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Review

Pericytes: Developmental, Physiological, and Pathological Perspectives, Problems, and Promises

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Pericytes, the mural cells of blood microvessels, have recently come into focus as regulators of vascular morphogenesis and function during development, cardiovascular homeostasis, and disease. Pericytes are implicated in the development of diabetic retinopathy and tissue fibrosis, and they are potential stromal targets for cancer therapy. Some pericytes are probably mesenchymal stem or progenitor cells, which give rise to adipocytes, cartilage, bone, and muscle. However, there is still confusion about the identity, ontogeny, and progeny of pericytes. Here, we review the history of these investigations, indicate emerging concepts, and point out problems and promise in the field of pericyte biology.

Introduction

Although Eberth described their presence in 1871 (Eberth, 1871), the discovery of pericytes is commonly assigned to the French scientist Charles-Marie Benjamin Rouget, who two years later described a population of contractile cells surrounding the endothelial cells of small blood vessels (Rouget, 1873). Zimmermann later called these cells "Rouget cells" and also coined the term "pericytes," alluding to their location in close proximity to the endothelial cells (Zimmermann, 1923). Between 1920 and 1950 numerous other publications described pericytes, some of them, however, questioning the contractility of the cells (reviewed by Sims, 1986). Part of these discrepancies probably had experimental reasons, but they may also reflect pericyte heterogeneity and confusion about cell identities. Today, it is clear that different cell types occupy the periendothelial compartments, yet that their correct identification is still challenging (reviewed by Krueger and Bechmann, 2010).

The currently accepted definition of a mature pericyte as a cell *embedded within the vascular basement membrane* (BM) came with the application of electron microscopy (reviewed by Sims, 1986). As discussed below, this definition is difficult to apply in situations of active angiogenesis. Another commonly applied defining criterion is the presence in microvessels, i.e., capillaries, postcapillary venules, and terminal arterioles. Also, this definition has been challenged by observations of subendothelial pericyte-like cells in large vessels (reviewed by Díaz-Flores et al., 2009).

The periendothelial location of pericytes is frequently confused with the periendothelial location of vascular smooth muscle cells (vSMCs), fibroblasts, macrophages, and even epithelial cells. Although the field has generally adopted the view that pericytes belong to the same lineage and category of cells as vSMCs, it should be remembered that there is no single molecular marker known that can be used to unequivocally identify pericytes and distinguish them from vSMCs or other mesenchymal cells. The multiple markers that are commonly applied are neither specific nor stable in their expression.

As a result, the term pericyte is frequently used in the literature to denote any microvascular periendothelial mesenchymal cell. In fact, most published papers on pericytes do not investigate whether they share BM with the endothelium and, to a large part, do not succeed in distinguishing pericytes from other perivascular cells. Current work on mesenchymal stem cells in the blood vessel wall and their possible relation to pericytes is one such area that we discuss further below. In fairness, the definition of a pericyte by criteria that requires ultrastructural analysis for identification is not practical.

Thus, as a compromise pericytes are usually defined—or described—using a mixture of criteria including location, morphology, and gene/protein expression pattern. Below, we highlight ambiguities concerning these criteria to help the reader value a particular concept or result. We attempt to provide a broad but brief base for the reader about the anatomy and ontogeny of pericytes and of the signaling pathways involved in pericyte recruitment and communication with the endothelium. These are topics that have been covered in greater depth in other recent reviews (Armulik et al., 2005; Gaengel et al., 2009). We go into more detail concerning the physiology and pathophysiology of pericytes, which are areas where significant advances have been made recently. Whereas uncertainties concerning cell lineages and identities remain, there is sufficient available information to consider a scenario in which pericytes play critical physiological roles in vascular development and homeostasis, as sources of fibrogenic cells in pathological situations, and as a possible reservoir of stem or progenitor cells for adult tissue repair.

Morphological Considerations

Shape and Location—a Continuum of Mural Cell Phenotypes

Pericytes appear to be ubiquitously present in blood microvessels, but not normally in lymphatic capillaries. In certain developmental abnormalities, or pathological situations, lymphatic capillaries may attract ectopic pericytes (Petrova et al., 2004).

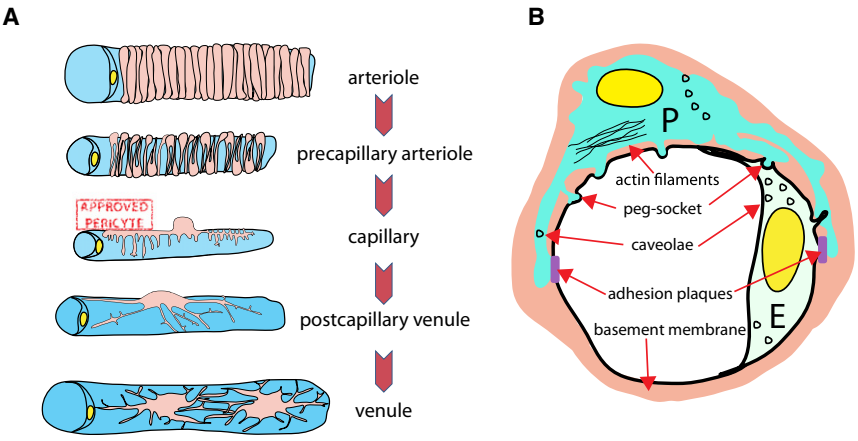

Figure 1. Pericyte Anatomy

(A) A continuum of mural cell cyto-architecture from arteriole to venule. A single vSMC layer around arterioles and precapillary arterioles encircles the entire abluminal side of the endothelium. vSMCs around arterioles have a flattened, spindle-shaped appearance with few cytoplasmic processes, whereas around precapillary arterioles the cell bodies are distinctly protruding and extend several processes encircling the endothelium. Pericytes investing capillaries have a nearly rounded cell body that gives rise to a few primary processes running on the endothelium in the length of the capillary. The primary processes give rise to secondary perpendicular processes. The tips of secondary processes attach firmly to the endothelium. On postcapillary venules the mural cell body flattens and gives rise to many slender, branching processes. vSMCs covering venules have a relatively big, stellate shape cell body with many branching processes, which, unlike arteriolar vSMCs, do not wrap circularly around the endothelium.

(B) Ultrastructural characteristics of pericytes and pericyte-endothelial interactions. Pericytes are rather anonymous in transmission electron microscopy. The mature capillary pericyte (P) has a discoid nucleus that is surrounded by a small amount of cytoplasm containing protein-producing organelles and mitochondria. Microtubules stretch along the primary and secondary cytoplasmic extensions. Intermediate filaments composed of desmin and vimentin are mostly concentrated within in the primary extensions. Dense bands of microfilaments containing actin, myosin, and tropomyosin are concentrated beneath the plasma membrane, in particular the inner surface membrane facing the endothelium. The outer, abluminal pericyte surface often shows numerous caveolae. Despite being separated by the shared BM, pericytes and endothelial cells (E) make numerous direct contacts of different type: schematically depicted are peg-socket contacts and adhesion plaques.

Pericytes extend primary cytoplasmic processes along the abluminal surface of the endothelial tube. These processes usually span several endothelial cells and occasionally bridge neighboring capillary branches. At capillary branch points, which commonly harbor a pericyte soma, primary processes are often found to extend along each branch, conferring a cellular Y-shape. Thin secondary processes extend from the primary processes. These are usually perpendicular in their orientation relative to the primary branches, thereby partially encircling the vessel (Figure 1A).

Ultrastructure

The pericytes are enveloped in a BM that is continuous with the endothelial BM. Pericytes probably contribute products to the BM, and in vitro analysis demonstrates that pericyte-endothelial interaction regulates BM assembly (Stratman et al., 2009, 2010). Mature pericytes thus become embedded *within* a capillary BM, a feature that makes it possible to identify primary and secondary pericyte processes by transmission electrom microscopy in ultrathin sections (Sims, 1986). It is unclear to what extent mature pericytes are always fully BM embedded. The literature contains many descriptions of incomplete or even absent BM coverage (reviewed by Díaz-Flores et al., 2009). The relationship between the pericyte and the microvascular BM is hard to see in embryonic tissue or in pathological situations, where angiogenesis is active and the BM in a state of synthesis or turnover. Here, the distinction between pericytes and other perivascular mesenchymal cells is particularly problematic.

The majority of the pericyte-endothelial interface is separated by a BM (Figure 1B). However, at discrete points, the two cell types contact each other at holes in the BM. The number and size of pericyte-endothelial contacts may vary between tissues, but up to 1,000 contacts have been described for a single endothelial cell. The contacts are of *peg-socket* type, in which pericyte cytoplasmic fingers (pegs) are inserted into endothelial invaginations (pockets). Other contact morphologies have been described, including sites where the two membranes come very close together (*close* or *occluding* contacts). These are located at the edge of the pericyte processes and possibly play an anchoring role. Another type of contacts, referred to as *adhesion plaques*, show microfilament bundles attached at the pericyte plasma membrane and electron-dense material in the opposing endothelial cytoplasm. They contain fibronectin and resemble adherence junctions ultrastucturally. Possibly, these are the sites where N-cadherin-based connections are formed between endothelial cells and pericytes (Gerhardt and Betsholtz, 2003; Gerhardt et al., 2000). Gap junction-like structures have also been reported at contacts between endothelial cells and pericytes (Díaz-Flores et al., 2009). These observations are somewhat anecdotal, however, and functional evidence for gap junctions between endothelial cells and pericytes exists in vitro (Larson et al., 1987), but not in vivo. As discussed below, the production of TGFβ by endothelial and mural cells may require their contact through gap junctions.

Pericyte Abundance

The pericyte density varies between different organs and vascular beds, and the proportion of the endothelial abluminal surface that is pericyte covered varies as well. The central nervous system (CNS) vasculature is generally regarded as being the most pericyte covered, with a 1:1–3:1 ratio between endothelial cells and pericytes, and an approximately 30% coverage of the abluminal surface (Mathiisen et al., 2010; Sims, 1986). Significantly lower ratios have been reported for some other tissues, e.g., human skeletal muscle, which has been stated to have a 100:1 endothelial-to-pericyte ratio (reviewed in Díaz-Flores et al., 2009; Shepro and Morel, 1993). Although there is undoubtedly variation, very low figures like this one are based on singular reports and may be questioned since much higher ratios have been described in skeletal muscle in other studies (Tilton et al., 1979). If true, it would also imply that many endothelial cells would be without pericyte contact, a situation that has

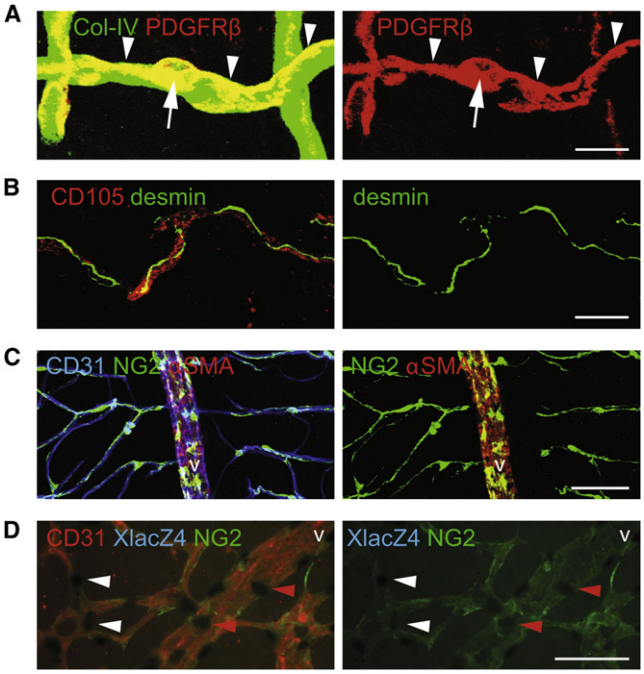

Figure 2. Examples of Pericyte Identification Using Different Markers

(A) The vascular BM of a brain capillary identified by collagen IV (Col-IV, green) and pericytes surrounding the endothelium by PDGFRβ (red) immunostaining. Note that the pericyte is embedded in vessel basement membrane, as seen by the yellow appearance on the merged image. The white arrow points to a typical pericyte cell body and the arrowheads indicate pericyte processes that stretch horizontally along the capillary.

