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Brain pericytes: emerging concepts and functional roles in brain homeostasis

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Short title: Brain pericytes and brain homeostasis

Abstract

Brain pericytes are an important constituent of neurovascular unit. They encircle endothelial cells and contribute to the maturation and stabilization of the capillaries in the brain. Recent studies have revealed that brain pericytes play pivotal roles in a variety of brain functions, such as regulation of capillary flow, angiogenesis, blood brain barrier, immune responses, and hemostasis. In addition, brain pericytes are pluripotent and can differentiate into different lineages similar to mesenchymal stem cells. The brain pericytes are revisited as a key player to maintain brain function and repair brain damage.

Key words: angiogenesis, blood brain barrier, mesenchymal stem cell, microvasculature, pericyte

I. Introduction

Pericytes in the microvasculature were first described by Rouget et al. (Rouget 1873; Rouget 1879). Zimmerman et al. termed the cells “pericytes”, since they are situated in the peri-endothelial space in the capillary (Zimmerman 1923). The cells have been long recognized as the cells that support the capillary, equivalent to arterial smooth muscle cells. However, increasing studies have unveiled a variety of physiological as well as pathological roles of these mysterious cells.

Pericytes are ubiquitously present in the capillaries in almost all tissues and organs. However, pericytes are most rich in the retina and brain (Frank *et al* 1987; Shepro and Morel 1993). The density of the pericytes is considered to be several-fold higher in the brain than in other organs, thus implying the importance of these cells in the brain (Frank *et al* 1987; Shepro and Morel 1993). During the preceding decade, it became evident that the cells regulate capillary flow, angiogenesis, the blood brain barrier, and immune responses in the brain (Hirschi and D'Amore 1996; Balabanov and Dore-Duffy 1998; Thomas 1999; Rucker *et al* 2000; Allt and Lawrenson 2001; Lai and Kuo 2005; Dore-Duffy 2008; Krueger and Bechmann 2010). Pericyte dysfunction may cause serious central nervous system (CNS) disorders. Moreover, recent progress in stem cell research has revealed that pericytes have the potential to differentiate into different cell lineages. These cells may play a pivotal role in repair and regeneration of brain tissue, since they have similar properties to mesenchymal stem cells.

There have been excellent reviews concerning the functional roles of pericytes (Hirschi and D'Amore 1996; Balabanov and Dore-Duffy 1998; Thomas 1999; Rucker *et al* 2000; Allt and Lawrenson 2001; Lai and Kuo 2005; Dore-Duffy 2008; Krueger and Bechmann 2010). We herein update the current understandings for pericyte physiology and pathophysiology, particularly in the brain.

II. Morphology

Brain pericytes are predominantly located in the capillaries. They are scarcely distributed in the arterioles and venules in the brain. The lumen of the capillaries is formed by endothelial cells, and pericytes surround their abluminal surface. The pericyte has prominent oval nuclei and several long extending processes. Primary processes elongate in the longitudinal axis along the capillaries, and secondary processes branch along the circumferential axis and penetrate into the basal membrane. The endothelial cells and pericytes closely contact each other by interdigitation. In addition, these cells are intimately connected

with ‘peg and socket’ contacts, adhesion plaques, gap junctions, or tight junctions (Hirschi and D'Amore 1996; Rucker *et al* 2000; Allt and Lawrenson 2001). By means of these close interactions, they mutually communicate and transmit intercellular signals.

III. Cell Markers

There has been no specific marker to identify brain pericytes. The absence of specific immunochemical markers has hampered the progress of pericyte research. Generally, pericytes have been identified with a combination of positive and negative cell markers. The markers of the cells might vary, depending on the specificity of the species (human or other species), tissue (brain or other tissues), localization (pre-, mid-, or post-capillary), differentiation (immature or differentiated), and developmental stages (embryo or adult). For instance, microvascular pericytes derived from the mouse embryo brain and the human brain do not express α-smooth muscle actin, whereas pericytes derived from the chicken embryo brain do express this protein (Gerhardt *et al* 2000). Kir6.1 is highly expressed in pericytes in the brain but is undetectable in pericytes in the skin and heart (Bondjers *et al* 2006).

The cell markers which have been suggested for pericytes are as follows: α-smooth muscle actin, desmin, chondroitin sulfate proteoglycan marker NG2, RGS-5, CD140a, CD140b (PDGFR-β), aminopeptidases A and N, nestin, Sca-1, CD34 (pluripotential), 3G5 (monoclonal Ab ganglioside antigen), high molecular weight melanoma-associated antigen, γ-glutamyl transpeptidase, alkaline phosphatase, butyrylcholinesterase, FcR, CD4, CD11b, MHC class I, II, and vimentin (Table 1) (Nayak *et al* 1988; Kunz *et al* 1994; Balabanov and Dore-Duffy 1998; Thomas 1999; Guillemin and Brew 2004; Armulik *et al* 2005; Bondjers *et al* 2006; Nisancioglu *et al* 2008; Paquet-Fifield *et al* 2009). Pericytes share cell markers with smooth muscle cells and with mesenchymal stem cells. The diverse expression pattern may be due to microenvironment-specified differentiation. Among various markers, PDGFR-β and NG2 are widely used to detect pericytes along with the localization at pericapillary space in the brain.

IV. Functional Roles

1. Regulation of microcirculation

(1) Contractile activity

Pericytes in the brain express contractile filaments and associated proteins, such as α-isoform or

muscle specific actin, myosin, tropomyosin, and cGMP-dependent protein kinase. Desmin or vimentin may be also expressed in the cells (Hirschi and D'Amore 1996; Rucker *et al* 2000; Allt and Lawrenson 2001; Bandopadhyay *et al* 2001; Bergers and Song 2005). Some studies reported that contractile proteins are expressed in pre- and post-capillary pericytes but rare in midcapillary pericytes (Nehls and Drenckhahn 1991; Boado and Pardridge 1994). However, it is also suggested that α-smooth muscle actin is strongly expressed in a significant number of mid-capillary pericytes (Bandopadhyay *et al* 2001). Although the expression level of these contractile proteins has been variously reported in brain pericytes, the amount of pericyte muscle actin is considered to be intermediate between endothelial cells and smooth muscle cells (Boado and Pardridge 1994).

The brain pericytes express the receptors for a variety of vasoactive substances, including adrenergic agonists, cholinergic agonists, histamine, serotonin, angiotensin II, endothelin-1, atrial natriuretic peptide, and ATP (Table 1) (Lee *et al* 1989; Takahashi *et al* 1989; Chakravarthy *et al* 1992; Ferrari-Dileo *et al* 1996; Matsugi *et al* 1997; McGinty *et al* 1999; Sakagami *et al* 1999; Pallone *et al* 2000; Pallone and Huang 2002). Patch clamp experiments revealed the expression of P_2X_7, P_2Y_4, ET_A, PDGFR-β, and the muscarinic receptor in freshly isolated pericytes (Puro 2007). Intracellular Ca^{2+}, a key signal triggering contraction molecule, increases in response to various vasoactive substances in brain pericytes. Cultured retinal pericytes contracted or relaxed in accordance with the changes in intracellular Ca^{2+} (Wakisaka *et al* 2001; Kamouchi *et al* 2004; Oishi *et al* 2007). Electrophysiological studies also showed that muscarinic stimulation elevated intracellular Ca^{2+} levels and increased Ca^{2+}-activated Cl^- currents in freshly isolated rat retinal pericytes (Sakagami *et al* 1999). Spontaneous surges of Ca^{2+} or Ca^{2+} transients were observed in a portion of the pericyte during electrical neuron activity *in vivo* using an animal model (Hirase *et al* 2004). In cultured human brain microvascular pericytes, extracellular protons induced Ca^{2+} oscillation via reverse-mode Na^+/H^+ exchanger 1 (Nakamura *et al* 2008). Various ionic channels such as ATP-sensitive K^+ channels, Ca^{2+}-activated K^+ channels, inwardly rectifying K^+ channels, voltage-dependent K^+ channels, and L-type voltage-dependent Ca^{2+} channels are expressed in brain pericytes (Li and Puro 2001; Puro 2007). Therefore, brain pericytes can contract following the increase in intracellular Ca^{2+}, which is induced by vasoactive substances. The interplay between Ca^{2+} signals and membrane potentials finely modulates the contraction of the pericytes.

(2) Regulation of the capillary flow

Pericytes are tightly attached to endothelial cells in capillaries. Adhesion plaques are important for transmitting the contraction of pericytes into the capillary diameter. The contraction of the pericytes along the circumferential axis leads to a reduced capillary diameter. It is generally accepted that capillary flow is predominantly regulated by the change in the diameter of precapillary arterioles. Therefore, the question of whether pericytes must contract arises.

Quite recently, direct evidence has been provided in whole-retina and cerebellar slices that pericytes control capillary diameter. Pericytes likely contribute to blood flow modulation in response to changes in neural activity (Peppiatt *et al* 2006). Reactive oxygen species cause a sustained increase in Ca^{2+} in human brain microvascular pericytes (Kamouchi *et al* 2007; Nakamura *et al* 2009). It has been also shown in the mouse brain that oxidative-nitrative stress-retained pericytes contracted, even after recanalization of an occluded middle cerebral artery (Yemisci *et al* 2009). The diameter of the capillary is not wide enough to allow several red blood cells to freely pass. Therefore, a reduction in capillary diameter may result in decreased passage or in entrapment of red blood cells (Figure 1). Impairment of microcirculation and ischemia-reperfusion injury may be induced by oxidative stress-induced pericyte contraction (Yemisci *et al* 2009).

In the retina, pericytes play an important role in the regulation of retinal blood flow (Wu *et al* 2003; Lombard 2006; Yamanishi *et al* 2006). It is possible that brain pericytes control the capillary blood supply in response to the activity of neurons. During brain hypoxia, poor pericyte relaxation may cause a critical decrease in capillary flow which cannot compensate for the lack of oxygen. A mismatch in the capillary flow to neuronal activity results in impairment of microcirculation and potentially underlies several CNS disorders.

2. Angiogenesis

(1) Angiogenic process

It is well known that pericytes play critical roles in both angiogenesis and vasculogenesis (Hirschi and D'Amore 1996; Gerhardt and Betsholtz 2003; Armulik *et al* 2005; Bergers and Song 2005; Hall 2006; von Tell *et al* 2006). *In vivo* experiments with a knockout mouse model clearly showed that the cells are involved in the stabilization and maturation of the microvasculature. Pericyte loss induces morphological changes in the brain capillary. A mouse model in which recruitment of pericytes was genetically ablated presented with

endothelial hyperplasia, increased capillary diameter, and increased transendothelial permeability in the brain (Hellstrom *et al* 2001).

