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The role of pericytes in blood-vessel formation and maintenance[1]

Gabriele Bergers[2] and Steven Song

Department of Neurological Surgery, Brain Tumor Research Center and UCSF Comprehensive Cancer Center, University of California San Francisco, San Francisco, CA 94143, USA

Blood vessels are composed of two interacting cell types. Endothelial cells form the inner lining of the vessel wall, and perivascular cells—referred to as pericytes, vascular smooth muscle cells or mural cells—envelop the surface of the vascular tube. Over the last decades, studies of blood vessels have concentrated mainly on the endothelial cell component, especially when the first angiogenic factors were discovered, while the interest in pericytes has lagged behind. Pericytes are, however, functionally significant; when vessels lose pericytes, they become hemorrhagic and hyperdilated, which leads to conditions such as edema, diabetic retinopathy, and even embryonic lethality. Recently, pericytes have gained new attention as functional and critical contributors to tumor angiogenesis and therefore as potential new targets for antiangiogenic therapies. Pericytes are complex. Their ontogeny is not completely understood, and they perform various functions throughout the body. This review article describes the current knowledge about the nature of pericytes and their functions during vessel growth, vessel maintenance, and pathological angiogenesis. *Neuro-Oncology 7, 452–464, 2005 (Posted to Neuro-Oncology [serial online], Doc. 05-023, September 1, 2005. URL http://neuro-oncology.mc.duke.edu; DOI: 10.1215/S1152851705000232)*

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What Is a Pericyte?

Pericytes were described more than 100 years ago as perivascular cells that wrap around blood capillaries (*peri*, around; *cyte*, cell). They are also called Rouget cells after their discoverer, Charles Rouget, or referred to as mural cells or, because of their contractile fibers, as vascular smooth muscle cells (vSMCs)[3] (Hirschi and D'Amore, 1996). Electron-microscope analyses first revealed the morphological character of pericytes. In general, pericytes possess a cell body with a prominent nucleus and a small content of cytoplasm with several long processes embracing the abluminal endothelium wall (Figs. 1A.a and b). They are embedded within the basement membrane of microvessels, which is formed by pericytes and endothelial cells (Mandarino et al., 1993). Pericytes do not serve solely as scaffolding as historically thought, but communicate with endothelial cells by direct physical contact and paracrine signaling pathways. Gap junctions provide direct connections between the cytoplasm of pericytes and endothelial cells, and they enable the exchange of ions and small molecules. Adhesion plaques anchor pericytes to endothelial cells, while peg-and-socket contacts enable the cells to penetrate through discontinuities in the vessel basement membrane and touch each other (Rucker et al., 2000). These junction complexes support transmission of mechanical contractile forces from the pericytes to the endothelium and contain N-cadherin, cell-adhesion

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[2]Address correspondence to Gabriele Bergers, University of California San Francisco, Department of Neurological Surgery, 513 Parnassus Avenue, San Francisco, CA 94143, USA (bergers@itsa.ucsf.edu).

[3]Abbreviations used are as follows: α-SMA, alpha smooth-muscle actin; BBB, blood-brain barrier; bFGF, basic fibroblast growth factor; ECM, extracellular matrix; HSC, hepatic stellate cell; NG2, neuron-glial 2; PDGFβ, platelet-derived growth factor receptor beta; RGS, regulator of G protein signaling; ROP, retinopathy of prematurity; VEGF2, vascular endothelial growth factor 2; vSMC, vascular smooth muscle cell.

molecules, β-catenin-based adherent junctions, and extracellular matrix (ECM) molecules such as fibronectin (Gerhardt and Betsholtz, 2003).

Pericytes exhibit long cytoplasmic processes that not only can contact numerous endothelial cells and thus integrate signals along the length of the vessel, but can also extend to more than one capillary in the vasculature. Interestingly, cell-cell contact appears necessary for the activation of the latent growth factor TGF-β1, which induces pericyte differentiation in vitro (Orlidge and D'Amore, 1987), supporting the notion that direct cell contact is a crucial communication tool for vessel maintenance and formation. Pericytes also exhibit a number of characteristics consistent with muscle-cell activity and express contractile smooth-muscle actin. In contrast to small blood vessels, which are composed of endothelial cells surrounded by a basal lamina and loosely covered by single pericytes, larger vessels are coated with multiple layers of smooth-muscle cells and elastic and collagenous fibers (Cleaver and Melton, 2003). Smooth-muscle cells in large vessels support and regulate blood flow (Fig. 1A.d). Veins are irregularly covered by smooth-muscle cells and pericytes and have valves to prevent the backflow of blood, whereas arteries have strong, elastic vessel walls with dense populations of concentrically formed smooth-muscle cells to withstand the higher blood pressures (Cleaver and Melton, 2003). It has been reported that smooth-muscle cells, in contrast to pericytes, are not embedded in the basement membrane and might not directly contact the endothelium (Gerhardt and Betsholtz, 2003). However, the possibility cannot be excluded that physical contacts between smooth-muscle cells and endothelial cells are more erratic, and therefore may go unnoticed. Because of all of this, it is still not clear whether pericytes are smooth-muscle cells or cells with smooth-muscle-cell characteristics that can turn into smooth-muscle cells, which would suggest that pericytes and smooth-muscle cells represent phenotypic variants of the same lineage, or whether these cells even have a distinct progenitor. It is therefore very common that pericytes are referred to as vascular smooth muscle cells.

Molecular Markers of Pericytes

The challenges of defining a pericyte have not been made easier by the facts that no general pan-pericyte molecular marker has been found, and that one will probably never be discovered because of the diverse characteristics, functions, and locations of pericytes in various organs. There are, however, a few dynamic molecular markers that are present in pericytes, albeit not exclusively, and are commonly used for their detection. The expression patterns of these markers can vary in a tissue-specific manner or be dependent on the developmental or angiogenic stage of a blood vessel. Desmin and alpha-smooth-muscle actin (α-SMA) are contractile filaments, and regulator of G protein signaling 5 (RGS-5) is a GTPase-activating protein; all three are intracellular proteins. Neuron-glial 2 (NG2), a chondroitin sulfate proteoglycan, and platelet-derived growth factor receptor beta (PDGFRβ), a tyrosine-kinase receptor, are cell-surface proteins. Antibodies against these proteins (except RGS-5) are commonly used to identify pericytes in tissue sections (Fig. 1B). Desmin is a muscle-specific class III intermediate filament found in mature skeletal, cardiac, and smooth-muscle cells. Desmin-deficient mice are viable and fertile, but they develop progressive muscle weakness and dystrophic alterations in both cardiac and skeletal muscles (Li et al., 1996; Milner et al., 1996).

