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Recruitment and retention: factors that affect pericyte migration

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Abstract

Pericytes are critical for vascular morphogenesis and contribute to several pathologies, including cancer development and progression. The mechanisms governing pericyte migration and differentiation are complex and have not been fully established. Current literature suggests that platelet-derived growth factor/platelet-derived growth factor receptor- β , sphingosine 1-phosphate/endothelial differentiation gene-1, angiopoietin-1/tyrosine kinase with immunoglobulin-like and EGF-like domains 2, angiopoietin-2/tyrosine kinase with immunoglobulin-like and EGF-like domains 2, transforming growth factor β /activin receptor-like kinase 1, transforming growth factor β /activin receptor-like kinase 5, Semaphorin-3A/Neuropilin, and matrix metalloproteinase activity regulate the recruitment of pericytes to nascent vessels. Interestingly, many of these pathways are directly affected by secreted protein acidic and rich in cysteine (SPARC). Here, we summarize the function of these factors in pericyte migration and discuss if and how SPARC might influence these activities and thus provide an additional layer of control for the recruitment of vascular support cells. Additionally, the consequences of targeted inhibition of pericytes in tumors and the current understanding of pericyte recruitment in pathological environments are discussed.

Keywords

Pericytes; Smooth muscles cells; SPARC; TGF β ; Angiogenesis

Introduction

Pericytes are critical to appropriate vascular morphogenesis and contribute to several pathologies, including cancer development and progression [1]. The mechanisms governing pericyte migration and differentiation are complex and have not been fully established. In addition to their function as vascular cells, pericytes can function as mesenchymal progenitor cells that contribute to local fibrosis [2]. Thus, a greater understanding of the factors that affect pericyte migration might also provide regulatory insight on an important source of progenitor cells.

Unlike normal vasculature, blood vessels within tumors are leaky, tortuous, and exhibit abnormal pericyte coverage [3–6]. However, pericytes are a significant part of the tumor microenvironment [7], as they contribute to vascular function and also serve as a potential pool of stromal progenitor cells. In contrast to other cellular components of tumor stroma, little is known about the biology of their recruitment and interaction with other cells in the tumor micro-environment [1]. Furthermore, the therapeutic efficacy of inhibition of pericyte function in tumors is not clear. This is in contrast to the effect of targeted inhibition of endothelial cells (ECs) or cancer-associated fibroblasts (CAFs), which can provide a therapeutic advantage [8]. Antiangiogenic therapy is believed to be effective at treating some types of human cancer by selectively ablating vessels that lack pericyte coverage. This can provide an acute and potentially transient resolution of the poor transport of macromolecules through tumor vasculature [9]. However, this ‘vascular normalization’ likely does not persist in most solid tumors upon chronic angiogenic suppression. Inhibition of pathways that participate in pericyte recruitment in combination with anti-angiogenic strategies has in some pre-clinical models shown increased efficacy of tumor control compared to antiangiogenic therapy alone [10]. These studies demonstrate that modulation of pericyte behavior might have clinical benefit. However, ablating pericytes has also been implicated in promoting tumor invasion and metastasis [11]. Thus, further development of pericyte-targeted therapeutic approaches requires a better understanding of the biological factors that control pericyte behavior.

Ontogeny of pericytes

endothelial cells (ECs) have intimate contact with mural cells, which participate in the deposition of a perivascular extracellular matrix (ECsM). Among mural cells, pericytes are ubiquitously present and constitute a heterogeneous population of cells in close contact with ECs. Unlike ECs, pericytes do not form a continuous sheath or contribute directly to the luminal surface of the capillary network. Instead, they are found as single cells, distributed at discontinuous intervals along the shared basement membrane [12]. Pericytes are physically embedded in the basement membrane between the extracellular environment and the capillary tube [13]. These cells protrude plasma membrane extensions over endothelial tubes, are believed to provide survival signals to the underlying endothelial layer, and are required for stable vessel function. Furthermore, the development and function of the vascular network relies in part on the presence of pericytes [14].

