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PERICYTES (A BIRBRAIR, SECTION EDITOR)

Pericytes in Vascular Development

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Abstract

Purpose of Review Pericytes are essential components of capillaries in many tissues and organs, contributing to vessel stability and integrity, with additional contributions to microvascular function still being discovered. We review current and foundational studies identifying pericyte differentiation mechanics and their roles in the earliest stages of vessel formation.

Recent Findings Recent advances in pericyte-focused tools and models have illuminated critical aspects of pericyte biology including their roles in vascular development. Pericytes likely collaborate with endothelial cells undergoing vasculogenesis, initiating direct interactions during sprouting and intussusceptive angiogenesis. Pericytes also provide important regulation of vascular growth including mechanisms underlying vessel pruning, rarefaction, and subsequent regrowth.

Summary A phenotypic transition likely occurs as pericytes shift from roles in vascular development to supporting vessel maturation, homeostasis, and physiology. We provide a forward-looking perspective on pericyte-focused studies of vascular development and applications aimed at basic and clinically relevant insights into pericyte biology.

Keywords Pericyte · Endothelial cell · Vascular development · Angiogenesis · Vasculogenesis · Mural cell differentiation

Introduction

Vascular pericytes have recently attracted significant attention as therapeutic targets in a wide range of pathologies including Alzheimer's disease, cancer, and tissue fibrosis [1]. Our understanding of their roles in these clinically relevant scenarios will be strengthened by a deeper knowledge of their embryonic origins and their fundamental contributions to vascular development processes under normal, physiological conditions [2]. For instance, treatment for diseases that arise from compromised vascular barrier function will likely benefit from uncovering the roles of pericytes in maintaining vessel wall integrity during angiogenic remodeling and in quiescence [3]. Moreover, clinical management of vascular occlusive diseases such as coronary and peripheral vascular diseases will move forward with increased insight into the transcriptional programming of pericytes and how they might be induced to contribute to vascular smooth muscle cell (vSMC) expansion during arteriogenesis and collateralization.

Until recently, vascular development studies have focused almost exclusively on endothelial cells and their roles in blood vessel formation, logically following their central role as the "basic building blocks" for the vascular system [4]. Pericytes, although known to intimately associate with quiescent microvasculature, have remained relatively under-studied in establishing their unique contributions to building nascent vascular networks. The field of vascular biology has likely only just begun to identify how pericytes may directly affect developmental processes that have previously been attributed only to the endothelium such as vasculogenesis and angiogenesis. Pericytes may also be influenced in specific ways by neighboring endothelial cells during vessel formation. It is therefore critical to understand the dynamic interactions between pericytes and endothelial cells at multiple levels during the earliest developmental stages to delineate their individual contributions to vascular development [5]. A key aspect of this interplay is identifying the earliest time point at which both cell types emerge from their respective precursors during

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embryonic cell differentiation. This timing is especially relevant to pericyte biology, as they potentially share developmental origins with other cell types that may inform our understanding of their emerging roles in vascular physiology (i.e., contractility and relationship with vSMCs) and disease, specifically in vessel "dropout" and potential for fibrosis or fibroblast conversion [6].

With this rationale for understanding pericytes in vascular development in view, here we present recent advances in illuminating pericyte biology in the context of previous studies that have laid the foundation for these discoveries. Specifically, we discuss the cellular origins of pericytes as they differentiate from more primitive, intermediate cell types (Fig. 1). We further consider that, as pericytes emerge from these precursors, they begin contributing to the earliest stages of vessel formation, perhaps as early as vasculogenesis, but certainly in angiogenic remodeling and vessel maturation. Lastly, we present recent studies that suggest pericytes may transition developmentally from being directly involved in structurally forming new vessels to distinct functional roles underlying vessel homeostasis and physiology.

