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Neurovascular Unit: a Focus on Pericytes

Inês Sá-Pereira · Dora Brites · Maria Alexandra Brito

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Abstract The blood–brain barrier (BBB) is a highly specialized system that controls the exchanges between the blood and the central nervous system (CNS). This barrier shields the CNS from toxic substances in the blood and provides nutrients to CNS, thus playing an essential role in the maintenance of homeostasis. The anatomical basis of the BBB is formed by the endothelial cells of brain microvasculature, with elaborated tight and adherens junctions, which together with pericytes, the basement membrane, and astrocytes, as well as neurons, microglia and oligodendrocytes form the neurovascular unit. The interaction between all these components guarantees a proper environment for neural function and a restricted permeability and transport. Pericytes were initially reported by Rouget in 1873 and since then they have been recognized as an important component of the BBB, despite the difficulty of their identification. Diverse functions have been assigned to pericytes, including a role in BBB properties, hemostasis, and angiogenesis, as well as a contractile, immune, and phagocytic function. These cells are also seen like multipotent cells and so with a great potential for therapy. Here, we review the neurovascular unit composition and the interplay between the diverse components, addressing pericytes with a particular detail.

Keywords Blood–brain barrier · Endothelial cells · Neurovascular unit · Pericytes

Abbreviations

ABC	ATP-binding cassette
α-SMA	α-smooth muscle actin
Ang	Angiopoietin
AJ	Adherens junction
BBB	Blood–brain barrier
BM	Basement membrane
BMVEC	Brain microvascular endothelial cell
CAM	Cell adhesion molecule
CNS	Central nervous system
ECM	Extracellular matrix
EC	Endothelial cell
ER	Endoplasmic reticulum
GFAP	Glial fibrillary acidic protein
GLUT-1	Glucose transporter-1
ICAM-1	Intercellular adhesion molecule-1
IL	Interleukin
JAM	Junctional adhesion molecule
MHC	Major histocompatibility complex
MMP	Matrix metalloproteinase
MRP	Multidrug resistance-associated protein
NVU	Neurovascular unit
OPC	Oligodendrocyte precursor cell
PDGF-β	Platelet-derived growth factor-β
P-gp	P-glycoprotein
RGS-5	G-protein signaling-5
TEER	Transendothelial electrical resistance
TGF-β	Transforming growth factor-β
TJ	Tight junction
VCAM-1	Vascular cell adhesion molecule-1
VEGF	Vascular endothelial growth factor
VEGFR2	Vascular endothelial growth factor receptor 2
ZO	Zonula occludens

I. Sá-Pereira · D. Brites · M. A. Brito (✉)
Research Institute for Medicines and Pharmaceutical Sciences (iMed.UL), Faculty of Pharmacy, University of Lisbon, Av. Prof. Gama Pinto, 1649-003 Lisbon, Portugal
e-mail: abrito@ff.ul.pt

D. Brites · M. A. Brito
Department of Biochemistry and Human Biology, Faculty of Pharmacy, University of Lisbon, Lisbon, Portugal

Introduction

The blood–brain barrier (BBB) is a complex and dynamic interface between the blood and the brain, formed by endothelial cells (ECs) of microvasculature, supported by a basement membrane (BM), and surrounded by

pericytes and astrocyte endfeet. The existence of interactions between the vascular and neural cells that are important for brain homeostasis led to the concept of a functional unit known as neurovascular unit (NVU) [1–4]. Pericytes are generally included in the context of the BBB, but their role in the NVU homeostasis is somehow dismissed. In this review, we present updated concepts about the NVU, individually addressing the characteristics of each of its components and the specific interactions among them that assure BBB properties and central nervous system (CNS) homeostasis. Furthermore, we explore the features of CNS pericytes, which are still intriguing cells with unclear roles that appear to be involved in pathological processes and that are looked as promising targets for therapeutics.

Neurovascular Unit

It was in 1885 that Paul Ehrlich injected vital dyes into the circulatory system and observed that all organs except the brain and spinal cord were stained [5]. Later on, Edwin Goldmann, an Ehrlich's student, injected trypan blue into cerebrospinal fluid and noticed that it only stained the CNS [6]. These evidences pointed to the existence of a barrier separating the CNS and the circulation that was named *bluthirnschranke* (blood–brain barrier) by Lewandowsky in 1900 who further noticed the absence of pharmacological actions of bile acids and ferrocyanide in the CNS [7]. However, this barrier was related with ECs and their tight junctions (TJs) only with the advance of electron microscopy [8].

All organisms with a developed CNS have a BBB that shields the CNS from toxic and harmful substances in the blood and from free paracellular diffusion of water-soluble molecules, especially through endothelial TJs. The BBB allows the uptake of water-soluble nutrients, metabolites, and required molecules into the CNS microenvironment and exports exogenous or dangerous compounds from the brain to the bloodstream through specific transport systems expressed by brain microvascular ECs [1, 4, 9]. The BBB also limits the entry into the brain of red blood cells and leukocytes in normal conditions [9].

The only regions where there is no BBB are those that regulate autonomic nervous system and endocrine glands of the body since blood vessels allow diffusion of blood-borne molecules across the vessel wall [10]. This barrier is mainly formed by ECs, astrocyte endfeet, BM and pericytes. All these elements, together with microglia and neurons, are part of the functional NVU [1, 4, 9]. More specifically, a differentiated BBB is composed by the highly specialized ECs, surrounded by a BM in which a large number of pericytes are embedded. These last cells are also covered by the BM, which is ensheathed by astrocytic endfeet (Fig. 1a). The close proximity of the several components of the NVU schematically represented in Fig. 1a can be depicted in the images of brain sections shown in Fig. 1b, where different cell types can be identified.

The interactions of ECs with the other components of the BBB provide a stable environment for neural function [4, 10]. On the other hand, the TJs between ECs, together with the enzymes and diverse transport systems, make the transport across the BBB strictly limited and translate a restricted permeability [1, 3, 4]. Thus, the BBB is also seen as a limiting factor and obstacle for the delivery of therapeutic agents and drugs into the CNS [3, 9]. Presently, the BBB endothelium is further regarded as an important relay station that plays a key role in the signaling between blood and brain compartments, in both directions, by different pathways, as we recently reviewed [4]. Moreover, common microvessels and the ramifications of nerve and glial cells mediate the connection between adjacent NVUs, thus establishing the intercommunication throughout the brain parenchyma.

Endothelial Cells

The first in vitro model of the BBB was developed by Ferenc Joó and his co-workers [11], consisting of viable brain microvessels isolated from rat brains, as shown in Fig. 2a. Such microvessels are composed of cerebral endothelial cells and pericytes sharing a common basement membrane, as depicted in Fig. 2b that shows the ultrastructure of a brain capillary cross-section on an electron micrograph.

ECs of brain capillaries, located at the interface between the blood and the brain, are considered the anatomical basis of the BBB due to their morphological, biochemical, and functional properties that are unique and distinguishable from all other ECs in the body [12–14]. Between adjacent ECs, there are elaborate intercellular junctions [4, 14, 15]. These correspond to overlapping sections of two ECs processes where there are several regions with dense material, known as "kissing points", as shown in the inset of Fig. 2b from a brain capillary, in Fig. 2c from cultured brain ECs, and as schematically represented in Fig. 2d. The brain capillary endothelium is 50–100 times tighter than peripheral microvessels [16]. Moreover, it has 0.2 to 0.4 μm thickness in the vicinity of intercellular junctions and 1.5–2 μm at the region of the cell nucleus [9, 14]. As shown in Fig. 2b, c, and d, ECs present smooth oval nuclei with uneven distribution of chromatin substance, a few caveolae and caveolae-like invaginations on the luminal side, and organelles like endoplasmic reticulum and mitochondria [14].

Compared to ECs in other tissues, the BBB endothelium has longer TJs [17], sparse pinocytic vesicular transport systems [15], and no fenestrations in their cytoplasm [18]. Moreover, these cells have a negative surface charge that repulses negatively charged compounds [19, 20]. Brain

