization. *Ang1*- or *tie2*-null mice die at midgestation from cardiovascular failure. These embryos show defective angiogenesis, and their blood vessels have poorly organized BM and show reduced coverage and detachment of pericytes.[75,77,79] Conversely, the overexpression of Ang1 leads to an expanded and stabilized, leakage-resistant vasculature.[80,81] The importance of Ang1 as a pericyte-derived, microvessel-stabilizing signal was also demonstrated by the ability of recombinant Ang1 to partially rescue the vascular defects in the retina attributable to pericyte loss.[82] Together, these studies point to Ang1 as a pericyte-derived signal that mediates maturation and quiescence of the microvascular endothelium. However, they do not necessarily explain the mural cell deficiencies in *ang1* and *tie2* knockouts. These cellular defects could be indirect, possibly reflecting loss of an endothelium-derived VSMC inductive signal, such as TGF-β (see above) or heparin-binding epidermal growth factor (HB-EGF). Iivanainen et al suggested that binding of Ang-1 to Tie-2 results in upregulation of endothelial HB-EGF, which promotes VSMC migration by binding to ErbB1 and ErbB2.[83] If the downstream HB-EGF/ErbB signaling controlled by Ang1/Tie2 would be crucial for pericyte recruitment, one would expect to see defective pericyte recruitment in *hb-egf/egfr*–knockout animals as well. However, this has not been reported. Instead, mice defective in HB-EGF develop severe heart failure caused by the cardiac valve and the ventricular chamber defects similar to *erbb1* and *erbb2* knockouts.[84]

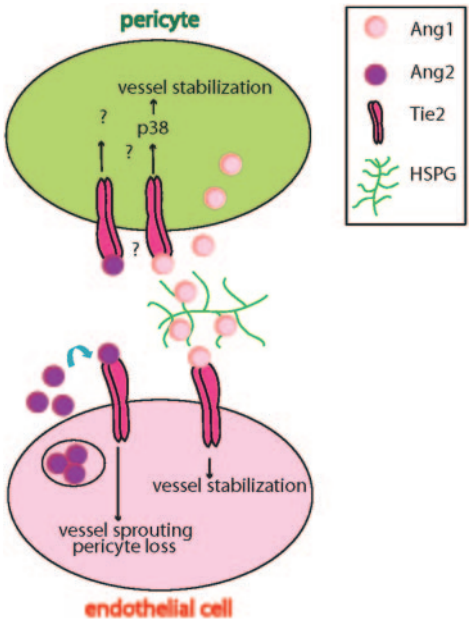

Figure 4. Ang1/Tie2 signaling is required for vessel stabilization. Signals originating from binding of pericyte-derived Ang1 to Tie2 lead to stabilization of endothelial cells. Ang2 antagonizes Tie2 signaling in endothelial cells, leading to vessel destabilization. The presence of Tie2 receptors on pericytes is still a controversial issue, but in vitro experiments suggest that it may have a role also in these cells.

Ang2 is an antagonistic ligand for Tie2 in endothelial cells,[85] but possibly an agonistic ligand for Tie2 in mesenchymal cells.[86] Ang2 overexpression mimics Ang1 or Tie2 deficiency, and Ang2 deficiency does not disturb prenatal vascular development but leads to defects in the eye vasculature (hyaloid vessel persistence) and intestinal lymphatics postnatally.[87] Ang2 is expressed mainly in endothelial cells and upregulated endothelial-derived Ang2 marks the onset of angiogenic sprouting in tumors.[88,89] In these cells, Ang2 is stored in specialized secretory vesicles and can be rapidly released on stimulation[90] (Figure 4).

Although the endothelial expression of Ang2 would suggest an autocrine route of signaling in the endothelium, effects on pericyte coverage are indicated. Ang2 overexpression in both xenografted and natural tumors correlates in-