Pericyte Differentiation

The neural crest (i.e., embryonic ectoderm germ layer) has been consistently described as a source for vascular pericytes [7], especially for those that contribute to the vasculature in the head region and specifically in the central nervous system (CNS). In fact, pre-migratory neural crest cells labeled via a Sox10-Cre construct were fate-mapped and found to yield pericytes tightly associated with vessels in the adult thymus [8], a consistent finding in parallel studies [9]. Pericytes have also been assigned a mesoderm/mesenchymal origin, but they have further been suggested to transition into mesenchyme from epithelial precursors (i.e., via epithelial-to-mesenchymal transition, EMT) [2]. Tissue-specific mesothelium for instance has been implicated in giving rise to vascular mural cells including pericytes. More specifically, this single-layer of epithelium has been observed to generate vascular mural cells in the lung [10], gastrointestinal system [11], and in the heart [12–15]. In the liver, the septum transversum mesenchyme (STM) has been shown to yield hepatic stellate cells (HSCs) and perivascular mesenchymal cells (PMCs) located adjacent to the vasculature [16]. While these populations include vSMCs and portal/central vein fibroblasts, it is unclear (i) if vascular pericytes in the liver are generated unilaterally by these cells (i.e., HSCs and PMCs), and (ii) the relative pericyte abundance in the morphologically heterogeneous microcirculation of the liver (e.g., on bile duct microvessels vs. sinusoidal capillaries, which are known to be fenestrated to facilitate filtration, among other functions). Another unique source of pericytes was recently identified in the skin vasculature where tissue myeloid progenitor cells were found to differentiate into pericytes [17]. Endothelial cells have also been described as a source for cardiac pericytes and vSMCs [18], though other double-labeled animal models [19] will be useful in further corroborating these findings.

This potential overlap between pericyte and vSMC origins has often suggested that pericytes lineages might be extrapolated from tracing vSMC differentiation. Pericytes and vSMCs eventually associate with distinct locations in a vascular network, and they are believed to be functionally and morphologically dissimilar, though their origins and differentiation programs may overlap to a certain extent [18, 20]. An example of this shared lineage yielding divergent populations was recently identified in the development of coronary artery SMCs [21••]. Specifically, pericyte recruitment into the mouse heart vasculature occurred around embryonic day 11.5 (E11.5) via the platelet-derived growth factor-BB (PDGF-BB) pathway. Notch signaling from the neighboring endothelium then promoted vSMC differentiation [22–25]

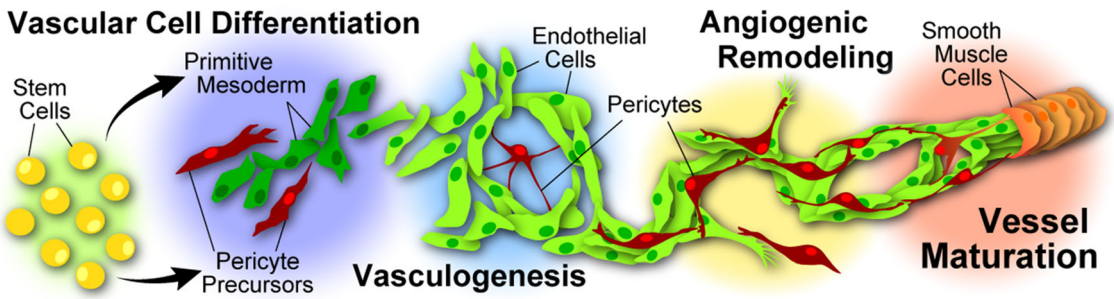

Fig. 1 Schematic illustrating a simplified progression for blood vessel development from vascular cell differentiation to vasculogenesis, subsequent angiogenic remodeling, and lastly vessel maturation and homeostasis. During the early stages of vascular formation, primitive mesoderm (dark green) and pericyte precursors (red) arise from pluripotent embryonic stem cells (yellow). Endothelial cells (green) emerge from mesodermal angioblasts and begin constructing basic vascular structures during vasculogenesis, perhaps with the involvement of mural cells including pericytes. These primitive blood vessels undergo angiogenic remodeling, recruiting pericytes to actively growing vessel networks. A subset of pericytes may further differentiate (red to orange) to contribute to arteriolar vascular smooth muscle cells (orange), as vessel maturation nears completion, and the microvasculature reaches quiescence and homeostasis

and development from a subset of these pericytes, while another pericyte sub-population remained associated with the coronary microcirculation [21••]. This paradigm may exist in other tissues where pericytes have been identified at early developmental time points. For instance, Jung et al. recently observed pericytes (and/or their precursors) invested in the capillary wall of blood vessels in the E10.5 mouse brain, and these cells expanded their coverage within the CNS microcirculation through the rest of embryonic and postnatal development [19]. Similarly, in extra-embryonic tissues such as the developing mouse yolk sac, PDGF receptor-β (PDGFRβ)-expressing pericytes (and/or pericyte precursors) were found alongside Tie2-positive capillary endothelium as early as E8.5 [26].

