Pericyte Dynamics during Angiogenesis: New Insights from New Identities

Peter C. Stapor Richard S. Sweat Derek C. Dashti Aline M. Betancourt
Walter Lee Murfee

Department of Biomedical Engineering, Tulane University, Lindy Boggs Center, New Orleans, La., USA

Key Words

Pericyte · Angiogenesis · Capillary sprouting · Phenotype

Abstract

Therapies aimed at manipulating the microcirculation require the ability to control angiogenesis, defined as the sprouting of new capillaries from existing vessels. Blocking angiogenesis would be beneficial in many pathologies (e.g. cancer, retinopathies and rheumatoid arthritis). In others (e.g. myocardial infarction, stroke and hypertension), promoting angiogenesis would be desirable. We know that vascular pericytes elongate around endothelial cells (ECs) and are functionally associated with regulating vessel stabilization, vessel diameter and EC proliferation. During angiogenesis, bidirectional pericyte-EC signaling is critical for capillary sprout formation. Observations of pericytes leading capillary sprouts also implicate their role in EC guidance. As such, pericytes have recently emerged as a therapeutic target to promote or inhibit angiogenesis. Advancing our basic understanding of pericytes and developing pericyte-related therapies are challenged, like in many other fields, by questions regarding cell identity. This review article discusses what we know about pericyte phenotypes and the opportunity to advance our understanding by defining the specific pericyte cell populations involved in capillary sprouting.

© 2014 S. Karger AG, Basel

Introduction

Vascular pericytes were first discovered in 1873 by the French physiologist, Charles Marie-Benjamin Rouget, and defined as ‘Rouget’ cells. Zimmerman introduced the term pericyte in 1923, describing them as a periendothelial support cell that wraps around the length of microvessels [1]. While the presence of pericytes in the microcirculation has been long-documented, their functional roles and importance in microvascular physiology have been largely underinvestigated. In more recent years, clarification of their versatile functionality has thrust them into the spotlight. Due to their putative influence on endothelial cells (ECs), pericytes have become a research area of growing interest and are increasingly being evaluated as potential targets for proangiogenic or antiangiogenic therapies.

Vascular pericytes are still defined morphologically as periendothelial support cells that elongate and wrap along ECs [2]. Through transbasement membrane interactions, pericytes are thought to regulate capillary diameter and physically influence EC behavior [3]. During capillary sprouting, vascular pericytes are recruited by ECs via platelet-derived growth factor (PDGF)-B/PDGF receptor- β (PDGFR- β) paracrine communication [4, 5]. We do know that the presence of pericytes is critical for normal angiogenesis [6]. However, the inability to distin-

guish specific pericyte subpopulations has limited (1) our understanding of how pericytes directly influence EC dynamics and (2) the development of pericyte-targeted angiogenic therapies. For example, pericytes have been shown to both stabilize and promote capillary sprouting. This apparent dual role motivates the following questions. Do specific subtypes of pericytes exist? Do pericytes change their phenotype in order to perform specific functions? In EC biology, specialized cell types are exemplified by the discovery of tip cells versus stalk cells during angiogenesis [7]. The tip cell paradigm highlights the potential for two neighboring cell types to be delineated by their phenotype and function. In the context of pericyte biology, determining the spatial and temporal phenotypic differences along angiogenic vessels versus mature vessels represents an equal opportunity to add new insight and perspective.

Common pericyte markers include smooth muscle α -actin (SMA), desmin, vimentin, PDGFR- β and neuron-gial antigen 2 (NG2). However, these markers identify multiple cell populations along the entire hierarchy of microvascular networks and are not angiogenesis-specific [3, 8, 9]. What is equally challenging is that pericyte marker expression varies across a network and per tissue. In this review, we propose a new paradigm for pericyte biology wherein transient expression patterns by pericyte subtypes during angiogenesis can potentially be used to distinguish pericyte functionality. In the first half of this review, we briefly summarize the current understanding of pericyte physiology and function. In the second half, we introduce the potential for defining functionally distinct pericyte subpopulations, by discussing the temporal expression dynamics of two neural molecules, NG2 and class III β -tubulin, by pericytes in the adult microcirculation.

Pericyte Dynamics Involved in Angiogenesis

Vascular pericytes are critical players in the microcirculation and their various functions are comprehensively covered by multiple reviews [3, 10–13]. However, the mapping of specific pericyte functions and downstream mechanisms to specific pericyte dynamics remains elusive. Pericytes are defined morphologically as cells within the basement membrane that elongate along the long axis of capillaries, and wrap around ECs with shorter processes [3, 14]. Direct interdigitations protrude from pericytes into ECs at their interface, and endothelial processes reciprocate [15]. Pericytes are functionally associated with

the regulation of blood vessel diameter, vessel permeability, EC proliferation, angiogenesis and even leukocyte recruitment [11, 14, 16–18]. Recent evidence shows that pericytes mediate capillary diameter by contraction in response to electrical or neurotransmitter stimulation [19], implicating pericytes as highly localized modulators of blood flow. Lack of pericyte recruitment leads to endothelial hyperplasia and increased vascular permeability [6, 20], associating pericytes with vessel stability and maturation.

Work in the pericyte biology field has established several mechanisms that regulate pericyte-endothelial communication [3, 11, 13]. The prominent signaling pathways are PDGF-B/PDGFR- β , angiopoietin 1 (Ang1)/Tie2 and transforming growth factor- β (TGF- β) [21], which regulate pericyte recruitment, EC viability and mural cell differentiation, respectively [22]. Pericytes can also influence the local extracellular matrix (ECM) to guide endothelial migration by modulating deposition of basement membrane proteins [23]. As pericytes have been linked to microvascular function, it makes sense that their coverage is heterogeneous among organs depending on the needs of the local tissue environment. In the brain, for example, a high density of pericytes tightly regulates the blood-brain barrier as a selectively permeable fortification which prevents fluctuations in the sensitive environment of the central nervous system (CNS). Pericyte coverage in skeletal muscle, however, may be as low as one pericyte for every 100 ECs [2]. Liver (stellate cells) and kidney pericytes are specialized to aid parenchymal functions. Recently, pericytes of both organs have garnered attention for their central role in liver and kidney fibrosis [24–26].

