Histology and Histopathology
From Cell Biology to Tissue Engineering

Pericyte morphology and function

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Summary. The proper delivery of blood is essential for healthy neuronal function. The anatomical substrate for this precise mechanism is the neurovascular unit, which is formed by neurons, glial cells, endothelia, smooth muscle cells, and pericytes. Based on their particular location on the vessel wall, morphology, and protein expression, pericytes have been proposed as cells capable of regulating capillary blood flow. Pericytes are located around the microvessels, wrapping them with their processes. Their morphology and protein expression substantially vary along the vascular tree. Their contractibility is mediated by a unique cytoskeleton organization formed by filaments of actin that allows pericyte deformability with the consequent mechanical force transferred to the extracellular matrix for changing the diameter. Pericyte ultrastructure is characterized by large mitochondria likely to provide energy to regulate intracellular calcium concentration and fuel contraction. Accordingly, pericytes with compromised energy show a sustained intracellular calcium increase that leads to persistent microvascular constriction. Pericyte morphology is highly plastic and adapted for varying contractile capability along the microvascular tree, making pericytes ideal cells to regulate the capillary blood flow in response to local neuronal activity. Besides the vascular regulation, pericytes also play a role in the maintenance of the blood-brain/retina barrier, neovascularization and angiogenesis, and leukocyte transmigration. Here, we review the morphological and functional features of the pericytes as well as potential specific markers for the study of pericytes in the brain and retina.

Key words: Pericyte, Neurovascular unit, Functional hyperemia, Alpha smooth muscle actin, Blood flow regulation, Blood-brain/retina barrier

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Pericyte phenotype and regulation of capillary diameter

The central nervous system (CNS) is made of about 86 billion neurons (Azevedo et al., 2009), using 20% of the total body energy (Watts et al., 2018). A proper neuronal function requires the restoration of the ATP stores after neuronal activation (Attwell and Laughlin, 2001). The supply of oxygen and glucose needed to produce ATP is delivered to the tissue via microcirculation. A fine flow regulation that directs blood to the activated neuronal groups while reducing it to inactive areas is essential for the brain and retina, where neighboring groups of neurons function independently from each other (Alarcon-Martinez et al., 2020). This process is named neurovascular coupling (Roy and Sherrington, 1890; Attwell et al., 2010; Newman, 2013; Yu et al., 2019a; Alarcon-Martinez et al., 2020) or, in physiological terms, functional hyperemia (Attwell et al., 2010; Newman, 2013). The anatomical substrate of functional hyperemia is the neurovascular unit, consisting of neurons, glial cells, endothelia, smooth muscle cells and pericytes (Attwell et

al., 2010; Newman, 2013; Dalkara and Alarcon-Martinez, 2015) (Fig. 1A). Each component of the neurovascular unit communicates with others by a complex signaling, and their contribution to neurovascular coupling is being increasingly recognized in health and disease (Yemisci et al., 2009; Attwell et al., 2010; Hamilton et al., 2010; Hall et al., 2014; Biesecker et al., 2016; Kisler et al., 2017a; Alarcon-Martinez et al., 2019).

Due to their location around the capillaries, pericytes have been proposed as cells that are able to modify the capillary diameter, regulating the amount of blood flowing through the microcirculation during functional hyperemia (Hall et al., 2014; Rungta et al., 2018; Alarcon-Martinez et al., 2020). Indeed, pericytes have been shown to express the contractile proteins needed to regulate blood flow (Wallow and Burnside, 1980; Herman and D'Amore, 1985; Hamilton et al., 2010; Hall et al., 2014; Rungta et al., 2018; Alarcon-Martinez et al., 2018, 2019, 2020) such as filamentous actin (F-actin) (Kureli et al., 2020) and alpha smooth muscle actin (α-SMA) (Alarcon-Martinez et al., 2018; Kim et al., 2020), including in humans (Yu et al., 2019b) (Fig. 1B). Thus, vasoactive mediators released in response to neuronal activity can change vessel diameter by modifying the conformation of contractile proteins in pericytes (Puro, 2007, 2012; Alarcon-Martinez et al., 2019; Kureli et al., 2020).