These and other vascular development studies highlight a challenge present across many lineage tracing studies and that is the positive identification of the terminally differentiated cell type of interest, here being pericytes. As stated above, PDGFRβ is a well-accepted marker of pericytes [2], but this cell surface receptor is also present on vSMCs and brain glia and can therefore present challenges in confident identification of pericytes in many tissues [27]. Vascular pericytes are also known to express neural glial antigen-2 (NG2, gene name: *Cspg4*, *Chondroitin Sulfate Proteoglycan-4*), which can be found on oligodendrocyte precursors (OPCs) in the brain, again confounding interpretation of cell identity [28]. An additional obstacle in pericyte identification is the presence of numerous cell types in peri-endothelial/capillary locations throughout the microcirculation including fibroblasts, macrophages, and epithelial cells [29–31]. One helpful distinction between pericytes and these cells is that non-vascular cell types rarely, if ever, integrate themselves within the vascular basement membrane (vBM). Thus, this unique configuration of ECM proteins (e.g., Type IV Collagen (Col-IV), laminins, perlecan) surrounding microvascular endothelia and pericytes can offer another criteria for their identification [32]. Ultimately, pericytes are the best identified through a combination of pericyte and endothelial markers, rigorous assessment of their morphology (which may include some slight heterogeneity [33, 34]), and their location within microvascular networks. Coupling these benchmarks with next generation sequencing approaches and single-cell analysis will sharpen existing pericyte-specific tools, and allow greater insight into critical questions such as when terminally differentiated pericytes first emerge during embryonic development and how they expand their coverage along the rapidly developing vasculature.

Wide-ranging signaling mechanisms have been implicated in orchestrating pericyte differentiation during embryogenesis. Cell surface PDGFRβ conveys intracellular signals after binding PDGF-BB ligands that are secreted by angiogenic endothelium [35], and these signals are believed to play an instrumental role in the differentiation, proliferation, and recruitment of both pericytes and vSMCs [36]. Similarly, transforming growth factor-β (TGFβ) is also important for mural cell differentiation from primitive mesenchyme, requiring the conversion of latent TGFβ to an active form via a dynamic interplay between Connexin43 (Cx43) gap junctions at the mural cell-endothelial interface, integrins, and matrix metalloproteinases (MMPs) [37••]. Angiopoietin-Tie signaling has been described as affecting pericyte differentiation and recruitment, though the exact role of this pathway in driving the pericyte lineage remains unclear [4, 38]. Genetic knockout studies such as those for Tie2 and Ang1 have suggested that pericytes emerge and home to nascent vessels despite developmental abnormalities in other tissue compartments [39, 40]. Recent observations involving the reprogramming of human induced pluripotent stem cells (iPSCs) into pericyte-like cells have suggested the importance of serum-containing media [41, 42] and perhaps the presence of basic fibroblast growth factor (bFGF or FGF2) [20]. However, a study by Guimarães-Camboa et al. raised an important caution when interpreting pericyte differentiation and identity in experiments involving cell manipulations ex vivo [43]. This message is reinforced by data indicating that initiation and maintenance of the vascular pericyte identity in a variety of contexts may be tightly linked to a sustained physical association with the microvascular endothelium [44].

Pericytes in the Earliest Stages of Vessel Formation

As discussed above, pericyte differentiation has been linked to direct contact with terminally differentiated endothelium and to endothelial cell-derived cues such as PDGF-BB. These observations suggest a developmental time-line in which endothelial cells differentiate first and likely commence the de novo formation of blood vessels (i.e., vasculogenesis) prior to the initiation of pericyte differentiation and subsequent association with the endothelium [45, 46]. This working model will likely require further validation, as it is currently unclear whether vascular pericytes (and/or their precursors) may actually: (i) differentiate concurrently with the emergence of endothelial cells, and (ii) participate in the earliest stages of blood vessel formation including vasculogenesis and subsequent developmental processes. Pericytes may temper endothelial cell proliferation for example [47, 48], promoting endothelial survival independent of vascular endothelial growth factor-A (VEGF-A) as vessels transition to a more quiescent state [49, 50].