Alterations in pericyte coverage are not surprisingly linked to multiple pathologies. In the case of diabetic retinopathy, the disassociation of pericytes from ECs has been associated with uncontrolled angiogenesis [12]. Decreased pericyte coverage in the brain causes neurodegeneration [27]. This is similar to a tumor growth scenario, in which loose pericyte coverage leads to vascular dysfunction [28, 29]. Pericytes contribute to angiogenesis during tumor vascularization. However, loss of pericytes or abnormal pericyte function in tumors increases vessel permeability and decreases vessel integrity, which enhances tumor metastatic potential [28, 30]. These examples illustrate the dual role of pericytes in regulating angiogenesis and vessel integrity.

During angiogenesis, pericyte dynamics include recruitment, ECM modulation, paracrine signaling and direct interactions with ECs (fig. 1) [3]. They have also been

shown to lead sprouting ECs and bridge the gaps between two sprouting segments in some cases [31, 32]. ECs of newly forming vessels recruit pericytes by secreting PDGF-B [4, 5]. Manipulating pericyte investment by altering recruitment to the endothelium can cause lethal microvascular dysfunction, deviate vascular patterning during development and affect the formation of new vessels during physiological and pathological angiogenesis [3, 33]. As previously mentioned, this can be attributed to pericyte regulation of EC proliferation. Recent work has also identified pericytes as important players in regulating basement membrane organization. During developmental vascular assembly, pericytes catalyze deposition of basement membrane proteins including collagen IV, laminin and fibronectin [11]. Pericyte actomyosin-mediated contraction can modulate the local mechanical environment for adjacent ECs [34]. EC behavior can also be mediated by direct connections between the two cell types via N-cadherin adhesions [13, 35], pericyte NG2 binding to $\beta 1$ integrins on ECs [29, 36] and, potentially, connexin 43 [37, 38]. Furthermore, pericyte-EC interactions induce changes in the expression of ECM-binding integrins by both cell types, which are necessary for investment in newly formed matrix [39]. Once vascular tubes are assembled, the pericyte-derived tissue inhibitor of metalloproteinase-3 (TIMP-3) prevents proteolysis of matrix proteins [40].

Because of their necessity in microvascular remodeling and proximity to ECs, pericytes have emerged as a cellular target to manipulate angiogenesis [41–43]. For example, the inhibition of PDGFR- β in tumors depletes pericytes and disrupts tumor vasculature, which curbs tumor growth [44]. Conversely, increasing pericyte coverage via Ang2 inhibition can represent an antiangiogenic tumor therapy [45]. Pericytes have been implicated in preventing tumor metastasis [28, 30], and depletion of pericytes in vascular retinopathy leads to hypervascularization and blindness [33]. The therapeutic potential for pericytes can also be exemplified by considering the similarities between pericytes and mesenchymal stem cells (MSCs) [46–51] (a concept that will be revisited in the next section) and appreciating that the delivery of MSCs represents a viable approach for pericyte-targeted angiogenic therapies [43]. The success of this approach has been well documented [43]. Therapeutically delivered MSCs have been convincingly shown to acquire pericyte characteristics and stabilize aberrant angiogenesis in various models of normal and pathological vessel remodeling. Still, the exact mechanisms that regulate pericyte and MSC physiology in these scenarios remain unclear, and

Fig. 1. Pericyte dynamics involved in angiogenesis including recruitment, ECM modulation and growth factor presentation (secretion and binding). During recruitment, nearby pericytes or interstitial cell populations (i.e. fibroblasts or resident precursor cells, depicted as the blue cell) migrate to the EC-lined capillary sprout. Pericytes can also directly interact with ECs via the NG2 transmembrane protein binding to $\beta 1$ integrins to influence their behavior [29, 36, 89]. Each dynamic represents a specific pericyte function critical for regulating capillary sprouting.

understanding the pericyte (and MSC) subtypes that contribute to angiogenesis and vessel stabilization will open new avenues for therapeutic manipulation of the microvasculature.

While it is clear that pericyte-EC interactions play a crucial role in establishing and maintaining a normal microcirculation, questions about pericyte mechanisms, especially during angiogenesis in the adult, remain. Determining phenotypic differences between the pericytes on angiogenic vessels and mature vessels may provide insight as to the varying functions of pericytes across the microvasculature. However, pericyte-specific phenotypes remain elusive, and recent studies document the overlap between pericytes and multiple other cell types. Interestingly, pericytes have even been suggested to display multipotency similar to stem cells. Many questions certainly also remain regarding pericyte lineage, yet in the

Fig. 2. Pericyte marker heterogeneity across a microvascular network. Examples of NG2 and SMA labeling along arterioles (A), venules (V) and capillaries (C) in the mesenteric networks of adult rats during angiogenesis. NG2 labeling identifies SMA-positive SMCs along arterioles but not SMA-positive SMCs along venules. At the capillary level, pericytes are positive for NG2 and negative for SMA. Scale bar: 25 μ m.

midst of any debate, the recognition of their phenotypic dynamics offers a valuable opportunity to discover new pericyte spatial and temporal identities that define specific cell subpopulations.

Pericyte Identity

Pericyte Phenotypic Markers

Pericyte heterogeneity and plasticity have been major barriers to further elucidating scenario-specific pericyte mechanisms. Common pericyte markers include NG2, SMA, desmin, vimentin and PDGFR- β , but their expression is not specific to pericytes [3]. Desmin and PDGFR- β are expressed by SMCs and interstitial fibroblasts [52]. SMA identifies vascular SMCs and myofibroblasts [53]. NG2 can identify macrophages, glial cells and various types of tumor cells [36, 54, 55]. Additionally, the expression of these markers and their overlap vary among pericyte populations along the hierarchy of a network (fig. 2) [9, 56]. Further complicating the issue of pericyte identification, expression of the various markers differs, depending on species, tissue and even developmental stage. As just one example, pericytes along microvessels in a developing chicken embryo stain positive for SMA, but this is not the case for mouse retina [3]. Since other cell types express all of the above markers, a pericyte cannot be ubiqu-

itously identified by any one marker. Observational data suggest that at least a population of pericytes is derived from interstitial fibroblasts [57], and, consequently, pericytes are often thought of as a plastic cell type somewhere between SMCs and fibroblasts.

The phenotypic state of a pericyte, indeed, seems to correlate with pericyte maturation. In the adult rat mesentery, pericytes along capillaries lacked SMA compared to assumingly more contractile cells along precapillary arterioles and postcapillary venules [9, 56]. As another example, Song et al. [42] demonstrated that only a subpopulation of PDGFR- β + perivascular progenitor cells in tumors expressed more mature pericyte markers (i.e. NG2, desmin or SMA). In support of their progenitor nature, the isolated PDGFR- β + cells were able to differentiate into the more mature NG2+ or SMA+ phenotypes after culture. When cultured along with ECs, the progenitor cells displayed the ability to differentiate into desmin+ cells. The results of their study support the concept that pericyte specific phenotypes are dependent on their local environment and suggest the need to better understand the temporal and spatial functional importance of pericyte subpopulations during angiogenesis. Recognition of pericyte phenotype dynamics represents an opportunity to expand our understanding of how pericyte markers relate to different cell states.