Pericytes are found around blood capillaries, precapillary arterioles, postcapillary venules, and collecting venules, and as a result are morphologically heterogeneous and distinct in different organs [15]. Pericyte density varies between organs and vascular beds, and the proportion of the endothelial abluminal surface that is pericyte-associated is often distinct between vascular beds [1]. Morphologically, pericytes range from fattened or stellate-shaped solitary cells with multiple cytoplasmic processes surrounding the capillary endothelium, to that of more rounded and compact cells. The latter is usually in contact with a large vessel area, while the former is not. For example, some mesangial cells in the kidney are thought to be a type of pericyte that form a branched multicellular core around which the glomerular capillaries wind [15, 16]. Pericytes are typically defined by location and interaction with ECs as demonstrated by electron microscopy. Concomitant with location, pericyte expression of distinct proteins has been evaluated. Due to the heterogeneity of pericytes, there are many molecular markers, such as alpha smooth muscle actin (α SMA), non-muscle myosin, tropomyosin, desmin, nestin, platelet-derived growth factor receptor- β (PDGFR- β), aminopeptidase A, aminopeptidase N (CD13), sulfatide, or nerve/glia antigen-2 (NG2) proteoglycan that have been used to identify pericytes [17]. However, heterogeneity of marker expression by pericytes in distinct vascular beds is common. For example, pericytes

on capillaries express desmin but typically are negative for α SMA. Pericytes localized to venules express desmin and α SMA [17, 18].

Pericytes in the tumor microenvironment

Angiogenesis entails a complex cascade of molecular events that depend on EC migration, proliferation, and the stabilization of newly formed vessels [19, 20]. This process is required for normal development and the progression of many disease states. The development of a functional vasculature requires the tubular organization of ECs and their maturation as structurally stable and functionally adjustable capillary networks. Pericytes promote EC survival which is critical for vessel stabilization and maturation. Pericytes have multiple functions relevant to the development and maintenance of tumor vasculature. They contribute to vascular development, stabilization, maturation, and remodeling [15]. In normal physiological conditions, pericytes are in appropriate abundance and are tightly associated with the endothelial abluminal surface. In the tumor microenvironment, however, pericytes are less abundant and develop altered morphologies and marker expression [15, 21, 22]. It is likely that mural cell deficiency contributes to the abnormal functional properties of tumor vessels, such as increased vessel leakiness [19].

The initial stage of angiogenesis begins with pericyte – EC dissociation followed at later stages by EC migration, proliferation, and subsequent endothelial tubulogenesis and vessel stabilization. During the initial stage, pericytes adopt an angiogenic phenotype morphologically evident as bulging cells with shortened cytoplasmic processes [11]. Detachment of pericytes from the vessel wall is a critical early step in the angiogenic process that facilitates EC migration and tubulogenesis (Fig. 1). Furthermore, a consequence of pericyte detachment from the vascular basement membrane is increased vessel permeability and leakage of plasma proteins that serve as a provisional matrix for EC and pericyte migration [23]. A better understanding of the variable expression of different pericyte markers has revealed the presence of pericytes in the immature vascular plexus, leading to the view that pericyte-mediated signaling may also be essential for the growth phase of angiogenesis [24].

Regulation and mechanics of pericyte migration

The recruitment of and interaction with pericytes are crucial for the stabilization of nascent endothelial tubes. Pericyte interaction with ECs, stromal cells, and the ECM is required in physiological events such as EC sprouting [25, 26]. Specifically, factors such as vascular endothelial growth factor (VEGF) and angiopoietin-1 (Ang1) activate ECM-degrading enzymes including matrix metalloproteinases (MMPs) which modify the ECM and facilitate EC migration [27]. Specialized ECs regulate the sprouting of vessels in physiological and pathological situations. A subtype of ECs, termed tip cells, lead the new sprout while stalk cells follow the tip cell. The stalk cells proliferate and form a lumen, while the nonproliferating tip cells continue to lead the persistent sprout [28].