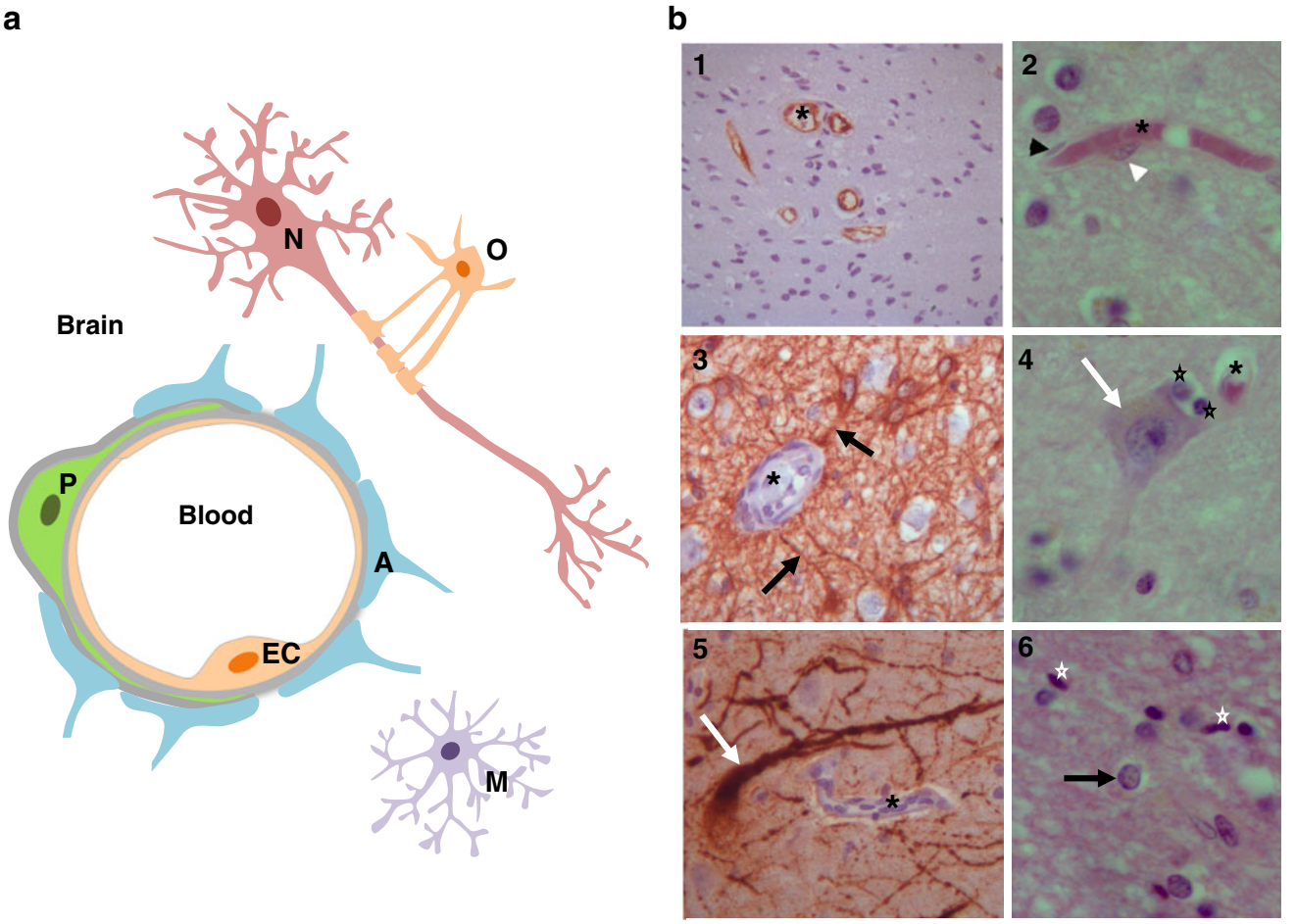

Fig. 1 Constitution of the neurovascular unit. **a** Schematic representation showing an endothelial cell (EC) surrounding a blood vessel and ensheathed by the basement membrane (gray line), a pericyte (P) and astrocyte (A) foot processes; close to the vessel, a microglial cell (M), a neuron (N), and an oligodendrocyte (O) are seen. **b** Histological sections of the brain parenchyma showing blood vessels (asterisk) surrounded by the different cell types (black arrowhead, endothelial cell; white arrowhead, pericyte; black arrow, astrocyte; white arrow, neuron; black star, oligodendrocyte; white star, microglia. 1, CD34 staining of endothelial cells; 2, hematoxylin–eosin (HE) staining showing an endothelial cell (flat cell with an elongated nuclei) and a pericyte with a voluminous cell body and a more round nuclei; 3, staining of glial fibrillary acidic protein, a characteristic cytoskeleton intermediate filament protein of astrocytes (brownish coloration), showing the foot processes extending towards a blood vessel; 4, HE staining showing a neuron with its cell body containing the nuclei and the beginning of the axon, as well as the nuclei of two close oligodendrocytes; 5, staining of neurofilaments, a characteristic intermediate filament protein of neurons (brownish coloration), showing the extension of neurites throughout the brain and particularly around a blood vessel; 6, HE staining where microglial cells are visible among other cells, as astrocytes. Original magnification: 2, 4, and 6, ×1,000; 1, 3, and 5, ×400

microvascular ECs (BMVECs) also have a great number and volume of mitochondria that enhance their energy potential for enzymes and transport systems activity [21]. ECs are equipped with specific transport systems and receptors (Fig. 3a, b), which have a unique pattern that facilitates the uptake of nutrients and hormones required for brain function [4, 9]. Solute carrier family of transport proteins assures the uptake of water-soluble molecules like glucose by the glucose transporter-1 (GLUT-1) [4, 9, 22], which expression is shown in Fig. 3b. Members of the ATP-binding cassette (ABC) transporter family, such as P-glycoprotein (P-gp) (Fig. 3b) and multidrug resistance-associated proteins (MRP), move out of the brain harmful hydrophilic and hydrophobic molecules [23]. The expression of P-gp is one of the specialized characteristics of BMVECs [24, 25]. ECs also express enzymes that can modify and change a range of molecules, which otherwise could pass through the BBB and affect neuronal function [1, 9]. Enzyme concentration is high in cerebral microvessels and includes γ-glutamyl transpeptidase, alkaline phosphatase, and aromatic acid decarboxylase [1]. Besides all these, BMVECs have other active pumps that help in the regulation of concentrations of ions, metabolites, and xenobiotics in the brain [4].

To eliminate spaces between ECs and prevent free paracellular diffusion of blood-borne substances into the brain parenchymal space, BMVECs of capillaries and postcapillary venules have junctional complexes that include, not

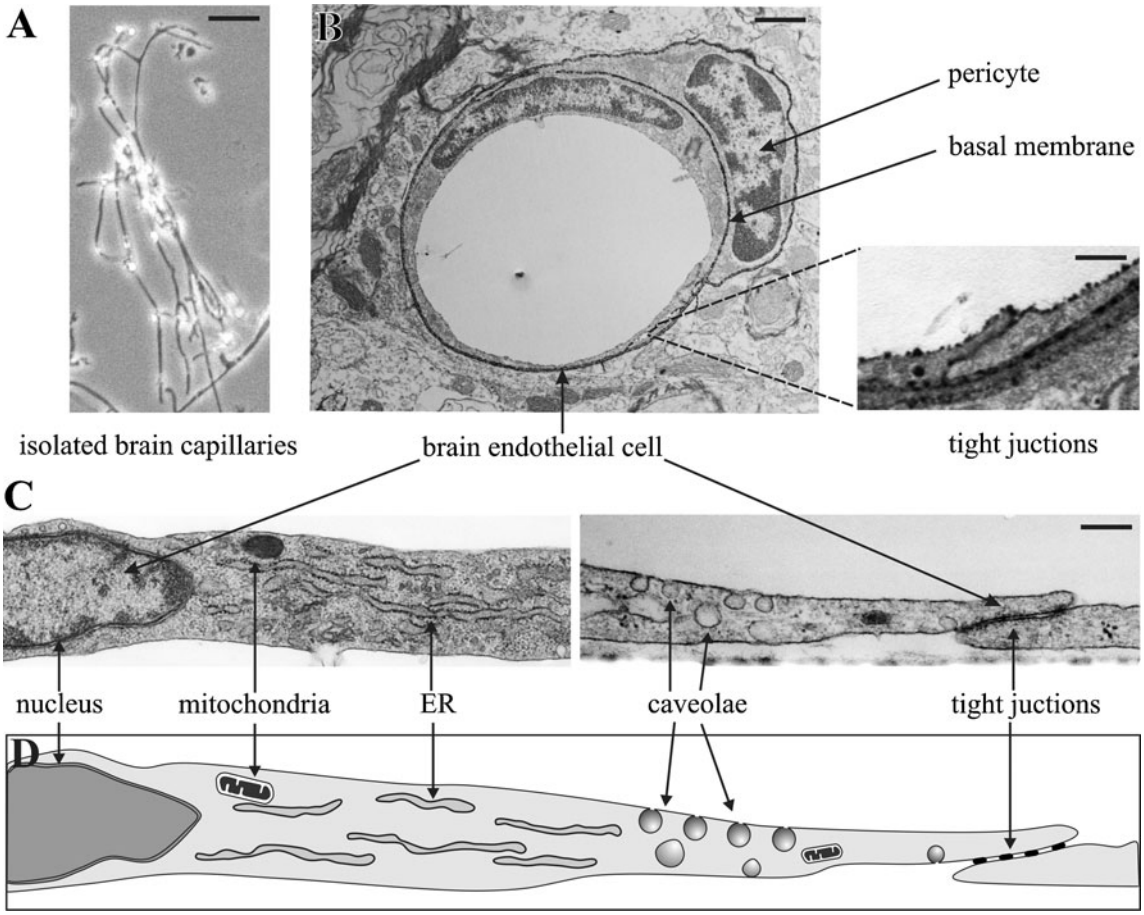

Fig. 2 The anatomical structure of the blood–brain barrier. Isolated rat brain capillaries viewed by phase contrast microscopy (**a**); cross-section of a capillary in a rat brain, transmission electron microscopy (**b**); ultrastructure of primary rat brain endothelial cells in culture by electron microscopy (**c**); and a schematic drawing of the morphological details (**d**). ER endoplasmic reticulum. *Bars*: **a** 20 μm, **b** 1 μm, *insert* 500 nm, **c** 500 nm. This figure was kindly provided by Szilvia Veszelka, Ágnes Kittel, and Mária A. Deli. Reproduced from [14], with permission of Bentham Science Publishers Ltd

only TJs, but also adherens junctions (AJs), as shown in Fig. 3a, b. The first are located on the apical region of ECs and AJs are below TJs. TJs proteins include the transmembrane proteins claudins, occludin, junctional adhesion molecules (JAMs) and the cytoplasmic proteins zonula occludens (ZO) [4, 26]. The claudins family includes by now 27 members [27], which have 20–27 kDa and four domains. Occludin was the first TJ protein discovered. It has 65 kDa and four domains like claudins but with a different amino acid sequence [28]. JAMs are proteins with approximately 40 kDa that have been identified in 1998. They belong to the immunoglobulin superfamily and have a single transmembrane domain [29]. There are three JAMs, including JAM-1 that is predominantly expressed in the brain [30]. JAM-1 is involved in cell-to-cell adhesion and takes part in the formation of TJs as an integral membrane protein together with occludin and claudins. Recent data obtained in our lab revealed the presence of another transmembrane TJ protein in BMVECs, named tricellulin, which is distributed along bicellular TJs and also found at tricellular TJs [31]. Finally, cytoplasmic proteins include ZO-1 that ensures the linkage of TJs to the cytoskeleton. AJs include cadherins that have a plasma membrane-spanning domain and a cytoplasmic domain associated with catenins, the other AJ proteins [32]. These last proteins make the link between cadherins and actin cytoskeleton [4]. AJs also include the nectin–afadin complex where afadin, also known as AF-6, anchors nectins to the cytoskeleton [33]. Although TJs are the primary seal between ECs, in BMVECs TJs and AJs are intermingled and AJs also contribute to the properties of the BBB endothelium [4].Thus, junctions limit the paracellular flux of hydrophilic molecules but not of small lipophilic substances such as O_2 and CO_2, which can diffuse freely across plasma membranes along their concentration gradient [10].