Although heterogeneous in morphology, in general,

pericytes have a prominent soma with several large processes (primary processes or branches) and small projections that emerge from the primary processes (secondary processes) covering the microvessels, including pre-capillary arterioles, capillaries and post-capillary venules (Takahashi et al., 1997; Hartmann et al., 2015a). Pericyte processes exhibit 2 main phenotypes: i) thin singular strands, twisting along the capillary lumen and forming helical structures (helical pericyte); ii) mesh-like sheath surrounding the whole vessel (mesh pericyte) (Sims, 1986; Hartmann et al., 2015a; Alarcon-Martinez et al. 2018). There is a relationship between pericyte morphology and the location of their soma along the microvascular tree (Hartmann et al., 2015a; Alarcon-Martinez et al., 2018; Kureli et al., 2020): pericytes with their cell body located at the pre-capillary arterioles, upstream microvessels and bifurcation points of a microvessel (i.e. junctional pericytes) present mesh-like, circular staining pattern wrapping microvessels. On the other hand, pericytes with their somas on the straight parts of a capillary segment (i.e. mid-capillary pericytes) present a helical, strand-like staining pattern (Hartmann et al., 2015a; Alarcon-Martinez et al., 2018; Kureli et al., 2020). Interestingly, mesh pericytes express more frequently and intensely α-SMA than mid-capillary ones, suggesting a higher contractibility for junctional than for mid-capillary pericytes (Hartmann et al., 2015a; Alarcon-Martinez et al., 2018). Recently, Alarcon-
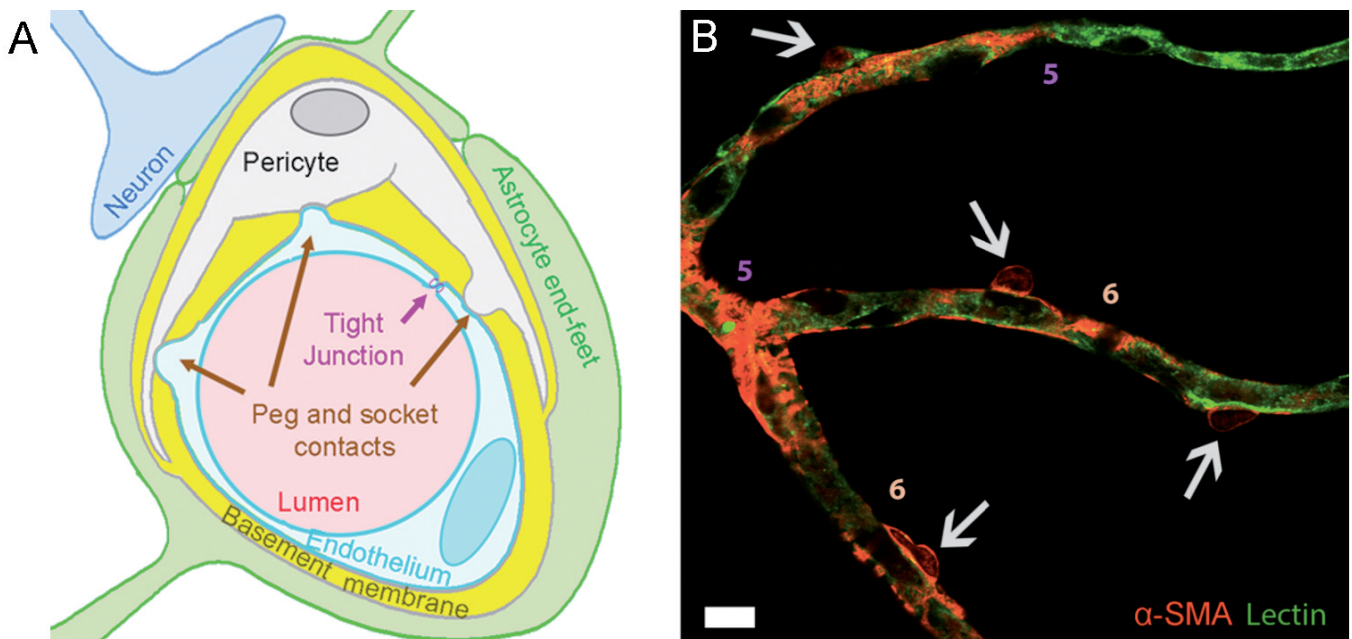
Fig. 1. The neurovascular unit. **A.** The neurovascular unit is formed by neurons, endothelia, smooth muscle cells, pericytes, the basement membrane encircling endothelia and pericytes, and astrocyte end-feet surrounding the microvessel. Peg and socket type contacts have been reported between endothelia and pericytes. Reproduced from Dalkara and Alarcon-Martinez, 2015 with permission. **B.** High order retinal capillaries visualized with lectin (green; numbers indicate the branch order) showed associated pericytes with α-SMA immunolabeling (red; arrows) after the prevention of α-SMA depolymerization *in vivo*. Reproduced from Alarcon-Martinez et al., 2018, with permission. Scale bar: 10 μm.

Martinez and colleagues have discovered a new type of pericyte with an atypical morphology (Alarcon-Martinez et al., 2020). In addition to the primary and secondary processes, these pericytes also present fine tubular processes as previously reported *in vitro* in other cell types (Rustom et al., 2004). They emerge from the pericyte soma and connect to the pericytes located on neighbouring capillaries, forming the interpericyte tunneling nanotubes (IP-TNTs) (Fig. 2). IP-TNTs contain organelles and express α-SMA to regulate the diameter of the connected capillaries by calcium signaling through the nanotube (Alarcon-Martinez et al., 2020). Of note, IP-TNTs resemble previously reported intervascular structures in fixed tissue (Cammermeyer 1960; Kuwabara and Cogan, 1960; Reissenweber and Pessacq, 1971; Williamson et al., 1980; Leibnitz and Bär, 1988; Brown, 2010; Mendes-Jorge et al., 2012). However, unlike IP-TNTs, these structures were positive for endothelial cell markers and negative for the α-SMA labels (Hughes et al., 2000; Mendes-Jorge et al., 2012), and their nuclei were found to be located in the middle of the process or even absent (Connolly et al., 1988; Mendes-Jorge et al., 2012), forming empty tubes made of basement membrane (Brown, 2010).