Considering pericyte identity or, more appropriately, the lack of identity, forces one to appreciate their phenotypic heterogeneity and overlaps with other cell types. The versatility and plasticity of pericytes is maybe best exemplified by their relationship with MSCs. The fate of various stem cell populations in vivo [42, 58–61] suggests that MSCs can differentiate into pericytes. Intriguingly, pericytes have also been implicated to be a source of multipotent stem cells [46] and have the ability to differentiate into osteogenic [47], macrophage/dendritic [48] and neural lineages [49]. The derivation of stem cells from the perivascular niche in multiple tissues [50, 51] further supports the stem cell nature of pericytes. Regardless of the origin or fate of pericytes, elucidating their specific roles in vessel stabilization versus capillary sprouting requires further spatial and temporal characterization of pericyte identities during angiogenesis.

In the rest of this review, we will focus on how we can apply what we know about the overlaps between pericytes and neural cells to discover new pericyte subpopulations and their potential specialized functions during angiogenesis.

Pericyte Marker Overlap with Neural Cells

In recent years, NG2, a marker of glial progenitor cells in the CNS, has become the most common marker for pericytes [62]. NG2 is a membrane spanning chondroitin proteoglycan and its expression in the CNS identifies oligodendrocyte progenitor cells [36] during development. NG2+ progenitor cells are also capable of producing astrocytes depending on the culture environment in vitro [36] with such a capability being confirmed for a small population of astrocytes in vivo [63]. In the microcirculation, NG2 identifies perivascular cells along arteries (fig. 2), capillaries and capillary sprouts, and is transiently upregulated along remodeling venules during angiogenesis [8, 64]. The identification and use of NG2 as a pericyte marker highlights the emerging area of microvascular research focused on the link between vascular and neural patterning [65–69]. This link is supported at the molecular level when considering growth inhibitors in the CNS such as ephrins, semaphorins, NG2, neuropilins and Nogo [55]. In the vascular system, these molecules also appear to play regulatory roles in the guidance of capillary sprouts and have helped define the functional differences between endothelial tip versus stalk cells. Neural molecules, like ephrin B2, EphB4 and the neuropilins have also been largely used as the gold standards for identifying arteriole versus venous EC identity [65, 66, 70–72]. Altogether, a coordinated link between neural and vascular patterning is just beginning to be characterized, and this offers an exciting new perspective on the study of adult microvascular remodeling.

We have shown that NG2 expression in adult rat mesenteric microvascular networks identifies perivascular cells along capillaries and arterioles, but not venules [8]. During microvascular network growth, NG2 expression by pericytes is upregulated along venules. Interestingly, this upregulation of NG2 is transient, and temporally correlates with capillary sprouting [64]. The transient expression pattern of NG2 during angiogenesis stresses the need to understand different functions of pericytes on unstimulated (i.e. quiescent) versus angiogenic vessels (fig. 3) and is consistent with NG2 expression by various tissue-specific precursor cell types during development, including oligodendrocytes, immature chondroblasts, osteoblasts and keratinocyte progenitor cells [36, 54]. A temporal specific NG2 phenotype might also exist for developing cardiomyocytes and skeletal muscle [54]. More than a pericyte marker, NG2 seems to be a marker of a dynamic cell type and indicator of a cell state. NG2 is thought to be expressed by partially differentiated progenitor cells and is downregulated upon differentiation [36]. This general

Fig. 3. Do pericytes change their phenotype during angiogenesis? They play a role in vessel stabilization during unstimulated (non-angiogenic) scenarios and capillary sprouting during proangiogenic scenarios. The question remains whether a subpopulation of pericytes emerges to perform functions unique to angiogenesis.

description is consistent with the observation of NG2 expression by oligodendrocyte progenitor cells, but not oligodendrocytes in the CNS during development and consistent with the view that pericytes are plastic cell types with progenitor potential. Our laboratory has also observed strong NG2 immunolabeling along nerves in adult rat mesenteric networks [8, 73]. Nerves were identified by typical nerve morphology and by the expression of protein gene product 9.5 (PGP 9.5), tyrosine hydroxylase, neurofilament and class III β -tubulin. Whether NG2 is actually expressed by the peripheral axons in the rat mesentery has still to be investigated. Work by Rezajooi et al. [74] suggests that NG2 expression might not be associated with the axons at all, but rather perineurial or endoneurial fibroblasts involved in axon ensheathment. Nonetheless, the expression of NG2 by glial progenitor cells and along nerves in the adult further highlights the overlap between vascular pericytes and neural cell types. And the common use of NG2 as a pericyte marker and its characterization raises the question of whether other neural phenotypic markers identify pericytes during angiogenesis.

Class III β -tubulin is a potential candidate and, like NG2, is another marker of neural progenitor cells in the

Fig. 4. Pericyte differentiation during angiogenesis. This represents an example of the phenotype change of pericytes from an unstimulated (nonangiogenic) to an angiogenic scenario in adult rat mesenteric microvascular networks. In unstimulated microvascular networks, class III β -tubulin labeling is nerve-specific and does not identify perivascular cells along PECAM-positive arterioles, venules (V) or capillaries (c). During angiogenesis, perivascular cells along all vessels change their phenotype to become class III β -tubulin-positive (arrows). The transient upregulation of class III β -tubulin by pericytes during angiogenesis highlights the potential for specialized pericyte subpopulations. Scale bars: 25 μ m.

CNS and peripheral nerves in the adult [75, 76]. Class III β -tubulin is one of seven β -tubulin isotypes that forms α/β -tubulin heterodimers with six α -tubulin isotypes during microtubule assembly, and is most commonly used as a marker of neural phenotypes [77]. During development in the CNS, class III β -tubulin is transiently expressed by glial precursor cells, and in adults it is expressed by peripheral nerves [77]. Outside the nervous system, class III β -tubulin expression by tumor cells correlates with increased metastasis and resistance to tubulin-binding agents [78, 79]. All taken together, the expression patterns of class III β -tubulin are similar to NG2. So, it was not unexpected that class III β -tubulin does identify vascular pericytes. What was surprising was when and where class III β -tubulin identified pericytes. In unstimulated adult rat mesenteric networks, class III β -tubulin is nerve-specific and absent along arterioles, venules and capillaries [80]. After the networks are stimulated to undergo angiogenesis, class III β -tubulin is expressed by pericytes along these vessel types, and is subsequently downregulated to unstimulated levels after capillary sprouting [80]. These observations suggest that class III β -tubulin is a potential marker of angiogenic pericytes (fig. 4). To our knowledge, the regulator of G-protein signaling 5 (RGS-5) is the only other potential pericyte marker that has been reported to transiently correlate with an angiogenic or activated state [81].