Alpha smooth-muscle actin is one of the six mammalian isoforms of the cytoskeletal protein actin. The beta and gamma nonmuscle actins are present in all cells, whereas α-SMA is normally restricted to cells of the smooth-muscle lineages. Certain nonmuscle cells have been shown to transiently express α-SMA, specifically fibroblasts, which are then referred to as myofibroblasts (Ronnov-Jessen and Petersen, 1996). Interestingly, α-SMA appears to inhibit migration and mobilization of fibroblasts (Ronnov-Jessen and Petersen, 1996), which suggest that it can promote a differentiated phenotype.

RGS-5 is a member of the "regulator of G protein signaling," or RGS, protein family, a category that includes more than 25 proteins. RGS proteins serve as GTPase-activating proteins for Gα subunits of heterotrimeric G proteins, and they negatively regulate G protein–coupled receptor signaling. RGS-5 acts as a GTPase-activating protein for Giα and Gqα and attenuates the signaling induced by angiotensin II, endothelin-1, sphingosin-1-phosphate, and PDGF in cultured cells (Bondjers et al., 2003; Cho et al., 2003). It was identified as a novel marker for developing pericytes when comparison was made of the gene expression profiles of wild-type embryos and PDGF-B-knockout embryos, in which developing blood vessels in the central nervous system are almost completely devoid of pericytes. RGS-5-positive cells in the wild-type embryos were identified as pericytes, and the expression pattern of RGS-5 strikingly overlapped with the expression pattern of PDGFR-β and NG2. In contrast, the brain tissue of PDGF-B-deficient mice lacked RGS-5 expression (Bondjers et al., 2003). Just recently, RGS-5 was discovered as a marker for sites of physiological and pathological angiogenesis in adults. Elevated levels of RGS-5 in pericytes have been observed during wound healing and ovulation, and RGS-5 expression was substantially increased concomitant with neovascularization during tumor progression in mouse models of pancreatic islet tumors and glioblastomas (Berger et al., 2005), indicating a strong correlation between RGS-5 expression and active vessel remodeling (Berger et al., 2005). However, RGS-5 is not found in every tissue or tumor, which indicates tissue-specific regulation of RGS-5 (R. Ganss, personal communication; S. Song, unpublished observation).

NG2 chondroitin sulfate proteoglycan (also called high-molecular-weight melanoma-associated antigen, or HMWMAA; the mouse derivate is called AN2) is expressed on the surface of pericytes during vasculogenic and angiogenic processes (Stallcup, 2002). Historically, NG2 was postulated to be characteristic of