In the angiogenic process, the interaction between pericytes and endothelial cells is essential for vessel stability and maturation (Hirschi and D'Amore 1996; Gerhardt and Betsholtz 2003; Armulik *et al* 2005; Bergers and Song 2005; Hall 2006; von Tell *et al* 2006). The turnover of endothelial cells is slow in the capillary where pericytic coverage is rich. In contrast, detachment of pericytes from the abluminal surface of the capillary initiates the stimulation of endothelial cells. Activated endothelial cells degrade the extracellular matrix and dissociate from the basement membrane. The cells then migrate and proliferate into the interstitial space and form lumens (Figure 2). The tip of the newly formed vessel attracts pericytes, and the tubes are subsequently covered with pericytes, leading to vessel remodeling, maturation, and stabilization (Hirschi and D'Amore 1996; Gerhardt and Betsholtz 2003; Armulik *et al* 2005; Bergers and Song 2005; Hall 2006; von Tell *et al* 2006).

Another hypothesis is that pericytes stimulate and guide endothelial cells to form new vessels. Several studies have suggested that migrating pericytes precede angiogenesis and initiate the angiogenic process. Therefore, pericytes may contribute to the early growth of vessels as well as to the stabilization and maturation of newly formed vessels (Beck and D'Amore 1997; Hirschi *et al* 1999; Ozerdem and Stallcup 2003; Fukushi *et al* 2004; Virgintino *et al* 2007).

(2) Angiogenic factors

The interaction between pericytes and endothelial cells regulates migration, differentiation, and proliferation of both cell types. These cells can act directly or indirectly in an autocrine or paracrine manner, which regulates the maturation and stability of newly formed vessels. Signaling molecules, including growth factors and cytokines, are secreted from these cells and exert their actions by binding to cognate receptors (Figure 3). Intercellular communication between endothelial cells and pericytes is mediated by multiple signals (Armulik *et al* 2005; Braun *et al* 2007; Gaengel *et al* 2009). Three major receptor-ligand systems, transforming growth factor-β, angiopoietin, and platelet derived growth factor, regulate angiogenesis via the interaction of endothelial cells and pericytes. Other signaling pathways are also involved in angiogenesis and vasculogenesis (Table 1) (Armulik *et al* 2005). In addition to secreted molecules, cell surface proteins mediate signals through cell-cell or cell-extracellular matrix interactions (Figure 3).

In this section, the signaling pathways involved in vasculogenesis and tumor- or ischemia-related angiogenesis are extensively reviewed not only in the brain, but also in all tissues and organs. We focus on the roles of pericytes in their mechanisms. Although these angiogenic mechanisms are probably involved in the brain as well as in other tissues, further studies are required to elucidate whether these processes are also related to CNS pericytes.

1) Transforming growth factor-β

Signal transduction by transforming growth factor (TGF)-β pathway is essential for angiogenesis (Bertolino *et al* 2005). Genetic inactivation of the genes encoding TGF-β in mice has revealed the pivotal role of this pathway in angiogenesis. TGF-β family ligands are produced as dimeric precursor proteins, then are cleaved by proteases and secreted from endothelial cells. They bind to heteromeric complexes of type II and type I serine/threonine kinase transmembrane receptors in endothelial cells and pericytes. TGF-β interacts with TβRII and ALK5, but can also signal via ALK1 in endothelial cells. Endoglin can facilitate TβRII/ALK5 and TβRII/ALK1 signaling, which induce phosphorylation of Smad2/3 and Smad1/5. Activated Smads form complexes with Smad4 and translocate into the nucleus to induce gene transcription (Goumans *et al* 2009).

The interaction between endothelial cells and pericytes is required for production of latent TGF-β. An *in vitro* study revealed that coculture of bovine aortic endothelial cells and bovine retinal pericytes produces latent TGF-β (Sato and Rifkin 1989). It was shown that endothelial cells produce TGF-β1 and -2, while smooth muscle cells produce TGF-β1 (Sato *et al* 1990). The activation of TGF-β1 was mediated by plasmin, whereas TGF-β negatively controls plasmin activation by upregulation of plasminogen activator inhibitor-1 (Sato and Rifkin 1989; Sato *et al* 1990). Studies with immunoelectron micrographs revealed that TGF-β, urokinase plasminogen activator, and plasminogen colocalize at the sites of endothelial cell-pericyte interdigitations in newly-formed capillaries in human granulation tissues (Wakui *et al* 1997). Gap junction communication between endothelial cells and mesenchymal cells is required for TGF-β activation. Endothelial-induced mural cell differentiation may be mediated by activation of TGF-β through gap junction communication (Hirschi *et al* 2003).

TGF-β derived from endothelial cells has positive autoregulatory effects on the expression, synthesis, and release of TGF-β. In endothelial cells, TGF-β can promote differentiation via Alk5/Smad2/3 signaling, and proliferation and migration through Alk1/endoglin/Smad1/5 signaling (Armulik *et al* 2005; Gaengel *et al* 2009).

TGF-β produced by coculture of endothelial cells and retinal pericytes regulates endothelial functions. TGF-β1 potently induces VEGFR-1 expression in endothelial cells, which prevents vessel loss induced by oxygen (Shih *et al* 2003). TGF-β inhibits the endothelial cell growth (Antonelli-Orlidge *et al* 1989) and movement (Sato and Rifkin 1989), and induces apoptotic cell death of endothelial cells (Yan and Sage 1998).

TGF-β signaling is important for proper differentiation of pericytes. TGF-β induces the differentiation of C3H/10T1/2 mesenchymal cells towards the smooth muscle cell/pericyte lineage with changes in phenotype- and cell-specific markers (Hirschi *et al* 1998; Ding *et al* 2004). TGF-β1 increased α-smooth muscle actin in human brain pericytes (Verbeek *et al* 1994) and was required for capillary-like structures by mediating the differentiation to pericytes (Darland and D'Amore 2001). On the other hand, C3H/10T1/2 proliferation was not mediated by TGF-β in the presence of endothelial cells (Hirschi *et al* 1999). The ablation of TGF-β signaling by a TGF-β type II receptor with a truncated C-terminal intracellular kinase domain demonstrated that TGF-β1 signaling controls pericyte functions. TGF-β accumulated α-smooth muscle actin mRNA and protein in the bovine retinal pericytes but inhibited pericyte growth (Sieczkiewicz and Herman 2003). These results indicate that TGF-β signaling promotes differentiation of pericytes and inhibits proliferation of pericytes. However, a recent study showed contradicting data, thus suggesting that TGF-β was dispensable for the induction of vascular smooth muscle cells and pericytes (Lindskog *et al* 2006).

Hereditary hemorrhagic telangiectasia is caused by mutations in TGF-β signaling (Fernandez *et al* 2006). Mice lacking endoglin and ALK-1 showed arterial-venous malformation (Li *et al* 1999; Urness *et al* 2000; Sorensen *et al* 2003). Endoglin-deficient mice die from defective vascular development. In this mouse, vasculogenesis was unaffected, whereas development of smooth muscle cells was impaired (Li *et al* 1999). Endothelium-specific disruption of TGF-β/ALK5 signaling caused a failure of the adjacent mesothelial cells to differentiate into smooth muscle cells. Exogenous TGF-β1 rescued the phosphorylation of Smad2 and the differentiation of smooth muscle (Carvalho *et al* 2004). ALK1 inactivation of by gene targeting showed that ALK-1 was required for differentiation and the recruitment of vascular smooth muscle cells (Oh *et al* 2000). In the TGF-β type1 receptor-deficient mouse, hematopoiesis and development of hematopoietic progenitors remained intact. However, mesenchymal cells were not able to differentiate into smooth muscle cells with TGF-β signaling (Larsson *et al* 2001). Taken together, inactivation of TGF-β signaling impairs the differentiation of pericytes, which reduces the numbers of pericytes and smooth muscle cells and causes defective vessel formation.

2) Platelet derived growth factor-B

Studies on platelet derived growth factors (PDGFs) and their receptors (PDGFRs) have established the roles of PDGF-B/PDGFR-β pathways in vessel formation. The ligands are dimers of disulfide-linked polypeptide chains and act primarily as homodimers. PDGF-B is expressed in endothelial cells and PDGFR-β in pericytes (Andrae *et al* 2008).

Genetic ablation of the PDGF-B/PDGFR-β pathway revealed that these signals are a key regulator for maturation of new vessels. PDGF-B and PDGFR-β-deficient mouse embryos are lethal during late gestation due to vascular dysfunction in the brain. They lack microvascular brain pericytes and develop capillary microaneurysms, which rupture during the prenatal period (Lindahl *et al* 1997). In PDGF-B and PDGFR-β knockout embryos, newly formed vessels in the brain showed endothelial hyperplasia with increased vessel diameter and hyperpermeability due to lack of brain pericytes, although microvessel density, length, and number of branchings were normal (Hellstrom *et al* 2001). Endothelium-specific ablation of PDGF-B generated viable mice. However, the pericyte density was low, which is associated with retinal microvascular abnormalities (Enge *et al* 2002). When cerebral angiogenesis in mice was induced by implanting a sandwich system of basic fibroblast growth factor (bFGF)/PDGF gel, the pericytes were rich in neocapillaries as induced by PDGF (Jariyapongskul *et al* 2003). In tumor angiogenesis, PDGFR-β signaling is required for recruitment of progenitor perivascular cells and vessel maturation (Song *et al* 2005).

PDGF-B is highly expressed in migratory tip cells at the leading edge of an angiogenic sprout. On the other side, PDGFR-β is expressed in perivascular mesenchymal cells or pericyte progenitor cells. Endothelial cells of sprouting capillaries synthesize and secrete PDGF-BB and induce the migration and proliferation of PDGFR-β-positive pericyte progenitor cells during vascular development (Ball *et al* 2007). The induction of pericytes from the perivascular mesenchyme may be initially induced by PDGF-independent signals, such as TGF-β. However, PDGF-BB derived from endothelial cells is necessary to recruit and cause proliferation of pericyte progenitors. Therefore, spatiotemporal changes in PDGF-B/ PDGFR-β signals regulate microvasculature maturation by recruitment of brain pericytes (Lindahl *et al* 1997; Hellstrom *et al* 1999; Hellstrom *et al* 2001).