Under physiological conditions, pericyte recruitment around vascular ECs is controlled by multiple pathways (Fig. 2; Table 1): platelet-derived growth factor (PDGF)/PDGFR- β , sphingosine 1-phosphate (S1P)/endothelial differentiation gene-1 (EDG-1), Ang1/tyrosine kinase with immunoglobulin-like and EGF-like domains 2 (Tie2), Ang2/Tie2, transforming growth factor β (TGF β)/activin receptor-like kinase 1 and 5 (ALK1 and ALK5), Semaphorin-3A (Sema3A)/Neuropilin (Nrp), and matrix metalloproteinase (MMP) activity [29]. Interestingly, many of these pathways are affected directly by secreted protein acidic and rich in cysteine (SPARC), a matricellular protein that is expressed in remodeling tissue and has been implicated as an extracellular chaperone for ECM proteins and growth factor pathways [30, 31].

PDGF

The PDGF family is comprised of four polypeptide chains (PDGF-A, B, C, and D) encoded by four different genes that result in five dimeric isoforms (PDGF-AA, -BB, -CC, -DD, and -AB) that bind to one of three PDGF receptors (PDGFR- $\alpha\alpha$, PDGFR- $\beta\beta$, or PDGFR- $\alpha\beta$) [32]. Specific ligation induces receptor activation and stimulates downstream signaling cascades that promote cellular proliferation, migration, survival, and VEGF expression [33–35]. During angiogenesis, PDGF is released from angiogenic ECs [1]. In fact, the PDGF/PDGFR- β pathway was the first pericyte-centered pathway to be shown to be required for angiogenesis. Binding of PDGF-BB or PDGF-AB to PDGFR- β on the pericyte induces activation of Ras/Rho/Rac as well as protein kinase C (PKC), which cooperatively induce pericyte migration. Ras also activates FAK and eRK, which drive pericyte proliferation [12]. Lack of PDGF expression results in the failure of vessels to recruit pericytes, microaneurisms, and embryonic lethality thus documenting the critical function of pericytes and this pathway to the angiogenic process and tissue homeostasis [36].

S1P/EDG-1

Sphingosine 1-phosphate (S1P) is generated by phosphorylation of sphingosine by sphingosine kinase (SphK), and is degraded by S1P phosphatases and S1P lyases [37]. S1P regulates mural cell proliferation [38–41], migration [39, 41], response to PDGF and EGF [42], and differentiation [42]. Beside intracellular functions, S1P in mural cells interacts with its family of membrane-bound G protein-coupled receptors [43], with EDG-3 and EDG-5 as the most prominent [38–41]. The majority of S1P present in serum is secreted by mast cells, monocytes and activated platelets [37]. Expression and activation of EDG-1 (S1P1) on ECs enhances the production of ECM that promotes the recruitment of pericytes [29, 37]. In addition, activation of EDG-1 on pericytes facilitates their migration to ECs [37]. Defects in vasculature and discontinuous coverage of blood vessels by mural cells are seen following ablation of sphingosine kinases [44] or knockout of EDG-1 [29, 45]. This is due in part to interruptions in N-cadherin trafficking and association with adherens junctions [46, 47] presumably in ECs, as endothelial-specific knockout of EDG-1 mimics the phenotype of the mice globally deficient in the receptor [48].

In contrast, constitutive global deletion of EDG-5 (S1P2) enhances mural cell recruitment to tumor blood vessels, potentially through the production of soluble factors that promote vascular maturation and the loss of chemorepulsion mediated by EDG-5 on mural cell precursors [49, 50]. Consistent with this, activation of EDG-5 by S1P inhibits mural cell migration and spreading through inhibition of Rac [49, 50]. Inhibition of EDG-5 with antagonist JTe-013 can restore mural cell migration in response to PDGF [50]. However, genetic ablation of EDG-5 selectively in mural or ECs has not been reported, thus deciphering the direct effects of EDG-5 on pericytes versus its function in ECs is unclear at the present time.

