Pericyte Signaling in the Neurovascular Unit

Mark Fisher, MD

Abstract—Brain pericytes are intimately associated with capillary endothelial cells, separated only by basement membrane. Pericyte research has been hampered by absence of pericyte-specific immunochemical markers. The vast array of pericyte functions include contractility, immunologic, migratory, and angiogenic. Pericytes have stem cell potential, contribute to the integrity of the blood-brain barrier, and have a regulatory role for hemostasis in the brain. Improvement in pericyte identification is likely to lead to marked increase in appreciation of the role of pericytes in the neurovascular unit. (Stroke. 2009;40[suppl 1]:S13-S15.)

Key Words: pericyte ■ endothelium ■ astrocyte ■ neurovascular

Research on the blood-brain barrier (BBB) usually emphasizes the neurovascular unit, with particular focus on astrocyte-endothelial interactions. The pericyte is often overlooked. As Lai and Kuo have stated: "It is possible that the relatively undefined phenotypic properties and the lack of specific cellular marker for pericyte are the reason to preclude the inclusion of pericyte..." Indeed, it is not uncommon to encounter schematic illustrations of the neurovascular unit that make no mention of the pericyte. This brief overview will attempt to flesh out some important properties of brain pericytes.

Pericyte Identification

Pericyte morphology is characterized by a stellate appearance with long cytoplasmic processes (Figure 1). Pericytes may indent adjacent endothelial cells, and vice versa, in peg-and-socket contacts and encircle individual blood vessels. Pericyte granularity is associated with extent of cytoplasmic lysosomes, and human brain pericytes appear to be exclusively granular with high content of acid phosphatase.

Specific immunochemical markers are lacking for pericytes, which originate from mesoderm. Attempts to identify pericytes typically require a series of stains with a combination of positive and negative immunoreactivity. Pericytes are typically immunoreactive for α-smooth muscle actin, γ-glutamyl transpeptidase, alkaline phosphatase, nestin, vimentin, and the platelet derived growth factor-beta (PDGF-β) receptor. Pericytes lack immunoreactivity for von Willebrand factor and glial fibrillary acidic protein, markers for endothelial cells and astrocytes, respectively.

Recent work by Bondjers et al may shed new light on pericyte identification. Using brain microvessels from mice lacking PDGF-β or the PDGF-β receptor, brain pericytes expressed the ATP-sensitive potassium channel Kir6.1. This expression seemed to be limited to pericytes of the brain. If confirmed, this would represent an important advance in the routine identification of brain pericytes using immunohistochemical or molecular markers.

Pericyte Localization

Pericytes are present on the abluminal surface of endothelial cells of capillaries, as well as arterioles and venules. Pericytes cover 22% to 32% of cerebral capillary surface (more than skeletal and cardiac muscle, but less than retina), and postcapillary venules tend to have more pericytes than do capillaries. Capillary pericytes, contained entirely within basal lamina (basement membrane) on the endothelial cell abluminal surface, tend to be localized over endothelial tight

Figure 1. Immunoperoxidase staining of human pericytes from cell culture preparation, using rabbit antinestin antibody (Chemicon International; 1:200) and biotinylated secondary antibody (1:200). Original magnification ×400.
junction regions. One layer of basement membrane separates pericytes from endothelial cells, whereas another basement membrane layer serves to compartmentalize pericytes from astrocyte endfeet in the neurovascular unit, as shown in Figure 2.

**Pericyte Function**

Brain pericytes have a substantial range of functions, mostly defined in a variety of cell culture studies. Most of these functions are implied, based on in vitro observations. These include:

- **Contractile function:** Brain pericytes express α-smooth muscle actin, with more robust expression in precapillaries compared to mid- and postcapillaries. The distribution of α-smooth muscle actin as well as the expression of endothelin receptors by pericytes imply contractile function of pericytes with blood flow regulatory capabilities.

- **Immune and phagocytic function:** Brain pericytes constitutively express low levels of adhesion molecules (intercellular adhesion molecule-1 and vascular cell adhesion molecule-1), with pericyte expression upregulation induced by inflammatory cytokines; moreover, pericytes have the capacity to present antigen to T-lymphocytes. Robust expression of acid phosphatase by pericyte lysosomes implies phagocytic function of pericytes.

- **Migratory function:** Pericyte movement to endothelial cells during vascular development is mediated by endothelial-derived PDGF-β and PDGF-β receptors expressed by pericytes. Pericyte-to-endothelial migration is readily demonstrable in cell culture preparations using in vitro capillary-like structures, as shown in Figure 3. After traumatic brain injury, approximately 40% of brain microvascular pericytes migrate from a microvascular to perivascular location, probably mediated by pericyte expression of urokinase plasminogen activator receptor.

- **Angiogenic function:** Pericytes have an important regulatory role in angiogenesis, orchestrating initiation, sprout connection, and termination via expression of transforming growth factor-β (TGF-β), vascular endothelial growth factor, and angiopoietin-1 and -2.

- **Contribution to the blood–brain barrier:** Given the intimate association between brain capillary pericytes and endothelial

![Figure 2. Cellular elements of the neurovascular unit, with the pericyte (P) sharing basement membrane (BM) with capillary endothelial cells (E), and astrocytes (A) ensheathing the capillary. Reproduced with permission by S. Karger AG from Allt and Lawrenson.](image)

![Figure 3. Bovine pericytes, immunostained for smooth muscle actin, 5 hours after coculture with bovine brain capillary-like structures in vitro. Most pericytes have rapidly migrated to the capillary-like structures, and some pericytes cover the surface. (×120) Reproduced from Minakawa et al.](image)
cells, it is not surprising that the pericytes have a substantial role in development of blood–brain barrier tight junctions and paracellular permeability. This is mediated in part via pericyte expression of TGF-β1 and angiopoieten-1. Pericytes also contribute to basement membrane formation by synthesizing type IV collagen, glycosaminoglycans, and laminin.

Stem cell function: Brain pericytes respond to basic fibroblastic growth factor in vitro by expression of neuronal and glial cell markers, suggesting that CNS pericytes have capacity to serve as neural stem cells.

Pericyte as a Regulator of Brain Hemostasis
The unique cellular architecture of the brain microvasculature and neurovascular unit has implications in an area that traditionally has received little attention: the regulation of hemostasis. It is increasingly recognized that individual organs have the capacity to differentially regulate blood clotting, and the brain is no exception. Bouchard and colleagues have defined functionally active tissue factor (the primary generator of the coagulation cascade) on the surface of human brain pericytes, the latter having the capacity to coactivate coagulation factors IX and X and also provide the membrane surface necessary for the prothrombinase complex. Moreover, brain pericytes negatively regulate brain endothelial fibrinolysis, and amplify the antifibrinolytic effects of endotoxin. Pericytes also robustly express and secrete the potent serine protease inhibitor (and antithrombin) protease nexin-1. Brain pericytes thus have both pro- and anticoagulant activity.

Conclusion
Pericytes of the brain have long been a relatively overlooked cellular constituent of the neurovascular unit. Nevertheless, given the increasing recognition of wide-ranging functions of the pericyte and improvements in its cellular markers, the pericyte appears poised to take its rightful place in our understanding of the functioning blood-brain barrier.

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