(B) A 3D view of brain endothelial cells (CD105, red) and pericytes (desmin, green). Note the dissimilar appearance of pericytes compared to the one in (A), which is due to the different marker used for identification. Desmin is an intracellular intermediate filament protein, visualizing clustered intermediate filaments, whereas PDGFRβ is a transmembrane cell surface protein displaying the pericyte membrane contour.

(C) Triple immunostaining of endothelium (CD31, blue) and mural cells (NG2-green; αSMA -red) in the retina. vSMCs covering the vein (v) are positive for αSMA and occasionally also for NG2, whereas pericytes surrounding the capillaries are αSMA negative and NG2 positive.

(D) Epifluorescent image of retinal vessels of a promoter trap transgenic mouse (XlacZ4) where pericyte nuclei are identified by X-gal staining (dark blue) and cell bodies by NG2 staining (green). Endothelial cells are visualized by CD31 (red) staining. Note that all X-gal-positive cells are also positive for NG2. Red arrowheads point to vSMCs covering the vein (v) and white arrowheads indicate pericytes covering capillaries.

Scale bars represent 10 μm (A), 20 μm (B), and 50 μm (C and D).

otherwise only been observed in pathological situations and in specific gene-targeted mouse mutants (see below). A conservative estimate based on available information is that endothelial-to-pericyte ratios in normal tissues vary between 1:1 and 10:1 and that pericyte coverage of the endothelial abluminal surface ranges between 70% and 10% (reviewed by Sims, 1986). These differences are in part organ specific, and pericyte density and/or coverage appears to correlate positively with endothelial barrier properties (i.e., brain > lung > muscle), endothelial cell turnover (large coverage = less EC turnover) (Díaz-Flores et al., 2009), and orthostatic blood pressure (larger coverage in lower body parts) (Sims et al., 1994). These observations are thus consistent with a role of pericytes in regulating capillary barriers, endothelial proliferation, and capillary diameter.

Identification

Several recent reviews provide lists of molecular markers for pericytes (Armulik et al., 2005; Díaz-Flores et al., 2009; Krueger and Bechmann, 2010) (examples shown in Figure 2). However, not all markers are useful from a practical perspective. It is also important to remember that no single entirely pericyte-specific marker is known and that all markers currently used are dynamic in their expression and may be up or downregulated in conjunction with developmental states, pathological reactions, in vitro culturing, etc. A state-of-the-art identification of pericytes in tissue sections or whole-mount preparations therefore relies on a combination of well-preserved tissue morphology, counter-labeling of endothelial cells, and two or more pericyte markers. Table 1 provides a list of currently validated and often-used pericyte markers, as well as emerging and transgenic markers for murine studies.

Ontogeny of Pericytes

Early observations suggested that mural cells originate from mesenchymal cells that condense on the abluminal side of the endothelial tube (Clark and Clark, 1925), a scenario that has been reinforced through later studies (Drake et al., 1998; Hungerford and Little, 1999). However, while mature vSMCs have a similar morphology and marker expression profile throughout the vasculature, it is now clear from numerous lineage-tracing studies that they have several different developmental origins, making even a single vessel mosaic from a developmental point of view (reviewed in Majesky, 2007; Majesky et al., 2011). For example, vSMCs of the aorta and many of its proximal branches have at least four different developmental origins—secondary heart field, neural crest, somites, and splanchnic mesoderm—contributions that largely follow a segmental pattern of distribution.

Probably, the majority of the mural cells in the head region, including all parts of the CNS, are neural crest derived, as demonstrated in chick-quail chimeras (Bergwerff et al., 1998; Etchevers et al., 2001; Korn et al., 2002) and indicated by marker expression in mice (Heglind et al., 2005) (Figure 3). Recent studies on thymus development demonstrated that also the mural cells in the thymus are derived from neural crest (Foster et al., 2008; Müller et al., 2008).

The origins of vascular mural cells in the gut (Wilm et al., 2005), lung (Que et al., 2008), and liver (Asahina et al., 2011) have been mapped to the mesothelium, the single-layer squamous epithelium that lines the coelomic cavities and its organs. Coronary vessel mural cells in the heart appear to have a similar development. Here, the epicardial mesothelium is thought to give rise to cardiac mesenchymal cells, including coronary vSMCs and pericytes (Cai et al., 2008a; Dettman et al., 1998; Mikawa and Gourdie, 1996; Wessels and Pérez-Pomares, 2004; Zhou et al., 2008) (Figure 3).

Taken together, the above-mentioned work therefore suggests that a common principle may exist for the development of mural cells in coelomic organs. Here, mesothelial cells undergo epithelial-to-mesenchymal transition (EMT), delaminate, and migrate into the organs to produce their mesenchymal components, including fibroblasts, vSMCs, and, most likely, also the pericytes. Although the unequivocal identification of the latter is problematic, as dicussed, available literature

Table 1. Murine Pericyte Markers

Pericyte Marker	Gene Symbol	Examples of Other Cell Types Expressing the Marker	Comments	References
Validated Markers				
PDGFR-β (platelet-derived growth factor receptor-beta)	Pdgfrb	Interstitial mesenchymal cells during development; smooth muscle; in the CNS certain neurons and neuronal progenitors; myofibroblasts; mesenchymal stem cells	Receptor tyrosine kinase; functionally involved in pericyte recruitment during angiogenesis; useful marker for brain pericytes	Lindahl et al., 1997; Winkler et al., 2010
NG2 (chondroitin sulfate proteoglycan 4)	Cspg4	Developing cartilage, bone, muscle; early postnatal skin; adult skin stem cells; adipocytes; vSMCs; neuronal progenitors; oligodendrocyte progenitors	Integral membrane chondroitin sulfate proteoglycan; involved in pericyte recruitment to tumor vasculature	Ozerdem et al., 2001; Ruiter et al., 1993; Huang et al., 2010
CD13 (alanyl (membrane) aminopeptidase)	Anpep	vSMCs, inflamed and tumor endothelium; myeloid cells; epithelial cells in the kidney, gut	Type II membrane zinc-dependent metalloprotease; useful marker for brain pericytes	Dermietzel and Krause, 1991; Kunz et al., 1994
αSMA (alpha-smooth muscle actin)	Acta2	Smooth muscle; myofibroblasts; myoepithlium	Structural protein; quiescent pericytes do not express αSMA (e.g., CNS); expression in pericytes is commonly upregulated in tumors and in inflammation	Nehls and Drenckhahn, 1993
Desmin	Des	Skeletal, cardiac, smooth muscle	Structural protein; useful pericyte marker outside skeletal muscle and heart	Nehls et al., 1992
New Markers Requiring Additional Validation				
RGS5 (regulator of G protein signaling 5)	Rgs5	Cardiomyocytes?; vSMCs	Regulate heterotrimeric G proteins by activating GTPase activity; angiogenic pericyte marker	Bondjers et al., 2003; Cho et al., 2003
SUR2 (ATP-binding cassette, subfamily C (CFTR/MRP), member 9)	Abcc9	Skeletal, cardiac, smooth muscle; renal tubular epithelium	Regulatory subunit of ATP-sensitive potassium channels	Bondjers et al., 2006
Kir6.1 (potassium inwardly rectifying channel, subfamily J, member 8)	Kcnj8	vSMCs	Associates with SUR2	Bondjers et al., 2006
Endosialin	Cd248	vSMCs, myofibroblasts; fibroblasts; T cells	Transmembrane cell surface glycoprotein; expression on pericytes is dynamic; downregulated during development	Christian et al., 2008
DLK1 (delta-like 1 homolog)	Dlk1	vSMCs; hepatoblasts in the developing liver; adipocyte progenitors	Transmembrane cell surface protein	Bondjers et al., 2006
Transgenic Markers				
XlacZ4		vSMCs, skeletal muscle progenitors during development	Expresses nuclear beta-galactosidase in pericytes and vSMCs; downregulated in association with injury	Tidhar et al., 2001
NG2 dsRED		vSMCs, oligodendrocyte progenitors	BAC-transgene; reposited in the Jackson Laboratory, stock #008241	Zhu et al., 2008

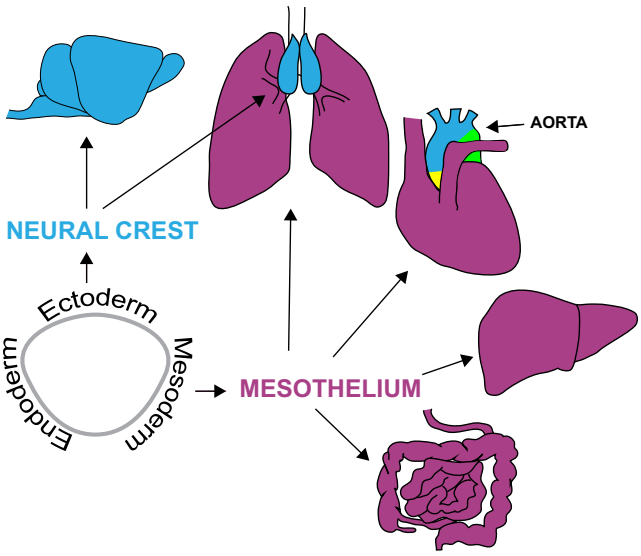

Figure 3. Developmental Origin of Mural Cells
Ectoderm-derived neural crest gives rise to vSMCs and pericytes in the CNS and thymus (light blue). Mural cells in coleomic organs are all mesoderm- and mesothelium-derived (violet). Epicardial mesothelium gives rise to mural cells in heart, lung mesothelium to pericytes in the lung, etc. Note that vSMC coverage around aorta has a multiple developmental origins, indicated by different colors (yellow, secondary heart field; light blue, neural crest; green, somite).