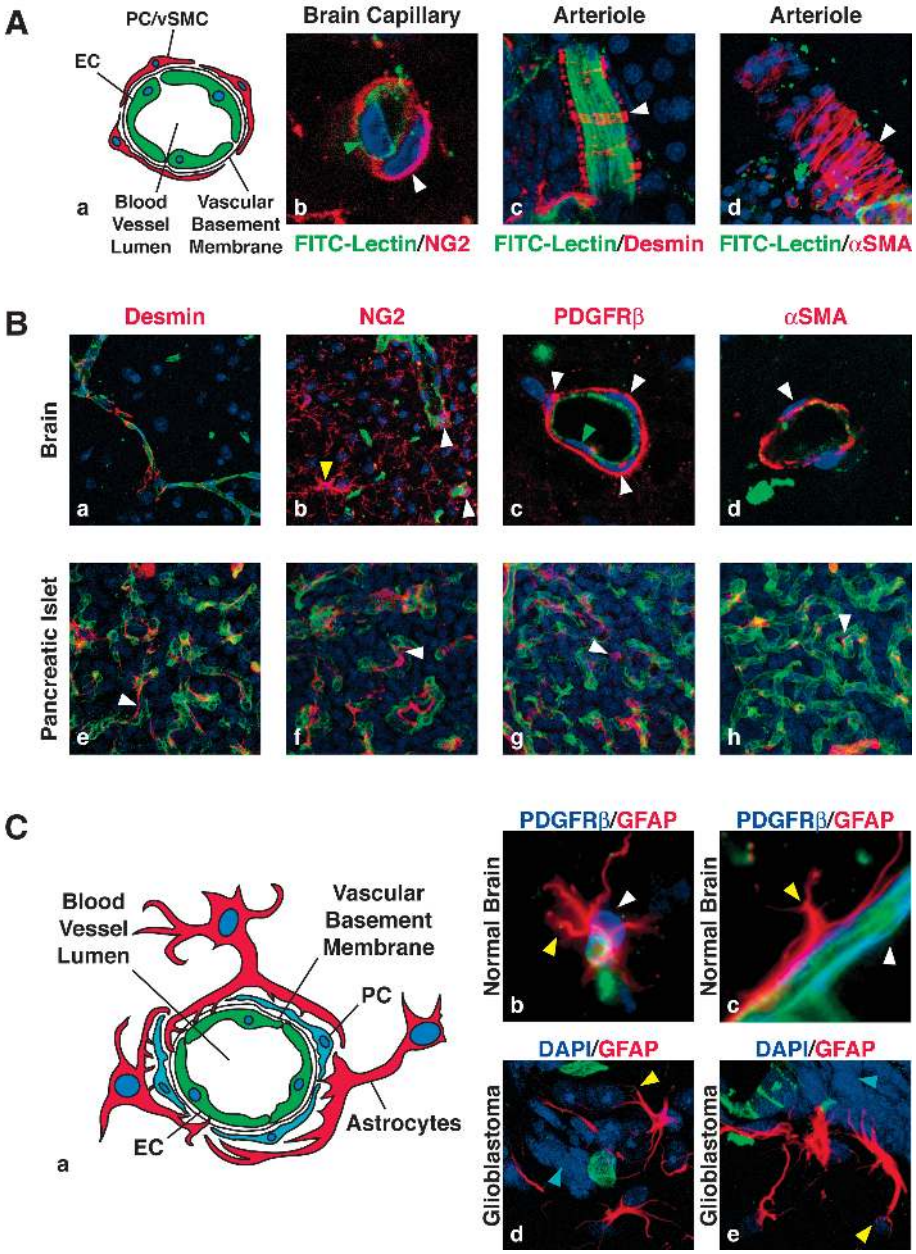

Fig. 1. Pericytes A. Capillaries are composed of endothelial cells (ECs; green) that form the inner lining of the wall with a surrounding basal lamina and pericytes (PC; red) that extend long cytoplasmic processes over the surface of the vascular tube (a, b). Larger vessels have several layers of pericytes and smooth muscle cells. Arterioles have strong elastic vessel walls (c) with dense layers of concentrically formed smooth muscle cells (d) to withstand the blood pressure. B. Immunohistochemical detection of pericytes exemplified in murine brain (a–d; red) and pancreatic islet tissues (e–h; red). NG2 is expressed in pericytes (b; white arrow), but also in microglia cells (b; yellow arrow) in the brain, whereas NG2 appears to be solely specific for pericytes in the pancreas (f; white arrow). α-SMA$^+$-pericytes (h) are less abundant in the pancreatic islets than NG2$^+$ or desmin$^+$ cells (e, f). Green arrows indicate EC and white arrows mark PC, while the yellow arrow points to microglia cells. Blood vessels in the tissues were visualized with FITC-labeled tomato lectin (green), and tissue sections were stained with red-labeled antibodies for desmin (A.c; B.a, e), NG2 (A.b; B.b, f), PDGFRβ (B.c, g), or α-SMA (A.d; B.d, h) to visualize pericytes and incubated with DAPI to identify nuclei. All pictures were captured on a confocal microscope at 63× magnification (A.b–d; B.a–h) with an additional 3× zoom (B.c, d) and additional 8× zoom (A.b). C. Brain capillaries consist of a continuous endothelium with complex tight junctions and interact with astrocytic foot processes and pericytes to constitute the BBB (a). Antibodies for glial fibrillary acidic protein (GFAP) detect astrocytes with their cytoplasmic processes in red (b–e), and antibodies for PDGFRβ identify pericytes in blue (white arrows; b, c). ECs are stained with both FITC-labeled tomato lectin (*Lycopersicon esculentum*) and FITC-labeled CD31 in green (b–e). Glioblastoma cells (in blue; cyan arrows) invade the brain parenchyma and migrate along blood vessels interrupting the interaction of normal astrocytes and the vasculature. Yellow arrows point to astrocytic foot processes; cyan arrows indicate glioblastoma cells and white arrows show pericytes. Pictures were captured on a confocal microscope at 63× magnification with an additional 4× zoom.

immature neural cells capable of differentiating into either glia or neurons and was therefore named neuron-glial 2. In support of this hypothesis, NG2 is found in the central nervous system in glial precursor O-2A cells, which give rise in vitro to either oligodendrocytes or type II astrocytes and lose NG2 expression during the differentiation process (Stallcup, 2002). NG2 exerts its biological activity partly by binding with high affinity to basic fibroblast growth factor (bFGF), PDGF-AA, and the kringle domains of plasminogen and angiostatin (Ozerdem and Stallcup, 2004). NG2 knockout mice are viable, but when pathological angiogenesis is induced in the adult mouse, such as ischemic angiogenesis in the mouse retina in response to hypoxia or bFGF-induced angiogenesis in the cornea, neovascularization is substantially reduced (Ozerdem and Stallcup, 2004).

The tyrosine-kinase receptor PDGFRβ is one of the most widely studied molecules expressed in pericytes and is discussed in detail in this review. Mice deficient in PDGFRβ or its ligand PDGF-B have severely reduced numbers of pericytes and subsequent hyperdilation of blood vessels, which causes edema formation and embryonic lethality. However, other cell types besides pericytes also produce PDGFRβ, such as fibroblasts, astrocytes, and certain tumor cells (Lindahl et al., 1997).

The Diverse Functions of Pericytes

Pericytes have been associated mainly with stabilization and hemodynamic processes of blood vessels. Their functions are, however, much more diverse. They can sense angiogenic stimuli, guide sprouting tubes, elicit endothelial survival functions, and even exhibit macrophage-like activities.

Pericyte Contractility and Regulation of Blood Flow

Similar to the smooth-muscle cells of larger vessels, pericytes can produce vasoconstriction and vasodilation within capillary beds to regulate vascular diameter and capillary blood flow (Rucker et al., 2000). The first line of evidence for this function came from the identification of contractile proteins such as α-SMA, tropomyosin, and myosin in pericytes. These filaments are also produced in smooth-muscle cells and therefore greatly contribute to the confusion between the definition of a smooth-muscle cell and a pericyte. Several molecules that regulate pericyte contractile tone have been identified. For example, pericytes possess receptors for both cholinergic and adrenergic (α-2 and β-2) receptors. The β-adrenergic response in pericytes leads to relaxation, whereas the α-2 response is antagonistic and produces contraction (Rucker et al., 2000). Other vasoactive substances that bind to pericytes are angiotensin II and endothelin-1. Interestingly, the expression of endothelin-1 is induced by endothelial cells, which also make nitric oxide, a potent vasodilator that promotes vessel relaxation by a cGMP-dependent mechanism. These molecules function as paracrine signals, regulate pericyte contraction and relaxation, and provide part of the evidence that endothelial cells and pericytes interact in the regulation of blood flow (Rucker et al., 2000). Finally, oxygen levels also regulate pericyte contraction. It has been shown that hyperoxia increases pericyte contraction in vitro, whereas elevated levels of carbon dioxide induce relaxation. These data suggest that vessels dilate when oxygen is needed but constrict if the levels are sufficient, thereby coupling the rate of blood flow to the metabolic state. Although pericyte contraction in vivo is more difficult to prove, ultrastructural morphometric techniques have demonstrated the compression of endothelial-cell membranes by apposing pericytes. Pericyte contraction was measured in response to vasoactive substances in skeletal muscle (Hirschi and D'Amore, 1996; Tilton et al., 1979).

Tissue-Specific Functions of Pericytes

Pericyte density differs in respect to the function of vessels and organs in which they are found. Pericytes are quite abundant on small venules and arterioles but are rather sparse on capillaries. Although it is unknown how pericytes choose their exact location on the vessel wall, they are not randomly located but functionally determined. It has been noted that they preferentially cover endothelial cell junctions, specifically during inflammation (Sims, 2000). Pericyte density is dependent on blood pressure levels, and it is an interesting observation that pericytes in humans are more abundant further down the torso and legs, at sites where increased pressure is necessary to pump the blood upward (Sims, 2000). Pericyte coverage of blood vessels also varies among the tissues. This is not necessarily surprising, because vascular cells acquire specialized characteristics in different organs, based on the functions of each organ. In several organs, such as the brain, liver, and kidneys, pericytes have been shown to perform specific functions and have therefore been given additional names in these organs.

Brain. The highest density of pericytes in the body is found in vessels of the neural tissues, such as the brain and the retinas. The reason for this is that endothelial cells in the brain form a continuous endothelium with complex, tight junctions, and they interact with astrocytic pedicels and with numerous pericytes to create the blood-brain barrier (BBB), which protects brain cells from potentially toxic blood-derived factors (Fig. 1C) (Ballabh et al., 2004; Cleaver and Melton, 2003). Pericytes play an essential role in the integrity of structural vessels and the BBB. Vessel degeneration is observed in hereditary cerebral hemorrhage with amyloidosis (Verbeek et al., 1997), and pericytes have been shown to protect hypoxia-induced BBB disruption in vitro (Hayashi et al., 2004). Most interesting is that pericytes in the brain can perform macrophage-like activities, thus providing an immunological defense. This phenotype has raised the hypothesis that pericytes can act as precursor cells of macrophages in the brain, and there are several observations to support this idea (Thomas, 1999). Like macrophages, pericytes take up small and

soluble molecules by pinocytosis, thereby cleaning the extracellular fluid as part of the BBB. Pericytes also have phagocytic activity (Thomas, 1999). The first evidence of phagocytic activity in pericytes was revealed when, following histamine treatment, pericytes gradually took up carbon over a course of several months (Majno et al., 1961). Congruent with these observations, systemic injections of protein tracers into immature mice lead to an accumulation of the tracer in pericytes of the brain and spinal cord (Kristensson and Olsson, 1973). Pericytes display several types of phagocytotic activity. They express scavenger receptors, which have broad-ligand-binding specificity, and are crucial in routine scavenging of many different molecules (Thomas, 1999); pericytes in culture can ingest various macromolecules, including polystyrene beads (Balabanov et al., 1996). In addition, pericytes express Fc receptors, which are essential for antibody-antigen complex recognition to trigger antibody-dependent phagocytosis (Balabanov et al., 1996). Furthermore, pericytes express numerous macrophage markers such as CR3 complement receptor, CD4, class I and II major histocompability complex molecules, and ED-2 (Balabanov et al., 1996).