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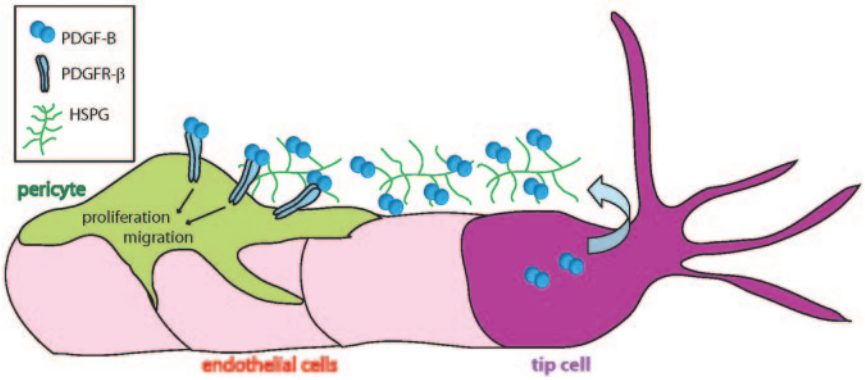

Figure 5. PDGF-B/PDGFR-β signaling is necessary for pericyte recruitment during angiogenesis. PDGF-B is synthesized and secreted by the migratory *tip cells* at the leading edge of angiogenic sprouts. Binding of PDGF-B to HSPG is important for localization of PDGF-B to the vicinity to the developing vessel. Pericytes, which express PDGFR-β, are dependent on of endothelium-derived PDGF-B for proliferation and migration.

versely with pericyte coverage.[88,89] In diabetic rats, Ang2 upregulation in the retina preceded pericyte loss from retinal microvessels (role of pericytes in diabetic microangiopathy is further discussed below) and direct injection of Ang2 into the eye induced pericyte loss from retinal vessels.[91] These results support the concept that Ang2 antagonism of Ang1-mediated Tie2 signaling leads to vessel destabilization involving detachment or loss of pericytes.

Tie2 might have direct effects in mural cells, a view that has gained support from in vitro studies showing that cultured VSMC-like cells express Tie2 and respond to Ang1.[92,93] In the aorta ring sprouting assay, Ang1 increases both endothelial sprout formation and mural cell recruitment, but only the latter effect was inhibited by p38 mitogen-activated protein kinase inhibitors, suggesting that Ang1 may have direct effects on both endothelial cells and mural cells in this system[94] (Figure 4).

In summary, Ang1 and Ang2 are expressed by different cell types in the microvessel wall and mediate opposing effects on Tie2 signaling and vessel stabilization. Available data support a concept of a default vessel-stabilizing paracrine Ang1/Tie2 stimulatory loop, which is interrupted by induction of an autocrine vessel destabilizing Ang2/Tie2 inhibitory loop in conjunction with onset of angiogenesis. Whether these functions reflect primary signaling events in the endothelial cells and secondary mural cell loss or whether angiopoietins have direct and complementary effects on both cell types requires further study.

PDGF-B/PDGFR-β Signaling in Mural Cell Recruitment

The PDGF-B and PDGFR-β pathway plays a critical role in the recruitment of pericytes to newly formed vessels.[95,96] During angiogenesis, sprouting endothelial cells secrete PDGF-B, which signals through PDGFR-β expressed by mural cells, resulting in proliferation and migration of mural cells during vessel maturation. The knockout of *pdgfb* and *pdgfrb* leads to similar phenotypes and perinatal death caused by vascular dysfunction.[97,98] The primary cause of the phenotype is lack of pericytes,[15,99,100] which has secondary consequences for the endothelium, leading to endothelial hyperplasia, abnormal junctions, and excessive luminal membrane folds.[101] These defects and the accompanying vascular dysfunction probably cause a compensatory upregulation of vascular endothelial growth factor (VEGF) A levels, which is observed in *pdgfb* and *pdgfrb* knockouts. Increased VEGFA may, in turn, promote further abnormalities, including vascular leakage and hemorrhage.[101]

Pericytes are initially induced in the absence of PDGF-B signaling, but the pericyte population fails to expand and spread along the microvessels.[15] Thus, the PDGF-B/PDGFR-β pathway is critically important for expansion of the pericyte population and possibly also for pericyte migration along the growing vessel (Figure 5). This might explain why the most affected vascular tissue in *pdgfb* and *pdgfrb* knockouts is that of the CNS, which lacks vasculogenesis-competent mesenchyme and, hence, PDGF-independent de novo induction of mural cells.[15]

The endothelium is a critical source of PDGF-B– for PDGFR-β–positive mural cell recruitment, as demonstrated by endothelium-specific ablation of *pdgfb*, which leads to pericyte deficiency.[19,102] Deletion of *pdgfb* in 2 other major cellular sources of PDGF-B, neurons, and hematopoietic cells had no effect on the vasculature.[103–105]