Our characterization of NG2 and class III β -tubulin labeling patterns in adult rat mesenteric microvascular networks suggests that class III β -tubulin⁺ pericytes are also NG2⁺ [80]. NG2⁺ cells, however, are not necessarily class III β -tubulin⁺. This is dramatically supported by the

observation that NG2⁺ SMCs along arterioles and pericytes along capillaries in unstimulated networks [8] do not express class III β -tubulin [80]. NG2 and class III β -tubulin expression by vascular pericytes exemplifies a link (at least phenotypically) between neural and vascular cell types. Yet, the differences in expression patterns between the two molecules highlight the still unknown answers regarding specific pericyte subpopulations, especially when considering subpopulations expressing or lacking other phenotypic markers such as SMA (fig. 3).

Human brain pericytes in vitro also express class III β -tubulin (fig. 5). The positive expression of class III β -tubulin by tumor cells and pericytes in vitro suggests a potential issue with using just class III β -tubulin expression as an indicator of a neural phenotype. For example, stem cell differentiation into nerves has been confirmed, in part, based on class III β -tubulin expression [82, 83]. However, our observations show that class III β -tubulin can be expressed by pericytes and is not nerve-specific. We have also confirmed that human placenta-derived pericytes, human mesenchymal stem cells, and mouse embryonic stem cells also express class III β -tubulin in vitro (data not shown). Since mesenchymal stem cells might be a source of vascular pericytes in adult tissues [58] and, vice versa, vascular pericytes can be induced to exhibit multipotent stem cell activity [49], we speculate that class III β -tubulin is indeed more than a nerve marker in vivo and potentially identifies a precursor cell population. Regardless, class III β -tubulin represents an exciting biomarker of cells in an active state and, in the context of pericytes, represents a potential phenotype related to the functions involved in angiogenesis.

Fig. 5. Pericytes in vitro express class III β -tubulin. **a** Commercially available human brain-derived vascular pericytes from ScienCell positively labeled for class III β -tubulin when cultured in pericyte medium containing 2% fetal bovine serum, 1% pericyte growth supplements and 1% 100 $\times$ penicillin/streptomycin. Scale bar: 50 μ m. **b** Higher magnification images display class III β -tubulin localization in tubule structures. Scale bar: 20 μ m. **c** All human brain-derived pericytes coexpressed the pericyte marker NG2. Scale bars: 50 μ m.

Do Pericyte Subpopulations Play Functional Roles?

The concept of specialized subpopulations of vascular pericytes forces the integration of studies using pericyte markers to identify pericytes and studies focused on elucidating the functional roles of those markers. NG2 is sometimes referred to as a marker of angiogenic pericytes along capillaries [31, 62] because it typically colocalizes with pericytes along all capillaries and capillary sprouts. In support of the angiogenic classification, we have demonstrated that NG2 is dramatically upregulated along venules during angiogenesis, implicating its involvement in capillary sprouting [64]. Importantly, NG2 is more than just a marker, and its identification led to its discovery as a regulator of capillary sprouting. NG2-binding of PDGF-AA and basic fibroblast growth factor (bFGF) [84, 85], as well as the kringle domains of plasminogen, which is important for the activation of latent TGF- β [86], implicates

its role as a regulator of local angiogenic growth factor presentation. NG2 expression has also been shown to orient with actin stress fibers in a linear array and has been linked to downstream Rho-Rac signaling [54, 87]. Another possible role for NG2 is manipulation of the local ECM environment as a receptor for type VI collagen [88]. The soluble form of the proteoglycan promotes EC motility and angiogenesis via engagement of galectin-3 and $\alpha 3 \beta 1$ integrin [89], suggesting a direct interaction between NG2-expressing perivascular cells and neighboring ECs. Transmembrane signaling between the two cell types is further supported by NG2 knockdown pericyte-EC coculture models. Pericyte-specific NG2 inhibition can reduce $\beta 1$ integrin activation by ECs and EC tube formation, junction formation and permeability [29].

The functional importance of this proteoglycan has been demonstrated in vivo with the use of *ng2*^{-/-} mice and functional blocking antibodies [90, 91]. Genetic deletion

Fig. 6. Class III β -tubulin regulates pericyte migration. **a–c** Class III β -tubulin and DAPI nucleic acid labeling in the human brain-derived pericytes 6 h after a scratch wound was applied. Scale bars: 200 μ m. Inhibition of class III β -tubulin was confirmed qualitatively by the reduction in class III β -tubulin-positive cells (**c**) compared to the control groups (**a, b**). **d** Quantification of scratch closure for the control sham, control siRNA and class III β -tubulin target siRNA groups. At 72 h (24 h transfection + 48 h for gene suppression to manifest), each monolayer was scratched and imaged. After 6 h, scratches were imaged again, and the change in the scratch area was blindly quantified for 8 wells per group ($n = 8$ per group). Scratch wound closure fractions were compared using a one-way ANOVA followed by a Student Newman-Keuls pairwise comparison test. * $p < 0.05$ represents significant differences from

the control sham group. + $p < 0.05$ represents significant differences from the control siRNA group. **e** Quantification of the fraction of class III β -tubulin-positive cells inside the initial scratch area compared to the general population outside the scratch area ($n = 8$ per group). Statistical comparison between the 2 groups was made using a Student's t test. CTRL = Control. * $p < 0.05$ represents a significant difference. **f** Example of class III β -tubulin and DAPI nucleic acid labeling along the leading edge of a scratch wound in the target siRNA group. Class III β -tubulin-positive versus class III β -tubulin-negative cells were more prone to migrate into the wound area. The asterisks indicate class III β -tubulin-negative cell nuclei. Arrows indicate class III β -tubulin-positive cells. Scale bar: 50 μ m.

or blockade of NG2 in hypoxia-induced microvascular growth in the retina and bFGF-induced growth in the cornea resulted in fewer vessels [91]. NG2 antibody inhibition in rat mesenteric microvascular networks has been shown to inhibit bFGF and vascular endothelial growth

factor (VEGF)-induced sprout formation [92]. During tumor angiogenesis, NG2 has been shown to sequester angiostatin, increasing the tumor growth rate [93]. Further advocating the functional role of NG2, tumors in mice with pericyte-specific NG2 deletion display a highly

dysfunctional and destabilized vasculature, which leads to increased vessel permeability and tumor hypoxia [29].