ECs also adhere to the BM through transmembrane proteins that are classified into three families of cell adhesion molecules (CAMs): selectins, immunoglobulin superfamily,

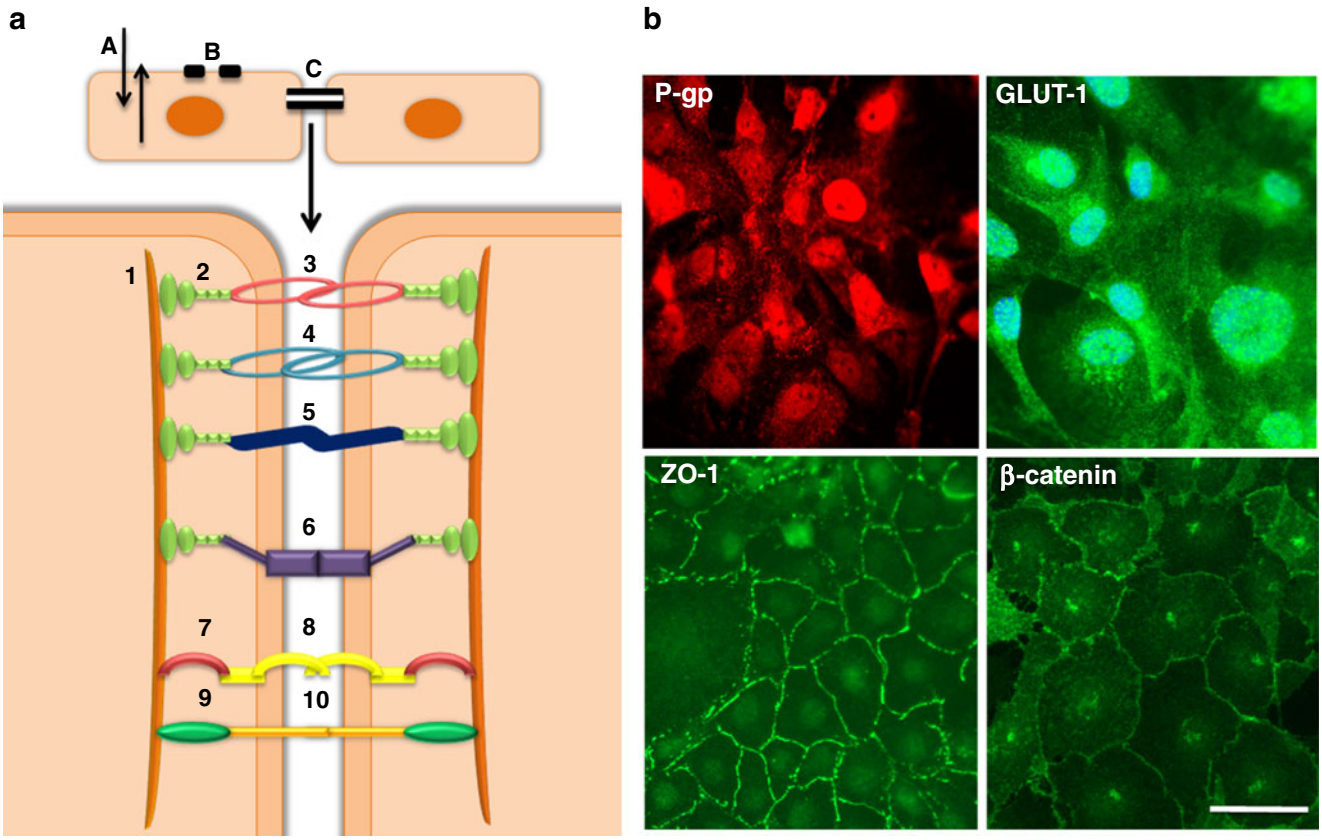

Fig. 3 The main characteristics of endothelial cells and their junctional complexes. **a** Schematic representation showing two adjacent endothelial cells with: transport systems (A), specific receptors (B), and tight and adherens junctions (C): 1, actin filament; 2, zonula occludens; 3, claudin; 4, occludin; 5, junctional adhesion molecule; 6, tricelullin; 7, catenins; 8, vascular endothelial cadherin; 9, afadin; 10, nectin. **b** Immunofluorescence images showing the expression of the transporters P-glycoprotein (P-gp) and glucose transporter-1 (GLUT-1), the tight junction protein zonula occludens-1 (ZO-1), and the adherens junction protein β-catenin. *Scale bar*, 40 μm

and integrins [4]. Therefore, these molecules contribute to BBB integrity and barrier properties [13].

Basement Membrane

The BM is an essential component of the BBB that surrounds ECs and pericytes and presents a duplicature that separates the pericyte from the EC and the astrocyte end-feet [34], as schematically represented in Fig. 1 and shown in Fig. 2b. Its formation and maintenance is assured by ECs, pericytes, and astrocytes [9]. The BM does not act as a significant barrier to the diffusion of small molecules, but its anchoring function plays an important role in the integrity of cerebral microvasculature and, thus, on BBB stability and properties [1], contributing to tissue/cell organization, stability, and differentiation [35, 36]. The BM appears to be also involved in pericyte function and differentiation, since pericytes encased in the BM or exposed to its proteins do not usually differentiate [37].

Like all BMs, it is formed by tightly interwoven protein sheets of 20–200 nm thickness [38], constituted by structural proteins (collagen and elastin), specialized proteins (fibronectin and laminin), and proteoglycans, organized in three apposed laminas [4]. The collagen type IV is a covalently stabilized network polymer and is one of the proteins most important for structural integrity of small vessels (Fig. 4). However, collagen type IV is dispensable for initiation of the BM assembly during early development [39]. The laminins, with a branched structure [35], present in CNS BM are the laminin α4 and α5 produced by ECs [40]. Together, the collagen type IV and the laminin form two overlapping polymeric networks [41]. Moreover, perlecan is the predominant heparan sulfate proteoglycan in the BM [13]. The attachment of the BM to the cells is done primarily by laminins to cell surface sulfated glycolipids and transmembrane receptors [35] (Fig. 4). Laminins are also critical for the organization and scaffolding of BM [35, 42], while collagen type IV enhances the stability of the membrane [35, 39]. All these matrix molecules form an extensive and complex extracellular matrix (ECM) [41] that is increasingly recognized to play an important role in signaling mechanisms [43].

Cell anchoring to the BM and the link between the ECM and the underlying cytoskeleton are mediated by proteins found on the matrix-proximate faces of ECs, as well as in

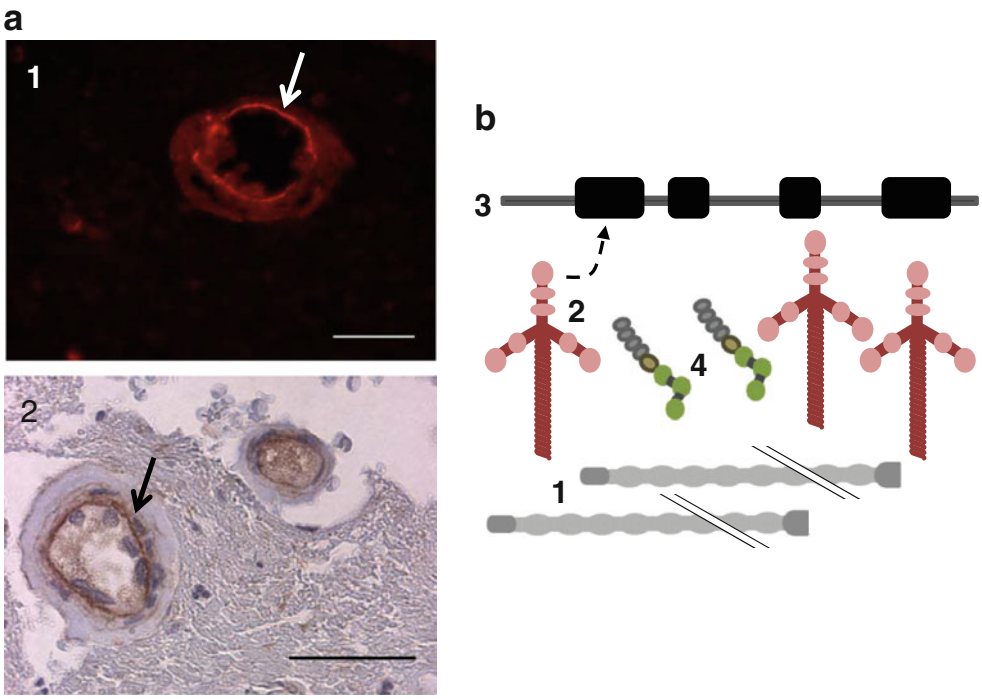

Fig. 4 Basement membrane (BM) and its main elements. **a** Immunofluorescence (1, red) and immunohistochemical (2, brownish coloration) analysis of collagen type IV in human hippocampus sections. Arrows point to collagen IV, the main component of the BM. Scale bars, 40 μm. b Schematic representation of the BM constitution: collagen type IV (1), laminins (2) that are principally responsible for the attachment of basement membrane to the cell receptors (3), and the perlecan (4) that is the predominant heparan sulfate proteoglycan in the endothelial cell BM (schematic representation adapted from Yurchenco and Patton [35])

other brain cells, known as CAMs and matrix adhesion receptors [35, 36, 43]. In addition to the important role in cell anchoring to the ECM, CAMs like integrins mediate endothelial signaling, cell migration, and brain capillary tube formation during angiogenesis [44]. However, the functional significance of these CAMs and receptors is only starting to be unraveled. The fact that significant alterations in both cellular adhesion receptors and their matrix ligands occur during focal cerebral ischemia supports their functional significance in the normal state, so that it was proposed that matrix adhesion receptors are essential for the maintenance of the integrity of the blood–brain permeability barrier and that modulation of these receptors contributes to alterations in the barrier during brain injury [36, 45].

Matrix metalloproteinases (MMPs) have the capacity to digest the BM, consequently reducing anchoring of brain ECs, and to affect TJ integrity that leads to alteration in BBB properties [43]. MMP-2 and MMP-9 are two gelatinases capable of proteolyzing some BM compounds including type IV collagen, elastin, fibronectin, and the proteoglycan aggrecan. MMP-2 can also cleave laminin. Serine proteases, cysteine proteases, and heparanase are other families of proteases that can affect the BM [36].

The BM can become thicker or thinner in response to stress stimuli and in certain pathological conditions [37]. Such alterations can be directly related with expression of proteases and migration of pericytes away from their vascular location [46]. Proteases can be secreted by EC and pericytes; and glial cells and pericytes may further induce ECs to produce MMPs, namely through cytokines like interleukin (IL)-6 [46]. The disruption of BM can promote alterations in the cytoskeleton of ECs that affect TJs and the barrier's integrity [4]. Kernicterus, the most severe pathological condition resulting from severe unconjugated hyperbilirubinemia [47], is one of the pathological conditions characterized by alterations of the BM and disruption of the properties of the BBB. In fact, studies performed in our lab showed that blood vessels in brain parenchyma present a thinner basement membrane and a decreased immunoreactivity to collagen type IV, which are accompanied by hyperpermeability and extravasation of albumin and erythrocytes from blood vessels (Brito et al., unpublished observations). Moreover, our unpublished in vitro studies showed that unconjugated bilirubin increases the activity of endothelial MMP-2 and MMP-9, the secretion of IL-6 by both ECs and pericytes, as well as the permeability of brain microvascular endothelium. Therefore, disruption of the BM characteristics appears as one of the players in the impairment of the barrier properties of BBB endothelium, where proteases and the cytokine IL-6 are also involved.