Pericyte Involvement in Vasculogenesis

As differentiating angioblasts within the primitive mesoderm give rise to endothelial cells, these newly derived cells begin to organize and coalesce into primitive vascular structures

during the vasculogenic phase of blood vessel development. Before these primordial vessels expand into an interconnected network, they contain hematopoietic cells and/or their precursors, inspiring their classification as "blood islands," such as observed in developing mouse yolk sac [51–54]. As stated earlier, PDGFRβ+ pericytes have been observed alongside the developing mouse yolk sac vasculature [26], but it is unclear how involved these pericytes are in early vessel development, or if they are recruited to vessels after these primitive "tubes" have completed an initial phase of assembly and lumenization, which has been documented within in vitro co-culture studies [55]. Given that pericytes establish gap junctions with endothelial cells during early development and beyond [37, 56–60], it is intriguing to speculate that, even at the earliest stages of vessel formation, pericytes may provide initiating and/or reinforcing cues for endothelial cell polarity [61], i.e., in establishing apical-basolateral surfaces and lateral edges. This potential role for pericytes is consistent with recent work implicating pericytes in maintaining vessel barrier function [62] by promoting synthesis and localization of junctional proteins along these endothelial lateral edges (i.e., tight junctions: ZO-1, claudin-5, occludin) and modulating vesicular transport, most notably at the blood-brain barrier (BBB) [63].

Pericytes appear to be present along the endothelium at the earliest stages of vessel formation. For example, in chick-quail graft experiments, mesenchymal cells (neural crest-derived) organized as mural cells around the mesoderm-derived, endothelial cell-lined tube of the aortic arch primordium, and adjacent primitive capillaries [7], yet we are still clarifying the precise time-line for, and mechanisms by which, pericytes engage with developing vascular networks. Pericytes may increase their numbers during embryonic development largely independent of vessel-association, and then subsequently home to vessel networks that are actively expanding via sprouting angiogenesis and "splitting" intussusception. Alternatively, or perhaps concurrently, mural cells including pericytes may first invest within the walls both major vessels [64, 65] and microvessels during vasculogenesis and then proliferate based on direct chemical and mechanical signals from the endothelium. Addressing these fundamental questions regarding early vessel formation and pericyte biology will likely have important therapeutic consequences, as a deeper understanding of the mechanisms underlying pericyte proliferation for example will be important in targeting pericyte expansion in disorders associated with their dysfunction or "dropout" from the vessel wall such as in Alzheimer's disease [66–69] and diabetic retinopathy [70–73]. Thus, incorporating recent pericyte-targeted tools into developmental models will usher in new insights into pericyte dynamics during vascular development and their roles in blood vessel physiology and pathology.

Pericyte-Endothelial Cell Interactions During Angiogenesis

Across numerous developing tissues and organs, the growth of new blood vessels from pre-existing microvasculature or angiogenesis occurs primarily, if not exclusively, on the capillary level where pericytes are most abundant. In particular, microvascular endothelial cells that sprout and initiate outward migration from a parent vessel secrete PDGF-BB [74, 75], presumably to stimulate neighboring pericytes into synchronized expansion and chemotaxis [76, 77]. The PDGF-BB ligand can anchor in the neighboring extracellular matrix (ECM) via binding by heparin sulfate proteoglycan-2 (Hspg2, or perlecan) [78]. A recent study by Dubrac and colleagues further demonstrated that PDGF-BB-induced pericyte migration is also dependent on NCK1 and NCK2 adaptor proteins, specifically in retinal neovascularization [79]. Heparin-binding EGF-like growth factor (HB-EGF) is another endothelial-derived signal that can promote pericyte recruitment to the vessel wall [80], though this cue may be more prevalent during vasculogenic tube formation. As highlighted by the ECM association of several pericyte-targeted signals, the specialized ECM known as the vascular basement membrane (vBM), and the individual components therein, may be as important in mediating pericyte-endothelial cell crosstalk during angiogenesis as soluble growth factors and other cytokines [34]. Endothelial cells deposit Col-IV, for instance, which acts as a scaffold for the incorporation of other vBM constituents such as fibronectin, laminin isoforms, perlecan, and nidogen-1 [55]. Collectively, these endothelial-derived cues create a local microenvironment that actively enhances pericyte migration along extending vessel sprouts and promotes the recruitment, investment, and retention of pericytes along these newly forming vessel branches [81]. Nevertheless, more work will be necessary to establish exactly how pericytes migrate and invest systemically throughout the microcirculation, i.e., via migration to specific vessel locations and then undergoing investment, or by attaching to the nearest blood vessel, propagating, and investing along the microvasculature as they expand in number.