PDGF-BB is secreted from the endothelial cell surface and diffuses into the matrix. PDGF-BB binds to heparin and heparan sulfate proteoglycans in the pericellular matrix, and the high concentration of

PDGF-BB is retained in the vicinity of the developing vessel. Consequently, PDGFR-β-positive pericyte progenitor cells migrate and proliferate along the growing vessel. The gradient of PDGF-BB formed by heparan sulfate proteoglycans is beneficial for localized regulation of pericytes at the leading edge of newly formed sprouts. It has been shown that reduction of N-sulfation attenuates PDGF-BB binding *in vitro*, and leads to pericyte detachment and impaired pericyte migration but not proliferation in the brain (Abramsson *et al* 2007). Therefore, appropriate structural features of heparan sulfate appear to be important to retain PDGF-BB and activate PDGFR-β signaling in the brain (Abramsson *et al* 2007).

The overexpression of PDGF-BB upregulates stromal cell-derived factor (SDF)-1α expression in endothelial cells in tumors. SDF-1α increases the motility of pericytes, and the recruitment of these cells was inhibited by blockade of the SDF-1α/CXCR4 axis. Therefore, the SDF-1α/CXCR4 signaling axis may be important for PDGF-BB-induced pericyte recruitment within a tumor (Song *et al* 2009). In caveolin-1 deficient mice, tumor angiogenesis was promoted with a marked reduction in pericytes. In addition, the migration and outgrowth of smooth muscle cells/pericytes following PDGF-B was impaired. Therefore, caveolin-1 plays critical roles in the recruitment of pericytes via PDGF signaling (Dewever *et al* 2007). A recent study indicated that there were two cohorts of PDGF receptors in pericytes from neonatal foreskin. One was primarily confined to lipid rafts, specifically the caveolae, and the other was localized to non-raft compartments. These receptors were activated by soluble PDGF-B and membrane bound PDGF-B, which mediate distinct cellular functions. Cytoskeletal reorganization was only induced by soluble PDGF-B (Sundberg *et al* 2009). The recruitment of pericytes may be determined by the signal balance between the PDGF-B concentration gradient and the localization of PDGFR-β on the cell membrane.

3) Angiopoietin

Angiopoietin/Tie2 signaling is important for vessel stabilization. Angiopoietin-1 causes tyrosine phosphorylation of the Tie2 receptor, thus resulting in downstream intracellular signaling. Angiopoietin-2 and its isoform act as antagonistic ligands for Tie2, and are considered natural Tie2 signaling antagonists.

The expression of angiopoietin-1 and angiopoietin-2 differs depending on the cell types in the microvessel wall. Dermal pericytes and endothelial cells exhibit a reciprocal expression of these molecules (Sundberg *et al* 2002). Angiopoietin-1 is expressed in the mesenchymal cells surrounding the endothelium, which can differentiate into pericytes (Patan 1998) and is produced by pericytes (Davis *et al* 1996; Sundberg

et al 2002; Wakui *et al* 2006). Angiopoietin-2 is expressed in endothelial cells, whereas it may also be expressed in pericytes (Wakui *et al* 2006; Shim *et al* 2007). Angiopoietin-1 and angiopoietin-2 mediate opposite effects on Tie2 signaling via paracrine and autocrine mechanisms (Armulik *et al* 2005).

The Tie2 receptor is predominantly expressed in endothelial cells (Dumont *et al* 1992; Sundberg *et al* 2002; Wakui *et al* 2006). Although the expression of Tie2 receptors on pericytes still remains controversial, Tie2 likely regulates these cells (Armulik *et al* 2005). In cultured retinal pericytes, the Tie2 receptor was functionally active (Cai *et al* 2008). In tumor neovascularization, Tie2 expression was observed in three distinct cell types: endothelial cells, proangiogenic cells of hematopoietic origin, and pericyte precursors of mesenchymal origin (De Palma *et al* 2005).

Pericyte-derived angiopoietin-1 binds to Tie2 receptors on endothelial cells and causes constitutive activation of the Tie2 receptor. Angiopoietin-1/Tie2 signaling promotes the survival of endothelial cells and maintains the endothelial cells in a quiescent resting state. In addition, angiopoietin-1/Tie2 induces the storage of dimeric angiopoietin-2 in endothelial cells. When the endothelial cells are activated, stored angiopoietin-2 is secreted and inhibits angiopoietin-1/Tie2 signaling. Chronic exposure of endothelial cells to angiopoietin-2 leads to apoptosis in the absence of cytokines (Sundberg *et al* 2002; Armulik *et al* 2005; Fiedler and Augustin 2006).

Angiopoietin-1/Tie signaling contributes to vessel maturation and stabilization in association with the extracellular matrix and pericytes. Tie1-deficient mice exhibited leaky vessels with edema and hemorrhage (Sato *et al* 1995). Pericytes were scarce in mice lacking angiopoietin-1 or Tie2. The ultrastructural analysis showed that endothelial cells did not properly recruit or associate with pericytes in these mice (Suri *et al* 1996). Transgenic overexpression of angiopoietin-2 had similar effects to those of angiopoietin-1 and Tie2 deficiency (Maisonpierre *et al* 1997). Upregulation of angiopoietin-2 appears to be important for pericytes loss in diabetic retinopathy (Hammes *et al* 2004). Therefore, angiopoietin-1/Tie2 signaling is required for vessel stabilization and angiopoietin-2 antagonizes Tie2 signaling in endothelial cells, leading to vessel destabilization. Mutual interaction between angiopoietin-1 and angiopoietin-2 regulates angiogenic cascades triggered by angiogenic stimuli (Sundberg *et al* 2002; Armulik *et al* 2005; Fiedler and Augustin 2006).

It is not yet fully understood precisely how the angiopoietin-Tie signal is involved in the recruitment of pericytes. The angiopoietin/Tie2 signal may be coupled to PDGF/TGF-β–mediated chemotaxis, proliferation, and differentiation of smooth muscle cells (Vikkula *et al* 1996). It was suggested that Tie1 and Tie2 are

required not for the recruitment of pericytes, but for the maintenance or survival of endothelial cells during late gestation (Puri *et al* 1999). Vasculogenesis proceeded normally in embryos lacking both Tie1 and Tie2. Mosaic analysis revealed that Tie1 and Tie2 were essential for the maintenance and survival of endothelial cells. Loss of Tie2 expression correlated with rapid endothelial cell apoptosis in hemorrhagic regions of the embryo (Jones *et al* 2001). Therefore, loss of pericyte recruitment caused by a loss of angiopoietin-1/Tie signaling may be due to endothelial cell apoptosis.

4) Vascular endothelial growth factor

VEGF is a powerful inducer of angiogenesis through endothelial cell activation. Pericytes secrete VEGF and modulate endothelial cell function (Hoeben *et al* 2004). Although VEGF stimulates the proliferation and migration of endothelial cells, the effects of VEGF on pericytes are less clear. VEGF and its receptor family members are expressed in both human dermal microvascular endothelial cells and bovine retinal pericytes, suggesting autocrine and paracrine mechanisms for regulating angiogenesis (Yonekura *et al* 1999).

VEGF likely regulates pericyte functions, because VEGF receptors are expressed in cultured retinal pericytes (Nomura *et al* 1995; Takagi *et al* 1996). In retinas from the neonatal rat, exogenous VEGF accelerated pericyte recruitment in the preformed endothelial network (Benjamin *et al* 1998). On the contrary, in tumors lacking myeloid-cell-derived VEGF-A, the vasculature was less torturous and had increased coverage of pericytes (Stockmann *et al* 2008). Blockade of VEGFR-2 increased the pericyte coverage of non-regressed brain tumor vessels via angiopoietin-1 upregulation, and degraded the basement membrane through matrix metalloprotease activation (Winkler *et al* 2004). Studies have recently demonstrated a novel VEGF mechanism for disruption of pericyte function and inhibition of vessel maturation. VEGF could induce the receptor complex consisting of VEGFR-2 and PDGFR-β. The activation of VEGFR-2 by VEGF suppressed PDGFR-β signaling by assembling the receptor complex, which caused decreased pericyte coverage in vascular sprouts and vessel destabilization (Greenberg *et al* 2008). Therefore, VEGF can regulate angiogenesis by stimulating endothelial cells and by affecting pericyte recruitment.

5) Regulator of G-protein signaing-5

Regulator of G-protein signaling-5 (RGS-5) acts as a GTPase activating protein for Giα and Gqα.

RGS-5 attenuates angiotensin II-, endothelin-1-, sphingosine-1-phosphate-, and PDGF-induced ERK-2 phosphorylation (Cho *et al* 2003). RGS-5 is important regulator of pericyte-endothelial interaction and the vascular maturation (Mitchell *et al* 2008). The expression pattern of RGS-5 is overlapped with that of PDGFR-β. Therefore, RGS-5 is a potential angiogenic pericyte marker at sites of physiologic and pathologic angiogenesis (Berger *et al* 2005). RGS-5 was specifically induced in pericytes activated by an angiogenic switch. RGS-5 was upregulated when nascent vessel sprouts acquired pericyte coverage during neovascularization (Mitchell *et al* 2008). Therefore, RGS-5 can be used as a quantitative measure of pericyte coverage (Mitchell *et al* 2008). RGS-5 deficient mice were generated to determine this protein's function in tumor angiogenesis. However, pericyte coverage and association with endothelial cells were unaffected in RGS-5-deficient tumors. In mice lacking RGS-5, development of vasculature was normal with proper pericyte coverage (Nisancioglu *et al* 2008). On the contrary, RGS-5 deficient pericytes presented more mature phenotype than those in wild type. Therefore, the loss of RGS-5 may be associated with pericyte maturation, and thus result in the vascular normalization of tumor vessels (Hamzah *et al* 2008).

6) Matrix metalloprotease

Pericellular proteases are essential for angiogenesis. Matrix metalloproteases (MMP) belong to a family of zinc-dependent endopeptidases that digest specific extracellular matrix components and aid the activation of proteins such as other MMPs and growth factors. MMP-9 is necessary for the recruitment of pericytes to cover endothelial cells and stabilize newly-formed microvessels (van Hinsbergh *et al* 2006). A recent study showed that membrane-type MMP (MT-MMP) was important for tube formation of lumens by endothelial cells. Proteolysis by MT-MMP1 generated a vascular guidance tunnel for the migration of endothelial cells (Stratman *et al* 2009).