Neurons

Neurons are characterized by a cell body, containing the nucleus and numerous organelles, an axon and numerous dendrites, as shown in Fig. 1. They present cytoskeleton proteins, as neurofilaments, which are organized as 10-nm-thick filaments in axons and dendrites [48]. It has been estimated that nearly every neuron in the human brain has its own capillary, as illustrated in Fig. 1, which attests the importance of the close neuronal–vascular relationship for normal brain functioning [9]. There are complex mechanisms of communication between neurons, astrocytes, and cerebral vessels within the

NVU in order to spatially and temporally adjust blood supply to the needs in energy and oxygen of neurons. The control and modulation of regional and local flow in the absence of ischemic injury is, thus, dependent upon neurovascular coupling [49]. The temporal and topographical coincidence of neuron injury and microvessel response during focal ischemia has suggested that neuron–microvessel interactions could be bidirectional [50].

There are many neurons that directly innervate ECs and astrocytic processes, like noradrenergic [51], serotonergic [52], cholinergic [53], and GABA-ergic neurons [54]. Furthermore, there are evidences that neurons can regulate the blood vessel's function through induction of the expression of enzymes unique of ECs in response to metabolic requirements [55]. Thus, neurons may have an important role on the BBB phenotype but little is known about this. On the other hand, ECs and a BBB well-developed are important to create a stable environment to neural function [2]. Evidences about the role of neurons in BBB properties begin to arise by studies such as that of Minami [56], which showed that the presence of neurons increases the transendothelial electrical resistance (TEER) and decreases the permeability of ECs in an in vitro model of the BBB. However, the underlying mechanisms are still to be established. Clarification of such mechanisms might provide novel tools for intervention, which assumes a particular relevance since neurovascular dysfunction has been increasingly associated with neurodegeneration [9, 57, 58].

Astrocytes

Astrocytes are glial cells whose endfeet ensheath the BM on the outer surface of the BBB endothelium, as shown in Fig. 1. They are characterized by their numerous processes containing the cytoskeleton intermediate filament, glial fibrillary acidic protein (GFAP), visible in Fig. 1b. In fact, the presence of 40 large GFAP-positive processes that radially and symmetrically extend in all directions from the soma and the coverage of five different blood vessels by a cortical human protoplasmic astrocyte were described [59]. These cells cover more than 99% of the endothelium [1], contributing to the BBB properties and development and to the unique endothelial phenotype. These roles are mediated by the expression and release of soluble factors [1, 4] and are possible due to their anatomic proximity to ECs [60]. The astrocytes interacting with ECs enhance TJs and reduce gap junctional area [61], thus demonstrating that these cells have an important role in the restricted permeability and in the integrity of the BBB. Accordingly, studies by Siddharthan et al. [62] and by Malina et al. [63] showed that the presence of astrocytes elevated the TEER and decreased the permeability of the BBB endothelium. The interactions between astrocytes and ECs are also essential in regulating brain water and electrolyte metabolism under physiologic and pathologic situations [9].

Besides the importance of the interactions between astrocytes and BMVECs for BBB properties, astrocytes are also essential for proper neuronal function and, thus, for a functional NVU. To this fact accounts the many fine processes of astrocytes, extending beyond the large ones, which result in an individual astrocytic domain that is thought to contain 300–600 neuronal dendrites [64] and 10^5 synapses in the rodent cortex and hippocampus [65]. In the human cortex, a single astrocyte might sense the activity and regulate the function of more than one million synapses within its domain [65]. Thus, astrocytes assume a central position for dynamic signaling within the NVU, and emerging evidence implicates these glial cells as one of the key players in coordinating the neurovascular coupling [65]. In fact, astrocytes can signal vascular smooth muscle cells of blood vessels in order to regulate the cerebral blood flow in response to moment-to-moment changes in local neuronal activity. Astrocytes also contribute to a variety of functions of neurons, including synapse formation and plasticity, energetic and redox metabolism, and synaptic homeostasis of neurotransmitters and ions [66]. Moreover, accumulating evidence suggests that alterations of neuron–glia interactions are associated with the development of neurodegenerative diseases [66]. So, nowadays, astrocytes are no longer seen as merely supporting glial cells but rather as pivotal intervenients in the homeostasis of the NVU.

Microglia

Microglia are a distinct class of glial cells that constitute the brain's immunocompetent cells and therefore are critically involved in various injuries and diseases [66]. Over time, their nature has been discussed, but now it is accepted that microglia are ontogenetically related to cells of the mononuclear phagocyte lineage, in contrary to the other cells of CNS [67]. Monocytes enter into the brain during embryonic development and differentiate into brain resident microglia displaying surface antigens of macrophages [68].

Microglia are characterized by two principal forms according to the brain conditions. In physiologic situation, the resting microglia have small bodies and long, thin processes, known as ramified morphology, which correspond to the vigilant form that is able to promptly recognize homeostatic disturbance in the CNS [67]. In case of pathology, microglia become activated and may assume a phagocytic morphology, characterized by an amoeboid morphology with short processes. The evolution from one form to another is associated with changes in cytokine release and surface antigens, corresponding to distinct phenotypes [9, 68]. The microglial reactivity to an insult is complex since these cells may exhibit distinct phenotypes along time or even simultaneously. This was observed in our hands by exposure to unconjugated bilirubin, where an early inflammatory response was followed by a phagocytic phenotype [69]. The phagocytic phenotype is usually regarded as a mechanism to

eliminate neurotoxic substances from brain parenchyma, such as blood-borne albumin that crosses microvessels when there is an increased permeability of the BBB [70].

Since microglia are located in perivascular space, it is conceivable that their interactions with ECs may influence BBB's properties. However, this is a still unclear issue that has led to contradictory findings in the literature. In fact, Willis [71] proposed that activation of microglia, similarly to astrocytes, directs TJ proteins to paracellular domains and restores BBB integrity after chemically stimulated loss of BBB integrity. On the other hand, it was shown that tumor necrosis factor-α released from activated microglia leads to BBB dysfunction, thus revealing the barrier impairment in neuroinflammation [72]. Therefore, further studies are needed to clarify the role that microglia may have in BBB properties, which is presently under investigation in our lab.

Oligodendrocytes

Oligodendrocytes are the glial cells responsible for the formation of myelin sheets in the CNS, which are important for the conduction of the neuronal stimuli. They are characterized by a small cellular body, shown in Fig. 1, and by several processes that enwrap diverse axons from different neurons. Oligodendrocytes synthesize large amounts of myelin membrane components, which include lipids and myelin specific proteins, and, latter on, wrap it around axons in the CNS. The result is a multilayered stack of membranes that are tightly attached at their cytosolic and external surfaces, known as the myelin sheet. One main function of myelin is to insulate the axon and to enable a rapid conduction of action potentials that provides the basis for fast processing of information [73].

According to the developmental program of the neurons to be myelinated, oligodendrocyte precursor cells (OPCs) enter terminal differentiation and transform into myelin-forming oligodendrocytes. However, in some pathological conditions, such as multiple sclerosis, there may be an extensive destruction and loss of oligodendrocytes, as well as an inability of OPCs to differentiate into mature myelinating oligodendrocytes [74]. In these conditions, there is a failure in myelin ensheatment of axons, which become unwrapped and more vulnerable to environmental stressors. Moreover, the saltatory conduction is disabled, which leads to loss of neuronal function [74]. Interestingly, this neurodegenerative disease is characterized by BBB impairment, as observed in Alzheimer's disease, among other pathologies [9, 57, 58]. However, as far as the NVU is concerned, oligodendrocytes have been systematically dismissed, despite the evidences of the functional interaction of oligodendrocytes with neurons and the well-established neurovascular coupling mentioned above. Thus, we propose that these myelinating cells should be included in the cell types of the NVU, as indicated in the schematic representation of the NVU shown in Fig. 1.

Pericytes

It was in 1873 that Charles Rouget [75] described for the first time a population of branched cells on the capillary wall of the hyalloid of the frog and regarded them as contractile elements distinct from migratory leucocytes. Vimtrup [76], studying capillaries in tails of different young living larvae, noted that the contraction of capillaries begins at these cells, spreading in both directions, and coined these cells as Rouget cells. According to Krueger and Bechmann [77], it was Zimmerman that introduced the term pericytes, fifty years after Rouget's description. The name pericytes arises from "peri-" around and "cyto-" cell and reflects their location at the abluminal side of the microvessels [34]. In fact, Zimmerman demonstrated that pericytes are present around capillaries in a wide range of species including fish, amphibians, reptiles, birds, and mammals; they are continuous with smooth muscle cells of arteries and veins; present highly branched with distinctive cytoplasmic processes within each capillary bed; and the contraction of these cells controls capillary permeability. Moreover, Zimmerman distinguished three subgroups of pericytes, depending on the type of vessel pericytes are located at, but already considered the existence of a continuum with countless forms of differentiation [78]. Pericytes have also been called the mural cells of the capillaries, as they are non-endothelial cells enclosed within the BM of microvessels [79]. These cells are presently known as perivascular cells with multifunctional activities, particularly in the maintenance of BBB properties and in CNS homeostasis [77, 80] as will be discussed below.

Localization and Distribution of Pericytes

Pericytes are located on the abluminal surface of ECs and luminal to astrocyte end-feet, as schematically represented in Fig. 1. Pericytes are closely related with ECs, arising over endothelial TJ regions, with one layer of BM between them (Fig. 5), as stated above. Another layer of BM surrounds pericytes [37], lying between them and the astrocyte endfeet [77].