The influences of NG2 on pericyte and EC dynamics highlight the association between a pericyte phenotype and function. However, spatially correlating NG2 expression by pericytes to dynamics involved in angiogenesis is not so straightforward. For instance, SMCs along quiescent arterioles also express NG2, and one generally assumes that the functions of SMCs along arterioles differ from the functions of pericytes along capillary sprouts. Nonetheless, considering the functional roles of NG2 and its coordinated angiogenesis-specific expression patterns supports that pericytes change their phenotype to play specific functions during angiogenesis. To further explore this possibility, let us reconsider our recent discovery of class III β -tubulin as an angiogenic pericyte marker. Maybe not surprisingly, the experimental results do support a functional role for class III β -tubulin.

Class III β -tubulin appears to play a role in pericyte migration, which is necessary for pericyte recruitment during the sprouting process. Cultured human brain-derived pericytes express class III β -tubulin in vitro (fig. 5). Given that MSCs in culture also express class III β -tubulin [83] and that pericytes and MSCs share phenotypic similarities, this result might have been expected and we have also observed class III β -tubulin expression by human-derived placental pericytes in vitro (data not shown). Considering pericyte class III β -tubulin expression in vitro together with our observations of class III β -tubulin upregulation by pericytes in adult rat mesenteric microvascular networks during angiogenesis (fig. 4 [80]) raises questions regarding (1) the cell state in vitro and (2) the potential differences between pericytes in vivo versus in vitro. Both issues will prove critical for applying the knowledge we have gained from in vitro studies.

To investigate the functional role of class III β -tubulin, we inhibited its expression by specific siRNA treatments and measured its effect on pericyte invasion in an in vivo scratch-wound assay. Commercially obtained human brain-derived pericytes (ScienCell) were grown to confluence and treated with no siRNA, control (nontargeting) siRNA or class III β -tubulin siGENOME SMARTpool siRNA from Dharmacon/Thermo Scientific for 24 h. siRNA inhibition of class III β -tubulin decreased the percentage of cells with observable class III β -tubulin immunolabeling (fig. 6c) and relative quantities of RNA compared to control groups based on qRT-PCR (data not shown). Inhibition of class III β -tubulin reduced cell migration into the area void of cells in a monolayer scratch test (fig. 6). The scratch-wound area closure fraction for

the class III β -tubulin siRNA group was 35% smaller compared to the nontargeting control siRNA group (0.45 ± 0.04 vs. 0.69 ± 0.06 ; $p = 0.002$) and 27% smaller than the sham control group (0.45 ± 0.04 vs. 0.62 ± 0.02 ; $p = 0.009$; fig. 6d). In the class III β -tubulin siRNA treatment groups, cells were both positive and negative for class III β -tubulin, indicating that the siRNA knockdown effect was not homogeneous. Class III β -tubulin+ cells were frequently found leading migration into the scratch area in the target siRNA groups (fig. 6e, f). The effect of class III β -tubulin inhibition on the scratch wound closure is consistent with reports by others [94] that have shown that class III β -tubulin in vitro can influence cell motility and proliferation. Our results in the context of pericytes suggest that expression of class III β -tubulin by pericytes during angiogenesis would influence their ability to be recruited to newly forming capillary sprouts. Undoubtedly, further follow-up studies including various pericyte sources and additional assays are required, but the preliminary results do motivate the need to understand the different roles of specific pericyte subpopulations. If we can define specific pericyte subpopulations, as in the endothelial tip versus stalk cell scenario, then we might be able to differentiate the importance of one pericyte type as opposed to another.

Summary

Pericytes are essential for angiogenesis and vessel maturation. Their specific mechanisms during these processes remain unclear due to the heterogeneity of pericyte identities across individual microvascular networks and different tissues. Although this has been a barrier in the past, transient pericyte phenotypes during capillary sprouting suggest that subpopulations perform specific angiogenic functions. The transient expression of NG2 and class III β -tubulin implicate two neural phenotypes in pericyte function during angiogenesis. Correlating known angiogenic dynamics with such subpopulations represents an opportunity to pinpoint specialized pericyte functions based on phenotype, or else target pericyte mechanisms for therapeutic benefits. Accordingly, defining specific pericyte subpopulations involved in angiogenesis versus vessel maturation will be advantageous, and maybe compulsory, for future studies that aim to elucidate pericyte mechanisms. As we attempt to focus on specific spatial and temporal pericyte subpopulations, the need for tunable model systems will emerge. The development of such in vivo and ex vivo systems that enable

pericytes to be dynamically probed within intact microvascular networks will help advance our understanding of pericyte identity [29, 92]. In the meantime, the discoveries of different pericyte subpopulations offer new foundations for the future.

Acknowledgements

This review is based on a lecture that was presented by the author at a symposium on vascular remodeling, organized by DZHK (German Centre for Cardiovascular Research), partner site Munich Heart Alliance, Munich, Germany, in February 2013. The work was supported by the Tulane Center for Aging and NIH 5-P20GM103629-02 to W.L.M. The authors would also like to thank Dr. Becky Worthylake for her generosity and insightful discussions regarding siRNA inhibition protocols.