These findings altogether suggest that pericyte morphology is highly plastic and adapted for contractile capability, making pericytes ideal cells to regulate the capillary diameter analogous to upstream vascular smooth muscle cells (vSMCs).

Pericyte ultrastructure: organelles and intracellular organization

Pericytes in the retina and brain have been studied by electron microscopy for several years (Maynard et al., 1957; Hogan and Feeney, 1963; Allsopp and Gamble, 1979; Murakami et al., 1979; Sims, 1986; Ushiwata and Ushiki, 1990). These ultrastructural studies have shown that pericytes have round nuclei with heterogeneous electron dense areas, suggesting an unevenly distributed chromatin (Maynard et al., 1957; Pavelka and Roth, 2015; Nahirney et al., 2016; Guérin et al., 2019; Alarcon-Martinez et al., 2020), concentrating toward the periphery of the nucleus (Hogan and Feeney, 1963; Sims, 1986; Pavelka and Roth, 2015) (Fig. 3). Pericytes are enclosed by basement (abluminal-oriented side) and plasma (luminal-oriented side) membranes (Hogan and Feeney, 1963; Alarcon-Martinez et al., 2020), which are significantly thicker in humans and monkeys compared to rodents (Hogan and Feeney, 1963). Interestingly, in humans and monkeys, there are substantial, large spaces or cavitations filled with electron dense material between these membranes (Hogan and Feeney, 1963). Pericytes have a cytoplasm with long processes, containing organelles such as large mitochondria, endoplasmic reticulum, glycogen and ribonucleoprotein granules, vesicles, and lysosomes (Hogan and Feeney, 1963; Sims, 1986; Bianchi et al., 2016; Nahirney et al., 2016; Alarcon-Martinez et al., 2020). On the luminal

side, the pericyte membrane is tightly in contact with the endothelium and is punctured by cytoplasmic pericyte protrusions projecting into endothelial indentations forming the structures known as peg-socket junctions or contacts (Allsopp and Gamble, 1979). On the abluminal side, pericytes are in close contact with glial cells, even showing fenestrations in their basement membrane at the contact site with Müller cells in human retinas (Hogan and Feeney, 1963). To generate sufficient force and transfer this to the extracellular matrix for constricting the capillary, pericytes have a cytoskeleton formed by filaments of actin (Wallow and Burnside, 1980; Herman and D'Amore, 1985). Significant accumulations of actin bundles are preferentially situated at the endothelial (i.e. luminal) side (Wallow and Burnside, 1980), binding to adhesion plaques (hence, connecting cytoskeleton with extracellular matrix) located at the contact points with endothelial cells (Bianchi et al., 2016; Nahirney et al., 2016). This actin lattice allows direct transmission of the mechanical force generated by pericytes to the extracellular matrix, leading to microvessel diameter
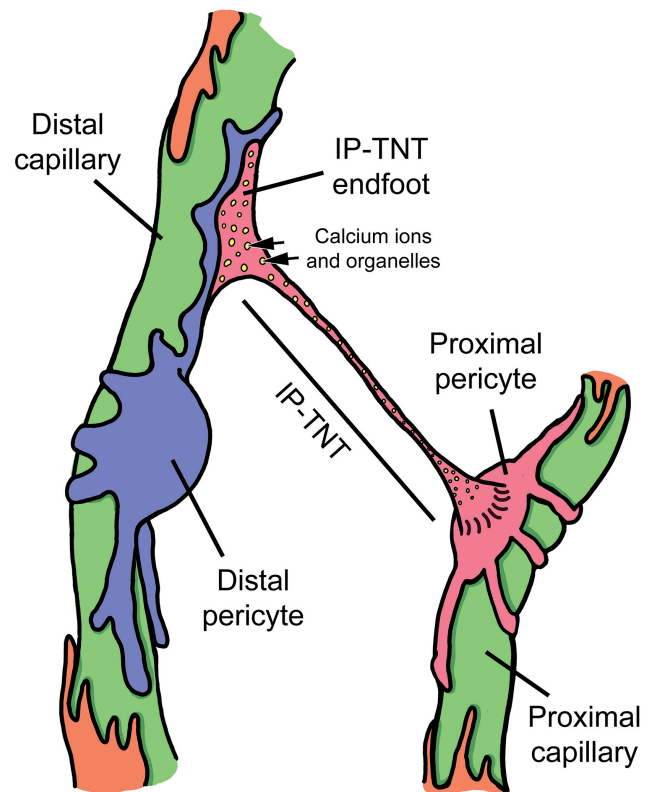
Fig. 2. Inter-pericyte tunnelling nanotubes. Interpericyte tunnelling nanotubes (IP-TNTs) are fine tubular processes connecting pericytes located in neighboring capillaries. IP-TNTs emerge from the soma of a proximal pericyte (red), forming a tubular process that contacts with a distal pericyte (blue). IP-TNTs contain organelles and allow communication between the connected pericytes by calcium signaling, as previously reported by Alarcon-Martinez et al., 2020.