Pericytes are distributed intermittently along the wall of pre-capillary arterioles, capillaries, and post-capillary venules [46, 77], varying their abundance according to microvessel types [81, 82]. In fact, pericytes raise at post-capillary venules and tend to disappear and to be replaced by smooth muscle cells as the vein caliber increases. The degree of vascular coverage by pericytes varies with the tissue type and appears to correlate with the degree of tightness of the interendothelial junctions [83]. In fact, pericyte-to-endothelia ratio in the brain is higher than in other organs (1:3 compared with 1:100 in striated muscles) [82], and the pericyte coverage in retina is even higher that in brain capillaries [84, 85], in accordance with the suggested role of pericytes in the maintenance of the BBB and the blood–retinal barrier. There are also variations

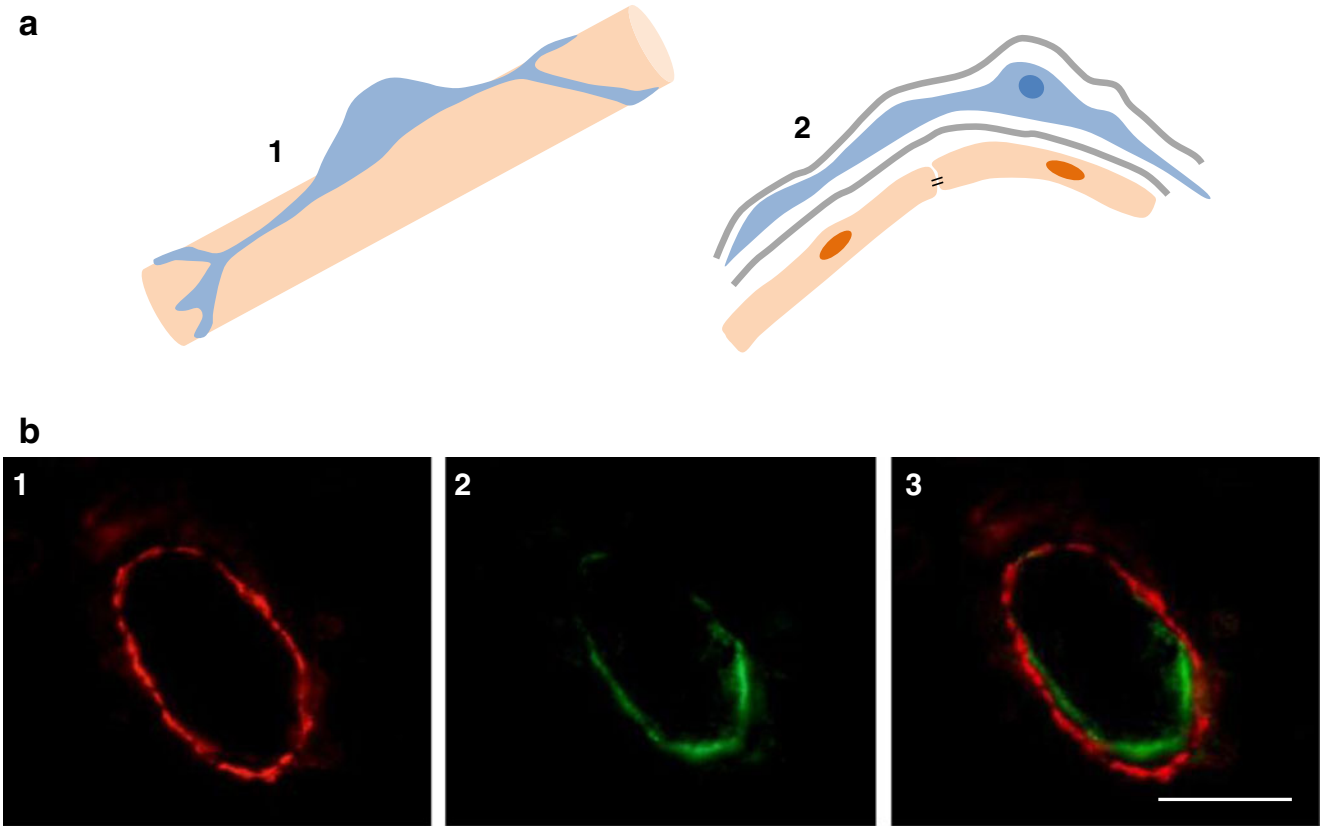

Fig. 5 Representation of pericyte ensheathment of endothelial cells. **a** Schematic representation of a pericyte (blue) ensheathing a blood vessel (orange) in a longitudinal view showing the long processes of a pericyte along a blood vessel (1) and a transversal view with a pericyte over endothelial cells and the basement membrane (gray) between them (2). **b** Double labeling immunofluorescence analysis of endothelial cells and pericytes in a paraffin section of human hippocampus. Pericytes were labeled for α-smooth muscle actin (1), endothelial cells for von Willebrand factor (2), and a merged image is shown (3). Scale bar, 20 μm

among species, as the average ratio of pericytes to ECs in the rat capillary is 1:5, whereas it is 1:4 in the mouse, and 1:3-4 in humans [37, 79]. Although it is recognized that CNS pericytes are extraordinarily numerous surrounding brain capillaries [86], the precise extension of vascular surface that is covered by pericytes is still unclear. In fact, Frank et al. [84] refer that pericytes cover 22–30% of cerebral capillary surface, whereas Dalkara et al. [82] refer 30–70% and Engelhardt and Sorokin [13] mention that they cover 99% of the abluminal surface of the capillary basement membrane in the brain. Due to the important role of pericytes in the BBB properties and, consequently, in brain homeostasis, this is surely an aspect that deserves further investigation.

Morphology of Pericytes

As shown in Fig. 6, brain pericytes are polymorphic cells, normally star-shaped [77, 79, 87, 88]. They have a spherical or oval cell body [34, 88] and a prominent round nucleus that differs from the elongated cigar-shaped nucleus of the EC [37] seen in Fig. 1b (panel 2) and schematically represented in Fig. 5a. Pericytes have long cytoplasmic processes oriented along the axis of the blood vessel, which give off secondary and terciary processes that engirdle the vascular wall as smaller circumferential arms [77, 82, 88], as illustrated in Fig. 5a. There may be up to 90 processes with a width of 300 to 800 nm per 100 μm of capillary length [9]. These figures attest the extension of the vasculature that is ensheathed by pericytes, which can be depicted by the pericyte vascular coverage shown in Fig. 5b. However, processes morphology tends to be heterogeneous and likely represents functional differentiation of pericytes [37, 46]. In fact, the pericyte processes may be large and broad, spanning a continuous and large surface of the vessel. Alternatively, they may form finger-like projections that are more confined and ensheath a more finite portion of the vessel surface. There may also be a retraction of projections, with protrusions of the cell away from the capillaries, which represents a migrating pericyte. This migrating pattern is associated with upregulation of cell surface proteases in pathological conditions and contrasts with the wrapping pattern that predominates in normal capillaries. It is also associated with the early stages of angiogenesis. A fourth pattern corresponds to the pericyte that is positioned

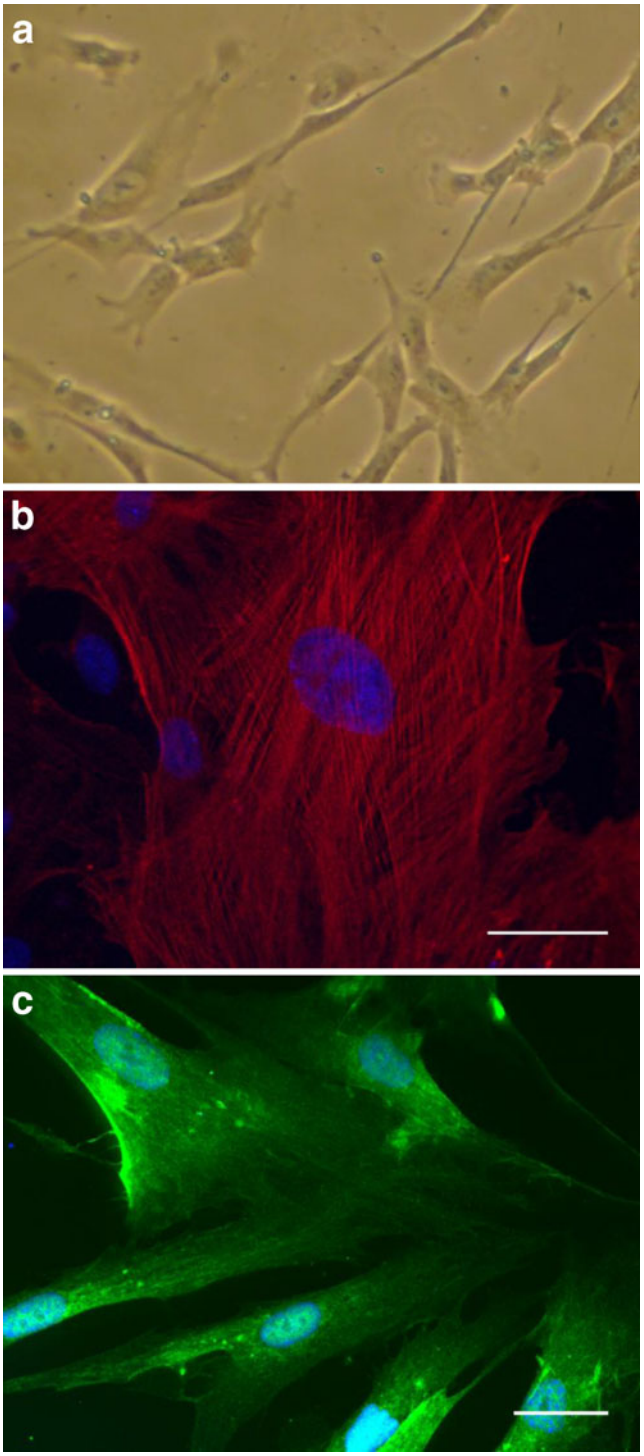

Fig. 6 Morphological features of human brain vascular pericytes in primary culture. **a** Phase contrast microscopy; original magnification, ×100. **b**, **c** Immunofluorescence analysis using the pericyte markers α-smooth muscle actin (**b**) and NG2 (**c**); nuclei were stained with Hoechst 33258 dye. *Scale bars*, 40 μm

longitudinally along the microvessel, which may reflect its migration along the microvascular wall or may reflect transition pericytes.

In contrast with ECs, pericytes contain large vacuoles and lysosomes, and its nuclear and cytoplasmic matrices are more electron-dense than those of the ECs [34, 89]. The extent of cytoplasmic lysosomes confers granularity to pericyte appearance, and human brain pericytes have a high content of acid phosphatase [87, 89].

Pericyte Identification and Characterization

The study of pericytes is difficult since they are not easy to extract from their location. It is further hampered by the lack of pericyte-specific markers, which is explained by the fact that pericytes are multipotent cells, as will be discussed below. Therefore, identification of pericytes requires a series of stains with a combination of positive and negative immunoreactivity.