References

- Zimmermann KW: Der feinere Bau der Blutkapillaren. *Z Anat* 1923;68:29–109.
- Shepro D, Morel NM: Pericyte physiology. *FASEB J* 1993;7:1031–1038.
- Gerhardt H, Betsholtz C: Endothelial-pericyte interactions in angiogenesis. *Cell Tissue Res* 2003;314:15–23.
- Hellstrom M, Kalen M, Lindahl P, Abramson A, Betsholtz C: Role of PDGF-B and PDGFR-beta in recruitment of vascular smooth muscle cells and pericytes during embryonic blood vessel formation in the mouse. *Development* 1999;126:3047–3055.
- Lindblom P, Gerhardt H, Liebner S, Abramson A, Enge M, Hellstrom M, Backstrom G, Fredriksson S, Landegren U, Nystrom HC, Bergstrom G, Dejana E, Ostman A, Lindahl P, Betsholtz C: Endothelial PDGF-B retention is required for proper investment of pericytes in the microvessel wall. *Genes Dev* 2003;17:1835–1840.
- Lindahl P, Johansson BR, Leveen P, Betsholtz C: Pericyte loss and microaneurysm formation in PDGF-B-deficient mice. *Science* 1997;277:242–245.
- Gerhardt H, Golding M, Fruttiger M, Ruhrberg C, Lundkvist A, Abramsson A, Jeltsch M, Mitchell C, Alitalo K, Shima D, Betsholtz C: VEGF guides angiogenic sprouting utilizing endothelial tip cell filopodia. *J Cell Biol* 2003;161:1163–1177.
- Murfee WL, Skalak TC, Peirce SM: Differential arterial/venous expression of NG2 proteoglycan in perivascular cells along microvessels: identifying a venule-specific phenotype. *Microcirculation* 2005;12:151–160.
- Nehls V, Drenckhahn D: The versatility of microvascular pericytes: from mesenchyme to smooth muscle? *Histochemistry* 1993;99:1–12.
- Ribatti D, Nico B, Crivellato E: The role of pericytes in angiogenesis. *Int J Dev Biol* 2011;55:261–268.
- Armulik A, Abramsson A, Betsholtz C: Endothelial/pericyte interactions. *Circ Res* 2005;97:512–523.
- Dulmovits BM, Herman IM: Microvascular remodeling and wound healing: a role for pericytes. *Int J Biochem Cell Biol* 2012;44:1800–1812.
- Gaengel K, Genove G, Armulik A, Betsholtz C: Endothelial-mural cell signaling in vascular development and angiogenesis. *Arterioscler Thromb Vasc Biol* 2009;29:630–638.
- Hirschi KK, D'Amore PA: Pericytes in the microvasculature. *Cardiovasc Res* 1996;32:687–698.
- Tilton RG, Kilo C, Williamson JR: Pericyte-endothelial relationships in cardiac and skeletal muscle capillaries. *Microvasc Res* 1979;18:325–335.
- Schonfelder U, Hofer A, Paul M, Funk RH: In situ observation of living pericytes in rat retinal capillaries. *Microvasc Res* 1998;56:22–29.
- Ayres-Sander CE, Lauridsen H, Maier CL, Sava P, Pober JS, Gonzalez AL: Transendothelial migration enables subsequent transmigration of neutrophils through underlying pericytes. *PLoS One* 2013;8:e60025.
- Stark K, Eckart A, Haidari S, Tirniceriu A, Lorenz M, von Bruhl ML, Gartner F, Khandoga AG, Legate KR, Pless R, Hepper I, Lauber K, Walzog B, Massberg S: Capillary and arteriolar pericytes attract innate leukocytes exiting through venules and 'instruct' them with pattern-recognition and motility programs. *Nat Immunol* 2013;14:41–51.
- Peppiatt CM, Howarth C, Mobbs P, Attwell D: Bidirectional control of CNS capillary diameter by pericytes. *Nature* 2006;443:700–704.
- Hellstrom M, Gerhardt H, Kalen M, Li X, Eriksson U, Wolburg H, Betsholtz C: Lack of pericytes leads to endothelial hyperplasia and abnormal vascular morphogenesis. *J Cell Biol* 2001;153:543–553.
- Folkman J, D'Amore PA: Blood vessel formation: what is its molecular basis? *Cell* 1996;87:1153–1155.
- Potente M, Gerhardt H, Carmeliet P: Basic and therapeutic aspects of angiogenesis. *Cell* 2011;146:873–887.
- Davis GE: Angiogenesis and proteinases: influence on vascular morphogenesis, stabilization and regression. *Drug Discov Today Dis Models* 2011;8:13–20.
- Humphreys BD, Lin SL, Kobayashi A, Hudson TE, Nowlin BT, Bonventre JV, Valerius MT, McMahon AP, Duffield JS: Fate tracing reveals the pericyte and not epithelial origin of myofibroblasts in kidney fibrosis. *Am J Pathol* 2010;176:85–97.
- Schrumpf C, Duffield JS: Mechanisms of fibrosis: the role of the pericyte. *Curr Opin Nephrol Hypertens* 2011;20:297–305.
- Greenhalgh SN, Iredale JP, Henderson NC: Origins of fibrosis: pericytes take centre stage. *F1000Prime Rep* 2013;5:37.
- Quaegebeur A, Segura I, Carmeliet P: Pericytes: blood-brain barrier safeguards against neurodegeneration? *Neuron* 2010;68:321–323.
- Gerhardt H, Semb H: Pericytes: gatekeepers in tumour cell metastasis? *J Mol Med (Berl)* 2008;86:135–144.
- You WK, Yotsumoto F, Sakimura K, Adams RH, Stallcup WB: NG2 proteoglycan promotes tumor vascularization via integrin-dependent effects on pericyte function. *Angiogenesis* 2014;17:61–76.
- Cooke VG, LeBleu VS, Keskin D, Khan Z, O'Connell JT, Teng Y, Duncan MB, Xie L, Maeda G, Vong S, Sugimoto H, Rocha RM, Damascena A, Brentani RR, Kalluri R: Pericyte depletion results in hypoxia-associated epithelial-to-mesenchymal transition and metastasis mediated by Met signaling pathway. *Cancer Cell* 2012;21:66–81.
- Ozerdem U, Monosov E, Stallcup WB: NG2 proteoglycan expression by pericytes in pathological microvasculature. *Microvasc Res* 2002;63:129–134.
- Ponce AM, Price RJ: Angiogenic stimulus determines the positioning of pericytes within capillary sprouts in vivo. *Microvasc Res* 2003;65:45–48.
- Hammes HP, Lin J, Renner O, Shani M, Lundqvist A, Betsholtz C, Brownlee M, Deutsch U: Pericytes and the pathogenesis of diabetic retinopathy. *Diabetes* 2002;51:3107–3112.
- Lee S, Zeiger A, Maloney JM, Kotecki M, Van Vliet KJ, Herman IM: Pericyte actomyosin-mediated contraction at the cell-material interface can modulate the microvascular niche. *J Phys Condens Matter* 2010;22:194115.