changes. Indeed, studies performed with atomic force microscopy disclosed the actin-based mechanical forces generated by cultured retinal pericytes (Lee et al., 2010), which were identified as wrinkles on malleable silicone rubber substrata and correlated with F-actin density changes (Lee et al., 2010). Recently, the role of F-actin polymerization in capillary pericyte contraction with the consequent constriction of capillaries has been confirmed *in vivo* (Kureli et al., 2020).

Correlative serial block-face focused ion beam scanning electron microscopy (FIB-SEM), an emerging technology, allows the correlation of previously identified fluorescent signals/structures with 3D scanning electron microscopy images (Guérin et al., 2019; Alarcon-Martinez et al., 2020; Hoffman et al., 2020). FIB-SEM has confirmed the distinctive ultrastructural features of pericytes having an electro-dense cytoplasm with long processes, which contain large mitochondria, vesicles, and endoplasmic reticulum (Guérin et al., 2019; Alarcon-Martinez et al., 2020). IP-TNTs have also been studied with FIB-SEM: Alarcon-Martinez and colleagues showed that IP-TNTs are enclosed by a basement and plasma membrane that emerge from the cytoplasm that surrounds the pericyte soma. Similar to the cytoplasm of other types of pericytes, the IP-TNTs contain mitochondria, vesicles, and endoplasmic reticulum, suggesting a passage for a potential cargo of organelles and signalling molecules (Alarcon-Martinez et al., 2020). IP-TNTs project to distal capillaries, connecting to downstream pericytes through actual direct membrane-to-membrane contacts such as gap junctions, allowing the transfer of signalling molecules like calcium ions (Alarcon-Martinez et al., 2020).

Histological markers for pericytes

Pericytes are found most densely in the brain and retina. When described in the 19th century by Eberth and Rouget, they pointed to the characteristic location of pericyte soma on the outside of capillaries, exhibiting a bump-on-a-log morphology. Their cytoplasmic processes wrap the microvessel wall, of which coverage differs along the course of the microvascular tree. Identification of pericytes by histopathology has always been a challenge. For many years, retinal digestion with trypsin and/or pepsin enzymes were widely used to study pericyte morphology and location in the retinal vasculature (Kuwabara and Cogan, 1960; Dietrich and Hammes, 2012; Mendes-Jorge et al., 2012). This method allows selective digestion of the retinal cells and, hence, isolation of the microvascular tree, which can then be visualized with periodic acid-Schiff (PAS) staining (Kuwabara and Cogan, 1960; Dietrich and Hammes, 2012; Mendes-Jorge et al., 2012). Thus, the typical morphology of the pericyte, unambiguously recognizable only on meticulous ultrastructural analyses before, was used as the "gold standard" criteria for their identification. This morphology-based identification has recently been complemented by the discovery of several cell-specific proteins that can be used to detect pericytes with immunohistochemistry.