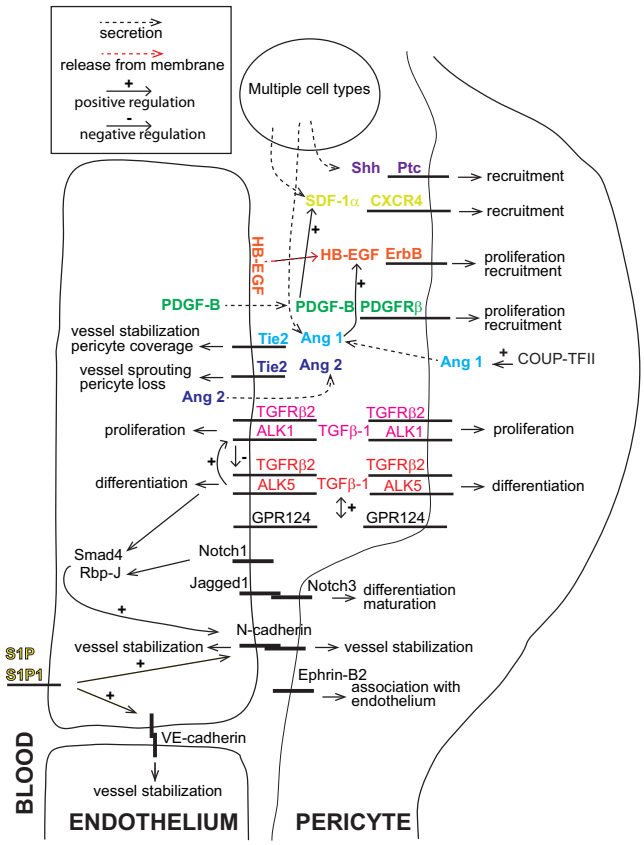

Figure 4. Signaling Pathways Mediating Mural Cell Recruitment, Differentiation, and Vascular Stabilization
Pericyte recruitment to the endothelium is mediated by multiple ligand-receptor complexes: PDGF-B/PDGFRβ, SDF-1α/CXC4R, HB-EGF/ErbB, Shh/Ptc, and Ang1/Tie-2. The cellular response to TGFβ/TGFβR signaling axis is dependent on the composition of the receptor and the relative level of the ligand. A ligand-receptor pair is indicated by the same color. N-cadherin and Notch-mediated vessel stabilization requires direct contact between a pericyte and an endothelial cell. Note that some signaling pathways are currently only proposed to be relevant for pathological angiogenesis (see text for details).

suggests common lineages for vascular mural cells in any given organ. The mentioned studies also point to close ontogenic relationships between mural cells and fibroblasts in many organs, supporting current ideas of such relationships also in pathological situations (discussed below). In spite of a mesothelial origin of pericytes in diverse organs such as heart, lung, liver, and gut, the signaling mechanisms that govern their recruitment into their final periendothelial location may be different. This is exemplified by the importance of PDGF-B/PDGFRβ signaling (further discussed below), which has been demonstrated in heart, lung, and gut, whereas the development of hepatic stellate cells (liver pericytes) occurs independently of PDGF-B/PDGFRβ (Hellström et al., 1999). A similar distinction has been made for neural-crest-derived pericytes, which depend on PDGF-B/PDGFRβ signaling in the CNS (Lindahl et al., 1997), but not in the thymus (Foster et al., 2008).

Whereas some insights have thus been obtained into the embryonic origin of pericytes in different organs, much less is known about how pericytes grow and spread along growing vessels in conjunction with developmental angiogenesis. Pericytes proliferate during angiogenesis in the CNS and in vitro, and the seeming lack of immature mesenchyme in the developing CNS would imply that new pericytes develop mainly by proliferation of pre-existing ones in this organ. However, to what extent new pericytes develop by division of pre-existing pericytes—by recruitment (proliferation, migration, and differentiation) from pre-existing vSMCs on neighboring large vessels and/or by differentiation from immature mesenchyme, or both—in most other organs are issues that are presently not definitively resolved. The same holds true for the question about ontogenetic relationships between pericytes and vSMCs. The continuum of mural cell phenotypes observed along terminal arterioles, capillaries, and postcapillary venules might suggest that these cells can differentiate into each other in conjunction with vessel growth and remodeling, but this also requires further investigation.

Signaling Pathways Implicated in the Development of Pericytes and Their Interaction with Endothelial Cells

As discussed above, the anatomical relationship between pericytes and endothelial cells suggests close interactions involving paracrine or juxtacrine signaling (Figure 4). Endothelial-pericyte signaling has recently been reviewed in detail (Gaengel et al., 2009). Below we cover briefly this area and point out recent advances in the understanding of how different intercellular signaling pathways play a role in vascular development and stability.

PDGF-B/PDGFRβ

PDGF-B is released from angiogenic endothelial cells and binds to PDGFRβ expressed on the surface of developing pericytes. As

a result, PDGFRβ-positive pericytes (or their progenitors) are corecruited with the angiogenic sprouts. PDGFRβ is expressed broadly on developing vSMCs, and PDGF-B probably plays a role also in the proliferation and differentiation of aortic and venous vSMCs. Knockout of the *pdgfb* or *pdgfrb* genes in mice results in virtually identical phenotypes and perinatal lethality resulting from vascular dysfunction caused by mural cell deficiency (Levéen et al., 1994; Soriano, 1994). The degree of this deficiency varies extensively between different organs, suggesting that other signaling pathways may play a similar role to that of PDGF-B/PDGFRβ in mural cell recruitment. For example, whereas the lack of pericytes in the brain, kidney, lung, heart, and skin of *pdgfb* or *pdgfrb* knockout mouse embryos is near total, the liver pericytes (stellate cells) are seemingly unaffected (Hellström et al., 1999; Lindahl et al., 1997). As discussed below, recent work suggests candidates for alternative endothelial-to-pericyte signaling pathways mediating pericyte recruitment.

PDGF-B expression is not uniform in the developing endothelium. Tip cells show higher *PDGF-B* expression than stalk cells. Pericytes are immediately attracted to emerging angiogenic sprouts and are usually lagging only slightly behind the tip cells (Gerhardt and Betsholtz, 2003). Developing arteries also express higher levels of *PDGF-B* mRNA than corresponding veins in correlation with the thickness of the mural cell coat. However, the importance of differential levels of *PDGF-B* expression in different endothelial cells is uncertain. The phenotype of a complete *pdgfb* knockout was rescued by re-expression of *PDGF-B* in endothelial cells from the Rosa26 promoter (Armulik et al., 2010), which would be assumed to provide similar levels of PDGF-B expression in all endothelial cells.

Once secreted, PDGF-B is normally bound to the extracellular matrix (reviewed by Andrae et al., 2008). This binding is conferred by a C-terminal retention motif (Ostman et al., 1991), which has affinity for heparin and heparan sulfate proteoglycans (HSPGs) (Abramsson et al., 2007; Kurup et al., 2006). Targeted deletion of the retention motif in mice ($pdgfb^{ret/ret}$) leads to hypoplasia and partial detachment of pericytes (Lindblom et al., 2003), suggesting that PDGF-B needs to be presented as a cell surface or matrix-bound factor in order to exert a proper signal for pericyte recruitment to the vessel wall. This scenario is supported by global reduction of N-sulfated heparan sulfate generated by knockout of the N-deacetylase/N-sulfotransferase (NDST)-1 enzyme, which leads to a delay in recruitment and partial detachment of pericytes (Abramsson et al., 2007). Probably, endothelial HSPGs are critical since pericyte-specific ablation of heparan sulfate did not interfere with pericyte recruitment into the developing CNS (Stenzel et al., 2009).

Although most studies have focused on embryonic development, there is also evidence that PDGF-B/PDGFRβ signaling plays a role postnatally. Constitutive activation of PDGFRβ by targeted insertion of an activating mutation in the *pdgfrb* locus in mice was shown to promote proliferation and inhibit differentiation in mural cells (Olson and Soriano, 2011), leading to postnatal phenotypes that are further discussed below. The importance of PDGF-B for pericyte recruitment to tumors is also discussed below. A recent report suggests that PDGF-B mediates mural cell recruitment and vessel maturation in response to the drug thalidomide in patients with hereditary hemorrhagic telangectasia and in mouse models of the disease (Lebrin et al., 2010). Thalidomide has previously been proposed to have antiangiogenic activities, but conflicting data have been reported regarding these effects and mechanisms have not been elucidated (Bauer et al., 1998).

TGFβ

TGFβ signaling has been implicated in the induction of mural cell formation from undifferentiated mesenchyme, in mural cell proliferation and differentiation, and in the regulation of endothelial cell proliferation and differentiation. Both endothelial cells and mural cells express TGFβ, but its activation from a latent proform appears to require collaboration between the two cell types (Sato and Rifkin, 1989). Likewise, TGFβ receptors are expressed on both cell types. Thus, the role of TGFβ signaling in vascular development, maintenance, and function is complex. It is difficult to sort out the primary roles of TGFβ signaling in each cell compartment, since they are interdependent, and as an inevitable consequence, affecting one will secondarily affect the other. A large number of studies ranging from in vitro models using single cell types or cocultures to in vivo work utilizing gain- and loss-of-function genetic approaches have demonstrated the pivotal role of TGFβ signaling, members of the TGFβ signaling pathway, and upsteam regulators and modifiers of TGFβ signaling in vascular development (reviewed by Gaengel et al., 2009).

Two distinct type I TGFβ receptors—activin receptor-like kinase (Alk)-1 and Alk-5—are expressed in both endothelial cells and mural cells and the two receptors appear to trigger different—even opposing—cellular effects (Goumans et al., 2002, 2003; Oh et al., 2000). Activation of Alk-5 in mesenchymal cells leads to phosphorylation of Smad2/3-promoting mitotic and migratory quiescence and differentiation into SMC. Activation of Alk-1 on the other hand leads to phosphorylation of Smad1/5 and the induction of target genes that promote cell proliferation and migration, and it opposes SMC differentiation (Chen et al., 2003; Goumans et al., 2002; Ota et al., 2002). In endothelial cells, a complex interplay between Alk-1 and Alk-5 signaling has been suggested, in which Alk-1 inhibits Alk-5, whereas Alk-5 at the same time is required for Alk-1 signaling.

Overall, Alk-5 seems to promote vessel maturation, whereas Alk-1 has the opposite effect. The net effect of TGFβ may depend on the relative levels of Alk-1/5 expression but also on the strength and duration of the TGFβ signal. Alk-1 signaling may dominate in the early phase of TGFβ stimulation, leading to cell proliferation and migration, whereas Alk-5 signaling dominates later, leading to cell differentiation and extracellular matrix production. Knockout of most of the different TGFβ signaling pathway genes in mice, e.g., *tgfb1* (Dickson et al., 1995), *alk1* (Urness et al., 2000), *alk5* (Larsson et al., 2001), *tgfbr2* (Oshima et al., 1996), *smad4* (Lan et al., 2007), *smad5* (Chang et al., 1999; Yang et al., 1999), and *endoglin* (Li et al., 1999), leads to embryonic lethality at midgestation with severe vascular abnormalities, including remodeling defects in the yolk sac vasculature, arterio-venous anastomoses, defective formation of mural cells, and—in some mutants—defective hematopoiesis. In humans, mutations in *ENDOGLIN*, *ALK1*, and *SMAD4* cause hereditary hemorrhagic telangiectasia, which are autosomal-dominant disorders in which vascular malformations arise through the formation of small-caliber vessel arterio-venous anastomoses (Berg et al., 1997; Gallione et al., 2004; McAllister et al., 1994).

The knockout of several different proteins appears to influence TGFβ signaling, thereby leading to vascular defects, including inappropriate formation of a mural cell coat. This includes connexins (Cx) 43 and 45, implicating the importance of gap junctions for TGFβ signaling in vascular development (Hirschi et al., 2003; Krüger et al., 2000). Studies on Cx43-deficient mesenchymal cells indicate that these cells had lost their ability to produce active TGFβ when cocultured with endothelial cells and as a result failed to differentiate into a mural cell phenotype. Likewise, several studies implicate integrins as critical regulators of TGFβ activation (Cambier et al., 2005). The role of integrins in this process may involve the recruitment of MMPs to activate latent TGFβ (Cambier et al., 2005) or the direct liberation of active TGFβ through conformational changes of the latent TGFβ complex (Wipff and Hinz, 2008), a process recently proposed to involve cell constriction-mediated tensile force across pro-TGFβ (Shi et al., 2011). Knockout of the orphan G protein-coupled receptor gene *grp124* in mice causes defective cerebral angiogenesis and hemorrhage (Anderson et al., 2011; Cullen et al., 2011; Kuhnert et al., 2010). TGFβ was demonstrated to stimulate GPR124 expression, and molecular profiling of the *gpr124* knockout vasculature revealed perturbed TGFβ pathway activation, together suggesting a close crosstalk between GPR124 and TGFβ (Anderson et al., 2011).