Liver. Pericytes also have specialized functions in the liver. Hepatic stellate cells (HSCs), also called Itoh cells (named after their discoverer, Toshio Itoh) (Suematsu and Aiso, 2001), are the pericyte equivalent in the liver (Sato et al., 2003). They are located between the parenchymal cell plates and the sinusoidal endothelial cells. In contrast to the continuous brain endothelium, liver endothelial cells are highly fenestrated and discontinuous. They line the hepatic sinusoids and mediate the exchange of metabolites between the portal blood, Kupffer cells, and hepatocytes and the processing of toxins (Abbott, 2002). Although a dense basement structure between hepatic epithelial cells and endothelial cells does not exist, HSCs have close contact with endothelial cells through incomplete basement-membrane components and interstitial collagen fibers. HSCs regulate the remodeling of the ECM by producing both ECM components and matrix metalloproteinases (Sato et al., 2003). HSCs are also involved in vitamin A metabolism and contain more than 80% of the total vitamin A in the body (Sato et al., 2003). Finally, HSCs are involved in the recruitment of inflammatory cells during hepatic-tissue repair and in fibrotic responses to liver diseases (Knittel et al., 1999; Sims, 2000).

Kidney. Pericytes of the glomerular capillaries in the kidney are called mesangial cells and account for approximately 30% of the glomerular cells. The cells are instrumental in the intussusceptive branching or splitting of a single invading vascular loop into several glomerular capillaries, which creates a significantly increased capillary surface area for blood ultrafiltration (Betsholtz, 2004). PDGF-B signaling appears to be critical for the development of mesangial cells, because mice deficient in PDGF-B or its receptor PDGFR-β lack mesangial cells and therefore form defective kidney glomeruli that have only one distended capillary loop (Betsholtz, 2004).

Pericyte Function in Vascular Development and Angiogenesis

Pericytes are not only involved in hemodynamic processes but also have an active role in vessel formation. Blood vessels develop early during embryogenesis and are derived from mesodermal precursors called angioblasts (Fig. 2A) (Carmeliet, 2004). Additionally, it is believed that endothelial cells share a common precursor with hematopoietic cells; this precursor is called a hemangioblast. This hypothesis of a common precursor is supported by the observations that developing hematopoietic and endothelial cells share common surface markers and that hematopoietic cells can bud from major embryonic blood vessels (Carmeliet, 2004; Cho et al., 2003; Ribatti et al., 2002).

Pericytes have a complex ontogeny, because they can develop from various cells, as a function of their location in the embryo. For example, pericytes can develop from the neurocrest in the forebrain and cardiac tracts (Bergwerff et al., 1998; Etchevers et al., 2002). Most commonly described and best understood, though, is their origin from mesenchymal stem cells (Creazzo et al., 1998). Under these circumstances, TGF-β1 appears to initiate differentiation of PDGFRβ$^+$ pericyte progenitor cells that are then chemotactically attracted by PDGF-B-secreting endothelial cells in the capillary plexus (Hellstrom et al., 1999). TGF-β1 also appears to drive the differentiation of neurocrest- or mesenchyme-derived progenitors into smooth-muscle-like cells (Chen and Lechleider, 2004; Darland and D'Amore, 2001). Furthermore, in vitro analyses revealed that there exists a common VEGFR2$^+$ vascular-progenitor cell type, derived from embryonic stem cells, which has the ability to differentiate into endothelial cells in the presence of VEGF or into vascular smooth-muscle cells when PDGF-B is added (Fig. 2A) (Carmeliet, 2004; Yamashita et al., 2000). Finally, there have been reports that pericytes can be generated from endothelial cells by transdifferentiation in a TGF-β3-dependent manner, as has been described in the dorsal aorta (Gittenberger-de Groot et al., 1999) and cardiac valves (Nakajima et al., 1997).

As soon as the primary capillary plexus has been assembled, it is refined into a functional network by angiogenesis—vessels undergoing extensive pruning and sprouting. Angiogenesis can include endothelial intussusception, endothelial cell bridging, vessel sprouting, or a combination of these processes (Fig. 2B). In vessel sprouting, angiogenic factors (e.g., VEGF) stimulate endothelial cells, which in turn start secreting several proteases to degrade the vessel basement membrane. This allows endothelial cells to invade the surrounding ECM and form a migration column that consists of proliferating and migrating endothelial cells. As demonstrated in the retina, this column is guided by a migrating endothelial cell at the very tip, which moves toward a VEGF gradient (Gerhardt et al., 2003). Studies of the corpus luteum have suggested that pericytes are also capable of guiding sprouting processes by migrating ahead of endothelial cells and expressing VEGF (Ozerdem and Stallcup, 2003; Ozerdem et al., 2001; Reynolds