Ang1/Ang2

Ang1, produced by vSMC and pericytes, binds to the Tie2 receptor expressed by ECs. The Ang1/Tie2 complex maintains and stabilizes mature vessels by promoting interactions between ECs and pericytes and by mediating cell–ECM interactions in vessel morphogenesis though the mechanisms of how this pathway functions is still not fully understood [51]. The formation of the Ang1/Tie2 complex results in the induction of heparin-binding epidermal growth factor-like growth factor (HB-EGF) expression, leading to mural migration via activation of epidermal growth factor receptors (EGFRs) erbB1 and erbB2 [12].

Ang1 is a critical factor for blood vessel stabilization after formation of a nascent parent EC tube. Ang1 induces vessel stabilization by inhibiting endothelial permeability and stimulating EC-dependent release of cytokines including TGF β and PDGF-B, which result in recruitment of pericytes to the nascent vessel [52]. Ang1-mediated recruitment of pericytes is enhanced by VEGF, which stimulates MMP-1 activity [12, 53]. Furthermore, Ang1 induces actin remodeling and migration in ECs in vitro as well as angiogenesis in matrigel plugs in vivo [54].

while Ang1 is expressed by pericytes, Ang2 is expressed by ECs and acts as an autocrine regulator of EC function [55]. In contrast to Ang1, Ang2 destabilizes vessels resulting in permeability and induction of dissociated cell–cell contacts [15]. Falcon et al. [56, 57] have demonstrated the rescue of normal vessel integrity as well as an enhanced pericyte recruitment in the absence of Ang2. Most notably, Ang2 inhibition led to the reduction of EC apoptosis and overall tumor vessel permeability, as well as the delay in tumor growth and development [56, 57]. Concurrently, the expression of Ang2 has also been implicated in driving metastasis [58] which correlates with the disruption of pericyte coverage. This strongly depicts the contrasting functionalities of Ang1 and Ang2; however, both are important regulators of vessel integrity and pericyte recruitment.

Sema3A/Nrp

The semaphorins are a complex family of membrane-bound and secreted proteins originally identified as axon guidance cues [59, 60]. This family of proteins signals through two major receptor families, Plexins and Neuropilins (Nrp) [59, 60]. Most secreted class 3 semaphorins, such as Sema3A, are known to bind to a holoreceptor complex consisting of Nrps as the ligand binding subunit and Plexins as the signal transducing subunit [59]. Sema3A is expressed in ECs and regulates EC migration and vessel remodeling in an autocrine manner [59, 61].

Recent reports have demonstrated that Sema3A expression and signaling through Nrp1 results in an enhanced vascular coverage through pericyte mobilization and the regulation of blood vessel remodeling [59]. Sema3A re-expression in vivo can increase pericyte coverage while reducing tumor growth and angiogenesis, giving rise to normalized and more functional blood vessels [59, 62]. Concurrently, several studies have identified Sema3A as a novel therapeutic target through its actions as an antiangiogenic and tumor-suppressing agent [62, 63]. Furthermore, recent work demonstrated that genetic deletion of Sema3A results in animals with severe renal vascular patterning defects [64], further highlighting the importance of Sema3A for vascular homeostasis.