Pericytes present contractile and cytoskeleton proteins and express surface antigens that contribute to their identification. Among them, there is α-smooth muscle actin (α-SMA) (Fig. 6b), the predominant contractile protein in CNS pericytes [90] that is localized at microfilament bundles [82]. However, pericytes of capillaries differ from those of larger vessels in their expression of contractile elements, and the expression of α-SMA varies according to the local, being more robust in pre-capillaries compared to mid- and post-capillaries [77, 79, 87, 91]. Moreover, not all pericytes express α-SMA, whereas vascular muscle cells also express this marker [90, 91], which illustrates the difficulty in the interpretation of data. In vitro, when freshly isolated, less than 30% are α-SMA-positive but 100% of pericytes become positive with time in culture, as they differentiate [37, 92], the reason why the detection of this contractile protein has been widely used to identify pericytes in vitro [93–95]. Regarding ECs, they are negative for α-SMA. Thus, double labeling for this contractile protein and an endothelial marker is frequently used to assess pericyte vascular coverage [80], as shown in Fig. 5b. Nevertheless, DeRuiter et al. [96] showed that ECs may transdifferentiate into cells expressing α-SMA, which render interpretation of data rather difficult. Pericytes also express the cytoskeleton proteins vimentin and nestin, whereas the presence of desmin has been reported by some authors [46, 79], but not by others [90, 97]. Among the surface markers of pericytes is the platelet-derived growth factor-β (PDGF-β) receptor, referred to mark 100% of cultured pericytes [79]. There are also the chondroitin sulfate proteoglycan NG2, shown in Fig. 6c and widely reported by others [79], which is also a neural progenitor cell marker [98]. Other markers are the VCAM-1 and ICAM-1, the pericytic aminopeptidase N, γ-glutamyl transpeptidase, and alkaline phosphatase, as well as the xLacZ4 transgenic reporter and the regulator of G-protein signaling-5 (RGS-5) [13, 77, 82, 87, 99]. However, most of these molecules are expressed in neighboring cells as well,

which render difficult the distinction from adjacent cells, such as oligodendrocytes and juxtavascular microglia [13, 77, 87]. On the other hand, CD146 is developmental and/or not expressed in all freshly isolated pericytes, whereas RGS-5 protein in brain is expressed during embryonic development, decreasing after birth and not expressed in normal adult CNS pericytes [79]. Covas et al. [100] also showed a sharing of diverse markers between mesenchymal cells and retinal pericytes including CD146 and NG2. Crisan et al. [101] confirmed the presence of NG2 and CD146 in brain pericytes, as well as in pericytes from skeletal muscle, myocardium, pancreas, bone marrow, abdominal fat, and placenta. The same authors showed that cultured pericytes express mesenchymal stem cells markers, including CD44, CD73, CD90, and CD105. Recently, Vandenhaute et al. [102] indicated that pericytes could be distinguished from smooth muscle cells by their different P-gp expression and γ-glutamyltranspeptidase activity, which opens new opportunities to distinguish pericytes from surrounding muscular brain cells. Taken collectively, it comes out that an unambiguous identification of pericytes and distinction from other cellular types is still not possible. This renders necessary further efforts in order to better characterize pericytes, an essential step to better understand their biology.

Origin of Pericytes

Despite extensive investigation, the origin of pericytes has still not been established and a specific pericyte precursor has not yet been clearly identified in developing organisms. Generally, it is considered that pericytes have a mesodermal origin or that, at least, one population of pericytes has such origin [79]. Accordingly, the reaction product of nucleoside diphosphatase, an enzyme that is bound to the plasmalemma in cells of mesodermal origin, is concentrated on the outer surface of the plasmalemma. There are also studies indicating that pericytes are derived from the neural crest, supporting a neuroectodermal origin for brain pericytes. These apparently contradictory theories about the origin of pericytes may be explained by a presumptive transition of ectoderm to mesoderm and may be related to reprogramming of embryonic stem cells [79].

By stimuli such as transforming growth factor-β (TGF-β), pericyte precursor cells would be recruited to sites of development by chemotactic attraction to PDGF-β-secreting cells [79]. It is possible that they migrate into the tissue during the later stage of vascularization then assuming their characteristics. Their precursor cells settle on newly formed capillary sprouts and differentiate into pericytes as they become enclosed within the basal lamina [103]. It was further proposed that there is a blood-borne precursor positive for both pericyte and macrophage, which infiltrates the neuropil under pathologic conditions. In fact, there are data suggesting that at least some pericytes derive from the monocyte lineage [77]. Hess et al. [104] found hematopoietic stem cell-derived cells engrafted in the brain after cerebral ischemia. These cells were not only microglia but also perivascular cells, located on the abluminal side of ECs, which point out that pericytes may also have origin in hematopoietic stem cells. Therefore, further studies are necessary to clearly establish the origin of brain pericytes.

Functions

In recent years, a variety of studies, mainly in cell cultures, set various functions of pericytes. These include contractile, immune, and phagocytic, as well as migratory and angiogenic functions. In addition, pericytes contribute to the BBB, perform a regulatory role in brain hemostasis, and are a source of adult pluripotent stem cells [79, 87, 105], as schematically represented in Fig. 7.

Contribution to BBB Properties

The short distance, about 20 nm, between ECs and pericytes [9], the high density of pericytes in the CNS, and the intimate association through gap junctions [106], peg-and-socket [107], and adhesion plaque junctions between these cells and ECs make pericytes as key players in the maintenance and stabilization of the BBB and in the development of BBB TJs, therefore contributing to the low paracellular permeability [108, 109]. Gap junctions allow communication

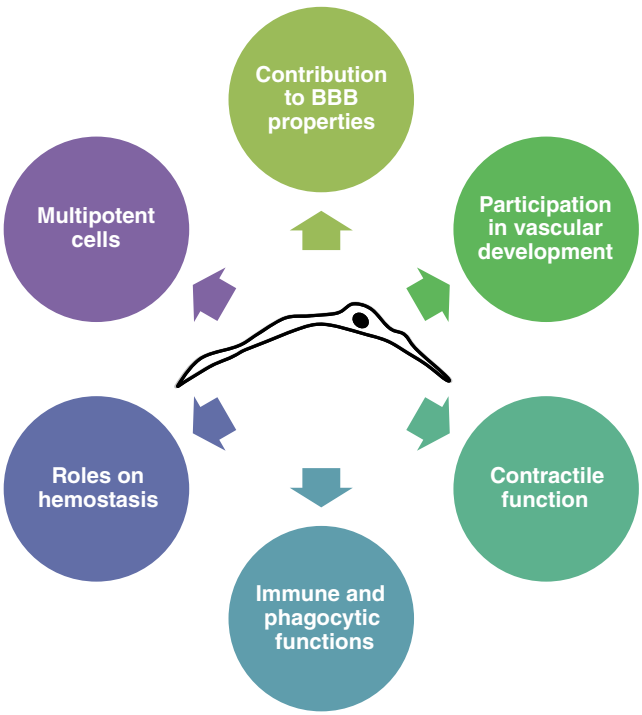

Fig. 7 Schematic representation of the main functions of pericytes

through exchange of ions and small molecules, and adhesion plaques support transmission of contractile forces from pericytes to other cells [106, 110]. Peg-and-socket contacts prevent pericytes penetrating through the basal membrane and contacting with other cells and vessels [107]. It was thought that these functions related to the BBB stabilization were in charge of astrocytes, but today we find data describing the development of endothelial TJs independently of astrocytes [77]. Al Ahmad et al. [111] studied the role of astrocytes and pericytes in TJs and AJs formation and observed the essencial role of each cell in the establishment of BBB-specific TJ complexes in ECs. Thus, we must consider a dialogue between several populations of the NVU and the possible existence of compensatory mechanisms that may take over each other's role in case of impaired function [87]. The ECs are not the only ones that express TJ molecules. Pericytes express several TJ molecules including claudin-12, JAM, ZO-1, ZO-2, and occludin. These cells also express several barrier-related transporters like ABCG2, P-gp, MRP 1, and GLUT-1 [112]. Once again, pericytes are essential for BBB properties as permeability and, thus, to brain homeostasis.

Dohgu et al. [108] showed that pericytes participate in tightening the intercelular junctions and facilitating P-pg function in brain ECs by cell-to-cell contact and through production of soluble factors including TGF-β. Besides this factor, others are also derived from pericytes, such as the vascular endothelial growth factor (VEGF) that increases the permeability of brain ECs, the basic fibroblast growth factor that tightens the intercellular junctions and induces the expression of MRP and angiopoietin (Ang)-1, an anti-permeability factor. These factors are also produced by astrocytes, which suggest that the four factors are involved in the interaction between ECs, pericytes, and astrocytes under physiological and pathophysiological conditions. Using a triple co-culture BBB model, formed by brain pericytes, brain ECs, and astrocytes, Nakagawa et al. [109] showed that the contact between the three different cells increased the TEER and consequently tightened TJs. Recently, Armulik et al. [113] demonstrated pericytes' role at the BBB in vivo, correlating reduced pericyte densities with increased vessel diameter and reduced vessel density, and established a correlation between pericyte density and BBB permeability for a range of tracers of different molecular masses. Bell et al. [114] also showed a correlation between pericyte loss and BBB breakdown and, consequently, brain accumulation of serum proteins and several vasculotoxic and/or neurotoxic macromolecules. The BBB integrity can also depend on the differentiation stage of pericytes. Thanabalasundaram et al. [115] showed that pericytes treated with TGF-β express more α-SMA and secrete more permeability factors like VEGF, MMP-2, and MMP-9 than the same cells incubated with basic fibroblast growth factor. Thus, the last could stabilize the BBB integrity by increasing TEER. The decrease of pericyte contractility may also interfere in BBB through TJ opening [116].

All these evidences turn possible that pericytes regulate brain's endothelial barrier by collaborating with astrocytes [108]. Recently, Daneman et al. [117] proposed a model for BBB formation, where ECs are induced to express BBB-specific genes by interactions with neural progenitors, and then the functional integrity of the BBB is regulated by pericytes during development and by astrocytes in adulthood. Pericytes also contribute to BM formation by synthesizing type IV collagen, glycosaminoglycans, and laminin [13, 87] and by inducing ECs to secrete BM components [118].

Participation in Vascular Development and Maintenance

During vertebrate embryo development, the first functional organ is the vascular system, whose growth must be continuous. The vasculature forms by vasculogenesis (new vessel formation from angioblasts or stem cells) and angiogenesis (sprouting, bridging, and intussusceptive growth from existing vessels). Mesenchymal cells differentiate into endothelial tubes that form a primitive blood vessel network from which new blood vessels develop. After formation of the first endothelial tubes, they become associated with mural cells that include pericytes and vascular smooth muscle cells [119].