As previously mentioned, pericytes are embedded in two membranes that help in distinguishing them from vSMCs, in addition to their typical eccentric nucleus. However, it is still challenging to distinguish them from adjacent microglia, oligodendrocytes precursor cells and macrophages, considering that pericytes present various morphologies along the vascular bed as detailed above. Pericytes have been shown to express platelet-derived growth factor receptor β (PDGFR-b), NG2 (Cspg4), CD13 (aminopeptidase N), vimentin, and the regulator of G protein signaling 5 (RGS5) in *in vitro* and *ex vivo* studies by means of immunohistochemistry (Armulik et al., 2011; Bhattacharya et al., 2020; Uemura et al., 2020). In addition to the above markers detected in both
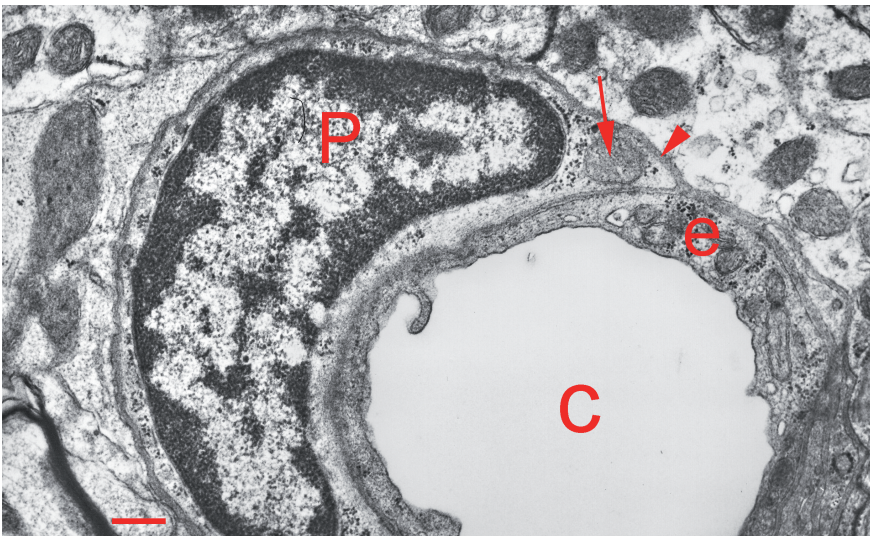
Fig. 3. An electron microscopy image of a blood capillary. A pericyte enclosed by the basement and plasma membranes (arrowhead) is shown. The pericyte is located around a capillary of a rat in the thalamic ventrobasal nucleus, containing a mitochondrion (arrow). The nucleus shows a heterogeneous electron dense area, concentrating toward the periphery of the nucleus. c, lumen; P, pericyte; e, endothelium; arrow, mitochondrion; arrowhead; pericyte membranes. Modified from figure 3.06 from the Atlas of Ultrastructural Neurocytology by Josef Spacek at SynapseWeb (https://synapseweb.clm.utexas.edu/306, Kristen M. Harris, PI). Scale bar: 500 nm.

vSMCs and pericytes, transgelin (SM22a), calponin1 (CNN1), desmin, and melanoma cell adhesion molecule (MCAM, or CD146) are also expressed in pericytes, though they are less prominently compared to vSMCs (Uemura et al., 2020). The expression profiles of pericytes become more distinctive toward downstream capillaries; unlike pericytes positioned proximally (i.e. near the arteriolar end) that express the above vSMC markers in lower amounts (Smyth et al., 2018; Vanlandewijck et al., 2018), expression of ATP-sensitive potassium-channel Kir6.1 (also called Kcnj8) and ATP binding cassette subfamily C member 9 (ABCC9) also known as sulfonylurea receptor 2 (SUR2), both of which are part of the same channel complex, along with the ligand delta homologue 1 (DLK1), is expressed in pericytes (Bondjers et al., 2006; He et al., 2016). Accordingly, when using the above markers, the position of the pericytes along the vasculature should be taken into consideration to avoid confusion with vSMCs. It should also be kept in mind that the expression of each marker may change with developmental stages as well as under pathological states (Armulik et al., 2011). For instance, during angiogenesis in embryogenesis or in pathological neovascularization seen in tumors, pericytes are shown to be labeled with CD248 (Endosialin/TEM 1) (Bagley et al., 2008) and DLK1 (Bondjers et al., 2006).

Although none of the available markers are entirely pericyte-specific, they can reliably identify pericytes when combined with morphological and location criteria summarized above. The expression of the mentioned markers is also heterogeneous, suggesting the presence of subpopulations of pericytes with different functions (Armulik et al., 2011; Santos et al., 2018). On the other hand, pericytes do not express the markers specific for other CNS cells such as GFAP (glial cells), CD31 or von Willebrand factor (endothelial cells), Iba1 (microglia) or NeuN (neuronal cells) (Brown et al., 2019), which may also indirectly help identification of pericytes. Despite all these tools, it is still indispensable to verify pericytes with additional markers such as those delineating the endothelia by its specific immunohistochemical markers or, more practically, using vessel markers such as fluorescently-labeled lectins (Robertson et al., 2015) as well as labeling nuclei with dyes such as Hoechst 33342 or DAPI.

The setting of the evaluation conditions (e.g. *in vitro* or *in vivo*), in addition to the fixation methods used, introduces a certain amount of variability to the determination of the expression of antigens. For example, some researchers failed to detect α-SMA in CNS capillary pericytes due to the use of paraformaldehyde or formaldehyde as the tissue fixative (Thavarajah et al., 2012). In contrast, rapid fixation with methanol at -20˚C to stabilize F-actin promptly before it depolymerizes or prevention of F-actin depolymerization by stabilizing agents like phalloidin led to the successful identification of α-SMA expression in mid-capillary pericytes, previously thought to be α-SMA immunonegative (Alarcon-Martinez et al., 2018). The identification of α-SMA in pericytes is crucial not only for histopathology, but also for physiology, because, considering the contractile function of this protein, it is an essential requirement to attribute a role to pericytes in blood flow regulation.

To study pericytes in the intact brain by high-resolution *in vivo* imaging, and also to avoid limitations of histological staining, several transgenic mice lines expressing fluorescent proteins under the control of pericyte-specific genes (e.g. NG2) have been generated (Hartmann et al., 2015b). Moreover, mice expressing two transgenic reporters, such as NG2 (Cspg4) (NG2–DsRed) and PDGFR-b (Pdgfrb-eGFP) (Jung et al., 2018) have also been developed since the use of a single reporter can be confounded by its expression in nearby neuroglial cells (He et al., 2016).