MMPs

Matrix metalloproteinases (MMPs) are a family of at least 26 endopeptidases that are associated with a number of invasive and metastatic human malignancies and are involved at multiple levels of tumor progression [65]. These proteinases, including MMP-2 and MMP-9 (gelatinases A and B, respectively), can degrade the ECM and basement membrane as well as cleave growth factors, cytokines, and growth factor binding proteins [66, 67]. MMPs have been linked to tumor cell invasion and metastasis and participate actively in remodeling the tumor microenvironment [68, 69]. In many cancers, stromal cells rather than neoplastic cells are the source of MMPs, including MMP-9 [70, 71] and MMP-13 [72]. The expression of these MMPs in stromal cells is in some cases stimulated by factors expressed by neoplastic cells [73, 74]. In particular, MMP-2 and MMP-9 can stimulate angiogenesis by promoting the invasion of the ECM by ECs and by increasing the bioavailability of VEGF [75]. MMPs are thought to participate in tumor vessel maturation, including pericyte recruitment. Mechanisms of this recruitment may include the direct promotion of pericyte proliferation

and protection against apoptosis, release of growth factors stimulating pericyte activation, and the promotion of pericyte migration by ECM degradation [12].

In human glioma and breast cancer, MMP-9 is expressed by vascular smooth muscle cells and in particular by pericytes at the proliferating tumor borders [76, 77]. In human neuroblastoma xenografts, pericyte coverage along tumor microvessels is decreased by 50 % in tumors grafted to MMP-9-deficient mice and transplantation with MMP-9-expressing bone marrow cells restores the formation of pericyte-associated tumor vessels [78]. Furthermore, implantation of tumor cells engineered to express high levels of active MMP-9 results in a tumor vascular network that has increased pericyte-associated vessels. In addition, forced expression of TIMP-3, a natural inhibitor of MMPs, results in decreased pericyte recruitment to blood vessels in neuroblastoma and melanoma tumor models [79]. The importance of MMPs to pericyte migration and EC association in normal tissue is supported by studies in mice deficient in membrane-type 1 matrix metalloproteinase (MT1-MMP), demonstrating a robust decrease in pericyte density concurrent with a disrupted vessel integrity in the brain [80]. Furthermore, active MMP-2 has been detected by immunolocalization in pericytes of telencephalic vessels of human embryos [81]. These observations are consistent with models of pathological neovascularization and vascular remodeling. For example, expression of MMP-1, -3 and -9 by vSMC was induced by arterial injury and exposure to basic fibroblast growth factor (bFGF) or PDGF [82, 83].

TGF β

Transforming growth factor β (TGF β) is a pleiotrophic cytokine expressed by ECs and pericytes during angiogenesis. TGF β is secreted in a latent complex which must be cleaved or otherwise manipulated to expose the active protein [84–86]. The precise cellular responses induced by TGF β depend on the specific TGF β receptors expressed and the level of ligand and receptor expression [87]. Receptors to active TGF β are ubiquitous throughout all tissues; therefore, the activation of latent TGF β and the bioavailability of active TGF β are regulated tightly in vivo [88–90]. Once activated, TGF β binds to TGF β receptor 2 (TGF β R2), which is critical for the activation of TGF β receptors activin receptor-like kinase 1 and 5 (ALK1 or ALK5) [91]. ALK1 and ALK5 expression by pericytes has remarkably opposite effects on pericyte recruitment and proliferation [91]: ALK1 activation stimulates pericyte recruitment while ALK5 activation results in cellular quiescence and vessel stabilization [92, 93]. The mechanisms that spatially regulate the activation of latent TGF β , ALK1, and ALK5 are not defined completely.

Transforming growth factor β (TGF β) signaling is required for the maintenance of contact between ECs and pericytes. TGF β promotes the expression and flux of basement membrane proteins [94–96], such that deficiency in TGF β results in disruption of the vascular network which is due in part to the disordered interaction between pericytes and nascent blood vessels. Concurrently, vascular networks lacking TGF β activity appear highly abnormal, lack pericyte coverage, exhibit tortuous vessels and areas of EC hyperplasia and vessel hemorrhaging, reminiscent of tumor vasculature. In mice, the abrogation of multiple factors in the TGF β cascade, including ALK1 and ALK5, results in embryonic lethality due to defective angiogenesis and flawed vascular development [96, 97].