Pericytes appear to have an important role in angiogenesis, participating in the three phases of the angiogenic process: initiation, sprout formation and migration, and maturation of new vessels and termination of angiogenesis [79]. This role has been demonstrated in models of brain injury and brain hypoxia that are strong stimuli for angiogenesis [120]. In ultrastructural studies, it was shown that pericytes are the first cells that respond to brain hypoxia and suffer morphological alterations in cats. These alterations were interpreted as initial steps of migration as the abluminal surface of the cells formed characteristic "peaks" pointing and extending towards the parenchyma. At the same time, the luminal BM between ECs and pericytes begins to thicken and the abluminal one thinned out [121]. Then, the elongation of pericytes and the disappearance of the basal lamina at the leading edge of migrating cells occur. Migratory pericytes express and show in the leading tips urokinase plasminogen activator and its receptor that are characteristic and mediate their activation and migration [89].

VEGF is very important in this process as it initiates vessel formation and activates a chain of molecular and cellular events that lead to mature vasculature [122]. VEGF, which production by pericytes is increased under hypoxia [123] and hypoglycemia [119] conditions, is crucial to communication with ECs. VEGF induces angiogenic sprouts, positive for the CD31 marker of mature and embryonic ECs,

to display α-SMA and desmin. This observation indicates that ECs transform into pericytes or smooth muscle cells and that VEGF plays an important role in this transformation. Furthermore, the number of pericytes covering new capillaries can be increased by VEGF [124]. Proof of the role of VEGF in angiogenesis is the fact that blockage of its receptor VEGFR2 can temporally normalize a tumor vessel structure [125]. Like VEGF, IL-6 is also an important cytokine produced by pericytes that is essential for EC recruitment and angiogenesis promotion [126]. Accordingly, we have recently observed that upon stimulation by unconjugated bilirubin, pericytes secrete VEGF and IL-6 (unpublished observations). We have also detected vessels with a poorly defined lumen, characteristic of immature blood vessels, together with an increased density of vessels, suggesting angiogenesis, in the brain parenchyma of a dead infant with kernictus [127]. Therefore, these observations raise the hypothesis that pericytes may also be involved in brain damage by bilirubin, which deserves further exploration in the future.

After formation, the nascent vessels are stabilized by recruiting mural cells, including pericytes. This stabilization process is regulated by at least four molecular pathways. The recruitment/differentiation of mural cells, namely pericytes, to sites of angiogenesis or neovascularization is mediated by the PDGF-β produced by ECs [79, 119, 128], presumably in response to VEGF [122] and by the corresponding PDGFR-β receptor expressed by pericytes [79, 119, 128]. The essential role of this factor was demonstrated by Hellström et al. [119] through PDGFR-β knockout mice that lacked pericytes along the vessels and by Abramsson et al. [128] who showed that the absence of an amino acid motif of PDGF-β produces defective investment of pericytes in the microvascular system. Angiopoietins are also important for vascular development and stabilization. Ang-1 produced by pericytes and perivascular cells binds to the endothelial receptor Tie-2, fostering vascular stabilization; Ang-2, expressed by ECs, binds to the same receptor and acts like a destabilizing factor [77, 122, 129] in the absence of VEGF, and is restricted to ECs in areas of vascular remodeling [122]. Thus, the development of vasculature remains unstable and immature until pericytes or its precursors are recruited [77, 129]. Like mentioned above, in the presence of VEGF, Ang-2 facilitates vascular sprouting [122]. Therefore, the presence of pericytes regulates negatively EC proliferation, determining their number, morphology, and microvessel architecture [119].

Vascular stability is sustained before pericytes arrive to their position, by binding of sphingosine-1-phosphate to its endothelial receptor [77]. After binding, the small GTPase Rac is activated in ECs [130] and N-cadherin, found in peg-socket contacts between ECs and pericytes, and vascular endothelial-cadherins that are normally found in junction complexes between neighboring ECs, are expressed [77]. TGF-β is a multifunctional cytokine that also promotes vessel maturation through stimulation of ECM production and differentiation of mesenchymal cells to mural cells [119, 122]. The Notch signaling, a highly conserved pathway in the vascular compartment, is involved in the remodeling of the primary vascular plexus into the hierarchy of mature vascular beds [78].

Termination of angiogenesis may be due to pericyte synthesis of angiostatic substances and components of the ECM, such as proteoglycans, collagen, and elastin [79]. This ECM is initially deposited at the parenchymal side of pericytes, but the pericyte progressively becomes surrounded by the basement membrane. It is then that the BBB is observed, leading to the hypothesis that this barrier is regulated, at least in part, by pericytes. The intact basal lamina may provide anchoring and structural integrity to the capillary but it may also be involved in signaling mechanisms that regulate pericyte function and differentiation. Changes in the basal lamina can be directly associated with pericyte expression of proteases and migration of pericytes from their vascular location, and can represent a vascular adaptation to changes in the environment [79]. On the other hand, deficient expression of ECM molecules may promote physiological angiogenesis [79]. Due to their important role in angiogenesis, pericytes may be suggested as a target to pharmacological therapy for tumors [77].

Taken collectively, pericytes are involved in EC stimulation and guidance, as well as in endothelial stabilization and maturation [78]. Pericytes provide guidance for endothelial movement and tube formation through secretion of mediators such as VEGF. Spreading ECs, in turn, stimulate pericyte precursor cell proliferation and migration by releasing VEGF and nitric oxide. Vessel stabilization occurs by pericyte investment and close interaction with ECs. Mature ECs secrete PDGF-β, which promotes proliferation and migration of pericyte precursor cells through activation of PDGF-β receptor expressed on the surface of pericyte progenitors. This mechanism leads to pericyte coverage of early endothelial tubes and vessel maturation further develops through Ang-1 and Notch-mediated signaling. Pericytes stabilize and reinforce the endothelial tube contributing to secretion of BM.

Despite the evidences pointing to pericytes' role in angiogenesis, this is a complex process that involves several cell types of the NVU. Pericyte–endothelial cross-talk, through peg-and-socket junctions and Gap junctions, is pivotal to physiological angiogenesis and to the adaptation to hypoxic injury [79]. In addition to the inter-digitations with ECs during the early phases of angiogenesis, pericytes also communicate with neurons during the maturation of newly formed vessels [79]. Astrocytes are also important for vascular development and both cells form endothelial

connections within the newly forming vessels, so that when pericytes and astrocytes are present in triple culture with ECs, tube formation occurs faster [46]. However, these different cells appear to have unique roles, as demonstrated in a three-dimensional in vitro model of the BBB [111]. In this model that more closely mimicks the interactions that occur in vivo, pericytes increased the number of tubes formed, thereby mediating a pro-angiogenic phenotype on ECs, whereas the presence of astrocytes seemed to alter vasculogenic and angiogenic-like processes in favor of vessel maturation. It is therefore conceivable that pericytes promote tube formation during early development to provide the developing CNS with optimal O_2 and nutrient supply, in accordance with the observations of Virgintino et al. [131] that in vivo pericytes are the first cells to interact with cerebral microvessels during early embryogenesis. Once neurogenesis declines and neuronal differentiation is initiated, newly differentiated astrocytes might inhibit the pericytic pro-angiogenic effect and induce BBB maturation [111]. Pericytes also induce proper localization of junctional proteins, lumen polarization, and functional activity of ABC transporters, similar to astrocytes, indicating that the presence of both cells is required to maintain optimal barrier characteristics [111].

In the adult brain, pericytes modulate capillary diameter by constricting the vascular wall [132], a process that may obstruct capillary blood flow during ischemia [133]. Thus, in addition to the pericytes' role in angiogenesis, pericytes also have important functions in the maintenance of brain vasculature and, so, on brain homeostasis as recently demonstrated by Zlokovic and co-workers [99]. In a series of elegant studies, these authors showed that a modest 20% loss in pericyte coverage can initiate vascular damage, whereas the doubled 40% loss in coverage results in a profound neurovascular insufficiency, changes in neuronal structure, and impairments in behavior, thus confirming a key role of pericytes in maintaining the integrity of the NVU.

Contractile Function

As already mentioned, Rouget [75] was the first to describe a pericyte and distinguished it from migratory leucocytes. This distinction was based in the elements found in pericytes, such as α-SMA, shown in Fig. 6b. However, not all pericytes express α-SMA, which supports the idea that the pericytes' constriction ability is not universal [90]. Pericytes also possess tropomyposin and myosin that contribute to their contractile capacity [134, 135], and some authos found the intermediate filament desmin also present in smooth muscle cells [46].

Pericytes can also express receptors for vasoactive substances such as prostacyclin [77, 87], angiotensin II, endothelin-1, catecholamines, and vasopressin. Ang-2 is a vasoactive peptide that most of the times causes vasoconstriction. Ferrari-Dileo et al. [136] showed that pericytes have specific Ang-2 binding sites. They also found binding sites for vasoactive intestinal polypeptide. van Zwieten et al. [137] discovered the presence of binding sites for vasopressin. The receptors for endothelin-1, other vasoactive peptide, were also identified [138] and include ETrA and ETrB. Dore-Duffy and Cleary [37], using rat brain after traumatic brain injury showed the increase of endothelin-1 and of the two receptors in both primary pericytes and capillaries. This fact, together with the increase of pericytes positive for α-SMA, may constitute the pathway that leads to the vasoconstriction and consequent decrease of blood flow that are characteristic of traumatic brain injury.

Pericyte tone in culture can be modulated by catecholamines (which are present in neurons innervating capillaries), with serotonin, histamine, and noradrenaline all constricting cultured pericytes [139]. In contrast, other vasoactive molecules produced by neurons and ECs, such as nitric oxide, cause the relaxation of pericytes through cyclic guanosine monophosphate [140]. Dilation of arterioles may also result from adenosine, a breakdown product of ATP that is produced during conditions of high metabolic demand. This way, dilation of blood vessels increases the blood flow that can be directed to areas of increased activity [139].

Like already mentioned, pericytes are associated to microvessels and have some features of smooth muscle cells, so that the absence of these last cells in microvessels turns pericytes their possible contractile substitutes [90]. In fact, the unique position of pericytes around ECs, with the primary processes aligned parallel to the vessel wall and the secondary processes encircling it, in addition to their contractile nature, is considered to contribute to the role played by pericytes in the regulation of blood flow at the microcirculatory level [82].