TGFβ pathway proteins also interact with other key vascular signaling pathways. Brain endothelium-specific knockout of *smad4* was recently shown to result in brain vascular defects, involving increased endothelial proliferation, vascular dilation, and reduced mural cell coverage (Li et al., 2011). This was connected to reduced vascular expression of N-cadherin, a cell adhesion molecule believed to mediate heterotypic cell contacts between endothelial cells and pericytes (Gerhardt and Betsholtz, 2003; Gerhardt et al., 2000). It was further demonstrated that TGFβ and Notch signaling in endothelial cells cooperate in the regulation of N-cadherin expression through direct interactions of their transcriptional effectors on the N-cadherin promoter (Li et al., 2011). Interestingly other signaling pathways may also converge at the regulation of vascular N-cadherin. Binding of plasma-borne bioactive lipid sphingosine-1-phosphate to the endothelial-specific G protein-coupled receptor $S1P_1$ (Edg1) is critical for the mural cell recruitment to the developing dorsal aorta and its proximal branches (Allende and Proia, 2002; Allende et al., 2003; Liu et al., 2000). Possibly, this effect is mediated by altered trafficking of endothelial cadherins VE- and N-cadherin (Lee et al., 1999; Paik et al., 2004). Thus, several signaling pathways known to be important for vascular maturation (TGFβ, Notch, and S1P) appear to regulate N-cadherin in heterotypic cell interactions between endothelial cells and pericytes.

Angiopoietin-1/Tie-2

Angiopoietin-1 (Ang-1) is expressed by perivascular mesenchymal cells (Davis et al., 1996), including pericytes (Sundberg et al., 2002), whereas its main receptor, Tie-2, is predominantly expressed on endothelial cells (Dumont et al., 1993). Thus, the suggested Ang-1/Tie-2 paracrine loop has reciprocal orientation in comparison with PDGF-B/PDGFRβ and has been proposed to mediate endothelial maturation and stability (Falcón et al., 2009; Gaengel et al., 2009) and reduce vascular leakage (Thurston et al., 1999). Tie-2 expression has also been demonstrated in subtypes of monocytes and macrophages (De Palma et al., 2005) and on certain pericytes (Cai et al., 2008b), potentially complicating the picture of cellular interactions.

Ang1 or *tie2* null mice develop cardiovascular defects and die in utero around midgestation (Dumont et al., 1994). Morphological analyses of *ang1* and *tie2* null embryos have demonstrated a lack of mural cells (Patan, 1998; Suri et al., 1996). In humans, mutations of the *TIE2* gene lead to venous malformation, a condition involving focal loss of venous mural cells (Vikkula et al., 1996). A role for Tie-2 in pericyte recruitment and/or maintenance is also suggested by the effects of local overexpression of the Tie-2 antagonist Ang-2 (potentially inhibiting Ang-1-mediated vascular stabilization), which leads to pericyte loss (Hammes et al., 2004).

How the mural cell defects arise in situations of ablated or inhibited Ang-1/Tie-2 signaling remains unclear, however. Analysis of mouse chimeras of normal and *tie2* null cells showed that pericytes were recruited to Tie-2-negative endothelium (Jones et al., 2001). Also, mice carrying signaling-deficient Tie-2 receptors showed pericyte recruitment to new vessels in spite of defective cardiac, hematopoietic, and endothelial development (Tachibana et al., 2005). Likewise, conditional knockout of *ang1* showed that this gene is not required for pericyte recruitment (Jeansson et al., 2011).

Thus, whereas Ang-1 and Tie-2 do not seem to be directly involved in pericyte recruitment, several publications support to the notion that pericyte-derived Ang-1 has important roles in blood vessel formation and/or stability. Ang-1 overexpression or administration results in increased vascular branching and remodeling of an immature vessel plexus into a higher-order hierarchical structure (Thurston et al., 2005; Uemura et al., 2002). Ang-1 expression (in pericytes) was recently demonstrated to be dependent on the chicken ovalbumin upstream promoter-transcription factor II (COUP-TFII), and conditional ablation of *couptf2* in adult mice led to compromised neoangiogenesis in tumors and matrigel plugs (Qin et al., 2010). In addition, Ang-1 expression from hematopoietic cells appears to have a critical role in angiogenesis, pericyte recruitment, and vascular stabilization, as demonstrated in *aml1* and hematopoietic cell-specific *zeb2* transcription factor gene knockout mice (Goossens et al., 2011; Takakura et al., 2000). A recent study demonstrated that cardiac-specific *ang1* deletion reproduced the full knockout phenotype and, moreover, that complete deletion after E13.5 was tolerated in the developing vasculature but produced defects in situations of injury or vascular stress (Jeansson et al., 2011). A tentative model consistent with most available data is that Ang-1 produced by mesenchymal cells, including pericytes, cardiomyocytes, hematopoietic cells, and possibly other cells types, provides stabilizing signals to the endothelium that reduce vascular permeability and increase higher-order structure, quiescence, and functionality of the vessel network during cardiac development and in conjunction with vascular injury.

Other Signaling Pathways Implicated in Endothelium-Pericyte Crosstalk

As discussed above, other signaling pathways than PDGF-B/PDGFRβ may exert a role as in mural cell recruitment in an organ-specific or compensatory way. One such pathway is heparin-binding epidermal growth factor (HB-EGF) signaling through EGF receptors (ErbBs). Several studies indicate that HB-EGF is essential for cardiovascular development (Iwamoto

et al., 2003; Nanba et al., 2006). HB-EGF has also been suggested to protect against the loss of pericytes from intestinal vessels following mesenteric artery occlusion, possibly by induction of pericyte proliferation and protection of the cells from oxidative stress (Yu et al., 2010). The role of HB-EGF in mural cell biology has been addressed mechanistically in vitro. In a cell coculture model, HB-EGF expressed by endothelial cell was shown to promote migration of vSMCs via ErbB1 and ErbB2 receptor expression on vSMCs (Iivanainen et al., 2003). In this model, HB-EGF expression on endothelial cells was enhanced by Ang-1, suggesting that Ang-1 may promote pericyte recruitment through HB-EGF. Analysis in more complex three-dimensional in vitro models of angiogenesis in extracellular matrices confirmed the importance of endothelium-derived HB-EGF and ErbB1 and ErbB4 for pericyte recruitment (Stratman et al., 2010). Moreover, the combined inhibition of PDGF-BB and HB-EGF in quail embryos led to reduced pericyte recruitment and vascular phenotypes reminiscent of those observed in mouse PDGF-B/PDGFRβ null embryos, suggesting that PDGF and EGF pathways may collaborate in pericyte recruitment (Stratman et al., 2010). Additional support for a crosstalk between PDGF and EGF signaling comes from studies of the ADAM17 protease, which releases active HB-EGF from its transmembrane precursor (Sahin et al., 2004). PDGFRβ stimulates ADAM17 in cultured fibroblasts, leading to proteolytic activation of EGFR ligands and stimulation of EGFR signaling (Mendelson et al., 2010). ADAM17 is required for vascular development and probably has a broad role in the activation of substrates important for endothelial development (Canault et al., 2010; Weskamp et al., 2010); however, it also seems critical for mural cell recruitment (Canault et al., 2010).

Another pathway recently implicated in pericyte recruitment is stromal-derived factor 1-a (SDF-1α)/CXCR4. SDF-1α promotes pericyte migration in vitro and in a tumor xenograft model in vivo (Song et al., 2009). Also for this signaling pathway a crosstalk with PDGF-B/PDGFRβ was suggested, since SDF-1α expression was shown to be stimulated by PDGF-B (Song et al., 2009). Additional evidence for an involvement of a SDF-1α/CXCR4 axis in pericyte recruitment was recently provided by the demonstration in vitro that SDF-1α acts synergistically together with hematopoietic cytokines SCF and IL-3 to mediate endothelial tube formation and maturation, including pericyte recruitment and vascular BM assembly (Stratman et al., 2011).

Analyses in mouse and avian embryos have demonstrated a role for sonic hedgehog (Shh) signaling in vasculogenesis (Vokes et al., 2004). Recent work also implicates Shh signaling in pericyte recruitment in plexus choroideus formation (Nielsen and Dymecki, 2010). The invading plexus choroideus pericytes express the Shh receptor Patched (Ptc), suggesting that pericytes are directly targeted by the Shh signal. The role of Shh/Ptc in plexus choroideus formation resembles the role for PDGF-B/PDGFRβ in the development of glomerular pericytes (mesangial cells) (Lindahl et al., 1998). Moreover, in both organs pericytes appear to play a critical role in the formation of a specialized capillary network dedicated for plasma filtration (in the formation of primary urine and cerebrospinal fluid, respectively). A possible role for Shh signaling in mural cell regeneration has been implicated through the demonstration that a population of vSMC progenitors present in the adventitial layer of the arterial wall is absent in Shh null mice (Passman et al., 2008). Recent studies also implicate Shh signaling in the recruitment of "perivascular support cells" in zebrafish embryos. Analyses of hedgehog (Hh) inhibitor-treated and Hh pathway mutant fish embryos demonstrated detachment of the support cells and brain hemorrhage (Lamont et al., 2010). Reduced Ang-1 expression is a possible mediator of the defects, suggesting interactions between the Hh and Ang-1/Tie-2 signaling pathways. Although the identity of the perivascular support cells and their relationship to mural cells remain poorly defined in zebrafish, recent work implicates the existence of mural cells in fish embryos (Santoro et al., 2009).

Notch signaling has major roles in endothelial cells and is critically required for arteriogenesis and angiogenic sprouting (reviewed in Chappell and Bautch, 2010; Sainson and Harris, 2008). Notch signaling also plays a role in mural cells and in endothelial-mural interactions. *NOTCH3* is mutated in the human stroke and dementia syndrome CADASIL (cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy), which involves focal degeneration of vSMCs (Joutel et al., 1996; Ruchoux et al., 1995). Analyses of *notch3* knockout mice have revealed an abnormal maturation of arterial vSMCs, which has been connected to reduced expression of a number of arterial vSMC markers, including PDGFRβ (Domenga et al., 2004; Jin et al., 2008). Increased expression of Notch3 in mural cells has also been shown to increase expression of certain vSMC markers (Liu et al., 2009). A critical Notch ligand in this context appears to be Jagged-1 (Jag-1) expressed on endothelial cells and induced in pericytes as part of an autoregulatory loop of Jag-1/Notch3 expression in these cells (Liu et al., 2009). Analysis of angiogenesis in the retina suggests a role for Notch3 in mural cell recruitment and implicates Ang-2 expression by the mural cells as a possible mediator of Notch3-induced vessel maturation (Liu et al., 2010). How this works is unclear, since other studies have implicated Ang-2 as a vessel-destabilizing factor, promoting pericyte loss (Hammes et al., 2004). In summary, however, there is accumulating evidence for a role of Jag-1 and Notch3 signaling in endothelial-mural cell interactions.