While pericyte definition relies practically on morphology and display of protein markers, revealing their gene profiles with single-cell RNA sequencing (scRNA-seq) has recently gained attention, and it is now considered a crucial tool to characterize a specific cell or tissue. Although transcriptomic studies of brain mural cells were not yet able to differentiate between pericyte subtypes reliably, they were able to disclose the differences in the expression profiles comparing vSMCs and pericytes, and also pericytes of different organs (He et al., 2016; Vanlandewijck et al., 2018). Some novel markers such as vitronectin (Vtn) and interferon-induced transmembrane protein 1 (Ifitm1) were identified to be specific for pericytes, after validation by *in situ* hybridization and immunohistochemistry, with the help of scRNA-seq studies in mural cells (He et al., 2016). Transcriptomics of mural cells in health and disease are promising in both understanding function and pathology (Francisco et al., 2020). However, the prediction of protein levels just by considering RNA levels is regarded as unreliable and not precise (Fortelny et al., 2017). Although using immunohistochemistry (Idikio, 2009) or reporter transgenic mice have some drawbacks, they have the advantage of seeing the physiology or pathology *in situ*, colocalizing the proteins or cells, and, hence, are still regarded as powerful techniques.

Physiological roles of pericytes

Form follows function: pericytes enwrapping microvessels with their processes prompted the idea since their discovery that they might have a contractile function and regulate microcirculatory blood flow. However, this hypothesis was questioned because the flow to the tissue is largely regulated upstream to microcirculation in peripheral organs. The latter view found support from studies that failed to detect α-SMA immunoreactivity in capillary pericytes in tissue sections (Nehls and Drenckhahn, 1991; Taylor et al., 2010; Hill et al., 2015; Wei et al., 2016; Grutzendler and Nedergaard, 2019) contrary to the studies using pericyte cultures, which consistently reported the presence of several

contractile proteins and the associated proteins, including α-SMA in pericytes (Herman and D'Amore, 1985; DeNofrio et al., 1989; Lee et al., 2010). On the other hand, functional studies performed on both cultures and explants of cerebral and retinal tissues showed that pericytes contracted in response to several vasoconstrictor agents as well as electrical stimulation (Herman and D'Amore, 1985; Kelley et al., 1987; Peppiatt et al., 2006; Puro, 2007; Mishra et al., 2016). Recent *in vivo* studies using 2-photon microscopy have confirmed these *in vitro* observations by showing that pericytes contract or dilate in response to vasoactive mediators as well as physiological stimuli (Fernández-Klett et al., 2010; Hall et al., 2014; Biesecker et al., 2016; Kisler et al., 2017b; Rungta et al., 2018). Cortical and retinal capillaries dilated in almost synchrony with arterioles during functional hyperemia, supporting the hypothesis that blood flow in the central nervous system is additionally regulated at the capillary level, possibly to match the heterogeneous distribution of metabolic demand between nearby neuron groups (Hall et al., 2014; Kornfield and Newman, 2014; Biesecker et al., 2016; Rungta et al., 2018). The higher pericyte: endothelial cell ratios in the cerebral and retinal microvasculature compared to the other organs point to a distinctive function of pericytes in these tissues (Frank et al., 1987; Shepro and Morel, 1993).

The discrepancies between histological and physiological findings have recently been clarified by using rapid tissue fixation methods that prevented depolymerization of α-SMA seen during tissue processing with slowly acting agents like PFA (Alarcon-Martinez et al., 2018, 2019). Actin is constantly de- and re-polymerized in almost all cells with a dynamic turnover between its monomeric globular (G-actin) and polymeric filamentous (F-actin) forms, whereas de novo protein synthesis has a slow pace (Yamin and Morgan, 2012). While α-SMA immunopositivity is not significantly affected by depolymerisation during tissue fixation in vSMCs and mesh pericytes in upstream capillaries that are rich in α-SMA, the small pool of α-SMA in downstream helical pericytes is depleted by rapid depolymerisation (unaccompanied by repolymerization due to lack of ATP) during tissue processing and, hence, evades detection. This issue has been convincingly demonstrated in the retina, where the layered structure of retinal vessels allows imaging of all microvessel orders in the same plane (Alarcon-Martinez et al., 2018; Yu et al., 2019b; Kim et al., 2020). However, it awaits confirmation from the brain tissue where microvessels irregularly branch in various directions, requiring the use of thick tissue slabs to be able to image all orders, which limits fixative and antibody penetration. In addition, rapid fixation of brain sections with methanol or acetone is challenging due to their high lipid content.