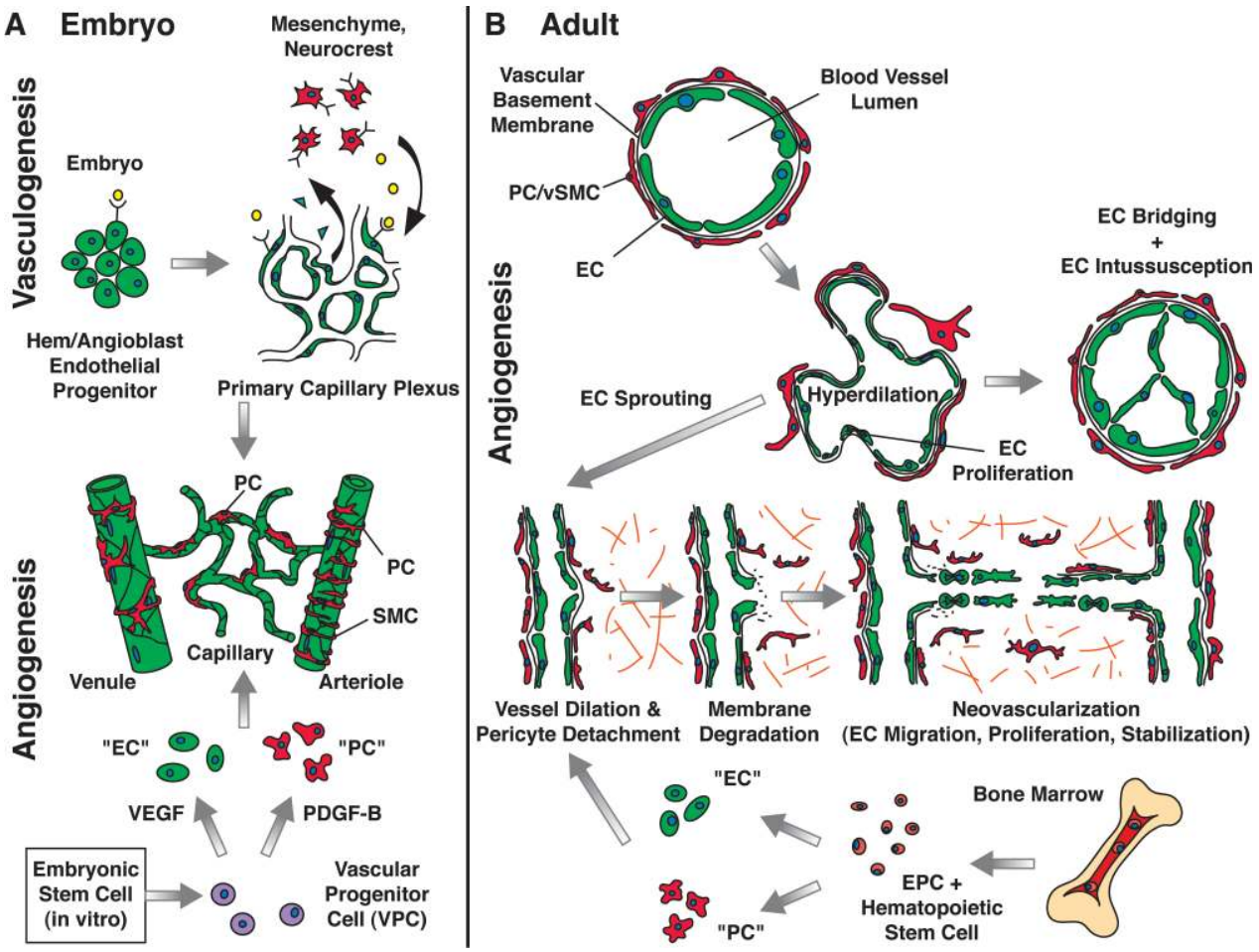

Fig. 2. Pericytes in vasculogenesis and angiogenesis A. Endothelial cells (ECs) and pericytes/vSMCs (PC) arise from different precursor cells. ECs develop from angioblasts or hemangioblasts in the embryo, while pericytes/vSMCs are derived form mesenchymal stem cells or neurocrest cells. In vitro data indicate that there exists a common vascular progenitor derived from embryonic stem cells that can give rise to EC in the presence of VEGF, and to PC in the presence of PDGF-B. In the embryo, endothelial cells first assemble into a simple capillary network. Vessels then sprout and prune (angiogenesis, B), become stabilized by pericytes/vSMCs that are recruited by PDGF-B-secreting endothelial cells, and segregate into the different vessel types. Arterioles exhibit a high density of circumferentially oriented SMCs and thicker EC walls to withstand the blood pressure. Venules, like capillaries, have irregularly arranged pericytes with multiple cytoplasmic processes and are composed of thinner EC walls with valves to prevent backflow of blood. B. New vessels are formed from existing blood vessels by endothelial cell bridging, intussusceptions, and/or sprouting. This is in general preceded by pericyte detachment from the vessel wall and subsequent vessel hyperdilation. When vessels form new sprouts, the vascular basement membrane is first degraded to enable EC to move into the ECM. This is accompanied by EC proliferation and migration toward an angiogenic stimulus. ECs can be either guided by EC tip cells expressing high levels of PDGF-B or by pericytes. Immature, newly formed vessels cease the proliferation, and ECs adhere to each other, form a lumen and become encircled by a basement membrane with recruited pericytes. ECs, and also PCs, can be recruited from the bone marrow, specifically in tumor angiogenesis. Green arrows indicate EC; white arrows point to PC.

et al., 2000). Newly formed sprouts cease proliferation behind this migration zone, adhere to each other, and form a new, lumen-containing vessel. Endothelial cells then secrete growth factors, partly to attract pericytes that envelop the vessel wall, and promote vessel maturation. Although several factors like S1P-1 (sphingosine-1-phosphate-1) and the angiopoietins have been implicated in the recruitment of pericytes and in vessel maturation (Jain, 2003), it is PDGF-B that appears to be the crucial player in the recruitment of pericytes to newly formed vessels (Betsholtz, 2004). During angiogenesis in embryos and adults, PDGF-B is expressed by the sprouting capillary endothelial cells, whereas its receptor, PDGFRβ, is localized on pericytes, which suggests a paracrine signaling circuit between the two cell types (Fig. 2A) (Enge et al., 2002; Hellstrom et al., 1999; Hirschi et al., 1999). It is believed that pericytes, because of their vessel-embracing position, are able to transfer angiogenic signals along the vessel length by contacting numerous endothelial cells. Endothelial cells and pericytes communicate by either direct contact or paracrine signaling, thereby also affecting each other's mitotic rate. Pericytes induce endothelial differentiation and growth arrest (Gerhardt and Betsholtz, 2003; Hirschi

et al., 1998; Sims, 2000). These ideas on pericyte and endothelial cell interactions are primarily based on the views that pericyte recruitment lags behind endothelial sprouting and that a window of pericyte absence allows for vascular plasticity resulting in growth, survival, remodeling, or regression of the endothelium, dependent on the presence or absence of angiogenic growth factors such as VEGF (Bondjers et al., 2003).

Finally, vascular polarity and arteriovenous vessel specifications are established. Although blood flow and pressure have been described as major determinants of arterial or venous vessel generation, genetic mutation studies of the *hedgehog*, *VEGF*, and *notch* genes in zebrafish and quail indicate that the fate of an endothelial cell may already be determined before blood circulation is initiated (Carmeliet, 2004). For example, *notch* and *gridlock* genes are expressed in precursors of arterial endothelial cells but not venous cells in zebrafish embryos (Lawson et al., 2001; Zhong et al., 2000). Ephrinb2, an Eph family transmembrane ligand, is expressed in arterial endothelial cells and pericytes, while its receptor, the tyrosine kinase EphB4, is predominantly found on the corresponding venous cells. Blood flow and pressure are also important factors in smooth-muscle cell differentiation in coronary arteries during arteriogenesis, and differentiation of smooth-muscle cells is delayed in coronary veins because of their lower blood pressure (Carmeliet, 2004). Taken together, endothelial cells and pericytes regulate vessel formation, maturation, and specification, all of which require the orchestration of many tightly regulated molecules.