SPARC

Secreted protein acidic and rich in cysteine (SPARC) is a matricellular protein that functions as an extracellular chaperone for collagen deposition and regulates pericyte behavior [6, 30, 31]. SPARC also influences the activity of multiple growth factors that participate in the angiogenic process [31]. We have found that tumors grown in SPARC-deficient (*Sparc*^{-/-}) animals develop blood vessels that have a reduced level of pericyte association when

compared to tumors grown in wild-type (WT) animals [6, 98, 99]. Careful evaluation of in vitro migration of pericytes isolated from wT and *Sparc*^{-/-} mice revealed that SPARC limits the activation of latent TGFβ in an endoglin- and integrin-dependent manner [6]. In particular, we found that pericytes express high levels of SPARC in normal adult mouse pancreas as well as in lesions of pancreatic ductal adenocarcinoma (PDAC) undergoing angiogenesis [6]. The absence of SPARC results in more efficient activation of latent TGFβ, which limits pericyte migration via the canonical TGFβ signaling cascade. We found that pericytes express endoglin, an accessory TGFβ receptor, and that endoglin and an αv integrin cooperate to activate latent TGFβ in an MMP-independent manner. SPARC interferes with the activation of latent TGFβ by binding directly to endoglin and preventing association of endoglin with αv integrin [6]. SPARC's capacity to block TGFβ activity in pericytes supports previous work showing that SPARC inhibits TGFβ-mediated SMAD phosphorylation and αSMA expression in fibroblasts [100]. Identification of SPARC expression by pericytes in resting and angiogenic vasculature suggests that SPARC activity is not regulated by expression alone. SPARC's function as an inhibitor of TGFβ activation and positive regulator of pericyte migration and recruitment must be spatially and temporally controlled during angiogenesis, as pericytes must eventually activate TGFβ signaling at the point of contact with nascent vessels. The constitutive activity of SPARC would interfere with vessel stabilization if expression was the only level of regulation. A potential method of regulation of SPARC activity is MMP-mediated degradation. SPARC is a substrate for multiple MMPs, including MMP3 [101]. MMP3 secretion and activation increases during angiogenesis, thus MMP3-mediated SPARC degradation may provide a mechanism to spatially and temporally regulate pericyte migration.

Secreted protein acidic and rich in cysteine (SPARC) has also been implicated in the regulation of PDGF receptor activation by directly interfering with the ligation of PDGFR by PDGF-BB and PDGF-AB [102, 103]. However, the consequences of SPARC inhibition of PDGFR activation are not clear. Additionally, the effect of SPARC on PDGFR activation has not been explored in vivo. Furthermore, we found that wT and *Sparc*^{-/-} pericytes respond equally well to PDGF-BB stimulation in vitro [6]. Finally, pericyte recruitment is reduced in *Sparc*^{-/-} animals and inhibition of TGFβ completely rescues the phenotype in vitro suggesting that pericyte migration, proliferation, and recruitment are multifaceted events.

Targeting pericytes

Pericytes migrate during the early phases of physiological angiogenesis to make way for growing sprouts [18], or in response to stress or injury [104]. Abnormal pericyte numbers may be an indication of altered vascular function as pericyte density has been linked to several normal and pathological conditions. Altered pericyte to EC ratios have been observed following traumatic brain injury [104], stroke [105], multiple sclerosis [106–110], diabetic retinopathy [111], aging [112, 113], Alzheimer's disease, formation of hypertrophic scars and keloids [114], cancer [115–118], hyperglycemia [119], pulmonary hypertension [120], and throughout development [121].