Ephrin-Eph receptor signaling plays major roles in endothelial cells but has also been implicated in mural cell biology. A mural cell-specific knockout of ephrin-B2 resulted in poor association of vSMCs and pericytes with vessels of different sizes (Foo et al., 2006). In capillaries, pericytes made only loose connections with the endothelial cells and were surrounded by abnormal extracellular matrix deposits. How Ephrin-B2 in mural cells acts to generate these defects is not fully clear. Eph receptor engagement in endothelial cells and mural cells may play a role (Salvucci et al., 2009), but migration defects in single Ephrin-B2-deficient mural cells in vitro point to important cell-autonomous functions of Ephrin-B2 in mural cells connected to the formation of focal adhesions (Foo et al., 2006). Interestingly, ablation of the focal adhesion protein α-parvin was recently demonstrated to lead to contraction or migration defects in mural cells and a defective mural cells association to vessels resembling the phenotype of mural cell-specific *ephrinb2* knockouts (Montanez et al., 2009).

Physiological Functions of Pericytes

Role of Pericytes and Brain Vascular Permeability and the Formation of the Blood-Brain Barrier

An abnormally increased vascular permeability is associated with many severe pathological conditions, including sepsis,

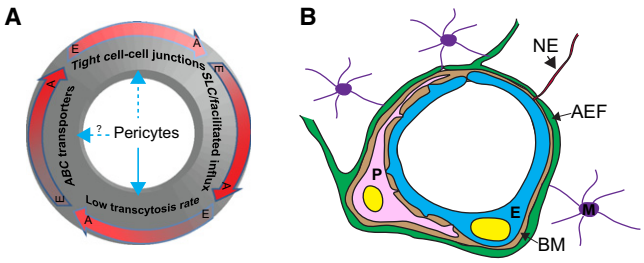

Figure 5. Pericytes at the Blood-Brain Barrier

(A) Traits of the mammalian BBB controlled by pericytes. The circle depicts four main traits of brain endothelium that collectively shield brain tissue: cell-cell junctions, SLC-transporters, low rate of transcytosis, and ABC transporters. The tightly sealed junctions restrict paracellular diffusion of most blood-borne substances. Transporters (SLC and ABC) mediate influx of essential polar molecules and efflux of xenobiotics. Transcytosis (adsorptive- or receptor-mediated) mediates selective passage of proteins. The traits develop at separate developmental stages depicted as bended arrows (E, embryo; A, adult), where gray color indicates relative immaturity and red relative maturity. The CNS pericytes are important for establishing the low transcytosis rate of brain endothelial cell, as indicated by a blue arrow. In addition, pericytes regulate the architecture of endothelial cell-cell, and possibly also the expression of ABC transporters.

(B) Cellular components of the neurovascular unit. Presented is a schematic cross-section of a brain capillary. The abluminal side of endothelium (E, blue) is faced by a pericyte (P, pink) with which it shares BM (light brown). The entire abluminal side of the vessel basement membrane is surrounded by astrocyte endfeet (AEF, green). The endothelial and/or pericyte surface is contacted by resident microglia (M, violet) and nerve endings (NE, red).

lung edema, and allergic reactions (reviewed by Wang and Dudek, 2009). The normal capillary bed also differs in its permeability in an organ-specific fashion. This primarily reflects differences in the specific characteristics of the endothelial cells. The vasculature of the CNS possesses characteristics that result in an "extreme" tightness of the vascular bed—the blood-brain barrier (BBB)—which is a functional term denoting that the healthy CNS vasculature is impermeable to the passive transport of cells, proteins, and bioactive compounds present in the blood. The BBB is a multicomponent system, the molecular and structural complexity of which has only recently become appreciated (reviewed in Abbott et al., 2010; Zlokovic, 2008) (Figure 5).

Recent work has uncovered a critical role for pericytes in the maturation and maintenance of the BBB (Armulik et al., 2010; Bell et al., 2010; Daneman et al., 2010). The brain vessels thus appear to harbor specialized pericytes, or alternatively they respond to pericyte signals differently compared to other vessels. Although pericyte diversity is still rather unexplored territory, there are already indications that certain pericyte markers may be brain specific (Bondjers et al., 2006; Heglind et al., 2005). As discussed above, the CNS vasculature shows the highest pericyte coverage among all analyzed organs. In addition, the brain vasculature is 100% covered by glial cell processes formed by astrocytes (astrocyte endfeet). The term *neurovascular unit* (NVU) is often used to denote the CNS vasculature and its structural and functional connections to the neural tissue. Besides endothelial cells, pericytes, and astrocyte endfeet, the NVU includes microglia and neurons, which both contact blood vessels by fine cytoplasmic processes (Figure 5). Therefore, when the CNS vasculature is studied, the cellular and molecular complexity of vessel-tissue interface needs to be taken into account. Currently used in vitro models of brain vasculature do not recapitulate the NVU complexity in vivo.

When and how do pericytes come into play in BBB development? Previous review literature discusses the idea that pericytes regulate the BBB at the level of endothelial junctions (Abbott et al., 2010, 2006; Cardoso et al., 2010; Lai and Kuo, 2005). This conclusion is based on in vitro coculture studies where pericytes were shown to increase the transendothelial electrical resistance (TEER) of endothelial cell monolayers (Al Ahmad et al., 2009; Dohgu et al., 2005; Hayashi et al., 2004; Hori et al., 2004; Nakagawa et al., 2009). To what extent TEER in vitro reflects the tightness of brain endothelial junctions and the BBB in vivo is unclear, however, as lung pericytes increase TEER in endothelial cultures as well (Dente et al., 2001). In addition, conflicting results on the effects of pericytes on TEER have been reported (Zozulya et al., 2008). The majority of published studies on the BBB are focused on the endothelial junctions, which are a critical but not the only endothelial component of the BBB. Additionally, brain endothelial transcytosis is suppressed (reviewed in Smith and Gumbleton, 2006). This has been documented, yet without an understanding of how this process is regulated and when during the development it is downregulated.

Two recent studies addressing the role of pericytes in the brain vasculature challenge the "in vitro view" of pericytes regulating the permeability of endothelial cell-cell junctions (Armulik et al., 2010; Daneman et al., 2010). These studies were performed on mouse models deficient in pericytes through genetic manipulation of the PDGF-B/PDGFRβ signaling pathway (see above). One of the studies, performed in a neurobiology lab (Daneman et al., 2010), asked which cell types are important for the development of the BBB during embryogenesis. The other study was performed in a vascular biology lab (Armulik et al., 2010) and addressed the function of pericytes in the adult mouse. Both studies independently concluded that pericyte deficiency caused increased brain vessel permeability, the extent of which correlated directly with the density of brain pericytes. They also found, somewhat unexpectedly, that the basis for the increased permeability was upregulated endothelial transcytosis (Figure 6). Finally, both studies demonstrated that the absence of pericytes did not result in a general loss of the brain endothelial molecular signature.

Currently it is unknown what type of endothelial transcytosis and which molecular pathway(s) are deregulated in the pericyte-deficient brain vessels. Microarray analysis of pericyte-deficient blood vessels identified several deregulated genes previously implicated in the regulation of endothelial permeability, including *vegfa*, *ang2*, and *adrenomedullin* (all up in the pericyte-deficient state) and *ang1* (down in the pericyte-deficient state) (Armulik et al., 2010; Daneman et al., 2010). The expression levels of *ang1*, *ang2*, and *vegfa* are consistent with the increased vascular permeability. Adrenomedullin, on the other hand, has well-documented protective effects on endothelial barrier function in several organs (reviewed in Temmesfeld-Wollbrück et al., 2007), and its strong upregulation in the pericyte-deficient state may reflect a compensatory mechanism. All of the abovementioned signaling molecules have been reported to regulate permeability by modulating endothelial cell-cell junctions; only VEGF has been connected to increased

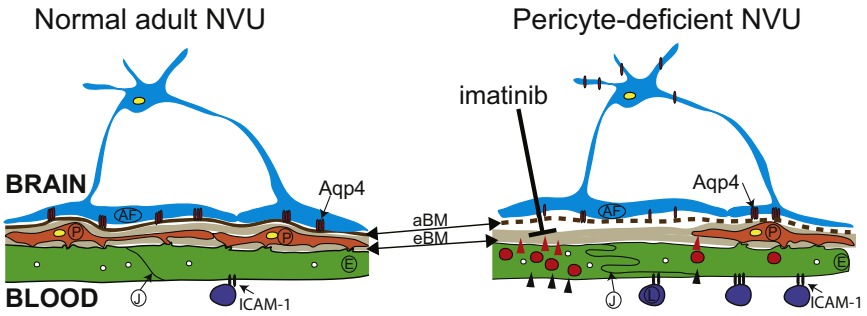

Figure 6. Changes at the NVU in the Pericyte-Deficient State

In the absence of pericytes (P, orange), the endothelium (E, green) shows convoluted junctions (J) and increased endothelial macrovesicular uptake (red vesicles; black arrowheads) and release their content to brain parenchyma (red arrowheads). Pericyte-deficiency affects polarization of astrocyte endfeet (AF, blue), shown by mislocalization of aquaporin-4 (Aqp4, red) and altered deposition of astrocyte-derived BM (aBM, dark brown). Deposition of endothelium-derived BM (eBM, light brown) is not affected in pericyte deficiency. Upregulation of leukocyte adhesion molecules (only ICAM-1 is depicted) on endothelium causes increased attachment of leukocytes (L, dark blue) in the lumen of pericyte-deficient blood vessels. The release of intravenously administered tracers into the brain parenchyma is inhibited by Imatinib treatment. Adapted from Armulik et al. (2010).

transendothelial permeability by inducing so-called vesiculotubular organelles (reviewed in Mehta and Malik, 2006). In general, the regulation of endothelial permeability is complex and dissection of the role of each candidate protein in pericyte-deficient mice will require further work.

Absence of brain pericytes seems to upregulate a transcytosis pathway that does not discriminate between different types of molecules and sizes (Armulik et al., 2010). In addition, the pericyte-deficient animals demonstrated defects in the polarization of astrocyte endfeet (Armulik et al., 2010), indicating that pericytes influence the development of the NVU at multiple levels. The treatment of pericyte-deficient mice with the drug Imatinib halted the extravasation of tracers without correcting pericyte coverage or astrocyte polarization defects. The administred tracers accumulated in endothelial vesicles, however, indicating that Imatinib blocked the endothelial release (exocytosis), not uptake (endocytosis), of the tracers (Armulik et al., 2010) (Figure 6). The specific target of the Imatinib involved in this process is currently unknown. Imatinib is a tyrosine kinase inhibitor with broad substrate specificity and several reported off-target effects (Cataldi et al., 2004; He et al., 2010; Netzer et al., 2003).

Interestingly, increased endothelial transcytosis has been reported to occur during several pathological conditions (reviewed in Nag et al., 2011). There are reports indicating that early BBB damage is associated with increased transcytosis, whereas the loss of endothelial junctional integrity takes place later (Nag et al., 2011). Coincidentally, pericytes have been described to leave the vessel wall during several pathological conditions (e.g., tumors, trauma, sepsis, diabetic microangiopathy, and fibrosis (further discussed below). The pathogenic significance of pericyte dropout from the vessel wall is currently unknown but one might speculate on a role in increased endothelial permeability. Recent work also suggests that progressive pericyte loss in the brain and subsequent increase of vessel permeability may promote neurodegeneration in aging mice (Bell et al., 2010).

Do Pericytes Regulate Blood Flow?