Not only the presence but also the level of PDGF-B is important for pericyte development. Mice heterozygotes for a *pdgfb*-null allele show reduced pericyte numbers compared with wild-type mice.[106] Different levels of chimerism for the recombined *pdgfb* allele in endothelium-specific knockouts produced to a range of PDGF-B–deficient states with up to 90% reduction in pericyte density.[102] The expression of PDGF-B is seen only at sites where active angiogenesis takes place.[15,101] In angiogenic sprouts in the developing CNS, PDGF-B expression is concentrated at the tip of the sprouts, which is also a site harboring actively proliferating pericytes (P. Andersson, H. Gerhardt, and C.B., unpublished observations, 2004). Thus, the endothelial expression of PDGF-B occurs at sites where active pericyte proliferation and recruitment takes place.

The importance of spatial control in regulation of PDGF-B expression is demonstrated by the deletion of its retention motif. This motif is required for the PDGF-B binding to heparan sulfate proteoglycans (HSPG). Mice that express retention motif–deficient PDGF-B have abnormal coverage of pericytes, which were partially detached.[20,107] Binding of PDGF-B to HSPG is most likely needed for localizing secreted PDGF-B in close vicinity to endothelial cells, where it promotes the recognition of PDGFB– by PDGFR-β–expressing cells (Figure 5). Accurately localized depots of PDGF-B might help pericytes to migrate along the microves-

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sel abluminal surface, similar to the requirement of matrix-associated VEGFA for endothelial cell migration along astrocytes in the postnatal retina.[9]

In a series of elegant articles, Soriano and colleagues have analyzed the relative importance of the different signaling pathways emanating from PDGFR-β in pericyte recruitment and function.[108–110] Using an allelic series of targeted *pdgfrb* mutations, where individual tyrosine residues alone or in combination are replaced with phenylalanine (F mutants) in PDGFR-β, they made the surprising observation that different signaling pathways appeared to be additive rather than specific for pericyte recruitment.[110] An additional surprise came from the finding that the most advanced mutants, where all major known downstream signaling pathways were disrupted, were viable even when the F7 allele occurred in hemizygous combination with the *pdgfrb*-null allele.[110]

In summary, the null, conditional, and subtle mutagenesis of *pdgfb* and *pdgfrb* in mice has demonstrated the crucial importance of this signaling pathway for mural cell recruitment during angiogenesis. Intriguingly, whereas the null mutants are invariably pre- or perinatally lethal, mutants with endothelium-specific loss of PDGF-B and signaling-defective PDGFR-β revealed that up to 90% pericyte reduction in selected capillary beds was still compatible with basic vascular functions and postnatal life. However, these mice develop severe eye abnormalities and kidney problems.[102,109,110]

S1P/Edg Signaling in Endothelial/Pericyte Interactions

Sphingosine-1-phosphate (S1P) is a secreted sphingolipid engaged in cell communication through certain G-protein coupled receptors denoted as $S1P_{1\ to\ 5}$. When added to cells, S1P triggers cytoskeletal, adhesive, and junctional changes, affecting cell migration, proliferation, and survival.[111]

Disruption of the *s1p1* (*edg1*) gene in mice causes mid/late-gestational lethality with vascular abnormalities involving defective VSMC/pericyte coverage of vessels.[112] A similar defect was noticed in double knockouts for *s1p2* and *s1p3*,[113] whereas other double, or triple *s1p1–3* knockouts showed more severe vascular defects and earlier lethality.[113]

Although $S1P_1$ expression was originally described in endothelial cells, these receptors are expressed widely in cultured cells, including both endothelial and mesenchymal cells. Using a *lacZ* reporter gene targeted into the *s1p1* locus, expression was demonstrated in embryonic vascular endothelium with the exception of veins, while widespread expression also in nonvascular cells was observed in adult tissues.[112] Consistent with the selective developmental expression in vascular endothelium, endothelial-specific knockout of *s1p1* recapitulated the mural sheath defects observed in the full *s1p1* knockouts, demonstrating that mural vessel coverage is directed by the activity of $S1P_1$ in the endothelium.[114] *S1p1* knockout in VSMC, on the other hand, had no adverse effects (referenced in[114]).