MMPs contribute to pericyte recruitment in various stages during angiogenesis (Chantrain *et al* 2006). Further study is required to determine the importance of proteases in pericyte-specific functions in angiogenesis. Similar to proteases, aminopeptidase A is present in activated pericytes in various pathological conditions associated with angiogenesis, whereas its expression was found to be low in the pericytes of quiescent vessels (Schlingemann *et al* 1996).

Endothelial cell-derived TIMP-2 and pericyte-derived TIMP-3 regulate capillary tube formation by inhibiting MT-MMP (Saunders *et al* 2006). Recruitment of pericytes into the endothelial cell-associated lumen

induced expression of TIMP-3 in bovine retinal pericytes. Consequently, the protease inhibited endothelial tube formation and blocked endothelial cell movement (Saunders *et al* 2006; Stratman *et al* 2009). Therefore, the balance between MMPs and TIMPs appears to control the stabilization of the capillary lumen.

7) Sphingosine-1-phosphate

Sphingosine-1-phosphate (S1P) is a lysophospholipid mediator which is synthesized by membrane sphingolipid metabolism by sphingosine kinases (SPHKs) 1 and 2. S1P is produced by red blood cells, activated platelets, and endothelial cells and is secreted into the plasma. S1P binds its G-protein coupled receptors $S1P_1$, $S1P_2$, and $S1P_3$, and triggers diverse effects in cardiovascular systems (Takuwa *et al* 2008).

The knockout of the $S1P_1$ receptor in mice blocked envelopment of nascent endothelial tubes with pericytes, which led to immature vessels (Allende and Proia 2002). Silencing $S1P_1$ by local injection of $S1P_1$ siRNA into tumors suppressed vascular stabilization and angiogenesis in the tumor (Chae *et al* 2004). The specific disruption of the $S1P_1$ gene in endothelial cells induced incomplete coverage with pericytes. The phenotype of the endothelium-specific disruption of $S1P_1$ was similar to that in $S1P_1$ receptor-deficient embryos. Therefore, pericyte coverage is regulated by $S1P_1$ receptor signaling in endothelial cells (Allende *et al* 2003).

The mechanism for vascular stabilization by S1P/$S1P_1$ signaling is likely mediated by the activation of N-cadherin in endothelial cells (Armulik *et al* 2005). Incomplete vascular maturation caused by pericyte deficiency in the $S1P_1$ knockout mouse was due to an inability to activate the small GTPase, Rac (Liu *et al* 2000). The $S1P_1$/Gi/Rac pathways in endothelial cells are required for proper N-cadherin trafficking and for strengthening N-cadherin-dependent cell-cell adhesion with pericytes in nascent blood vessels (Paik *et al* 2004). Cadherins are transmembrane proteins mediating cell-cell adhesion. N-cadherin is present in adhesion junctions on endothelial cells and contributes to pericyte adhesion (Hirschi and D'Amore 1996; Gerhardt and Betsholtz 2003). N-cadherin is recruited not at endothelial cell–cell junctions but at sites where endothelial cells meet pericytes (Dejana 2004). In the embryonic chicken brain, blocking N-cadherin function impaired pericyte adhesion and increased pericyte recruitment. N-cadherin mediated the adhesion between endothelial cells and pericytes and its disruption resulted in disturbed vascular morphogenesis in the brain (Gerhardt *et al* 2000). The lack of N-cadherin in genetically manipulated ES cells led to impaired pericyte covering of endothelial outgrowths, although sprouting angiogenesis was unaltered (Tillet *et al* 2005).

Therefore, S1P acts on $S1P_1$ receptors on endothelial cells and strengthens the contact and coverage with pericytes through the activation of N-cadherin expressed in endothelial cells.

8) Notch

The Notch signaling pathway is important for cell-cell communication. The Notch pathways are present both in the endothelium (Notch1, Notch4, Jagged1, Dll-1 and -4, Hes1, Hey1, and Hey2) and in the surrounding pericytes, which also express Notch3 (Scehnet *et al* 2007).

In normal development and tumor angiogenesis, Notch signaling regulates blood vessel sprouting and branching (Noguera-Troise *et al* 2006; Gridley 2007; Sainson and Harris 2007; Dufraine *et al* 2008). Notch signaling is important for vessel stabilization and maturation. Heterotypic Notch signaling from endothelial cells to pericytes plays a role in pericyte coverage (Sainson and Harris 2008).

The Notch ligand Dll4 is important for vessel maturation by affecting pericyte recruitment. The soluble form of the Dll4 extracellular domain or the targeted allele deletion resulted in a marked reduction in pericyte recruitment (Scehnet *et al* 2007). Although Notch signal inhibition with a secreted soluble form of Dll4 reduced pericyte activity, these cells were also absent in tumors expressing high Dll4. Therefore, up- or downregulation of Dll4 may cause similar responses (Li *et al* 2007). A recent study has shown that endothelial Jagged1 increases Notch3 and Jagged1 expression through Notch3 on mural cells. Endothelial cells induces pericyte differentiation through the activation and induction of Notch3, thus indicating that the Notch3/Jagged1 interaction between cells may be important for the maintenance of the differentiated pericyte phenotype (Liu *et al* 2009).

9) Endosialin

Endosialin is a transmembrane glycoprotein originally identified as the antigen recognized by the FB5 monoclonal antibody. Endosialin is expressed in the tumor vasculature. Recent research has shown a strong expression of endosialin in pericytes during active angiogenesis, and endosialin was upregulated during embryonic and tumor development (Bagley *et al* 2008). Endosialin was not expressed in the normal human adult brain but was expressed in tumor vessel associated pericytes (MacFadyen *et al* 2007; Christian *et al* 2008; Simonavicius *et al* 2008). Although the function of endosialin remains to be elucidated, this pericyte glycoprotein may be important in angiogenesis.

10) Integrin

The adhesion between endothelial cells and pericytes is an indispensable step in angiogenesis. Moreover, cell-cell and cell-matrix adhesive interactions are required for angiogenesis. Among the proteins involved in these interactions, the integrins are important for this process and mediate pericyte attachment with endothelial cells and the extracellular matrix. Integrins are heteromeric transmembrane glycoprotein adhesion molecules and consist of two distinct chains, the α and β subunits. Endothelial cells and pericytes express a subset of mammalian integrins including the fibronectin receptors α4β1 and α5β1; the collagen receptors α1β1 and α2β1; the laminin receptors α3β1, α6β1, and α6β4; and the osteopontin receptor α9β1. In addition, pericytes express the α7β1 (laminin receptor) and α8β1 (osteopontin receptor) integrins (Silva *et al* 2008).

Angiogenic sprouting and postangiogenic vessel survival in collagen were blocked by antibody against β1 integrins. Therefore, β1 integrins were considered to critically regulate the formation and survival of new vessels (Carnevale *et al* 2007). α4β1 integrin was expressed in endothelial cells in developing vessels. Conversely, proliferating pericytes expressed the α4β1 ligand vascular cell adhesion molecule (VCAM-1). The interaction between α4β1 integrin and VCAM-1 mediates endothelial cell and pericyte adhesion in developing vessels. Therefore, integrin α4β1/VCAM-1 signaling is critical for the correct interaction between endothelial cells and pericytes during neovascularization (Garmy-Susini *et al* 2005). Integrin-regulated angiogenesis appears to be mediated by pericyte function. Treatment of tumors with the αvβ3/αvβ5 integrin antagonist S247 decreased pericyte coverage of vessels (Reinmuth *et al* 2003). *In vitro* and *in vivo* models demonstrated that the NG2 proteoglycan in pericytes interacts with α3β1 integrin in the endothelial cells. Galectin-3 enhances integrin-dependent signals by forming a multimolecular complex with α3β1 integrin and NG2 proteoglycan. NG2 proteoglycan on the cell surface of pericytes stimulates the migration and morphogenesis of endothelial cells by activating transmembrane signaling through α3β1 integrin (Fukushi *et al* 2004). In the NG2 knock out mouse model, pericyte proliferation was reduced in the retina. Basic FGF-induced angiogenesis was also reduced in the NG2 null cornea (Ozerdem and Stallcup 2004). NG2 proteoglycan enhanced the proliferation of pericytes through activation of α3β1 integrin or the potentiation of growth factor signaling (Stallcup and Huang 2008).

The genetic ablation of integrin demonstrated the important roles of these molecules in angiogenesis

and vascular development. Inactivation of the gene encoding integrin β1 subunit in mice led to pericyte defects and postnatal lethality. Integrin β1-deficient pericytes failed to extend long processes and lacked proper interactions with endothelial cells. As a result, mutant pericytes spread poorly, although they were present in larger numbers (Abraham *et al* 2008). In α4 integrin-deficient mouse embryos, pericytes failed to migrate and tended to cluster at angiogenic branch points. Cultured α4 integrin-null pericytes growing on fibronectin showed impaired migration and motility regulated by mechanical signals. Pericytes normally express α4β1 integrin, and α4 expression was localized predominantly on pericytes associated with angiogenic vessels. Therefore, α4β1 integrins are likely required for pericyte spread along angiogenic vessels (Grazioli *et al* 2006). The interaction between endothelial cells and pericytes upregulated integrins α5β1, α3β1, α6β1 and the fibronectin and laminins basement membrane proteins. Close contact between endothelial cells and pericytes induced basement membrane formation *in vitro* and *in vivo* (Stratman *et al* 2009). Accordingly, integrins and their receptors stabilize the vessels by mediating the interaction between pericytes and endothelial cells or the extracellular matrix.

11) Ephrin

The Eph receptor tyrosine kinases (RTKs) are activated in response to the binding of ephrin ligands to extracellular regions. Ephrin-Eph signaling is important for the interaction between endothelial cells and pericytes (Augustin and Reiss 2003; Kuijper *et al* 2007).

EphrinB2, a ligand for Eph receptor tyrosine kinases, was expressed in pericytes in the capillary as development proceeded (Gale *et al* 2001). The expression of Eph-ephrin molecules in mural cells was critical for assembly of the vessel walls during maturation (Kuijper *et al* 2007). The pericyte-specific inactivation of ephrinB2 clearly demonstrated the important role of this molecule in angiogenesis. EphrinB2-deficient pericytes showed insufficient contacts with endothelial cells and failed to envelope the endothelial monolayer. EphB-ephrinB2 interactions between endothelial cells and pericytes and between adjacent pericytes appear to control the directional migration of pericytes and the pericyte coating on maturing vessels (Foo *et al* 2006). EphrinB2 signaling contributes to proper assembly of endothelial cells and pericytes during postnatal angiogenic remodeling. This function requires bidirectional signaling through EphrinB2 and EphB surface-bound receptors through cell-to-cell contacts, and is dependent on Src tyrosine phosphorylation of the intracytoplasmic domain of ephrinB2 (Salvucci *et al* 2009).