In vitro studies suggest that pericytes provide ECs with survival signals through cell–cell contacts [8]. As a result, ECs not associated with pericytes are more sensitive to growth factor withdrawal or inhibition [122]. Pericytes also contribute to vascular stability by limiting EC proliferation. Concurrently, the density of EC proliferation is reduced when vessels are covered by pericytes [123]. Furthermore, pericyte coverage correlates inversely with the incidence of metastasis [9]. Indeed, targeting of pericytes by the use of PDGFR inhibitors concurrently induces pericyte detachment and an enhanced sensitivity of ECs to various antiangiogenic regimens, including inhibition of VEGF signaling and metronomic

chemotherapy [124–126]. Interestingly, a number of clinically approved targeted agents, including sunitinib and sorafenib, incorporate PDGF and VEGF receptor-inhibitory action.

Previous work has demonstrated that targeting PDGFR- β , which is expressed by pericytes, resulted in reduction of blood vessel number and control or reduction in tumor size in models of islet, colon, and prostate cancers [125, 127–129]. The resulting tumor vasculature was comprised of vessels that lacked pericyte coverage and exhibited hyperdilation. Additionally, recent reports have demonstrated the invasive potential of neutrophils in the absence of tight EC–pericyte interactions. Neutrophils are a critical component of the adaptive immune response, utilizing actin-based motility to crawl through the body's tissue and find infected target cells [130]. Proebstl et al. [131] demonstrated that neutrophils follow other neutrophils within the venular wall. This was the first observation of neutrophil-specific directional cues along pericyte processes [131]. The resultant effect of this phenomenon was that clear hot spots were noted where multiple neutrophils were seen to exit venular walls through the same pericyte gap. These findings highlight the significance of tumor-associated pericytes to maintaining a functional vasculature, and provide reasons for furthering the understanding of the mechanisms of pericyte regulation.

Concluding remarks

Pericyte recruitment is a required step of the angiogenic cascade. Multiple signaling molecules, including PDGF-BB, Ang1, and S1P, act together to induce the mobilization of pericytes to nascent vessels. TGF β signaling at the EC/pericyte interface then blocks pericyte migration and induces basement membrane deposition. TGF β signaling is also required for maintaining contact between ECs and pericytes. Failure for pericytes to become properly associated with nascent blood vessels results in destabilization of the nascent vasculature. Morphologically, such vascular networks appear highly abnormal, and in addition to lacking pericyte coverage, they exhibit tortuous vessels and areas of EC hyperplasia and vessel hemorrhaging. Such vessels result from angiogenesis occurring in solid tumors; therefore, the functional significance of pericytes to tumor vasculature is a clinically relevant area of basic research.

One of the key functions of pericyte coverage in a vascular network is to provide growth factors to the underlying endothelium; these in turn induce survival signals that result in sustained vessel viability. One hypothesis is that in the presence of pericytes, vessels are not susceptible to depletion of exogenous growth factors in terms of their viability, and that, in the absence of pericyte coverage, they become reliant on exogenous growth factors. As a result, anti-angiogenic pruning of pericyte-deficient tumor blood vessels can result in a more efficient vascular network. This concept, termed 'vascular normalization', is thought to enhance the delivery of chemotherapeutics to tumors. However, there are conflicting reports on the acute effects of anti-angiogenic therapies on vascular function [132–135]. Furthermore, we have found that chronic therapy with anti-angiogenic strategies can dramatically reduce vascular function in the tumor microenvironment [136]. An attractive solution might be to inhibit growth factor pathways that induce angiogenesis and pericyte recruitment. Furthermore, as pericyte-mediated blood vessel stabilization appears to control the exit of tumor cells into the vascular system, this is an additional consideration for the development of anti-angiogenic strategies that also reduce pericyte recruitment. Recently developed second and third generation small molecule receptor tyrosine kinase inhibitors that show potent activity against multiple angiokines have shown striking therapeutic activity in preclinical models [136]. In addition, we propose that understanding the contribution of SPARC to the pericyte–EC interaction might illuminate novel biology that is exploitable for therapy.

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Fig. 1.