While pericyte elaboration along new vessels may be largely governed by the signals released from sprouting endothelial cells, pericytes may reciprocally influence angiogenic remodeling. For example, among other vBM components [82], pericytes deposit vitronectin within the vessel wall of the CNS microcirculation [83], which may be involved in clinical conditions such as cerebral autosomal dominant arteriopathy with sub-cortical infarcts and leukoencephalopathy (CADASIL) by accelerating white matter lesions during aging [84]. Furthermore, for pericytes to actively participate in sprouting angiogenesis, they would likely need to present alongside endothelial cells in the act of outward migration from an existing vessel, which is observed in the developing

mouse brain (Fig. 2, [34]) and retina [85], as well as in an aortic ring explant model [86]. Sprout-associated pericytes have also been suggested to influence the levels and distribution of pro-angiogenic cues including VEGF-A [87, 88] and certain microRNAs [89]. However, it is not clear if this is true in other organs/tissues or across all developmental windows, as there may be important time-delays between endothelial cell sprouting and pericyte recruitment to allow for greater plasticity in angiogenic remodeling [50]. Pericytes have also been observed bridging gaps between endothelial cells, both at the sprout "tip" and between endothelial cells of an incomplete vessel branch [90, 91]. These observations, alongside many others [82, 92–100], have further suggested that pericytes may also lead endothelial sprouting in various healthy and pathological conditions. A recent study from Errede et al. has further suggested that brain pericytes construct Col-IV-based tunneling nanotubes to guide endothelial cell filopodial extensions and perhaps even facilitate long-range communication between endothelial cells and pericytes on discrete vessel segments [101]. This study, as well as those described above, highlights the fact that we have much more to discover in establishing the dynamic interplay between vascular pericytes and the remodeling endothelium, as well as other neighboring cell types [102], during sprouting angiogenesis.

New vessel branches can arise from existing vasculature via another mode of structural remodeling known as intussusceptive, or "splitting," angiogenesis [103]. Introducing a bifurcation into a single vessel, thereby creating two parallel channels, involves the protrusion and subsequent expansion of a tissue pillar into the lumen of an existing vessel. Relative to sprouting angiogenesis, the mechanisms underlying intussusception remain understudied, as detecting these events by light microscopy can be challenging. Karthik and colleagues recently applied serial block face-scanning electron microscopy (SBF-SEM) to the zebrafish caudal vein plexus and found complex intussusceptive remodeling to refine the hierarchical nature of the developing vascular network [104]. While this group did not focus on the involvement of mural cells, and specifically of pericytes, previous observations have suggested that pericytes extend cytoplasmic processes along inter-endothelial cell junctions as trans-capillary pillars form [105, 106]. The molecular mechanisms governing pericyte interactions with intussusceptive angiogenic endothelium are largely inferred from the sprouting angiogenesis context [107], but as pericyte-focused tools continue to expand, models such as the zebrafish caudal vein plexus can be revisited to refine current models of pericyte involvement during intussusception.

Pericyte Contributions to Vessel Pruning and Regrowth

Pericytes have been implicated not only in vessel formation but also in the selective pruning of vasculature that may have been produced in excess or is no longer required for tissue perfusion [108]. Pericyte-mediated vessel regression, for example, has been shown to occur via calpain activation by CXCR3, which causes an involution of human dermal microvascular endothelial cells in an in vitro Matrigel assay [109]. Pericyte deposition of endosialin within the vBM can also promote vessel rarefaction via endothelial cell apoptosis, in part by limiting endothelial cell adhesion to the ECM [110]. In contrast, pericytes can affect vBM remodeling to limit vessel regression by blocking matrix metalloproteinases (MMPs). Pericytes secrete tissue inhibitor of metalloproteinase-3 (TIMP-3), for example, to block the activity of MMP-1 and MMP-10 and sustain nascent and established vascular structures [111–113]. Thus, remodeling of the local capillary microenvironment, especially targeting the vessel wall ECM, is a mode by which pericytes regulate vascular growth and maintenance of new microvessel branches. The physical association of pericytes with the endothelium has also been identified as an important modulator of capillary stability [114] and in turn vessel regression. In a model of hyperoxia, disrupted pericyte-endothelial cell associations contributed to retinal