Eph-ephrin interactions may be linked to other angiogenic signaling pathways. In hepatic stellate cells, the resident pericytes in liver, ephrinB2 played a role in angiogenesis as a downstream effecter of PDGF signaling (Semela *et al* 2008). EphB4 decreased the permeability of the tumor vessels via angiopoietin-1/Tie2 signaling at the endothelium/pericyte interface. The activation of EphB4 reverse signaling via ephrinB2 increased the expression and phosphorylation of Tie2 in endothelial cells and angiopoietin-1 in pericytes (Erber *et al* 2006). Eph-ephrin signals act as a negative regulator for sprouting angiogenesis by pericyte coverage. In EphA2-deficient mice, the coverage of pericytes in the tracheal capillary was lower due to defective interactions between endothelial cells and pericytes, whereas the responses to inflammation were exaggerated (Okazaki *et al* 2009).

12) Fibroblast growth factor

FGF binds to heparan sulfate proteoglycans in the extracellular matrix, which is required for the activation of FGFR signaling. Consequently, FGF acts locally in a paracrine manner. FGF-2 is known as basic FGF. Angiogenesis was stimulated by conditioned medium from pericytes, and was inhibited by an antibody to basic FGF. Therefore, pericytes may stimulate angiogenesis via secretion of an FGF-like molecule (Watanabe *et al* 1997). A previous study suggested that FGF and PDGF have positive feedback effects on each other's signaling. Combination therapy with both PDGF-BB and FGF-2 markedly stimulated collateral arteriogenesis in the ischemic hind limb model (Cao *et al* 2003). It has been shown that FGF-2 upregulates PDGFR-α and PDGFR-β in newly formed blood vessels (Cao *et al* 2003; Zhang *et al* 2009). FGF-2 enhances the expression of PDGFR-β in cultured pericytes (Kano *et al* 2005). Moreover, it was shown that FGF-2 induced expression of PDGF-α and PDGFR-β in endothelial cells, whereas PDGF-BB upregulated FGFR-1 in pericytes. PDGF-BB upregulated FGFR-1 promoter activity, which increased the responsiveness to FGF-2 (Nissen *et al* 2007). These results indicate that FGF-2 and PDGF-BB have synergistic effects on both signaling system by amplifying the mutual receptor expression. Both signals reciprocally increase their endothelial cell and pericyte responses, which results in disorganized neovascularization (Cao *et al* 2003; Nissen *et al* 2007).

(3) Origin of pericytes

Brain pericytes likely differentiate from the progenitor cells and proliferate during angiogenesis. The cells are generally accepted as being of mesodermal origin. It is assumed that pericytes are derived from the

bone marrow (Figure 4). Bone marrow supplies perivascular progenitor cells, which can differentiate into pericytes (Lamagna and Bergers 2006). Another possibility is that pericyte progenitor cells originate from preexisting vasculature or from local tissues (Figure 4).

1) Bone marrow

Previous studies indicated that hematopoietic stem cells and bone marrow-derived mesenchymal stem cells were incorporated into the perivascular sites of the tumor vessels (Rajantie *et al* 2004; Bababeygy *et al* 2008; Bexell *et al* 2009). In the vessels of pancreatic islet tumors, PDGFR-β+ pericyte progenitor cells were recruited from the bone marrow to perivascular sites and differentiated into pericytes (Song *et al* 2005). In vessels of Ewing's tumors, specific subpopulations of bone marrow-derived progenitor cells differentiated into cells which expressed pericyte markers (Reddy *et al* 2008). During the angiogenesis in Ewing's tumors, the tumor microenvironment affected the differentiation pathway of bone marrow-derived cells (Reddy *et al* 2008). In neovascularization experiments using subcutaneous FGF-2-supplemented Matrigel plugs, over half of the pericytes in the vessel-like networks arose from bone marrow progenitors. The newly formed vessels contained pericytes of both bone marrow and non-bone marrow origin (Tigges *et al* 2008).

In brain ischemia, pericyte progenitor cells may be mobilized and recruited from bone marrow into ischemic areas. It was reported that in murine brain ischemia, bone marrow-derived cells were recruited from the peripheral blood and contributed to the remodeling and stabilization of newly formed blood vessels. The cells derived from bone marrow had the characteristics of pericytes and were positive for VEGF and TGF-β (Kokovay *et al* 2006). More recently, novel mechanisms have been proposed concerning how pericytes are formed from bone marrow-derived cells. In stroke mouse models, fused cells of bone marrow-derived cell expressing specific markers for mature pericyte increased in the ischemic areas. These results indicated that pericytes were formed by cell fusion and the process participated in angiogenesis following stroke (Piquer-Gil *et al* 2009). Adult bone marrow is considered to be a rich reservoir of pericyte progenitor cells. Therefore, cells originating from the bone marrow likely play a role in angiogenesis induced by tumor and ischemia (Lamagna and Bergers 2006).

2) Local environment

Pericyte progenitors may also derive from the tumor microenvironment. A primary source of tumor

pericytes was suggested to be a rare population of stroma-derived mesenchymal progenitors characterized by Tie2 expression (De Palma *et al* 2005). It was shown that the aorta contains immature mesenchymal cells capable of differentiating into pericytes. A population of immature mesenchymal cells in the postnatal rat aorta were transformed into pericytes when they were exposed to PDGF-BB or cocultured with endothelial cells (Howson *et al* 2005). A rat aorta model of angiogenesis also demonstrated that the aorta wall contains Tie2+ non-endothelial mesenchymal cells in the intimal/subintimal layers. These cells are a potential source of mural precursor cells and may contribute to angiogenesis in response to angiopoietin (Iurlaro *et al* 2003). Thus, it remains to be determined whether pericyte progenitor cells are recruited from the bone marrow via peripheral circulation or if they originate from resident perivascular cells in the microenvironment.

3. Blood brain barrier

(1) Neurovascular unit and anatomical structure

The blood brain barrier (BBB) protects the brain from exposure to various toxic substances circulating in the blood (Smith and Gumbleton 2006; Cecchelli *et al* 2007; Bernacki *et al* 2008; Zlokovic 2008). The BBB is comprised of cerebral microvascular endothelial cells, pericytes, and astrocytes (Mathiisen *et al* 2010). These cells form a neurovascular unit together with neurons and extracellular matrix. Dysfunction of the neurovascular unit is one possible mechanism underlying disorders of the central nervous system (Hawkins and Davis 2005; Persidsky *et al* 2006).

Pericytes are important cellular constituent of the BBB. Recent studies have revealed that pericytes actively control vascular permeability at the capillary level in the brain. The close interaction between endothelial cells and pericytes is required for proper BBB function (Table 2). Lack of pericytes causes immature vessels and results in increased transendothelial permeability in the brain (Lindahl *et al* 1997; Hellstrom *et al* 2001). Accumulating evidence indicates that pericytes maintain the BBB (Balabanov and Dore-Duffy 1998; Thomas 1999; Lai and Kuo 2005; Persidsky *et al* 2006).

The anatomical structure of the capillary is stabilized by pericytes, since these cells encircle the capillary tube, which is composed of endothelial cells. Pericyte contraction mechanically tightens the junction between adjacent endothelial cells and thus protects it from overstretching. The decreased contractility of pericytes alters the physical barrier in the capillary by opening the inter-endothelial junction (Edelman *et al* 2006). In addition to anatomical structure, transport across the BBB is limited by physical (tight junction) and

metabolic (enzymes and diverse transport systems) barriers (Persidsky *et al* 2006). Pericytes actively control these barriers by interacting with endothelial cells.

(2) Mechanisms of BBB regulation

An *in vitro* model which contains brain endothelial cells cocultured with brain pericytes and astrocytes can mimic BBB function. The *in vitro* BBB model has revealed that pericytes are required to maintain BBB function (Hori *et al* 2004; Dohgu *et al* 2005; Parkinson and Hacking 2005; Hartmann *et al* 2007; Nakagawa *et al* 2007; Kim *et al* 2008; Zozulya *et al* 2008; Cardoso *et al* 2010). Close contact between pericytes and endothelial cells is essential for barrier functions. In a triple coculture model, in which endothelial cells and pericytes were cultured on both sides of the filter membrane and astrocytes at the bottom of the culture dish, pericytes were necessary to maintain higher electrical resistance and lower permeability (Nakagawa *et al* 2007).

The barrier functions are maintained by BBB cellular junctions, BBB transport systems, and enzymatic BBB. Pericytes regulate endothelial cell tight junction formation, blood-brain barrier differentiation, and permeability through tight junction and transendothelial cell transport (Balabanov and Dore-Duffy 1998). Therefore, pericytes actively control the BBB by altering the function and expression of proteins associated with the BBB (Figure 5).

1) BBB cellular junctions

Tight junctions are facilitated by tight junction proteins, such as occludins, claudins, and other junctional proteins (junctional adhesion molecule-A), a cytoskeleton linking protein such as ZO-1 or actin, and adherens junctions (Hawkins and Davis 2005; Bernacki *et al* 2008; Zlokovic 2008). Proteins related to the tight junction are regulated by pericytes. Contact of endothelial cells with pericytes induced the expression of ZO-1 in developing retinal vessels (Kim *et al* 2009). In the presence of pericytes and astrocytes, expression levels of occludin, claudin-5, and ZO-1 at the borders of rat brain endothelial cells were enhanced (Nakagawa *et al* 2009). Moreover, tight junction molecules such as occludin, claudin-12, ZO-1, and ZO-2 were expressed in peripheral nerve pericytes. Therefore, pericytes themselves may facilitate transport through barriers in cooperation with endothelial cells (Shimizu *et al* 2008).