How does $S1P_1$ signaling in endothelial cells affect mural cell recruitment? Studies by Paik et al suggest that $S1P_1$ signaling through Rac promotes trafficking of N-cadherin to polarized plasma membrane domains in endothelial cells, thereby strengthening contacts with mural cells (Figure 6).[115] N-cadherin-based adherence junctions are located to peg–socket contacts between endothelial cells and pericytes.[9] Evidence obtained from anti–N-cadherin antibody injection into chick brain[8] or short-interfering RNA inclusion in Matrigel plugs[115] suggests the functional importance for N-cadherin in these contacts. However, the importance of N-cadherin in endothelial cells may go beyond heterotypic endothelium–pericyte interactions. Luo and Radice demonstrated that endothelium-specific knockout of N-cadherin significantly decreased expression of VE-cadherin levels, leading to midgestational lethality from severely disturbed vasculature caused by endothelial-endothelial junction defects.[116] The finding that N-cadherin acts upstream of VE-cadherin in vascular morphogenesis, together with earlier studies implicating $S1P_1$ as a major regulator of fundamental endothelial cell functions,[117–119] suggests that the mural cell deficiency in $S1P_1$ knockouts has a complex pathogenesis involving disrupted endothelium/pericyte interactions in combination with cell autonomous endothelial defects (Figure 6).

Notch Signaling in the Vascular Wall

Notch signaling is critically important for the establishment of arterio-venous (A-V) identity in the developing vasculature.[120] Activation of Notch signaling in the endothelium induces arterial markers, such as ephrin B2, CD44, and neuropilin 1, and suppresses venous markers such as Eph B4.[121,122] Conversely, Notch repression in endothelial cells was recently shown to be important for the establishment of venous endothelial identity.[123]

Also, arterial and venous VSMC differ in their morphology and function. Interestingly, a recent study also demonstrates a role for Notch signaling in specification of the arterial characteristics of VSMC. The arterial SMC in *notch*$^{-/-}$ mice appear to have lost some of their arterial characteristics and have instead acquired the characteristics of venous SMC.[124] Importantly, the endothelium in *notch*$^{-/-}$ mice maintains the correct A-V identity. Notch3 is expressed by VSMC of small and medium-sized brain arteries,[125] and *NOTCH3* is mutated in CADASIL, a human stroke and dementia syndrome affecting the same type of vessels.[126] Although the pathogenesis of the vascular lesions in CADASIL is not fully understood, the studies of *notch3*$^{-/-}$ mice suggest that it might involve problems with the specification of correct VSMC identity.

Even though Notch is required for specification of A-V identity in endothelial and mural cells, this does not necessarily mean the involvement of juxtacrine signaling between the 2 cell types. The Notch ligands Jagged1 and Delta4 are both expressed by endothelial cells and appear to act primarily on Notch1 and Notch4 receptors also present on endothelial cells. The Notch3-dependent arterial VSMC differentiation does not correlate with the temporal expression of Jagged1 and Delta4 during development, and the mechanism of activation of Notch3, therefore, remains elusive. It appears that Notch signaling autonomously and independently specifies arterial characteristics in endothelial and mural cells.

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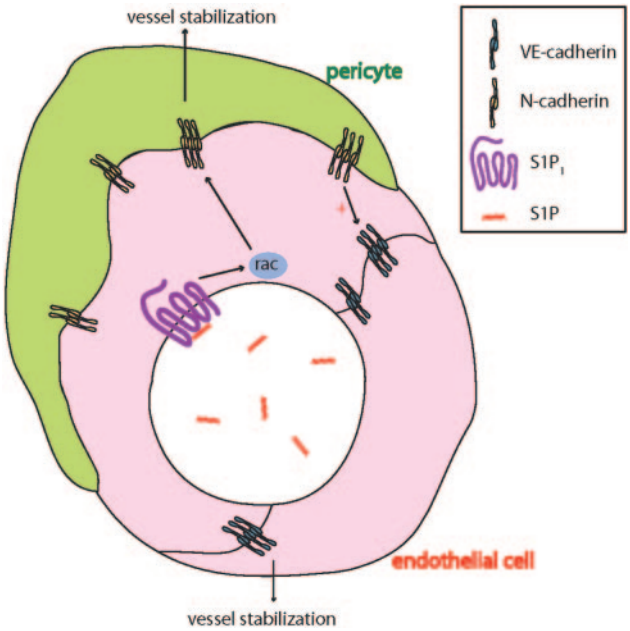

Figure 6. Endothelial S1P/S1P$_1$ signaling is important for establishing stable pericyte coverage. S1P$_1$-mediated vessel stabilization may be mediated through N-cadherin–based endothelial–pericyte contacts. Localization of N-cadherin on the plasma membrane on endothelial cells is dependent on activation of small GTPase rac through the S1P/S1P$_1$ pathway.