A recent study has disclosed that F-actin polymerization may also contribute to the contraction in downstream pericytes stimulated with noradrenaline (Kureli et al., 2020). An increase in F- to G-actin ratio may boost actomyosin cross bridging by extending the length of α-SMA filaments present, in addition to strengthening the submembranous beta-actin lattice, transferring the contractile force generated to the extracellular matrix for constricting the vessel (Cipolla et al., 2002; Gunst and Zhang, 2008; Lee et al., 2010; Yamin and Morgan, 2012). The extensive and almost circular coverage of the vessel wall by upstream pericytes and also at capillary bifurcations suggest a constrictive role similar to vSMCs, whereas the helical structure points to a modest tension, setting the tonus of downstream capillaries (Rungta et al., 2018; Gonzales et al., 2020; Kureli et al., 2020). This structural formulation is consistent with 2-photon studies, showing that the upstream microvascular unit diverts the blood to the microvessels dilated in response to neural activity, which may then be distributed among the downstream capillaries depending on the local energy demand by varying their resistance (Hall et al., 2014; Hill et al., 2015; Rungta et al., 2018). Further supporting a regulatory role of pericytes in microcirculation, transgenic mice designed to have a low number of pericytes on the brain microvessels exhibit an impaired response to functional activation (Kisler et al., 2017b).

Pericyte contraction is thought to be regulated by intracellular Ca^{2+} concentrations as in vSMCs (Wu et al., 2003; Kamouchi et al., 2004; Hamilton et al., 2010). In addition to *in vitro* studies, this has been recently confirmed *in vivo* by using genetically encoded calcium indicator, GCaMPs, during sensory stimulation or ischemia-induce pericyte contraction (Rungta et al., 2018; Alarcon-Martinez et al., 2019). A sustained intracellular calcium increase in pericytes during ischemia has been proposed to underlie the persistent pericyte contraction that impairs microcirculatory reperfusion after recanalization (no-reflow) (Alarcon-Martinez et al., 2019) (Fig. 4).

Pericytes also play a role in maintaining the blood-brain and blood-retina barrier (BBB or BRB) (Kaur et al., 2008; Cunha-Vaz et al., 2011). Pericyte coverage is essential for converting the inter-endothelial junctions to tight and adherens type junctions required for BBB function. During the formation of the cerebral and retinal microvessels, interactions between endothelial cells, pericytes, and astrocytes orchestrate the increased expression of the tight junction protein ZO-1 (Kim et al., 2009). PDGFB/PDGFR-β pathway plays a significant role in the formation and maintenance of BBB/BRB (Armulik et al., 2010; Trost et al., 2016). Indeed, inhibition of pericyte PDGFR-β signaling disrupts the recruitment of retinal pericytes to the newly forming vessels, hampering the development of a functional BRB and causing plasma exudation, edema and hemorrhages in the retina (Uemura et al., 2002). Transgenic mice lacking the retention motif of PDGFB display decreased pericyte coverage (Lindblom et al., 2003). However, unlike newly forming microvessels, the impairment of PDGFB/PDGFR-β signaling pathway does not

destabilize BRB in mature vessels but makes the BRB vulnerable to injury (Park et al., 2017).

Pericytes are also essential for neovascularization and angiogenesis, which require an orderly communication with endothelial cells and involve several signaling pathways (Gerhardt and Betsholtz, 2003; Armulik et al., 2011) (Fig. 5). PDGFB/PDGFR-β signaling plays a critical role in new vessel formation, and its defects cause pericyte loss, abnormal microvessels and microaneurysms in addition to BBB/BRB dysfunction (Lindahl et al., 1997; Betsholtz, 2004). TGF-β/TGFβR2 as well as Ang1/Tie2 signaling between pericytes and endothelia also play crucial roles in neovascularization and angiogenesis (Jeansson et al.,
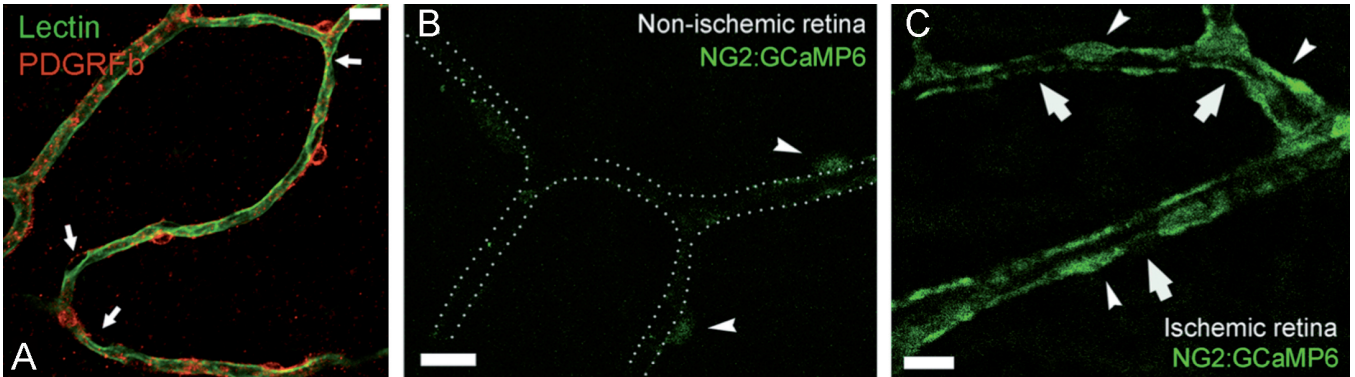