Extracellular matrices produced by pericytes improved brain endothelial barrier function as

estimated by the electrical resistances (Hartmann *et al* 2007). An *in vitro* BBB model revealed that angiopoietin-1 secreted from pericytes induced occludin expression in brain capillary endothelial cells via Tie2 receptor activation (Hori *et al* 2004). Dexamethasone increased angiopoietin-1 but decreased VEGF expression in brain pericytes, which may underlie mechanisms of glucocorticoid-induced BBB stabilization (Kim *et al* 2008). Pericyte-conditioned medium increased occludin and ZO-1 expression under normoxia and partially reversed decreased expression of occludin under hypoxic conditions. Because an angiopoietin-1 neutralizing antibody inhibited these effects, tight junction proteins are considered to be regulated by pericyte-secreted angiopoietin-1 (Wang *et al* 2007). An *in vitro* BBB model with cocultured brain capillary endothelial cells and pericytes revealed that the pericytes upregulated BBB functions through continuous production of TGF-β. Therefore, pericyte-derived TGF-β1 may be another regulator of BBB (Dohgu *et al* 2005). The level of TGF-β1 is decreased by cyclosporin A in brain pericytes, suggesting that cyclosporin A-induced dysfunction of the BBB may be mediated by TGF-β1 derived from pericytes (Takata *et al* 2007).

In addition to upregulation of tight junction proteins in endothelial cells, pericytes induce MMP secretion in endothelial cells, which contributes to hyperpermeability. Brain capillary endothelial cells co-cultured with pericytes resulted in an increased amount of endothelial MMP-9 and active MMPs (Zozulya *et al* 2008). Pericytes might trigger endothelial MMP production through cytokine mediators, including TGF, TNF, IL-6, and others (Virgintino *et al* 2007). MMPs can degrade basal lamina proteins such as fibronectin, laminin, and heparan sulfate (Zlokovic 2006). Therefore, secretion of MMP by pericytes contributes to the breakdown of BBB.

2) BBB transport systems

ATP-binding cassette transporters may be functionally regulated by pericytes. Diffusible factors from pericytes were sufficient to induce MRP6 expression in brain capillary endothelial cells (Berezowski *et al* 2004). In a co-culture model, brain pericytes upregulated barrier function and P-glycoprotein functional activity of brain endothelial cells via TGF-β1 signaling (Takata *et al* 2007). Transporters may also be expressed in peripheral nerve pericytes. The barrier-related transporters ABCG2, P-glycoprotein, MRP-1, and Glut-1 were detected in pericytes (Shimizu *et al* 2008). Immunogold cytochemistry electronmicroscopic studies revealed that P-glycoprotein was expressed at the luminal and abluminal membranes of pericytes in rat and human brains (Bendayan *et al* 2006).

4. Macrophage activity

Early investigators observed and discriminated between granular and agranular pericytes. Brain pericytes have cytoplasmic lysosomes, which account for their granular appearance. Macrophage components were shown to be present in brain pericytes. They had Fc receptors, exhibited antibody-dependent phagocytosis, and acted as antigen-presenting cells (Thomas 1999; Guillemin and Brew 2004). Moreover, it has been reported that these cells express macrophage scavenger receptors and likely act as scavenger cells (Balabanov and Dore-Duffy 1998; Thomas 1999). Pericytes can uptake soluble and small molecules from the blood or brain parenchyma through interstitial fluid. These cells transport materials by infoldings of the plasma membrane, endocytosis through pinocytosis or phagocytosis, and through receptor-mediated endocytosis (Balabanov and Dore-Duffy 1998; Lai and Kuo 2005). Therefore, brain pericytes may represent the first line of immunologic defense of the brain (Balabanov *et al* 1999; Guillemin and Brew 2004).

Pericytes migrating into the brain parenchyma may act in a similar manner to brain macrophages, such as macrophage infiltrating into the brain, perivascular macrophage, and microglia. The pericytes may be another myeloid cell type located adjacent to but distinct from the perivascular macrophage (Guillemin and Brew 2004). Brain pericytes act in the neuroimmune systems as brain macrophages in cooperation with various types of brain macrophages. Although the functional and cellular differences among brain macrophages are still poorly understood, pericytes may modulate immune system function by controlling NK cells, T cells, and B cells. Further study will define the immunomodulatory roles of pericytes in the brain.

5. Pluripotent activity

Pericytes are plastic and multipotent. Pericytes isolated from various tissues have the ability to differentiate into other mesenchymal cell types, such as smooth muscle cells, fibroblasts, adipocytes, chondrocytes, and osteocytes (Figure 5). A recent study has shown that mesenchymal stem cells reside in the perivascular position and have characteristics of pericytes (da Silva Meirelles *et al* 2008; Caplan 2009). Moreover, pericytes have been suggested to affiliate with mesenchymal stem cells (Caplan 2008; Crisan *et al* 2008). Although it is still unclear whether pericytes associate with mesenchymal stem cells, the perivascular zone may be the mesenchymal stem cell niche *in vivo*.

(1) Brain

Brain microvascular pericytes appear to be a source of adult multipotent progenitor cells, because they express markers characteristic of pericytes, neurons, and glial cells during culture with bFGF-containing media without serum. These data suggest that brain microvascular pericytes have neural stem cell capabilities (Dore-Duffy *et al* 2006). Perivascular cells isolated from mouse meninges not only expressed pericyte-specific markers but also stem cell markers. The cells displayed phenotypic characteristics of pericytes with a distinct developmental potential (Brachvogel *et al* 2005; Brachvogel *et al* 2007). Hematopoietic stem cells expressed pericyte progenitor markers as well as pericyte markers (Bababeygy *et al* 2008). Therefore, brain pericytes are able to differentiate into progenitor and mature cells with distinct phenotypes. The microvasculature may serve as a pool of mesenchymal stem cells in the brain.

(2) Other organs

Adipose-derived stromal cells located at the perivascular position express mesenchymal, pericytic, and smooth muscle markers. Although they exhibited pericyte properties, they were pluripotent and capable of differentiating into distinct lineages (Traktuev *et al* 2008). Pericytes were able to differentiate along the adipocytic and chondrocytic lineages *in vitro* and *in vivo* (Farrington-Rock *et al* 2004). PDGFR-β+ stromal vascular cells in adipose tissue had adipogenic potential, but PDGFR-β+ cells isolated from other organs did not display this adipogenic potential. The microvasculature in the adipose tissue may function as a niche for adipocytes (Tang *et al* 2008). Pericytes from microvascular walls in postnatal skeletal muscle were able to regenerate skeletal muscle. Therefore, pericytes represent myogenic precursors in human skeletal muscle (Dellavalle *et al* 2007; Morgan and Muntoni 2007). Skin pericytes have been shown to act as mesenchymal cells, and exhibited the capability to differentiate into different lineages, such as bone, fat, and cartilage. In addition, skin pericytes modified the microenvironment and promoted skin regeneration independent of angiogenesis (Paquet-Fifield *et al* 2009). Pericytes in various tissues may have mesenchymal stem cell potential (Caplan 2008; Crisan *et al* 2008).

Conclusion

Brain pericytes are considered to play a pivotal role in the maintenance of the brain function.

Malfunction of these cells may underlie the pathogenesis of CNS diseases through the dysregulation of microcirculation, angiogensis, blood brain barrier and neurogenesis.

Figure Legends

Figure 1. Pericytes control capillary flow.

Diameters of the capillary lumen are altered by contracture of surrounding pericytes. Capillary flow is controlled by dilatation (A) or contraction (B) of pericytes in response to physiological (e.g. neural activity) as well as pathological conditions (e.g. acidosis and reactive oxygen species) in the microenvironment.

Figure 2. Pericytes stabilize newly formed vessels.

In stabilized vessels, pericytes are tightly attached to the endothelial cells with interjacent basement membrane. The stabilized capillaries function as mature vessels and sustain the homeostasis between the blood and the parenchyma (A). When stimulated, the pericytes are detached from the perivascular position and capillary vessels are unstabilized (B). After pericyte detachment, endothelial cells are activated and the basement membrane and ECM are degraded. The stimulated endothelial cells migrate and proliferate into the ECM. The stimulated endothelial cells develop immature lumens, which hyperdilate and exhibit hyperpermeability. Newly formed vessels are restabilized by pericytes.

Figure 3. Angiogenic signaling in triangle of pericytes, endothelial cells, and extracellular matrix

Angiogenesis is finely modulated by the interaction between pericytes and endothelial cells. Pericytes and endothelial cells secrete a variety of angiogenic factors. These signaling molecules act on adjacent homo- and heterotypic cells via their specific receptors in an autocrine or paracrine manner (e.g. TGF-β/TGF-βRII-ALK1, ALK5, PDGF-B/PDGFR-β, angiopoietin-1,-2/Tie2, VEGF/VEGFR-2, sphyngosine-1-phosphate/$S1P_1$, FGF-2/FGFR). During angiogenesis, regulator of G-protein signaling-5 (RGS-5) may contribute to the signal transduction by regulating the activity of G proteins. Direct cell-cell communication through cell surface proteins or cell-ECM interactions are also involved in regulation (e.g. integrin, endosialin, N-cadherin, Notch). Matrix metalloprotease (MMP) degrades proteins in the extracellular matrix and modulates the interaction of the cells by its degradation. The relationship between pericytes, endothelial cells, and extracellular matrix proteins controls angiogenesis and consequently affects tissue

regeneration.

Figure 4. Pericytes in blood brain barrier

Pericytes directly or indirectly control the BBB. They actively regulate the expression of tight junctions (claudin and occludin) or zonula occludens in endothelial cells and strengthen barrier functions. They express BBB components, BBB transporters (ABC transporters), solute carriers (GLUT1 and SGUT), and BBB ectoenzymes. In addition, pericytes have phagocytotic activity and remove macromolecules in the perivascular space.

Figure 5. Origin and pluripotential of pericytes

Pericytes may be recruited from bone marrow-derived cells through the peripheral circulation. In addition, the cells may originate from the local environment. Pericytes appear to possess pluripotent activity and can differentiate into different lineages.