Factors involved in pericyte recruitment and retention during angiogenesis. Angiogenesis requires pericyte–endothelial cell (EC) dissociation, pericyte migration and proliferation. Pericyte mobilization is driven by several factors (PDGF, Ang1, ALK1, S1P, eGF, and Sema3A) and is negatively regulated by others (TGF β , Ang2, ALK5). The mobilized pericyte is recruited to a nascent vessel through the activity of factors such as SPARC, MMP9, and Sema3A, which results in vessel stabilization

Fig. 2.

Modulation of pericyte recruitment at the pericyte-EC interface. Pericyte recruitment to vascular endothelial cells (ECs) is controlled by a complex interplay between multiple signaling pathways. The schematic illustrates some of the better understood signaling cascades that affect pericyte recruitment. PDGF and EGF are released by ECs and bind to PDGFR-β and eGFR (respectively) on pericytes, stimulating pericyte migration and proliferation. S1P binds to EDG-1 expressed by pericytes and ECs, promoting pericyte migration. The Ang1/Tie2 complex enhances pericyte-EC interaction and induces the expression and release of EGF. Ang2 released by ECs binds to Tie2 on ECs and promotes pericyte-EC dissociation. TGFβ binds to TGFβR2, leading to the activation of ALK1 or ALK5. ALK1 activation stimulates pericyte recruitment while ALK5 activation results in cellular quiescence. Sema3A is released by ECs and forms a complex with Nrp1 and Plexin. This promotes enhanced EC migration and vessel remodeling. MMP9 and SPARC drive ECM remodeling, which facilitates growth factor activation and stimulates pericyte recruitment. SPARC, produced by ECs and pericytes, limits the activation of TGFβ and interferes with the ligation of PDGFR and eGFR to their respective ligands

Table 1
Factors affecting pericyte migration

Regulators of pericyte biology				
Molecule	Effect on migration/recruitment	Effect of deficiency	Other functions	References
PDGF	↑	Loss of pericyte recruitment to vessels, microaneurisms, embryonic lethality	Receptor activation can lead to proliferation, migration, survival, VEGF expression, activation of Ras/Rho/Rac, PKC, FAK, ERK	[1, 12, 33–36]
S1P/EDG-1	↑	Defects in vasculature and discontinuous coverage of blood vessels	Regulates cellular response to PDGF and eGF, enhances ECM production	[37–50]
Ang1	↑	Embryonic lethality due to cardiovascular failure, defective angiogenesis, reduced pericyte coverage	Critical factor for blood vessel stabilization, actin remodeling, migration	[12, 15, 51–54]
Ang2	↓	Normal vascular development, lymphatic defect	Angiogenic sprouting in tumors, inversely correlated with pericyte coverage	[15, 55–57]
Sema3A/Nrp	↑	Reduced vessel density, tortuous vessels	Induces vessel stabilization, enhanced pericyte recruitment and content, reduces tumor growth and angiogenesis	[59, 62, 63]
MMP9	↑	Reduced recruitment and vessel association of pericytes	ECM remodeling, growth factor cleavage, vessel maturation, protection against apoptosis	[12, 65–70, 73–75, 78, 79, 101]
TGFβ	↓	Disruption of the vascular network due to disordered interaction between pericytes and nascent blood vessels, deficiency of pericyte coverage	Ubiquitous, promotes expression and flux of ECM proteins	[87–90, 94–96, 137, 138]
ALK1	↑	Embryonic lethality, flawed vascular development, defective angiogenesis	Induces pericyte proliferation and recruitment to vessels	[97, 138]
ALK5	↓	Embryonic lethality, flawed vascular development, defective angiogenesis	Induces pericyte contractile protein expression, promotes ECM production, stabilization of nascent capillary tube	[97, 138]
SPARC	↑	Reduced vessel association of pericytes	Extracellular chaperone for collagen deposition, influences activity of multiple angiogenic growth factors, regulates MMP9-mediated pericyte recruitment, interferes with activation of latent TGFβ, regulates PDGF receptor activation	[6, 31, 98–103, 137]