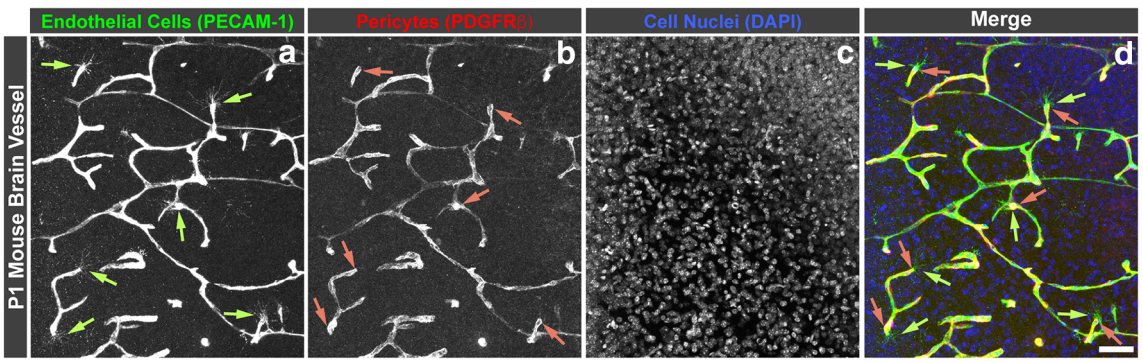

Fig. 2 Confocal image of sprouting endothelial cells labeled for platelet-endothelial cell adhesion molecule-1 (PECAM-1: A and green in D, indicated by light green arrows) with associated pericytes labeled for platelet-derived growth factor receptor-β (PDGFRβ: B and red in D, indicated by pink arrows) in a postnatal day 1 (P1) mouse brain. Cell nuclei are labeled with DAPI (C, and blue in D). All of the denoted pericytes appear to be positioned along emerging endothelial "tip" cells from the base of the filopodia, along the sprout, to the parent vessel. Scale bar in D, 50 μm

vessel regression and abnormal microvascular remodeling [50]. Pericyte detachment or absence has also been associated with the rarefaction of kidney capillaries [115] and with the malformation of retinal vasculature akin to defects observed in diabetic retinopathy [70] and in tumors [116]. This role for direct pericyte-endothelial contact in vessel stability may involve intercellular communication mechanisms such as those mediated by Cx43-based gap junctions [117], but the specific molecular cues regulating these interactions are still being established.

While pericyte loss has been associated with vessel pruning and rarefaction, pericytes have also been observed to persist in locations where capillary regression has occurred. These surviving pericytes appear to provide a scaffold for rapid revascularization of tissues that are transiently exposed to anti-angiogenic agents [118]. Interestingly, these pericytes also attach to and maintain vBM components such as Col-IV [119–121], perhaps so that these Col-IV "sleeves" may be re-used by endothelial cells in the event that vessel regrowth is stimulated. Not all of these pericytes remain in avascular locations after vessel regression, however, as some have been reported to migrate to surviving vessels, specifically in a model of VEGF receptor inhibition in the mouse retina [122].