Molecular Regulators in Pericyte Biology

PDGF was originally identified in platelets and serum in vitro as a mitogen for fibroblasts, glial cells, and smooth-muscle cells (Betsholtz et al., 2001). The PDGF family is composed of four ligands. PDGF-A, -B, -C, and -D form homodimers, and PDGF-A and -B can also heterodimerize. Extracellular proteolytic removal of an N-terminus fragment (the CUB domain) is a prerequisite for PDGF-C and -D activation (Betsholtz et al., 2001). PDGFs bind to a membrane-bound receptor tyrosine kinase consisting of two subunits, alpha and beta, which can homo- and heterodimerize. In general, the alpha unit binds A, B, and C ligands, whereas the beta subunit binds B and D ligands. The response of a particular cell to PDGF is dependent on its specific complement of PDGF receptors and the bioavailability of the various PDGF dimers (Betsholtz et al., 2001). In the embryo, PDGF-B expression is restricted to endothelial cells and megakaryocytes (Lindahl et al., 1997) and is highest in sprouting, immature capillaries, whereas PDGFRβ expression is found on the mesenchymal pericyte progenitor cells, which indicates a paracrine signaling pathway between endothelial cells and pericytes. In agreement, genetic ablations of PDGF-B or PDGFRβ in mice produce identical phenotypes and reveal the significant role of PDGFRβ signaling in pericyte proliferation and recruitment to blood vessels. PDGF-B- or PDGFRβ-null mutants die during late gestation from cardiovascular complications that include widespread microvascular leakage and edema, arterial smooth-muscle cell hypoplasia, and abnormal kidney glomeruli (Hellstrom et al., 1999; Leveen et al., 1994, Lindahl et al., 1997, 1998; Soriano, 1994). Interestingly, the cause of the microvascular dysfunction in the mutant mice is severe pericyte deficiency (Lindahl et al., 1997). Blood-vessel dilation and microaneurysms in mutant embryos correlated with severe reduction or even total loss of pericytes on the affected vessels, most prominently in the brain and heart (Hellstrom et al., 1999). It has also been shown that PDGF-B expression in the endothelium is critical for proper pericyte coverage on vessels, because pericyte deficiency is still observed when PDGF-B is deleted only in the endothelial cells of mice (Enge et al., 2002). However, it is important to note that the development of pericytes still can be induced when PDGF-B/PDGFRβ signaling is disrupted, but the cells are unable to expand and spread along the newly formed vessels because of their reduced proliferative capability and, likely, reduced migratory capability (Betsholtz et al., 2001).

Tissue-culture experiments have revealed that contact between endothelial cells and pericyte precursors leads to activation of TGF-β1, which in turn inhibits endothelial cell proliferation and migration (Orlidge and D'Amore, 1987; Sato and Rifkin, 1989), reduces VEGF-receptor 2 (flk-1) expression on endothelial cells (Mandriota et al., 1996), and induces differentiation of perivascular cells into pericytes (Hirschi et al., 1998, Ramsauer and D'Amore, 2002). TGF-β1 acts as a multifunctional cytokine in vessel formation, because it also induces differentiation of mesenchymal stem cells and neuro crest cells into pericytes (Chen and Lechleider, 2004; Ding et al., 2004). The functional implications of TGF-β1 in this process are highlighted by experiments with endoglin-knockout mice, which are nonviable as a result of defective vascular remodeling and smooth-muscle cell differentiation (Li et al., 1999). Endoglin is a TGF-β-binding protein and is also referred to as TGFβ type III receptor. Another paracrine signaling pathway important in vessel-wall formation is that of the angiopoietins and their Tie receptors. The receptor tyrosine kinase Tie2 is expressed in endothelial cells (Sundberg et al., 2002; Suri et al., 1996) and is stimulated by angiopoietin-1 (Ang-1) or angiopoietin-2 (Ang-2), which is secreted by surrounding mesenchyme and perivascular cells (Fig. 2A). Ang-1 is believed to be involved in vessel maturation; this is supported by recent data that Ang-1 was able to mature pericyte-deficient blood vessels in the retina (Uemura et al., 2002). On the other hand, its sibling Ang-2 has been proposed as a destabilizing factor in blood vessels, loosening the pericyte-endothelial contact points. When VEGF is present, Ang-2 promotes blood-vessel growth and sprouting, whereas in the absence of VEGF, Ang-2 leads to endothelial cell death and vessel regression (Hanahan, 1997; Maisonpierre et al., 1997). Mouse mutants lacking Tie2 or Ang-1 (Suri et al., 1996; Vikkula et al., 1996) have severe vascular defects and are unable to recruit pericytes, but it is more likely that the primary defects still occur in endothelial cells and

that the effects on the pericyte coat are secondary to a general derangement or dysfunction (Gerhardt and Betsholtz, 2003).

Pericytes in Vascular Disease

The vasculature is usually quiescent in the adult, and endothelial cell turnover can be measured in years in tissues that do not require ongoing angiogenesis. However, although angiogenesis is relatively rare, it does occur in wound healing and in the female reproductive system during the menstrual cycle and pregnancy. The neovascularization process resembles the embryonic process and involves the coordinated action of many molecules (Fig. 2). Angiogenesis also occurs under pathological conditions like diabetic retinopathy and tumor growth. The difference between physiological and pathological angiogenesis lies in the tightly regulated balance of proangiogenic and antiangiogenic factors. During physiological neovascularization, newly formed vessels rapidly mature, become stable, and cease proliferation, whereas blood vessel growth under pathological conditions loses the appropriate balance between positive and negative regulators. Vessels formed during pathological angiogenesis do not stop growing and are under constant reconstruction, leading to an aberrant vascular system.