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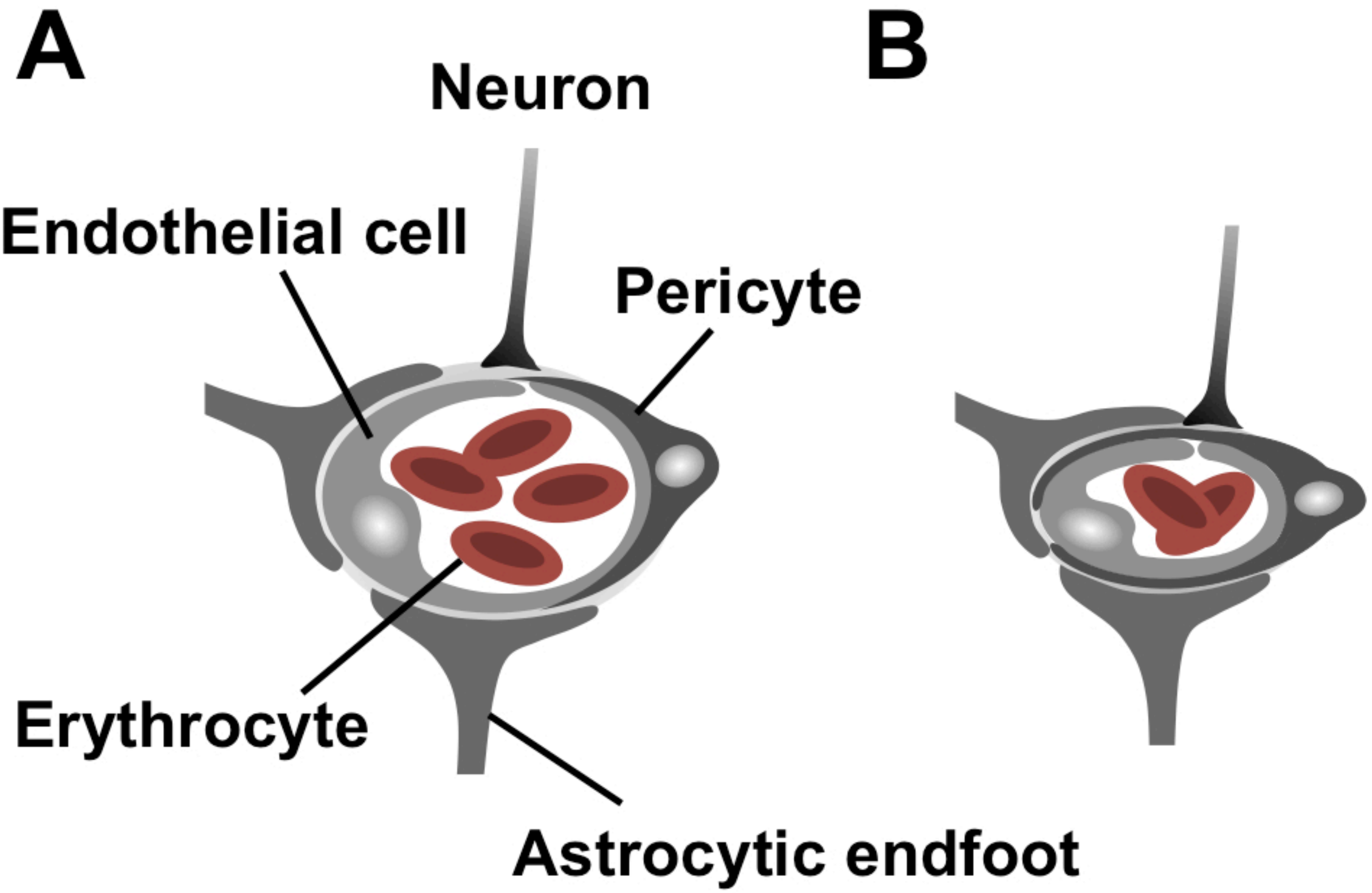
Table 1. Pericyte markers and factors involved in the pericyte functions

Cell markers	PDGFR-β, NG2, α smooth muscle actin, desmin, vimentin, RGS-5, 3G5, Kir6.1, nestin, Sca-1, aminopeptidases A and N, high molecular weight melanoma-associated antigen, alkaline phophatase, γ-glutamyl transpeptidase, butyrylcholinesterase, FcR, CD4, CD11b, CD34, CD140a, MHC class I·II
Cellular functions	
Regulation of capillary flow	ATP, cholinergic agonists, adrenergic agonists, histamine, serotonin, angiotensin II, endothelin-1, atrial natriuretic peptide, reactive oxygen species
Angiogenesis	TGF-β/TβRII-ALK1·ALK5, PDGF-B/PDGFR-β, heparan sulfate proteoglycan, SDF-1α, angiopoietin-1·2/Tie2, VEGF/VEGFR-2, RGS-5, MMP, TIMP, aminopeptidase A, sphyngosine-1-phosphate/$S1P_1$, N-cadherin, Notch/Jagged1/Dll4, endosialin, integrin family, NG2, EphrinB2/EphB, FGF-2/FGFR

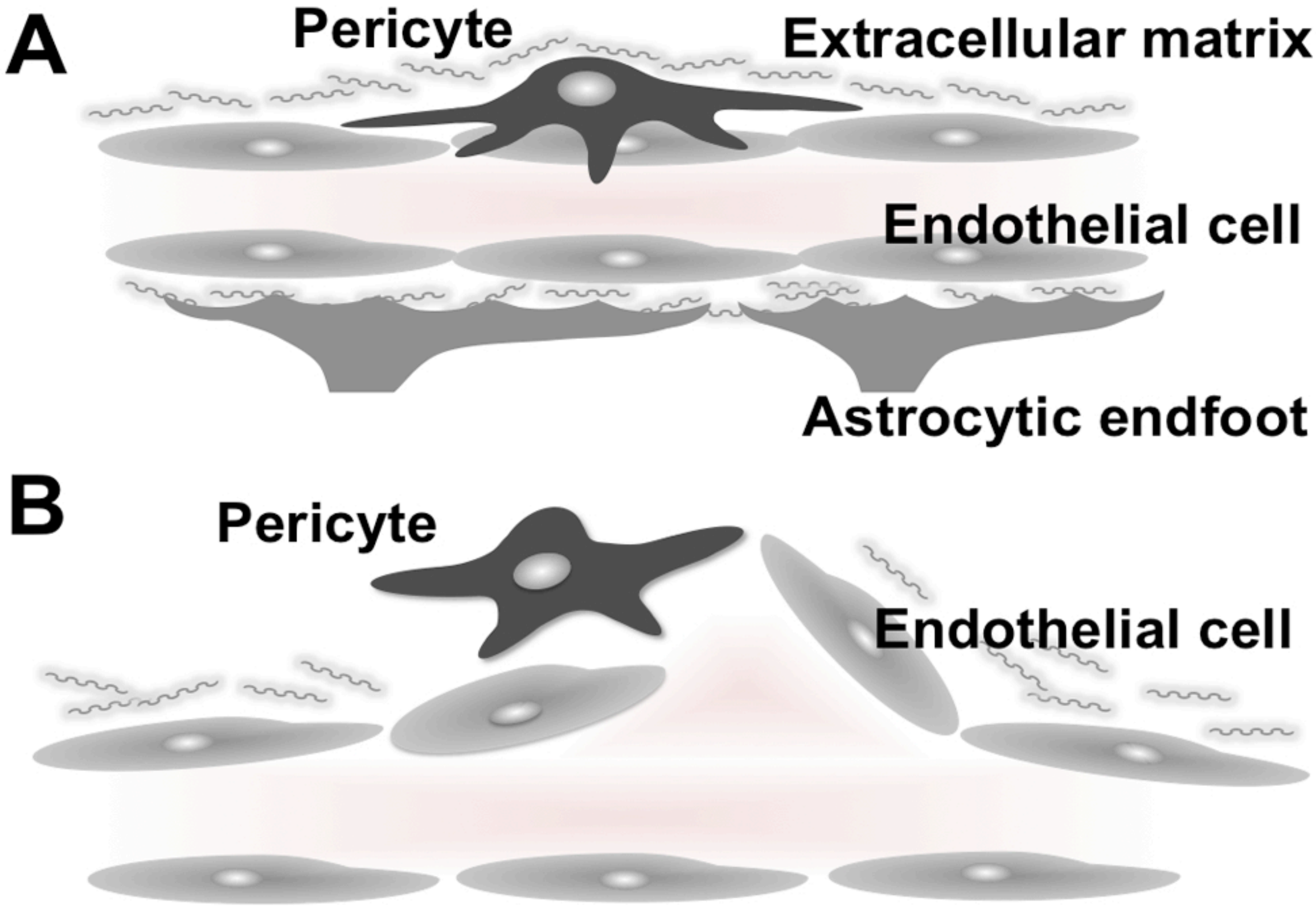
Table 2. Pericytes in the formation of blood brain barrier

Proposed mechanisms	References
Tightening inter-endothelial junction (physical barrier)	(Edelman *et al* 2006)
Production of extracellular matrices	(Hartmann *et al* 2007)
Regulation of endothelial tight junction proteins	(Kim *et al* 2009; Nakagawa *et al* 2009)
Pericytes strengthen barrier function by upregulating tight junction proteins via angiopoietin-1 (Hori *et al* 2004; Wang *et al* 2007; Kim *et al* 2008) and TGFβ-1 (Dohgu *et al* 2005; Takata *et al* 2007).	
Regulation of endothelial transport systems	(Takata *et al* 2007)
Regulation of endothelial MMP	(Virgintino *et al* 2007; Zozulya *et al* 2008)
Expression of tight junction proteins and barrier-related transporters in the pericytes	(Bendayan *et al* 2006; Shimizu *et al* 2008)