Capillary constriction, the first function suggested for pericytes, was largely based on indirect evidence (reviewed in Díaz-Flores et al., 2009; Hamilton et al., 2010; Puro, 2007). As imaging techniques have evolved, pericyte-mediated capillary constriction in response to vasoactive substances and neurotransmitters has been observed in some situations in vivo and in organ cultures ex vivo. For a detailed description of molecular pathways engaged in response to vasoactive molecules and neurotransmitters in a "generic" pericyte, leading to capillary constriction or dilation, the reader is referred to recent review articles on the topic (Attwell et al., 2010; Hamilton et al., 2010).

What is the physiological relevance of pericyte contractility? In the brain, the regional blood flow increases in response to increased local neuronal activity. This process, called functional hyperemia, is neurotransmitter mediated and occurs at the level of arterioles (reviewed in Attwell et al., 2010). The demonstration of pericyte-mediated constriction of capillaries ex vivo in cerebellar brain slices and retina preparations may suggest capillary-mediated control of blood flow in the CNS as well (Peppiatt et al., 2006). A recent study demonstrated pericyte-dependent capillary constriction in vivo using intravital microscopy (Fernández-Klett et al., 2010), however, without evidence for capillary-mediated functional hyperemia. Another study used ex vivo cerebral slices and in vivo imaging to suggest that pericyte contraction caused by oxidative stress after brain ischemia/reperfusion leads to capillary constriction (Yemisci et al., 2009), indicating that pericytes may regulate capillary blood flow in pathological situations.

Although the aforementioned studies may seem to provide evidence for pericyte contraction in blood flow regulation, a number of caveats exist in interpreting the data. First, convincing evidence for blood flow control elicited solely at the capillary level in vivo is still lacking. Second, while the identification of pericytes is problematic in fixed specimens due to the lack of specific markers (discussed above), it is even more challenging in living tissues. In a study of cochlear pericytes, the vasculature was visualized by labeling with DAF-2DA, an intracellular detector of NO (Dai et al., 2009), whereas pericytes were distinguished by the shape of their cell body. In the other studies, pericytes were also identified by their cell body morphology in beta-actin-GFP transgenic mice (Fernández-Klett et al., 2010), or without any labeling (Peppiatt et al., 2006; Yemisci et al., 2009). This raised concerns about the proper identification of pericytes and the type of microvessels studied (Vates et al., 2010). Third, it is unclear whether the long slender pericyte processes may generate enough force to constrict a capillary. Finally, different results have been recorded in the same organ in vivo and ex vivo (Dai et al., 2009). Clearly, the jury is still out concerning pericyte contraction and the regulation of blood flow in physiological and pathophysiological situations.

Are Pericytes Mesenchymal Stem Cells Involved in Tissue Regeneration?

A number of recent studies suggest that pericytes may constitute multipotent stem and/or progenitor cells, such as mesenchymal stem cells (MSCs) (Crisan et al., 2008b; Davidoff et al., 2004; Feng et al., 2011), white adipocyte progenitors (Olson and Soriano, 2011; Tang et al., 2008), muscle stem cells (Dellavalle et al., 2007), and even neural stem cells (Dore-Duffy et al., 2006). To discuss this issue, we need to go back 40 years, when MSCs were identified as fibroblast-like cells, called CFU-F (colony- forming unit fibroblastic) in human bone marrow cell cultures seeded at clonal density (Friedenstein et al., 1970). When heterotopically transplanted, these single-cell-derived clones generated cartilage, bone, adipose, and fibrous tissue. The cells were later named bone marrow mesenchymal stem cells (BM-MSCs), whereas cells with similar lineage potential for in vitro differentiation (adipocyte, osteoblast, chondrocyte) subsequently identified in other tissues were referred to as MSCs. The easy access to MSCs provides prospects for human regenerative medicine, but the identity and function of these cells in vivo remain unclear. Do MSCs strictly fulfill the criteria of a stem cell, i.e., do they self-renew, and can their progeny differentiate into a functional multiple cell types of a given tissue? The reader is referred to recent excellent reviews that discuss this topic (García-Gómez et al., 2010; Nombela-Arrieta et al., 2011).

Nearly ten years ago it was shown that MSCs reside in a vascular niche in bone marrow and dental pulp (Shi and Gronthos, 2003). Simultaneously, cultured pericytes were shown to differentiate in vitro into osteoblasts, adipocytes, chondrocytes, vSMCs, and skeletal muscle (Collett et al., 2003; Dellavalle et al., 2007; Doherty et al., 1998; Farrington-Rock et al., 2004). Together, these observations paved the way for the concept of a perivascular niche for MSCs (reviewed in Corselli et al., 2010). Recent work has extended this concept to several other tissues in addition to bone marrow and dental pulp and provided further support for the notion that that MSCs derive from pericytes (Crisan et al., 2008a; Crisan et al., 2008b). However, whereas there is no doubt that MSCs can be isolated from perivascular locations in most—perhaps all—organs (Crisan et al., 2008b; Dellavalle et al., 2007; Tang et al., 2008), the question remains whether they are identical to the cells that a vascular biologist would refer to as pericytes. Usually, conclusions are based on a small number of pericyte markers, such as NG2 and PDGFRβ, which somtimes occur in combination with endothelial markers, such as CD105 and CD34 (Corselli et al., 2010; Galvez et al., 2008). A detailed discussion about the marker expression in MSCs and pericytes has been reviewed elsewhere (Anjos-Afonso and Bonnet, 2011). For example, PDGFRβ was used as a marker to demonstrate a mural cell origin of white adipocytes (Tang et al., 2008) and muscle progenitors (Dellavalle et al., 2007). Consitutive activation of PDGFRβ by targeted mutagenesis of the endogenous gene inhibited adipogenesis, demonstrating the involvement of this pericyte marker protein in adipocyte differentiation (Olson and Soriano, 2011). However, as discussed above, PDGFRβ is also expressed by certain fibroblasts (Andrae et al., 2008; Heldin and Westermark, 1999), and it is therefore still not entirely clear whether perivascular mesenchymal stem or progenitor cells expressing PDGFRβ derive from pericytes proper, or from, e.g., perivascular fibroblasts. The adventitial layer of larger vessels has been demonstrated to contain stem cells that have the capacity to generate vSMCs (reviewed in Ergün et al., 2011; Hoglund et al., 2010). Moreover, in cases where cells have been sorted by FACS from blood vessels, the vascular preparations have included vessels of different caliber and type (i.e., not only pericyte-containing microvessels), and rigorous analyses on the homogeneity of the isolated cell population have not been performed. Finally, even if some pericytes may possess plasticity, it is unsolved whether all pericytes have the same stem or progenitor cell potential.

The lack of absolutely specific genetic tools for pericyte labeling limits interpretation of cell sorting data but also the prospects for conclusive fate mapping. A few studies have nevertheless been performed using pericyte-expressed Cre recombinase. *Pdgfrb-Cre* was used to trace white adipocytes to a mural cell origin, but as already underlined, PDGFRβ is expressed by other mesenchymal cell types as well. A recent study made use of *ng2-Cre* and tamoxifen-inducible *ng2-CreER* mice to address the ability of pericytes to generate odontoblasts (Feng et al., 2011). NG2 is also not a completely specific pericyte marker (Table 1), hence, *ng2-Cre* mice need to be further characterized before it can be concluded to what extent their Cre expression is restricted to (and efficient in) pericytes in different organs.

Some reports suggest that pericytes may have a broader plasticity than the classical MSC repertoire of fates. One such example is the testosterone-producing Leydig cells present in the testis mesenchyme. Pericytes have been proposed to act as a progenitor pool for the regeneration of Leydig cells following their experimental elimination (Davidoff et al., 2004). The new Leydig cells appeared close to vessels, and it was therefore concluded that the progenitor cells are vSMCs and/or pericytes. However, the perivascular Leydig cell progenitors expressed the neural/glial marker nestin, raising some doubt as to their identity. Leydig cells have been proposed to derive from PDGFRα-positive mesenchymal cells residing in the testis mesenchyme at perivascular locations (Gnessi et al., 2000). A more surprising finding, perhaps, is that CNS pericytes are able to generate self-renewing spheres in vitro with the capacity to differentiate into neural cell lineages (Dore-Duffy et al., 2006). Similar to other studies, however, the lack of defining pericytes markers complicates the interpretation also of this study. The purification of pericytes from isolated brain microvessels was based on markers (NG2 and nestin) also expressed on neural progenitor cells (Aguirre et al., 2004; Belachew et al., 2003; Lendahl et al., 1990). There is also strong evidence that neural stem cells reside in a perivascular niche (Tavazoie et al., 2008). Together with the issues of marker specificities, this raises doubt concerning the pericyte origin of the plastic neural cells residing in the vascular niche. On a more general note, this is also relevant for the interpretation of in vitro data on cultured pericytes, since these are usually derived from vascular preparations using counterselection for endothelial cells. Currently, therefore, the origin of both MSC and pericyte cultures remains unclear in most instances.

A growing number of studies nevertheless demonstrate that tissue resident stem cells reside in vascular niches, including neural, hematopoietic, and mesenchymal stem cells (Tavazoie et al., 2008, and reviewed in Corselli et al., 2010; Ehninger and Trumpp, 2011; Ergün et al., 2011). During embryonic

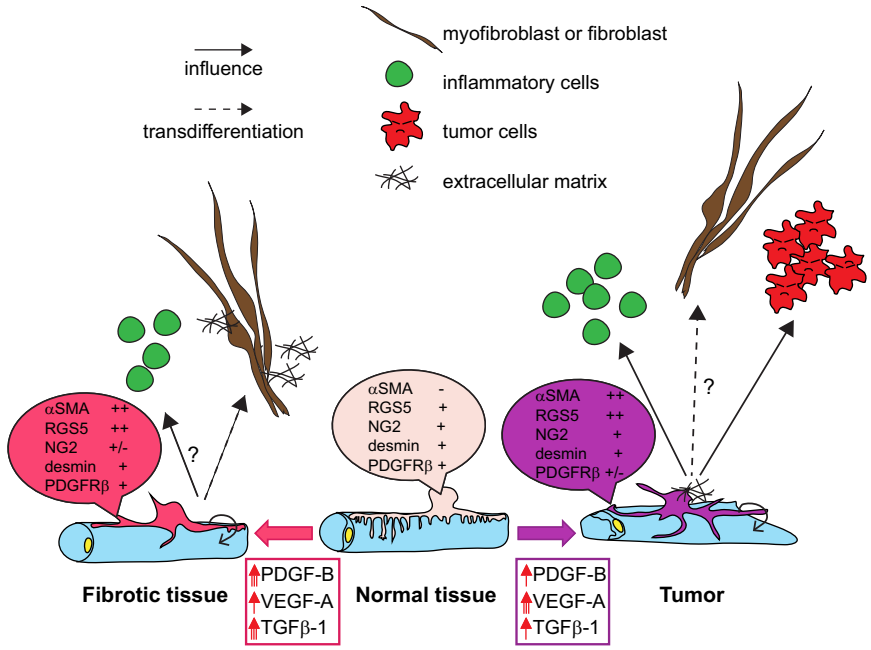

Figure 7. Hypothetic Roles of Pericytes in Pathology

During pathophysiological processes (inflammation, fibrosis, tumor growth), pericytes are activated by a combination of growth factors. In conjunction with fibrosis, pericytes display changed marker profiles (indicated in speech balloons), detachment from the BM, and transdifferentiation into myofibroblasts (dashed arrow). Detachment of pericytes causes increased vessel permeability (bended arrow). Myofibroblasts deposit collagenous extracellular matrix. In tumors, tumor pericytes exhibit altered marker expression (indicated in speech balloons), and modulate trafficking of inflammatory cells, tumor growth and metastasis (arrows), endothelial cell normalization, and vascular permeability (bended arrow). The extent of pericyte-mediated modulation of inflammatory cells in fibrosis, and pericyte transdifferentiation into fibroblasts or myofibroblasts in cancer, is proposed by many studies but is still largely unclear (indicated by "?").

development, the developing vasculature has been shown to have a role in tissue induction that goes beyond the simple supply of nutrients and oxygen (Lammert et al., 2001; Matsumoto et al., 2001). It is perhaps therefore not surprising that the adult vasculature harbors stem cells that are actively involved also in tissue repair. Recent studies on liver regeneration indicate the active role of liver endothelial cells and hepatic stellate cells in hepatocyte proliferation (Ding et al., 2010; Passino et al., 2007). During the healing of bone fractures, the invading vasculature was shown to be closely associated with osteoblasts (Maes et al., 2010). Also, human skin pericytes have been shown to enhance the regenerative capacity of human dermis in coculture models by expression of laminin α5 chain, a protein that has been shown to promote skin regeneration (Paquet-Fifield et al., 2009).