Pericytes in Vessel Maturation, Homeostasis, and Physiology

As the active formation of blood vessels transitions to establishing mature, quiescent microvascular networks, pericytes also shift from structural remodeling and expansion roles to supporting vessel physiology and homeostasis. After pericytes divide and migrate to establish sufficient coverage within the microcirculation [123], their proliferative capacity appears to diminish, as recent work by Berthiaume and colleagues suggests that selective ablation of quiescent brain capillary pericytes induces neighboring pericytes to extend cellular processes and re-establish vessel coverage without notable changes in pericyte proliferation [124]. These data suggest that established microvascular pericytes might be a post-mitotic cell type as seen with cortical neurons [125] and certain muscle cell types, though vessel remodeling stimuli re-engage mechanisms capable of inducing pericyte division [86], suggesting a level of retained plasticity. As pericytes establish defined locations within the microvasculature, they likely refine the vBM surrounding themselves and the endothelium to transform the provisional ECM constructed during vessel remodeling to a more mature basement membrane [83]. The vBM is a critical structure for the barrier function of the microcirculation in many tissues, seen most clearly in the BBB and associated vBM defects contributing to specific neurovascular pathologies [126–128]. Along with fine-tuning the vBM and reducing systemic proliferation, pericytes promote vessel maturation in part by becoming more invested in the vessel wall and downregulating mechanisms facilitating their migration [79, 129]. Reduced migration might also be essential for establishing more permanent cell-cell interaction with the endothelium, as ultra-structural observations have identified "peg-and-socket" contact points [130–133]; interestingly, the exact time course for these structures is not well-established, and they may actually be present early in vascular development, aligning with pericyte differentiation via endothelial gap junction formation [58–60, 117, 133, 134]. Pericytes are capable of deconstructing "peg-and-socket" junctions and restarting active migration along and outward from the vessel wall in certain scenarios such as during inflammation [135, 136] and in disease settings like diabetic retinopathy [72, 73, 137]. Thus, hallmarks of pericytes that are transitioning to quiescence while concurrently promoting vessel maturation and stability include a reduction in proliferation and migration while increasing deposition of vBM components that reinforce the microvessel wall.

In addition to mechanisms governing the phenotypic shift in pericytes from remodeling to more stable, pericyte location within a microvascular network also appears to be tightly regulated. For example, pericytes appear to establish non-overlapping domains such that no two pericytes cover the same "territory" along the microvessel wall [124], though the mechanisms underlying this coordinated coverage remain to be fully established. Pericytes at different locations throughout the microcirculation also appear to have distinct morphological characteristics [33••], with recent classifications including: (1) *ensheathing pericytes* that contain α-smooth muscle actin (αSMA) and wrap fully around pre-capillary arterioles, (2) *mesh pericytes* lacking αSMA and begin coverage of capillary-sized vessels, and (3) *thin-strand pericytes* also without αSMA but morphologically distinct with single cellular extensions along segments of the microvasculature [33••]. This topology and morphological heterogeneity suggests that, just as endothelial cells can acquire arterio-venous identity based on their location within the vasculature [138, 139], pericytes might also integrate chemical and mechanical cues to assume discrete phenotypic roles within the microcirculation. Consistent with this idea are observations of pericytes giving rise to vSMCs [21••] and perhaps contributing to arteriogenic remodeling [140] and collateral artery formation. Pericytes have also been reported to respond to vasoactive cues [141], propagate electrical signals within the microcirculation (e.g., via gap junction-mediated conducted vasomotion) [142–144], and perform vasomotor functions similar to vSMCs [145–150], though passive regulation of vessel diameter by pericytes has also been described [124, 151, 152]. These studies highlight the need for more innovative models [153, 154] and techniques [66, 155] to resolve this potential role for capillary pericytes across various tissues and organs.

Perspectives: Looking Ahead

Recognition of pericytes and their vital roles in forming and maintaining the microcirculation is continuing to grow. A notable example of this increased appreciation can be seen with the recent interest in establishing in vitro platforms to model pericyte-mediated vessel integrity and especially the BBB [156, 157]. Many of these models seek to differentiate human induced pluripotent stem cells (iPSCs) into various vascular cell types including pericytes [41, 42]. It will therefore be critical to build a more solid understanding of pericyte differentiation programs and the transcriptional and behavioral hallmarks of their progression into a mature, terminally differentiated cell type. Validating these and other pericyte cell lines will further require a more comprehensive understanding of the phenotypic heterogeneity within pericyte populations as they differentiate and contribute to the discrete stages within vascular development (Fig. 1). Lastly, greater insight into the various roles that pericytes acquire during blood vessel formation will be essential for "benchmarking" pericytes used in cell-based assays as well as for potentially targeting pericyte functions in certain disease conditions. Coupling recent advances in imaging and transcriptional profiling modalities with pericyte-focused tools and models will ensure that realizing many, if not all, of these goals is just on the horizon and very much within reach.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Human and Animal Rights All reported studies/experiments with human or animal subjects performed by the authors were performed in accordance with all applicable ethical standards including the Helsinki declaration and its amendments, institutional/national research committee standards, and international/national/institutional guidelines.

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