- 35 Paik JH, Skoura A, Chae SS, Cowan AE, Han DK, Proia RL, Hla T: Sphingosine 1-phosphate receptor regulation of N-cadherin mediates vascular stabilization. *Genes Dev* 2004; 18:2392–2403.
- 36 Stallcup WB, Huang FJ: A role for the NG2 proteoglycan in glioma progression. *Cell Adh Migr* 2008;2:192–201.
- 37 Fang JS, Dai C, Kurjiaka DT, Burt JM, Hirschi KK: Connexin45 regulates endothelial-induced mesenchymal cell differentiation toward a mural cell phenotype. *Arterioscler Thromb Vasc Biol* 2013;33:362–368.
- 38 Hirschi KK, Burt JM, Hirschi KD, Dai C: Gap junction communication mediates transforming growth factor-beta activation and endothelial-induced mural cell differentiation. *Circ Res* 2003;93:429–437.
- 39 Stratman AN, Malotte KM, Mahan RD, Davis MJ, Davis GE: Pericyte recruitment during vasculogenic tube assembly stimulates endothelial basement membrane matrix formation. *Blood* 2009;114:5091–5101.
- 40 Saunders WB, Bohnsack BL, Faske JB, Anthis NJ, Bayless KJ, Hirschi KK, Davis GE: Coregulation of vascular tube stabilization by endothelial cell TIMP-2 and pericyte TIMP-3. *J Cell Biol* 2006;175:179–191.
- 41 Ozerdem U: Targeting of pericytes diminishes neovascularization and lymphangiogenesis in prostate cancer. *Prostate* 2006;66:294–304.
- 42 Song S, Ewald AJ, Stallcup W, Werb Z, Bergers G: PDGFRbeta+ perivascular progenitor cells in tumours regulate pericyte differentiation and vascular survival. *Nat Cell Biol* 2005; 7:870–879.
- 43 Kelly-Goss MR, Sweat RS, Stapor PC, Peirce SM, Murfee WL: Targeting pericytes for angiogenic therapies. *Microcirculation* 2013, Epub ahead of print.
- 44 Bergers G, Song S, Meyer-Morse N, Bergsland E, Hanahan D: Benefits of targeting both pericytes and endothelial cells in the tumor vasculature with kinase inhibitors. *J Clin Invest* 2003;111:1287–1295.
- 45 Gerald D, Chintharlapalli S, Augustin HG, Benjamin LE: Angiopoietin-2: an attractive target for improved antiangiogenic tumor therapy. *Cancer Res* 2013;73:1649–1657.
- 46 da Silva Meirelles L, Caplan AI, Nardi NB: In search of the in vivo identity of mesenchymal stem cells. *Stem Cells* 2008;26:2287–2299.
- 47 Doherty MJ, Ashton BA, Walsh S, Beresford JN, Grant ME, Canfield AE: Vascular pericytes express osteogenic potential in vitro and in vivo. *J Bone Miner Res* 1998;13:828–838.
- 48 Balabanov R, Washington R, Wagnerova J, Dore-Duffy P: CNS microvascular pericytes express macrophage-like function, cell surface integrin alpha M, and macrophage marker ED-2. *Microvasc Res* 1996;52:127–142.
- 49 Dore-Duffy P, Katyshev A, Wang X, Van Buren E: CNS microvascular pericytes exhibit multipotential stem cell activity. *J Cereb Blood Flow Metab* 2006;26:613–624.
- 50 Tang W, Zeve D, Suh JM, Bosnakovski D, Kyba M, Hammer RE, Tallquist MD, Graff JM: White fat progenitor cells reside in the adipose vasculature. *Science* 2008;322:583–586.
- 51 Tigges U, Komatsu M, Stallcup WB: Adventitial pericyte progenitor/mesenchymal stem cells participate in the restenotic response to arterial injury. *J Vasc Res* 2013;50:134–144.
- 52 Nehls V, Denzer K, Drenckhahn D: Pericyte involvement in capillary sprouting during angiogenesis in situ. *Cell Tissue Res* 1992;270: 469–474.
- 53 Cushing MC, Liao JT, Jaeggli MP, Anseth KS: Material-based regulation of the myofibroblast phenotype. *Biomaterials* 2007;28:3378–3387.
- 54 Stallcup WB: The NG2 proteoglycan: past insights and future prospects. *J Neurocytol* 2002;31:423–435.
- 55 Sandvig A, Berry M, Barrett LB, Butt A, Logan A: Myelin-, reactive glia-, and scar-derived CNS axon growth inhibitors: expression, receptor signaling, and correlation with axon regeneration. *Glia* 2004;46:225–251.
- 56 Nehls V, Drenckhahn D: Heterogeneity of microvascular pericytes for smooth muscle type alpha-actin. *J Cell Biol* 1991;113:147–154.
- 57 Rhodin JA, Fujita H: Capillary growth in the mesentery of normal young rats. Intravital video and electron microscope analyses. *J Submicrosc Cytol Pathol* 1989;21:1–34.
- 58 Au P, Tam J, Fukumura D, Jain RK: Bone marrow-derived mesenchymal stem cells facilitate engineering of long-lasting functional vasculature. *Blood* 2008;111:4551–4558.
- 59 Ozerdem U, Alitalo K, Salven P, Li A: Contribution of bone marrow-derived pericyte precursor cells to corneal vasculogenesis. *Invest Ophthalmol Vis Sci* 2005;46:3502–3506.
- 60 Rajantie I, Ilmonen M, Alminaita A, Ozerdem U, Alitalo K, Salven P: Adult bone marrow-derived cells recruited during angiogenesis comprise precursors for periendothelial vascular mural cells. *Blood* 2004;104:2084–2086.
- 61 Amos PJ, Mulvey CL, Seaman SA, Walpole J, Degen KE, Shang H, Katz AJ, Peirce SM: Hypoxic culture and in vivo inflammatory environments affect the assumption of pericyte characteristics by human adipose and bone marrow progenitor cells. *Am J Physiol Cell Physiol* 2011;301:C1378–C1388.
- 62 Ozerdem U, Grako KA, Dahlin-Huppe K, Monosov E, Stallcup WB: NG2 proteoglycan is expressed exclusively by mural cells during vascular morphogenesis. *Dev Dyn* 2001;222: 218–227.
- 63 Zhu X, Bergles DE, Nishiyama A: NG2 cells generate both oligodendrocytes and gray matter astrocytes. *Development* 2008;135: 145–157.
- 64 Murfee WL, Rehorn MR, Peirce SM, Skalak TC: Perivascular cells along venules upregulate NG2 expression during microvascular remodeling. *Microcirculation* 2006;13:261–273.
- 65 Eichmann A, Le Noble F, Autiero M, Carmeliet P: Guidance of vascular and neural network formation. *Curr Opin Neurobiol* 2005; 15:108–115.
- 66 Weinstein BM: Vessels and nerves: marching to the same tune. *Cell* 2005;120:299–302.
- 67 Carmeliet P, Tessier-Lavigne M: Common mechanisms of nerve and blood vessel wiring. *Nature* 2005;436:193–200.
- 68 Adams RH: Molecular control of arterial-venous blood vessel identity. *J Anat* 2003;202: 105–112.
- 69 Larrivee B, Freitas C, Suchting S, Brunet I, Eichmann A: Guidance of vascular development: lessons from the nervous system. *Circ Res* 2009;104:428–441.
- 70 Shin D, Garcia-Cardena G, Hayashi S, Gerety S, Asahara T, Stavrakis G, Isner J, Folkman J, Gimbrone MA Jr, Anderson DJ: Expression of ephrinB2 identifies a stable genetic difference between arterial and venous vascular smooth muscle as well as endothelial cells, and marks subsets of microvessels at sites of adult neovascularization. *Dev Biol* 2001;230: 139–150.
- 71 Wang HU, Chen ZF, Anderson DJ: Molecular distinction and angiogenic interaction between embryonic arteries and veins revealed by ephrin-B2 and its receptor Eph-B4. *Cell* 1998;93:741–753.
- 72 Gale NW, Baluk P, Pan L, Kwan M, Holash J, DeChiara TM, McDonald DM, Yancopoulos GD: Ephrin-B2 selectively marks arterial vessels and neovascularization sites in the adult, with expression in both endothelial and smooth-muscle cells. *Dev Biol* 2001;230:151–160.
- 73 Stapor PC, Murfee WL: Spatiotemporal distribution of neurovascular alignment in remodeling adult rat mesentery microvascular networks. *J Vasc Res* 2012;49:299–308.
- 74 Rezajooi K, Pavlides M, Winterbottom J, Stallcup WB, Hamlyn PJ, Lieberman AR, Anderson PN: NG2 proteoglycan expression in the peripheral nervous system: upregulation following injury and comparison with CNS lesions. *Mol Cell Neurosci* 2004;25:572–584.
- 75 Draberova E, Del Valle L, Gordon J, Markova V, Smejkalova B, Bertrand L, de Chadarevian JP, Agamanolis DP, Legido A, Khalili K, Draber P, Katsetos CD: Class III beta-tubulin is constitutively coexpressed with glial fibrillary acidic protein and nestin in midgestational human fetal astrocytes: implications for phenotypic identity. *J Neuropathol Exp Neurol* 2008;67:341–354.
- 76 Katsetos CD, Legido A, Perentes E, Mork SJ: Class III beta-tubulin isotype: a key cytoskeletal protein at the crossroads of developmental neurobiology and tumor neuropathology. *J Child Neurol* 2003;18:851–866, disc 867.
- 77 Katsetos CD, Herman MM, Mork SJ: Class III beta-tubulin in human development and cancer. *Cell Motil Cytoskeleton* 2003;55:77–96.