Conclusions

Insight into the molecular mechanisms of endothelial–pericyte interactions has accelerated during the past 1 to 2 years, but although we are beginning to elucidate the molecular details of these interactions, we are still lacking understanding about many aspects of endothelial–pericyte communication. It is far from clear how pericytes stabilize vessels in the late stages of physiological angiogenesis and why this process fails in tumor vessels. Do pericytes have active functions in adult microvessel vessel homeostasis and maintenance, or do they merely mediate proliferative quiescence? Many questions related to the physiology and pathology of pericytes currently have no clear answers. Even though plausible, it is also not clear whether the different density, morphology, and marker expression of pericytes in different organs reflect differences in their physiological functions. Additional questions include whether pericytes possess stem or progenitor properties and if pericytes (or their absence) contribute to wound healing, pathological fibrosis, and diabetic microangiopathy.

Below is a brief description of some cases where recent insights have been made into pericyte pathophysiology, providing prospects for pericyte-directed therapy. For more detailed discussions, please refer to the cited reviews and original literature.

Tumor Pericytes As Putative Antiangiogenic Targets

Tumor vessels are heterogeneous in their pericyte coverage and antiangiogenic therapy directed against the endothelium appears to lead to ablation of the naked endothelial tubes, whereas the pericyte covered stretches are protected.[24] This has led to the idea that combinations of antiendothelial and antipericyte agents might act synergistically in antiangiogenic therapy. Bergers et al tested this concept by applying combinations of VEGF- and PDGF-pathway inhibitors in a transgenic model of pancreatic islet tumors. Indeed, they recorded complementary and synergistic antiangiogenic and antitumor effects.[127] Additional studies using animal models have shown that inhibition of PDGF signaling reduces interstitial tumor pressure and, thereby, enhances the effect of chemotherapy.[128,129] These studies indicate potential benefits of targeting pericytes in the treatment of tumors.

Pericytes and the Pathogenesis of Diabetic Retinopathy

Pericyte loss from microvessels is the earliest cellular deficiency noticed in diabetic retinopathy, yet both the cause of the pericyte dropout, and the potential causal role of this event in the pathogenesis of diabetic retinopathy, remains controversial. Animal model studies involving genetic or pharmacological PDGF-B/PDGFR-β–pathway inhibition have demonstrated that pericyte deficiency created in the absence of diabetes is sufficient to trigger states of retinopathy that are reminiscent of both nonproliferative and proliferative diabetic retinopathy in humans.[9,130] Diabetic pericyte dropout is mimicked by retinal Ang2 injection, and Ang2 haploinsufficiency appears to exert a protective effect on diabetes-induced pericyte dropout.[91] Together, these data suggest that pericytes may have a causal role in diabetic retinopathy and that pericyte protective agents (once developed) may have a therapeutic use in the early stages of diabetic retinopathy.

Pericytes and the Pathogenesis of Rare Disorders

Pericytes normally do not invest lymph vessels, although collecting lymphatics have associated SMC. Human lymphedema distichiasis is caused by mutations in the *FOXC2* gene, leading to lower levels of the Foxc2 transcription factor. Foxc2 is normally expressed by lymphatic endothelium. Both lymphedema distichiasis patients and *foxc2*$^{-/-}$ mice show ectopic recruitment of mural cells to lymph vessels, leading to focal narrowing of the lymphatics and lymph stasis.[131] Lack of Foxc2 appears to reprogram lymphatic endothelial cells to a more blood vessel endothelium–like phenotype, which includes diminished VEGFR3 expression, increased PDGF-B expression, and increased production of BM components. Also, TGF-β signaling might contribute to the increased pericyte recruitment because increased levels of endoglin was observed in *foxc2*$^{-/-}$ mice. These findings open the possibility of using agents that inhibit pericyte recruitment in the treatment of certain forms of hereditary lymphedema.

To date, no genetic human diseases have been described where the pericyte deficiency is the cause of the disease. However, it was recently reported that patients with Adams-Oliver syndrome have a pericyte deficiency.[132] The genetic cause(s) of Adams-Oliver syndrome remains to be established.

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