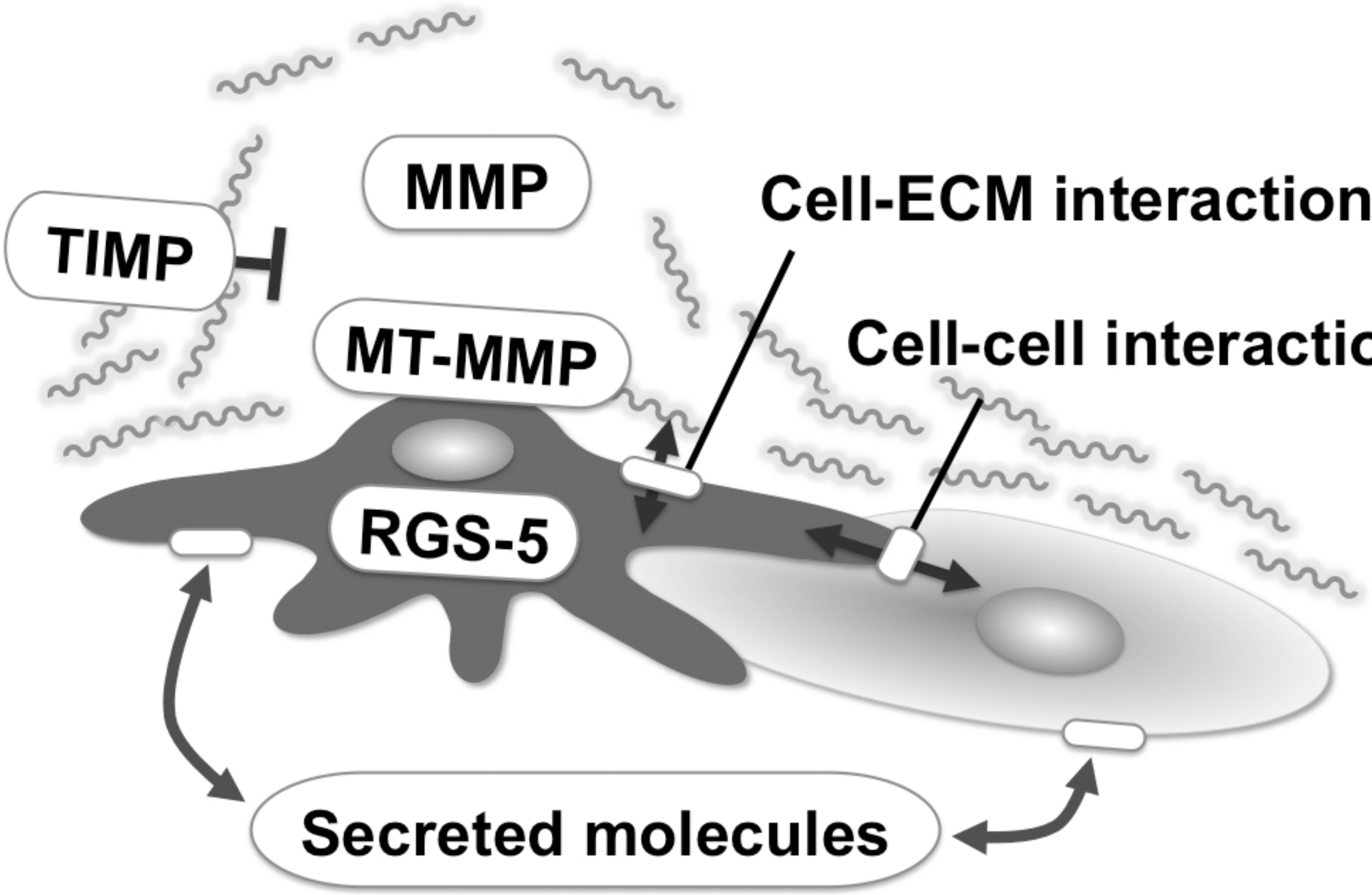
(Figure 1)


A
B
Neuron
Endothelial cell
Pericyte
Erythrocyte
Astrocytic endfoot

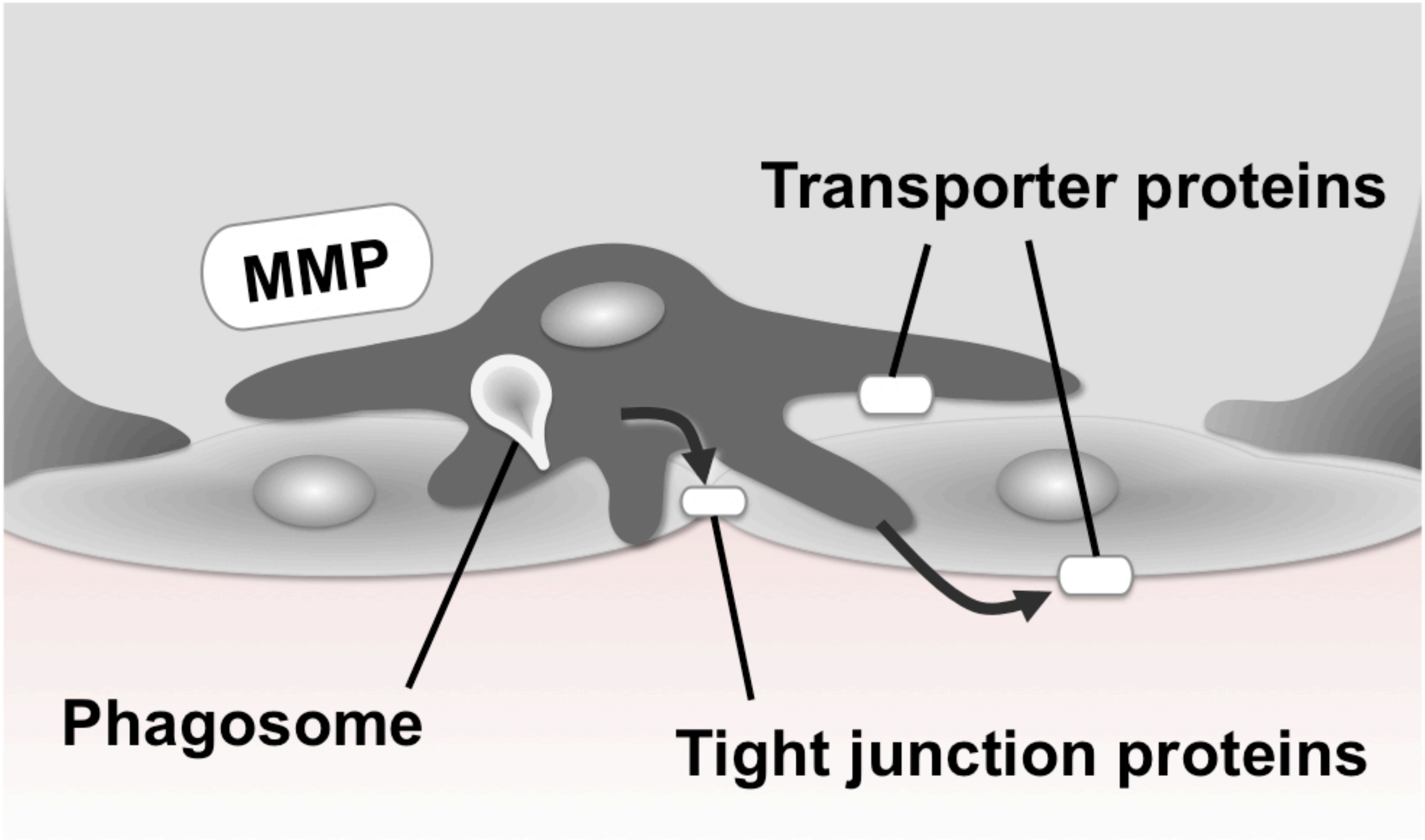
(Figure 2)


A
Pericyte
Extracellular matrix
Endothelial cell
Astrocytic endfoot
B
Pericyte
Endothelial cell

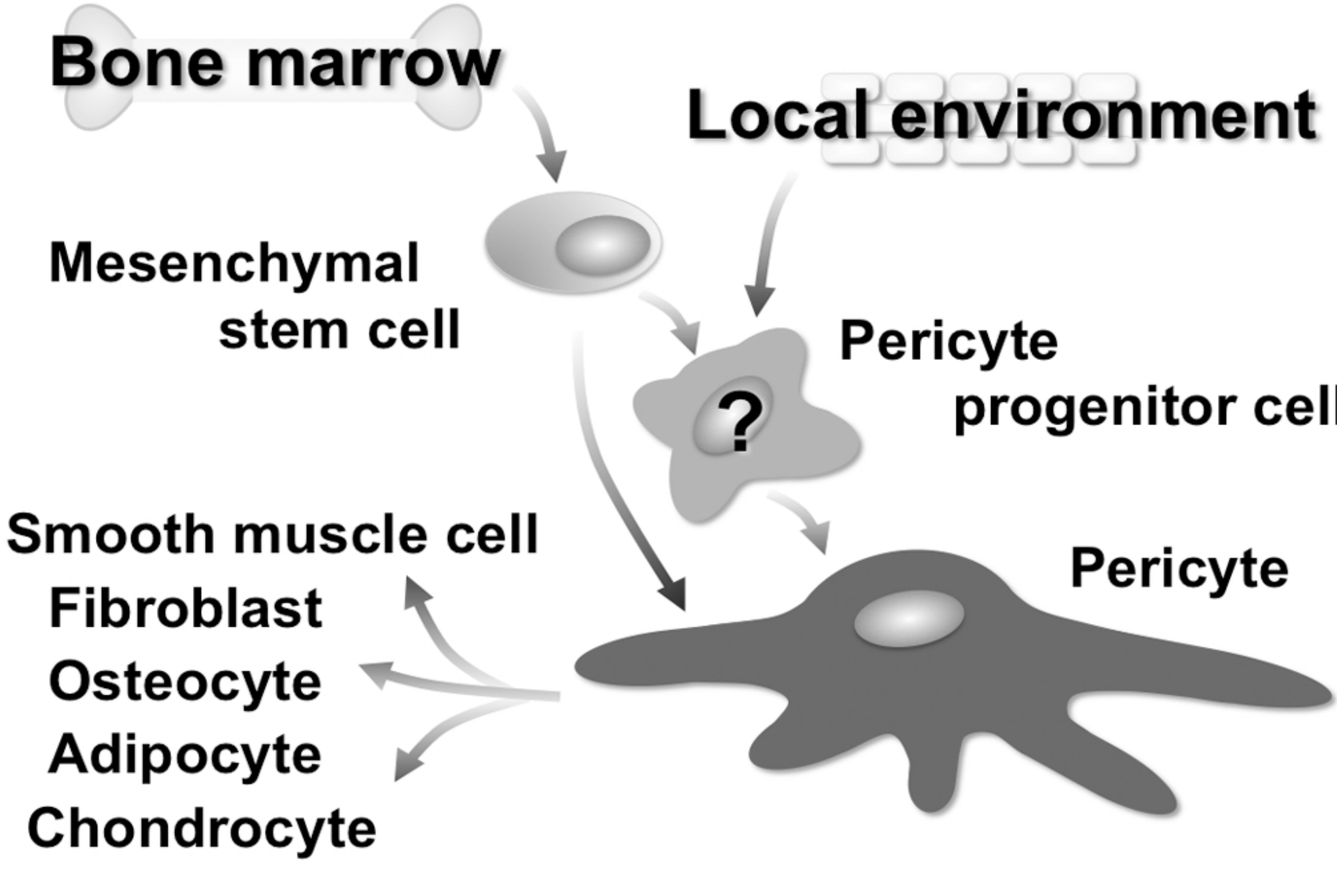
(Figure 3)


MMP
TIMP
MT-MMP
RGS-5
Cell-ECM interaction
Cell-cell interaction
Secreted molecules

(Figure 4)


Transporter proteins
MMP
Phagosome
Tight junction proteins

(Figure 5)


Bone marrow
Local environment
Mesenchymal
stem cell
Pericyte
progenitor cell
?
Smooth muscle cell
Fibroblast
Osteocyte
Adipocyte
Chondrocyte
Pericyte