Pericytes in Diabetic Retinopathy

The retina has the highest pericyte density in the body. Diabetic patients are prone to developing diabetic retinopathy, of which an early hallmark is the loss of pericytes in the retina (Cai and Boulton, 2002). Chronic hyperglycemia is believed to cause this "pericyte dropout." This has severe consequences because the vascular walls weaken and generate microaneurysms (Hammes et al., 2002; Wilkinson-Berka et al., 2004). Indeed, ultrastructural analyses of retinal microaneurysms reveal a consistent absence of pericytes, suggesting that the loss of vessel integrity due to the absence of pericytes may render vessels vulnerable to aneurysms. When progressive vascular occlusions in the human diabetic eye lead to blindness, the retina responds with either a progressive increase of vascular permeability, leading to macula edema, or the formation of new proliferating, immature vessels (Campochiaro, 2004; Hammes et al., 2002; Miller et al., 1997). Congruently, increased expression of VEGF-A and its receptors has been demonstrated in diabetic retinas (Benjamin, 2001). VEGF-A is also very likely responsible for the induced vascular leakage, and antagonists of VEGF and its receptors have been shown to reduce retinopathy in animal models (Aiello et al., 1995; Benjamin, 2001; McLeod et al., 2002; Robbins et al., 1998). Two major rodent models have been preferentially used to elucidate the exact mechanism of pericyte degeneration: the streptozotocin-induced diabetic model of early retinal damage by hyperglycemia (Kondo and Kahn, 2004) and the model of oxygen-induced proliferative retinopathy (resembling human retinopathy of prematurity [ROP]). Both of these models undergo the same critical steps of retinal neovascularization as in proliferative diabetic retinopathy (Hammes et al., 2002; Smith et al., 1994). Although the underlying mechanisms of pericyte loss in the retina are still unknown, mice that show impaired PDGF-B signaling when they are adults (endothelial-specific PDGF-$B^{ko/ko}$ mice, PDGF-$B^{ret/ret}$ mice) also develop retinopathy concomitant with severe pericyte loss (Betsholtz, 2004). Pharmacological inhibition of PDGF signaling with Gleevec (STI57, imatinib) promoted pericyte apoptosis and exacerbated angiogenesis by further inducing VEGF and VEGFR2 in the rodent ROP model (Wilkinson-Berka et al., 2004). First, these data support the concept that PDGF is required for pericyte growth and viability. Second, pericytes elicit survival functions for endothelial cells because induction of the survival factor VEGF is likely to be a response to the loss of pericytes. Further, the correlation between lack of pericytes and onset of neovascularization in retinopathy, as well as the pericyte association with developing capillaries and cessation of vessel growth, supports the concept that pericytes have suppressive influence of capillary growth.

Pericytes in Tumors

Angiogenesis in tumors leads to a chaotic, poorly organized vasculature with tortuous, irregularly shaped, and leaky vessels that are often unable to support efficient blood flow. Because of the imbalanced expression pattern of angiogenic factors, tumor vessels appear to be in a constant state of remodeling, which involves simultaneous formation and regression of vascular tubes (Bergers and Benjamin, 2003; Folkman, 2000). Just as tumor endothelial cells differ from the normal, quiescent endothelium, tumor pericytes also differ from normal pericytes. Figure 3 illustrates tumor pericytes, visualized with different markers, in three tumor types derived from transgenic or orthotopic mouse tumor models: glioblastomas, pancreatic islet carcinomas, and mammary carcinomas. In general, pericytes in tumors appear to be more loosely attached to the vasculature, and their cytoplasmic processes can extend into the tumor tissue (Fig. 3). They seem to be less abundant in some tumor tissues in comparison to the respective normal tissue (Fig. 1) and can change their pericyte expression profile (Fig. 3) (Benjamin et al., 1998; Morikawa et al., 2002). For example, pericytes in pancreatic islet tumors and glioblastomas appear to contain a higher number of α-SMA-positive pericytes than the respective normal tissue (Morikawa et al., 2002) (Figs. 1B and 3). In addition, tumor vessels differ among tumor types: The vasculature of mammary carcinomas is enormously enlarged and thickened, glioblastoma vessels appear thin walled and hyperdilated, and vascular tubes in islet carcinomas are more irregular but only slightly hyperdilated (Fig. 3). Pericyte coverage of blood vessels is also dependent on the tumor type. While islet carcinomas have reasonably dense pericyte coverage, glioblastomas and mammary carcinomas exhibit a more dramatic reduction in pericyte density when compared to the respective normal tissues (Fig. 3).