Pathological Roles of Pericytes

Are Pericytes a Fibrosis-Generating Cell Type?

Fibrosis is a common pathophysiological response of many tissues to chronic injury. Wound healing and tissue remodeling and repair are processes normally activated in response to injury to maintain the original architecture and integrity of tissues and organs. However, prolonged exposure to the chronic injurious stimuli causes deregulation of normal processes and result in an excess deposition of extracellular matrix, scar formation, and organ failure (reviewed in Kisseleva and Brenner, 2008). Fibrosis is caused by a cascade of events, including damage to the epithelium or endothelium, release of TGFβ1, recruitment of inflammatory cells, and activation of myofibroblasts as collagen-producing cells (Kisseleva and Brenner, 2008).

One of the critical steps in fibrosis progression is the activation of myofibroblast progenitors. Myofibroblasts are fibroblast-like cells that express αSMA, deposit pathological extracellular matrix, and are directly responsible for the extent of fibrosis. Identifying the origin of the myofibroblasts constitutes a challenge (Hinz, 2007). Currently, there are several hypotheses about the cellular origin of myofibroblasts, including resident fibroblasts, fibrocytes, epithelial cells (following EMT), bone marrow-derived cells, endothelial cells (following endothelial-to-mesenchymal transition), and pericytes (reviewed in Powell et al., 2011; Varga and Trojanowska, 2008).

The role of pericytes as myofibroblast precursors is suggested by studies of fibrogenesis in the liver (Fabris and Strazzabosco, 2011), kidney (Schrimpf and Duffield, 2011), and systemic sclerosis (SSc) (Mahoney et al., 2011; Wei et al., 2011). Recent studies have used genetic fate mapping to address the origin of myofibroblasts in fibrosis. While these studies excluded epithelial cells undergoing EMT as the fibrogenic cell population in liver fibrosis (Scholten et al., 2010), FoxD1-Cre mice were used to identify interstitial mesenchymal cells as the myofibroblast precursors in the kidney. These cells expressed PDGFRβ and CD73, prompting the suggestion that they may be pericytes (Duffield and Humphreys, 2011; Lin et al., 2008). Whereas PDGFRβ is an accepted pericyte marker, CD73 is reportedly expressed on leukocytes and endothelial cells (Stagg et al., 2011), however, raising some doubt about the conclusions. In summary, while the origin of myofibroblasts is far from being elucidated, accumulating evidence indicates that pericytes may constitute a source of myofibroblast progenitors (Figure 7).

Pericytes in Cancer Biology

Pericytes: an obligatory component of the tumor stroma. Tumors are not only composed of malignant cells. Neoplastic cells coexist with a variety of extracellular matrix components and cell types such as fibroblasts, myofibroblasts, endothelial cells, pericytes, and leukocytes. Together, these components, termed *tumor stroma*, build up the microenvironment in which the cancer cells proliferate. As tumors progress, the stromal compartment evolves through continuous paracrine interaction between the tumor cells and stroma cells, and among the different types of stroma cells themselves. It is now acknowledged that the tumor stroma is an essential component of tumor growth, invasiveness, and promotion of tumor dissemination

(Joyce and Pollard, 2009; Polyak et al., 2009). The recognition of the important functions of the stroma in tumor initiation, growth, and progression has led to the notion that the tumor microenvironment influences the therapeutic outcome, as well as provides opportunities for therapeutic targeting (reviewed by Pietras and Ostman, 2010).

Pericytes are a ubiquitous part of the tumor microenvironment (reviewed by Hanahan and Weinberg, 2011). Yet, in contrast to other tumor stroma cellular components, little is known about their recruitment, identification, and interaction with other stromal or tumor cells. Whereas targeting endothelial cells or cancer-associated fibroblasts might represent a therapeutic advantage (Pietras and Ostman, 2010), concomitant targeting of pericytes has unclear therapeutic effects (Hainsworth et al., 2005; Nisancioglu et al., 2010). In this chapter, we review the current understanding of tumor pericyte origin, recruitment, and functions, as well as discuss emerging areas in tumor pericyte research. Finally, we summarize our present understanding about hemangiopericytomas as tumors arising from pericytes.

Recruitment of pericytes to tumor vessels. As it occurs during physiological angiogenesis, pericytes are recruited into tumor blood vessels by PDGF-B/PDGFRβ signaling (Abramsson et al., 2002). In a mouse model of malignant melanoma, overexpression of PDGF-B by the tumor cells resulted in increased recruitment of pericytes and stabilization of the tumor neovasculature (Furuhashi et al., 2004). Tumors grown in *pdgfb*$^{ret/ret}$ mice show fewer pericytes in the tumor vasculature, as well as increased detachment of the fewer pericytes present (Abramsson et al., 2003; Nisancioglu et al., 2010). Interestingly, the amount of pericytes but not their proper attachment to the tumor blood vessels could be partially rescued when tumor cells were engineered to express PDGF-B (Abramsson et al., 2003), suggesting that an endothelial source of PDGF-B is important for the proper pericyte investment of tumor blood vessels.

Recent reports suggest a role for HB-EGF in the recruitment of pericytes to the tumor vasculature in a model of pancreatic cancer (Nolan-Stevaux et al., 2010) and of pericyte-expressed EGFR in the development of resistance of antiangiogenic therapy in a xenograft model of lung carcinoma (Cascone et al., 2011). Another study reports that SDF-1α may recruit tumor pericytes via CXCR4 (Song et al., 2009). These studies thus provide grounds to assume that other factors than PDGF-B might be directly involved in pericyte recruitment to the tumor vasculature. When three different tumor models were grown subcutaneously in the pericyte-deficient *pdgfb*$^{ret/ret}$ mice the reduction in pericytes observed was different for every tumor model studied (Nisancioglu et al., 2010), suggesting tumor-type-specific involvement of multiple pathways in pericyte recruitment.

What is the origin of tumor pericytes? Coinjection of mouse embryonic fibroblasts from XlacZ4 mice with tumor cells into a subcutaneous space resulted in recruitment of LacZ+ cells exclusively to perivascular locations in the tumors, suggesting that pericytes may be recruited from local immature mesenchymal cells (Abramsson et al., 2002). Transplantation of GFP+ bone marrow into irradiated mice led to GFP+ cell accumulation in perivascular locations in a variety of tumor models (Du et al., 2008; Rajantie et al., 2004). This process appeared to be dependent at least in part on SDF-1α expression (Song et al., 2005). Although located perivascularly, these NG2-positive cells expressed additional monocyte markers, such as CD45 or CD11b. Thus, whether these cells are indeed pericytes, or perivascular fibroblasts or infiltrating monocytes, remains uncertain.

Whereas pericytes are attracted to the tumor neovasculature by the same means as in developmental angiogenesis, pericyte investment of tumor blood vessels is clearly aberrant (Morikawa et al., 2002). The extent of pericyte coverage on tumor vessels is typically diminished as compared to normal tissues, and pericytes are loosely associated to the endothelial cells, with cytoplasmic processes that penetrate deep in the tumor parenchyma. The exact causes of the abnormal pericyte behavior in tumors are still unknown. Tumor hypoxia drives VEGF-A expression, and while VEGF-A is a potent mediator of endothelial sprouting and neovascularization, it is not efficient in generating a mature vascular network (Chen et al., 2007). Also, VEGF-A has recently been shown to be a negative regulator of pericyte function and vessel maturation (Greenberg et al., 2008). Thus, hypoxia-triggered proangiogenic factors might impair pericyte recruitment and investment of the tumor vasculature by keeping pericytes in an ''activated'' state (Raza et al., 2010).

Identification of pericytes in tumors is associated with the same concerns as during normal development. As mentioned earlier, tumor pericytes are loosely associated with endothelial cells, complicating identification of pericytes according to morphological criteria. Electron microscopy shows partial detachment of tumor pericytes (Baluk et al., 2005). Commonly used immunohistochemical markers for tumor pericytes include αSMA (Nisancioglu et al., 2010; Ozawa et al., 2005; Sennino et al., 2007), NG2 (Abramsson et al., 2003), PDGFRβ (Ozawa et al., 2005), desmin (Sennino et al., 2007), RGS5 (Berger et al., 2005; Nisancioglu et al., 2008), and the XlacZ4 transgenic mouse (Abramsson et al., 2002) (Figure 7). ASMA is often absent in quiescent pericytes in normal tissues (Table 1), notably in the CNS. However, αSMA is readily detected in pericytes in pathological conditions such as tumor angiogenesis, tissue fibrosis, and inflammation (Gerhardt and Betsholtz, 2003). TGFβ, which has been involved in smooth muscle cell maturation, is a potential driver of the expression of αSMA in tumor pericytes (Song et al., 2005).

Tumor pericytes as therapeutic targets. The tumor stroma influences the outcome of therapeutic approaches and may hence provide targets for therapeutical intervention (Pietras and Ostman, 2010). Recent analysis of the causes of resistance to anti-VEGF therapy points to an important role of tumor-infiltrating leukocytes in providing other angiogenic signals (Casanovas, 2011; Shojaei and Ferrara, 2008). Pericytes have also been proposed to protect the endothelium in situations of VEGF-A inhibition. VEGF-A ablation in tumors led to selective elimination of tumor blood vessels that lacked pericyte coverage (Benjamin et al., 1999). Double targeting of both endothelial cells and pericytes has been suggested to have better antitumoral effect than targeting of any of the cell types alone (Bergers et al., 2003; Erber et al., 2004). However, the pharmacological approaches used involved tyrosine kinase inhibitors, which are known for their promiscuity regarding molecular targets (Fabian et al., 2005). Thus, the enhanced antitumor effect observed may reflect inhibition of other signaling pathways than those intended. The approach for double targeting of endothelial cells and pericytes used in preclinical studies has also been tested

in an early clinical trial with negative results (Hainsworth et al., 2007). Also, other work in preclinical models has failed to demonstrate increased effects of endothelial targeting in the absence of pericytes (Nisancioglu et al., 2010). Depletion of pericytes instead led to an unexpected increase in tumor growth in some models (Nisancioglu et al., 2010; Sennino et al., 2007). A correlation between poor pericyte coverage of tumor vasculature with increased metastatic events has also been reported (discussed below). In summary, the question whether targeting tumor pericytes is of therapeutic advantage is still unanswered.