Fig. 4. Ischemia-dependent capillary constrictions are mediated by intracellular calcium increase in pericytes. **A.** Labeling of the vasculature with lectin (green) and pericytes with anti-PDGFRβ antibody (red) shows capillary constrictions in ischemic retinae (arrows). **B, C.** Ischemia leads to intracellular calcium increase in pericytes (green, arrows point to constrictions) identified in mouse retinae expressing the genetically encoded calcium indicator GCaMP6 specifically in pericytes (NG2:GCaMP6) (arrowheads). With permission of BioMed Central (BMC) as publisher (Alarcon-Martinez et al., 2019). Scale bars: 10 μm.
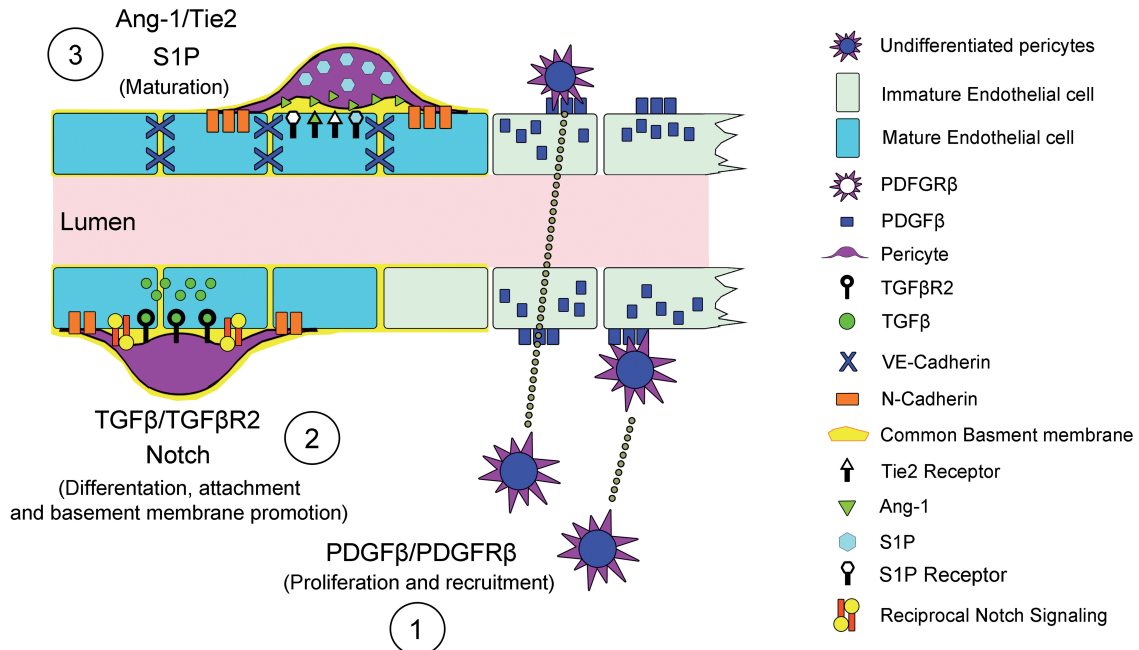

Fig. 5. The contribution of pericytes in angiogenesis. The recruitment of undifferentiated pericytes to newly formed vessels is supported by the interaction between PDGFβ and PDGFRβ. Once pericytes are at the vascular wall, Notch and TGFβ/TGFβR2 signaling differentiate mural cells and attach them to the newly formed vessels. The TGFβ/TGFβR2 interaction also promotes the formation of the common basement membrane and stabilizes newly formed vessels. Ang-1/Tie2 interaction promotes blood brain barrier formation. Finally, S1P and its receptor at pericytes promote interconnection between endothelial-endothelial (VE-cadherin) and pericyte-endothelial cells (N-cadherin) (Reproduced from Dalkara and Alarcon-Martinez, 2015 with permission).

2011; Caporarello et al., 2019).

Pericytes on post-capillary venules have a stellate morphology and do not express significant amounts of α-SMA (unlike large venules); hence, they are thought not to have a contractile function. Instead, they regulate leukocyte transmigration from blood to the tissue. In accordance with such an immune function, pericytes express several chemokines, cytokines and adhesion molecules (Rustenhoven et al., 2017). The intercellular adhesion molecule-1 expressed by pericytes has been shown to guide leukocytes through the space between pericyte processes toward the tissue by interacting with the integrins on leukocytes (Proebstl et al., 2012).

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