For all of these reasons, pericytes are thought to be

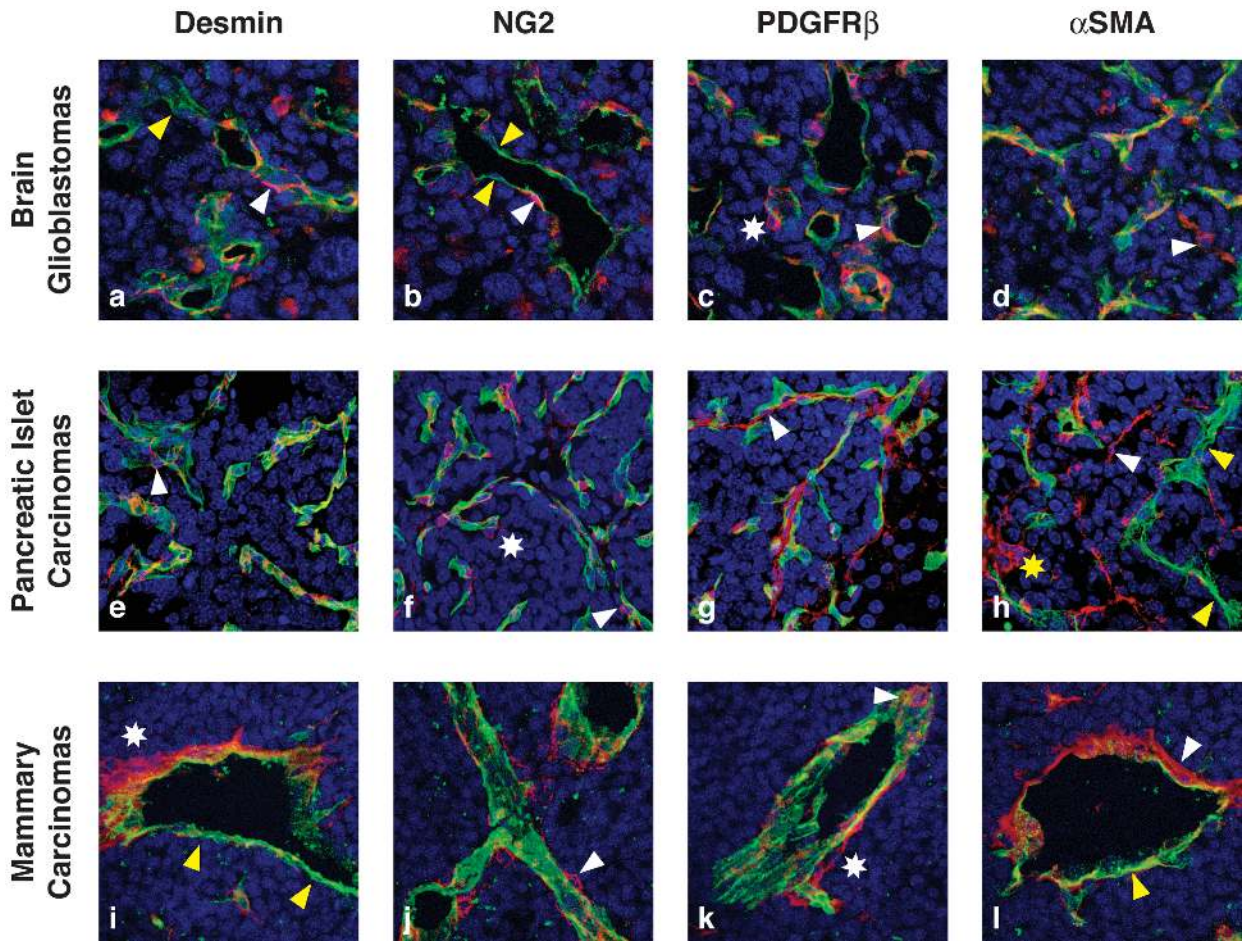

Fig. 3. Pericytes in tumors. Tumor sections from three mouse models of tumorigenesis were used to visualize pericytes in glioblastomas (a–d), pancreatic islet carcinomas (e–h), and mammary carcinomas. SV40 Tag/H-ras transformed astrocytes intracranially injected into athymic mice, generate glioblastomas (Blouw et al. 2003). Transgenic mice expressing SV40Tag under the control of the insulin promoter develop pancreatic islet carcinomas (Hanahan, 1984). MMTV-neu mice overexpress the neu-oncogene under the control of the MMTV promoter and develop mammary adenocarcinomas (Guy et al., 1992). Blood vessels in the tumors were visualized by FITC-labeled tomato lectin (*lycopersicon esculentum*) and immunostaining with an FITC-labeled CD31 antibody. Tumor tissue sections were then stained with red-labeled antibodies for desmin (a, e, i), NG2 (b, f, j), PDGFRβ (c, g, k), or α-SMA (d, h, l) and incubated with DAPI to identify nuclei. Tumor vessels (green) are very distinct from their normal counterparts (see Fig. 1) because they become irregularly shaped, hyperdilated, and enlarged. In addition, tumor vessels differ among tumors. The vasculature of mammary carcinomas is enormously enlarged and thickened, GBM vessels are thin and hyperdilated, and vascular tubes in islet carcinomas are more irregular but only slightly hyperdilated. Pericyte coverage of blood vessels is also tumor dependent. Islet carcinomas have more pericyte coverage than GBMs or mammary carcinomas. Tumor pericytes are in general more loosely attached (c, d, f, h, i, k; white asterisks), and in some tumors like GBM less abundant (a, b) when compared to normal tissue. In addition, other tumors like mammary carcinomas contain clusters of pericytes that appear not to be distributed properly (i, l). Pericytes in tumors intend to bridge between blood vessels and to extend their cellular processes toward tumor cells (h, i, l).

rather abnormal or dysfunctional in tumors and therefore, until recently, have been neglected as important contributors to tumor angiogenesis. The exact causes of abnormal pericyte behavior are still unknown, but may include imbalanced endothelial-cell/pericyte signaling circuits and/or a limited pool of recruitable pericytes (Abramsson et al., 2002). It is also notable that hematopoietic cells from bone marrow that expressed the pericyte marker NG2 were recently identified in close contact with blood vessels in xenograft Bl6-F1 melanoma tumor models (Rajantie et al., 2004) and in the transgenic model of pancreatic islet carcinomas (Song et al., 2005). This suggests that recruitment of bone-marrow-derived cells to sites of a growing vasculature is not limited to endothelial cells, but can also include pericytes.

It is surprising that normal vessels can apparently tolerate a substantial reduction in the density of pericytes, at least in mice; although a reduction in pericyte density produces functionally and structurally abnormal vessels, only pericyte reductions of >90% are lethal (Abramsson et al., 2003). This observation suggests that even small numbers of pericytes, as observed in tumors, can still be functional and important for vessel stability

and endothelial-cell survival. Recent experiments targeting pericytes in tumors support this view. Glioblastomas contain a substantial fraction of blood vessels that are not covered by pericytes (see also Fig. 3). It has been demonstrated that these vessels are more dependent on VEGF as an endothelial survival factor, because they are selectively eliminated when VEGF is withdrawn from the tumors (Benjamin et al., 1999). In contrast, glioblastomas or fibrosarcomas that overexpress PDGF-B exhibited an increased pericyte density around blood vessels (Guo et al., 2003). Blocking PDGFR signaling in a transgenic mouse model of pancreatic islet carcinogenesis (Rip1Tag2) with the receptor tyrosine kinase inhibitor SU6668 caused regression of blood vessels, which was due to the detachment of pericytes from tumor vessels, and thereby restricted tumor growth and stabilized the cancer (Bergers et al., 2003). Similarly, SU6668 detached and diminished pericytes in xenotransplant tumors and thereby restricted tumor growth (Reinmuth et al., 2001; Shaheen et al., 2001). The functional significance of PDGFRβ signaling in tumor pericytes was confirmed by studies in PDGF-B retention mice. PDGF-B$^{ret/ret}$ mice lack the C-terminal retention motif in PDGF-B that mediates PDGF-B binding to proteoglycans at the cell surface and in the ECM. These mice are viable but have fewer pericytes, as they lack proper recruitment and integration of pericytes within the vessel wall, particularly in the retina and kidney (Lindblom et al., 2003). Implanted tumors in PDGF-B$^{ret/ret}$ mice are hemorrhagic and contain few pericytes around the tumor blood vessels that become hyperdilated. It is notable that ectopic expression of PDGF-B in those tumor cells was able to increase pericyte density but failed to cause pericytes to attach more firmly to blood vessels, which indicates that localized PDGF-B from the endothelium is essential for proper pericyte adhesion to the vessel wall (Abramsson et al., 2003). These data suggest that tumors use the same signal mechanisms that are used in developmental angiogenesis. These results also imply that tumor pericytes, albeit less abundant and more loosely attached than normal pericytes, still regulate vessel integrity, maintenance, and function. The fact that tumor vessels without pericytes appear more vulnerable suggests that they may be more responsive to antiendothelial drugs. Indeed, combinations of receptor tyrosine kinase inhibitors that target endothelial cells and pericytes by blocking VEGF and PDGF signaling, respectively, more efficiently diminished tumor blood vessels and tumors than any of the inhibitors individually (Bergers et al., 2003). The same effect was achieved when PDGF inhibitors were combined with an antiangiogenic chemotherapy regimen that targeted endothelial cells (Pietras and Hanahan, 2005). Targeting PDGFR signaling disrupted pericyte support, while the antiangiogenic chemotherapy targeted the sensitized endothelial cells, collectively destabilizing the preexisting tumor vasculature. When considered together, these data provide evidence that pericytes are potentially important and functional vascular-cell components in tumors that elicit survival mechanisms to establish and maintain tumor vessels.

Summary

Collectively, the data described above are far from complete, but should rather accentuate that pericytes are essential components of the microvessel wall, with important metabolic, signaling, and mechanical roles that support endothelial cells in a manner that depends on tissue and angiogenic stage. Pericytes are also proposed to be important contributors to the regulation of pathological angiogenic processes like diabetic retinopathy and tumor angiogenesis. During tumor propagation, pericytes, in spite of having structural abnormalities, still appear to provide functions necessary for vessel maintenance and endothelial-cell survival. These findings have led to a new concept of antiangiogenic therapy: combined targeting of endothelial cells and pericytes to more efficiently diminish blood vessels and halt subsequent tumor growth.

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