Pericytes and tumor vessel normalization. In contrast to the healthy tissue vasculature, tumor vessels are highly abnormal structurally and functionally (Jain, 2005). Apart from aberrant pericyte coverage, tumor vessels are characterized by irregular shape and a disorganized architecture with highly dysfunctional and leaky endothelial cell layers. All these changes harm tumor perfusion, resulting in poor drug access to the tumor and impaired oxygen delivery. Together, the aberrant features of tumor blood vessels have been recently considered a hallmark of cancer (De Bock et al., 2011). The abnormal features are the consequence of persistent production of proangiogenic factors, notably VEGF-A.

Pharmacological blockade of VEGF-A signaling reverts—albeit transiently (Winkler et al., 2004)—the aberrant features of tumor vasculature, leading to vessel normalization. Tumor vessels thereby resemble the vessels of normal tissues, resulting in improved oxygen, drug, and nutrient delivery to the tumor (Jain, 2005). Of importance for this discussion is that the vessel normalization correlates with increased pericyte coverage and attachment to the vascular wall.

Tumor vessel normalization is desirable because it improves drug and chemotherapy delivery to the tumor. Because normalization appears to be a transient result of antiangiogenic therapy (Winkler et al., 2004), it might be advantageous to increase its duration, and hence we need to learn more about its mechanisms. Does tumor vasculature normalize as a consequence of improved pericyte abundance (and/or function) or does normalization cause more efficient pericyte recruitment as a bystander effect? A recent study provides some evidence that strengthens the case of pericytes as regulators of vascular normalization. Experimental pancreatic cancer grown in mice deficient for the pericyte marker RGS5 showed increased pericyte maturation and vascular normalization, which correlated with diminished vessel leakiness and hypoxia (Hamzah et al., 2008).

Emerging Concepts in Tumor Pericyte Research—Transendothelial Migration of Tumor Cells and Leukocytes

The tumor microenvironment has a major role in modulating the metastatic capacity of most cancers. Most of our knowledge on stroma-promoting metastatic events comes from studies on cancer-associated fibroblasts or infiltrating immune cells (Joyce and Pollard, 2009; Pietras and Ostman, 2010). However, pericytes may be involved as well (Gerhardt and Semb, 2008; Raza et al., 2010). Evidence for the contribution of pericytes to increased metastatic events comes from an experimental model of pancreatic cancer (Xian et al., 2006) as well as from human colorectal cancer patients (Yonenaga et al., 2005). An association between metastatic events and reduced expression of mural cell markers has been demonstrated across a wide range of human solid tumors, suggesting that low pericyte coverage may trigger metastasis and correlate to poorer prognosis (Ramaswamy et al., 2003). Beyond these correlations, the question about cellular and molecular mechanisms remains. It is still unclear whether pericytes actively promote metastasis or constitute a physical barrier to vascular dissemination and/or extravasation of tumor cells. The former has no plausible hypothesis as of yet. The latter could be explained because pericyte deficiency increases interstitial fluid pressure, which might promote a passive flow of detached tumor cells into the circulation through leaky endothelial cell layers. Moreover, pericytes could play a role in limiting the colonization of metastatic tumor cells at the site of extravasation. Metastasizing melanoma or lung carcinoma cells gain access to the brain and then remain in perivascular locations where they undergo apoptosis or grow into an established metastasis (Kienast et al., 2010). Pericyte dysfunction may hence alter the perivascular microenvironment facilitating the colonization and growth of metastatic cells in the target organ.

The transmigration of inflammatory cells to the site of inflammation entails a complex cascade of events that involve the inflammatory cells, cytokines, endothelial cells, BM, and pericytes. This process is comprehensively reviewed elsewhere (Nourshargh et al., 2010; Soehnlein and Lindbom, 2010). In brief, there are several physical barriers that the inflammatory cells must overcome in order to reach into the tumor or inflammatory site, including the endothelium, the BM, and the pericytes. By using in vivo imaging, recent studies suggest that inflammatory cells migrate across the vessel wall at permissive sites devoid of pericyte coverage (Voisin et al., 2010; Wang et al., 2006). Other reports implicate pericytes in the regulation of T cell exit from the thymus (Zachariah and Cyster, 2010) and in the regulation of leukocyte adhesion molecules in endothelial cells (Daneman et al., 2010) (discussed above). Recent findings suggest a role for tumor pericytes in regulating the transmigration of inflammatory cells. Tumors developed in mice deficient for the pericyte-specific gene RGS5 showed increased infiltration of $CD8^+$/$CD4^+$ T cells after adoptive transfer. These data suggest that pericytes may modulate the vasculature to adapt to different conditions, either facilitating or inhibiting the trafficking of immune cells (Hamzah et al., 2008). It was also recently demonstrated that PDGFRβ activation leads to upregulated expression of a battery of immune response genes in pericytes (Olson and Soriano, 2011). Thus, immune modulation and transmigration of inflammatory cells may be added to the growing list of pericyte mediated vascular functions of relevance for the design of antiangiogenic and vascular targeting strategies in cancer and other diseases (Figure 7).

Tumors Arising from Pericytes

Hemangiopericytomas (HPCs) were first described as tumors arising from the pericytes described by Zimmermann (Stout and Murray, 1942). HPCs primarily affect adults of 20–70 years of age (Enzinger and Smith, 1976). The most commonly affected anatomic sites for HPCs are lower extremities, retroperitoneum, and head and neck, notably in the supratentorial meninges. HPCs bear a close histomorphological similarity to solitary fibrous tumors, which makes them often indistinguishable (Gengler and Guillou, 2006; Park and Araujo, 2009). In fact, in the WHO classification, the concept of HPC as a vascular,

pericyte-derived tumor was abandoned in favor of a fibroblastic cell of origin (Fletcher, 2006).

Diagnosis of HPC is initially based on typical architectural vascular pattern associated with a population of mesenchymal cells that display no discernible differentiation under light microscope (Koch et al., 2008). The vessels form a vascular network with branching vessels of various diameters, with the smaller vessels partly compressed by the surrounding cellular proliferation. On the immunohistochemistry level, HPCs express αSMA and CD34, being negative for CD31, GFAP, and Mib-1. Under these criteria, HPC is classified as WHO grade II (Louis et al., 2007). Management of HPC is preferentially by surgical resection. It is notoriously difficult to predict the prognosis and clinical behavior of HPC (Gengler and Guillou, 2006), perhaps because at the pathology level it is difficult to define malignant characteristics. Different studies report disparate incidence of metastasis (10%–60%). Being a highly vascularized tumor, HPC has then become a target for antiangiogenic therapies. Combined therapy with Bevacizumab and temozolomide showed promising results (Park et al., 2011). Sorafenib and Sunitinib have also been employed with relative success (Mulamalla et al., 2008).

In summary, HPC is a rare mesenchymal tumor with unclear cellular origin, presumably pericytes. Diagnosis, management, and metastatic potential are ill defined, and despite some recent therapeutic improvements by employing antiangiogenic drugs (Park et al., 2010), a better understanding of the molecular pathogenesis of this malignancy is much needed.

Pericytes in Diabetic Retinopathy

Diabetic retinopathy is one of the most common complications of diabetes, with one-third of adult diabetic patients affected, and constitutes a leading cause of blindness (Cheung et al., 2010). Together with diabetic nephropathy it is considered the prototypical microvascular complication of diabetes. This view, however, has been recently widened to include the entire array of changes that occur during the disease (Gardner et al., 2011). The microvascular changes manifest as pericyte death and BM thickening with altered blood flow in the retina capillaries. These changes lead to capillary leakage—macular edema—and vessel occlusion and constitute the nonproliferative phase of diabetic retinopathy. Proliferative diabetic retinopathy develops secondary to capillary occlusion, which upregulates growth factors, notably VEGF-A. Proliferative retinopathy is defined by the growth of abnormal new vessels from the retina to the posterior surface of the vitreous or the iris.

It is currently accepted that diabetic retinopathy manifests first by vascular cell apoptosis, including pericyte detachment, thickening of the BM, vascular dysfunction, and permeability, all traits of the nonproliferative phase of the disease. The protease technique (Bresnick et al., 1977) used for the study of the retinal vasculature in fixed specimens consistently reveals the presence of pericyte ''ghosts,'' or pockets in the BM that appear to have harbored a pericyte (Kern et al., 2000). Other studies noted the absence of pericytes in some capillaries with intact endothelial cells and that vessels with microaneurysms tended to have no pericytes (Barber et al., 2011).

The cause of pericyte apoptosis in diabetic retinopathy is poorly understood. Some possible mechanisms link pericyte apoptosis with increased oxidative stress and NF-κB activation (Romeo et al., 2002). Disruption of the PDGF-B/PDGFRβ signaling axis has also been suggested to play a role in pericyte apoptosis. Endothelial cell ablation or reduced levels of PDGF-B have been shown to recapitulate the pericyte loss and aneurysms and some of the vascular changes that characterize diabetic retinopathy (Enge et al., 2002; Hammes et al., 2002). Interestingly, a recent study identified overexpression of PKCδ in pericytes following hyperglycemia. Activation of PKCδ phosphorylates p38α MAPK, resulting in increased expression of the tyrosine protein phosphatase SHP-1. This signaling cascade leads to PDGFRβ dephosphorylation and pericyte apoptosis (Geraldes et al., 2009). It is unclear how hyperglycemia induces PKCδ overexpression.

Another of the key attributes of the nonproliferative phase of diabetic retinopathy is the loss of blood-retinal barrier integrity leading to increased permeability and macular edema, which in turn precedes vascular proliferation (Ockrim and Yorston, 2010). Several cellular and molecular mediators have been implicated in the onset of macular edema, such as VEGF-A, advanced glycation end products, or TGFβ (Ehrlich et al., 2010). Given the similarities between the blood-brain barrier and the blood-retinal barrier, macular edema could be caused by pericyte dropout leading to increased endothelial transcytosis. Indeed, vesicular transport-related genes such as caveolin-1 and PV-1 were demonstrated to be consistently upregulated in the retinas of diabetic rats, whereas genes encoding for tight junctions such as occudin and claudin-5 showed only marginal downregulation in the initial diabetes period (Klaassen et al., 2009).

Concluding Remarks

An iterated concern in pericyte biology is the definition of the cell type. The problems entailed with pericyte identification all go back to what we mean with the term pericyte. The classical description—and definition—is that of a quiescent pericyte in a stable blood vessel of a normal adult organ. Yet pericyte precursors, or the pericytes of immature and remodeling vessels in the embryo and in pathological conditions, are also functional, as demonstrated by the effects of their alteration or absence in various experimental and pathological situations. Thus, the pericyte is a ''moving target,'' the description and definition of which will inevitably change and become more refined as research moves forward. Still, the current literature is laden with reports in which pericyte identification by morphology and marker expression is clearly substandard, work that should therefore be taken with a large grain of salt. Nevertheless, pericyte research is currently undergoing an explosion of activity in diverse areas of developmental and vascular biology and pathology. Emerging concepts include the physiological role of pericytes in the regulation of vascular permeability to solutes, molecules, and cells, proinflammatory responses, and vascular stabilization and normalization in tumors. The seemingly obligatory presence of mesenchymal and other stem cells in vascular niches pose questions about the identity of the stem cells–are some of them pericytes, or are perciytes functional niche cells, or both? Clearly, research on pericytes has accelerated in the past few years and our perspectives and knowledge about these cells will rapidly change over the years to come.

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