- 78 Gan PP, McCarroll JA, Po'uha ST, Kamath K, Jordan MA, Kavallaris M: Microtubule dynamics, mitotic arrest, and apoptosis: drug-induced differential effects of betaIII-tubulin. *Mol Cancer Ther* 2010;9:1339–1348.
- 79 Kavallaris M: Microtubules and resistance to tubulin-binding agents. *Nat Rev Cancer* 2010; 10:194–204.
- 80 Stapor PC, Murfee WL: Identification of class III beta-tubulin as a marker of angiogenic perivascular cells. *Microvasc Res* 2012;83: 257–262.
- 81 Berger M, Bergers G, Arnold B, Hammerling GJ, Ganss R: Regulator of G-protein signaling-5 induction in pericytes coincides with active vessel remodeling during neovascularization. *Blood* 2005;105:1094–1101.
- 82 Bertani N, Malatesta P, Volpi G, Sonogo P, Perris R: Neurogenic potential of human mesenchymal stem cells revisited: analysis by immunostaining, time-lapse video and microarray. *J Cell Sci* 2005;118:3925–3936.
- 83 Foudah D, Redondo J, Caldara C, Carini F, Tredici G, Miloso M: Human mesenchymal stem cells express neuronal markers after osteogenic and adipogenic differentiation. *Cell Mol Biol Lett* 2013;18:163–186.
- 84 Goretzki L, Burg MA, Grako KA, Stallcup WB: High-affinity binding of basic fibroblast growth factor and platelet-derived growth factor-AA to the core protein of the NG2 proteoglycan. *J Biol Chem* 1999;274:16831–16837.
- 85 Grako KA, Ochiya T, Barritt D, Nishiyama A, Stallcup WB: PDGF (alpha)-receptor is unresponsive to PDGF-AA in aortic smooth muscle cells from the NG2 knockout mouse. *J Cell Sci* 1999;112:905–915.
- 86 Goretzki L, Lombardo CR, Stallcup WB: Binding of the NG2 proteoglycan to kringle domains modulates the functional properties of angiostatin and plasmin(ogen). *J Biol Chem* 2000;275:28625–28633.
- 87 Lin XH, Dahlin-Huppe K, Stallcup WB: Interaction of the NG2 proteoglycan with the actin cytoskeleton. *J Cell Biochem* 1996;63:463–477.
- 88 Stallcup WB, Dahlin K, Healy P: Interaction of the NG2 chondroitin sulfate proteoglycan with type VI collagen. *J Cell Biol* 1990;111: 3177–3188.
- 89 Fukushi J, Makagiansar IT, Stallcup WB: NG2 proteoglycan promotes endothelial cell motility and angiogenesis via engagement of galectin-3 and alpha3beta1 integrin. *Mol Biol Cell* 2004;15:3580–3590.
- 90 Huang FJ, You WK, Bonaldo P, Seyfried TN, Pasquale EB, Stallcup WB: Pericyte deficiencies lead to aberrant tumor vascularization in the brain of the NG2 null mouse. *Dev Biol* 2010;344:1035–1046.
- 91 Ozerdem U, Stallcup WB: Pathological angiogenesis is reduced by targeting pericytes via the NG2 proteoglycan. *Angiogenesis* 2004;7: 269–276.
- 92 Stapor PC, Azimi MS, Ahsan T, Murfee WL: An angiogenesis model for investigating multicellular interactions across intact microvascular networks. *Am J Physiol Heart Circ Physiol* 2013;304:H235–H245.
- 93 Chekenya M, Hjelstuen M, Enger PO, Thorsen F, Jacob AL, Probst B, Haraldseth O, Pilkington G, Butt A, Levine JM, Bjerkvig R: NG2 proteoglycan promotes angiogenesis-dependent tumor growth in CNS by sequestering angiostatin. *FASEB J* 2002;16:586–588.
- 94 Ganguly A, Yang H, Cabral F: Class III beta-tubulin counteracts the ability of paclitaxel to inhibit cell migration. *Oncotarget* 2011;2: 368–377.