Yemisci et al. [133] showed that pericytes contract during ischemia and even after reopening of an occluded middle cerebral artery. Pericytes also cause segmental narrowing of capillaries, turn erythrocytes trapped in the capillary constrictions, and obstruct microcirculation. These authors also proved that peroxynitrite leads to pericyte constriction, wheareas the suppression of oxygen and nitrogen radical formation can reverse this situation. Ischemia induces pericytes to express α-SMA as well, and this upregulation is correlated with smaller capillary diameter [139]. Therefore, it appears that α-SMA can be induced within the capillary and that this upregulation may be related to the role of pericytes in focal regulation of capillary blood flow in acute stress response [79].

With all these evidences, it is considered that pericytes may have a contractile function and, consequently, blood flow regulatory capabilities, especially in pre-capillary arterioles [34, 77]. Pericytes may, thus, play a role in the distribution of capillary blood flow accordingly with the very focal demand by a small group of nearby cells, as a final step of flow regulation after the pre-capillary arteriole. This potential mechanism of flow regulation with fine spatial resolution may be important for tissues with high functional specialization such as the brain and retina [82]. Proof of that is the study of Fernández-Klett et al. [141] using real-time imaging of pericytes in the cerebral cortex of mice that showed that pericytes are really capable of regulating capillary red blood cell flow, which may have importance in CNS disorders. These results add support for a contractile role in pericytes of the CNS microvasculature, similar to that of vascular smooth muscle cells [90]. However, we need to have in mind that not all pericytes may be contractile and this capacity may vary along the vein axis, with the species, tissue, and development stage [77]. In addition, we also must take into account the differences that may exist in the expression of some proteins in vitro [82].

Immune and Phagocytic Function

Brain pericytes constitutively express low levels of adhesion molecules (ICAM-1 and VCAM-1), which have potential stimulatory activity in major histocompatiblity complex (MHC)-class II dependent antigen presentation [142]. Thus, pericytes may have the capacity to present antigens to T lymphocytes. Balabanov and Dore-Duffy [34] showed the response of primary rat CNS pericytes to interferon-γ with upregulation of the MHC-class II molecule and antigen presentation to primed lymphocytes. Brain pericytes also produce immunoregulatory cytokines like IL-1β and IL-6 [143]. TGF-β produced by pericytes may also function as immunoregulator at the BBB [34, 105].

Pericyte lysosomes express acid phosphatase that implies a phagocytic function [87]. Pericytes have components that are recognized by macrophage-selective monoclonal antibodies EBMS/11 and ED2 [144, 145]. Pericytes can uptake soluble and small molecules from the blood or brain parenchyma through interstitial fluid and transport materials by infoldings of the plasma membrane, endocytosis through pinocytosis or phagocytosis, and receptor-mediated endocytosis [34]. An earlier study showed vesicular transport by cerebral pericytes after hydrocephalic edema and proposed these cells as precursors of juxtavascular phagocytes [146]. After cerebral edema, these cells evidence vacuoles, phagosomes, and micropinocytic vesicles. In addition, when there is erythrocyte extravasation as a result of BBB injury, pericytes may be capable of ingesting whole erythrocytes [147].

Roles on Hemostasis

After vascular injury, pericytes may function in blood coagulation events that lead to thrombin formation due to their subendothelial location in the microvasculature. Accordingly, data obtained by Bouchard et al. [148] indicate that pericytes can activate and propagate the coagulant response through the extrinsic pathway and that the activities of the required enzyme complexes can be differentially regulated in response to agonist stimulation, thus supporting the concept that pericytes may play an important role in regulating coagulation events after cerebrovascular lesion. These authors further defined functionally active tissue factors on the surface of human brain pericytes that are the primary generator of the coagulation cascade. In contrast, the studies performed by Kim et al. [149] suggested that pericytes negatively regulate brain EC fibrinolysis, thus supporting the procoagulant activity. However, these studies indicated that pericytes are the principal in vitro source of the serpin protease nexin-1 that is known to have primarily antithrombin effects, therefore suggesting that these cells may provide endogenous anticoagulant activity. Moreover, these authors showed that pericytes decrease endothelial tissue plasminogen activator, a serine protease that processes plasminogen into proteolytically active plasmin, thus allowing fibrinolysis to occur. This effect appears to be mediated by a soluble-derived factor since it was observed in a non-contact ECs–pericytes co-culture model. So, it appears that pericytes are cells with both pro- and anticoagulant activities [87].

Multipotent Cells

Multipotent stem cells with grossly identical characteristics and developmental potentials have been obtained from multiple organs, such as skeletal muscle, bone marrow, skin, pancreas, fat, dental pulp, placenta, and umbilical cord [150]. Considering the widespread distribution of blood vessels in nearly all organs, it was proposed that perivascular cells constitute a stock of multilineage progenitor cells [150]. The pericyte is considered a relatively undifferentiated connective tissue cell in the capillaries or other small vessels, a mesenchymal stem-like cell associated with the walls of small blood vessels that provides support and can differentiate into several cell types [46]. In fact, it was already demonstrated that pericytes differentiate into fibroblasts, smooth muscle cells, and macrophage/dendritic cells, as well as into ECs, adipocytes, and chondrocytes, and are also a source of osteogenic progenitor cells [37, 46, 79, 105, 151]. Skin pericytes have also been shown to act like mesenchymal stem cells with the capacity to differentiate into several lineages, like bone, fat, and cartilage [152]. On the other hand, stem cells in other organs, such as Ito cells in the liver, are pericytes [37].

Multipotent cells from the bone marrow share some pericyte characteristics, such as the expression of α-SMA in culture. This and several other observations led to the concept that pericytes are multipotent stem cells [37, 79, 105] rather than mesenchymal stem cells. The multipotentiality of pericytes includes the differentiation in neural cells. One of the first evidences of the differentiation of pericytes into neuronal cells was the study of Yamashima et al. [153] that showed the differentiation of pericytes into neurons and glial cells after ischemia injury in the monkey hippocampus. The neural potential of primary pericytes subcultured from isolated rat CNS capillaries was also demonstrated by Dore-Duffy et al [105], who showed the differentiation of multipotent pericytes along multiple lineages including astrocytes, neurons, and oligodendrocytes. These authors further showed that pericytes also generate neurospheres and that there are capillary-generated neurospheres as well [79]. Interestingly, the latter are formed more quickly and differentiate sooner than the former, suggesting that ECs provide trophic support [79, 105]. This observation is in line with the stem cell potential within the neurogenic niches located in the subependymal zone and in the subgranular zone, where the close proximity with ECs is thought to play a key role in neurogenic activity [154]. CNS capillary pericytes from transgenic mice harboring a temperature-sensitive mutant of the SV40 virus target T-gene, known as Immortopericyte, are also pluripotent cells that can be induced to differentiate along mesenchymal and neuronal lineage at 37°C [155]. More recently, it was shown that non-CNS pericytes, obtained from the aorta, can also be induced to neural differentiation and that differentiated cells exhibit functional properties of neurons [156].

The capacity of pericytes to differentiate in such a large number of cell types, together with the fact that their broad stem cell potential goes beyond organ-specific production of progenitor cells, has raised an enormous interest in their use in therapy. Among such applications is the treatment of Duchenne muscular dystrophy for which pericytes are promising candidates for cell-based therapy protocols in patients. In fact, transplanted pericytes purified from human skeletal muscle, fat, pancreas, and placenta regenerate human myofibers in dystrophic mouse muscles more efficiently than do myoblasts, total unfractionated tissue-derived cells, or ECs. In addition to structural regeneration, functional recovery was observed in dystrophic mice treated with pericytes isolated from muscle biopsy specimens from healthy adults, as well as from patients with Duchenne muscular dystrophy, though these last showed marginal effects [157]. The pericytes' inherent actions on the vasculature, already described, render these cells ideal for cardiac repair. Indeed, their clinical potential as an effective donor cell population for cardiac therapy was demonstrated as transplantation of pericytes into acutely infarcted hearts in mice showed cardioprotective effects, with induction of angiogenesis and reduction of scar formation [150]. Pericytes are also protective in neurologic disorders, as evidenced in experimental autoimmune encephalomyelitis, following injection of pericytes and traffic to the brain of a significant number [79]. Finally, it is envisaged that CNS pericyte may be used as a source of purified viable multipotent stem cells with the potential of directed neurogenesis that can become important in the future for therapeutics [105]. In addition to cell therapy, it is conceivable that the signaling pathways that regulate pericyte proliferation and differentiation can be modulated. In this regard, the recent identification of the PDGF-β receptor signaling as an important in vivo regulator of their progenitor potential [158] opens new opportunities for modulation of pericyte differentiation as a tool to combat diseases and particularly brain disorders. Therefore, further studies are needed to assure the proper translation of experimental findings to the clinical use of pericytes as a cellular therapy for brain disorders.

BBB Dysfunction and Pericyte Involvement

Actually, there are numerous diseases associated with BBB dysfunction, including hypoxia and ischemia [159], multiple sclerosis [160], edema [161], Parkinson's and Alzheimer's diseases [162], epilepsy [163], tumors [164], and glaucoma [165]. BBB dysfunction can range from a simple transient opening of TJs to chronic barrier breakdown. Changes in transport systems and enzymes can also occur [3]. One of the consequences of the BBB breakdown is the increase of permeability that leads to the penetration of plasma into extracellular space of the brain causing vasogenic brain edema [166]. Blood components such as red blood cells and leukocytes can enter into the brain due to ischemic injury, intracerebral hemorrhage, trauma, neurodegenerative diseases, inflammation, or vascular disorder, thus promoting the production of neurotoxic products that can influence and compromise synaptic and neuronal functions [9]. It was suggested that the length of brain capillaries is reduced in neurodegenerative diseases, like Alzheimer's disease, which diminishes the transport of energy substrates and nutrients across the BBB, as well as the elimination of neurotoxins from the brain [9, 57]. As far as Alzheimer's disease is concerned, the accumulation of β-amyloid, besides inducing neurodegeneration, elicits toxicity to pericytes and capillaries [167].

The increase of the BBB permeability can be due to chemical mediators that are released in pathologic conditions and include glutamate, aspartate, taurine, ATP, nitric oxide, IL-1β,

histamine, thrombin, platelet-activating factor, and free radicals among others. Some of the latter are released by ECs and the endothelium itself responds to the released agents [10]. Pericytes can produce a range of mediators in response to pathology. In fact, the use of lipopolysaccharide to mimic infection induced the production of nitric oxide, cytokines, and chemokines, reflecting an inflammatory response by these cells [168]. Results from our lab also showed an increased expression of endothelial nitric oxide synthase and enhanced production of nitrites, the end product of nitric oxide, together with the already referred production of the cytokines IL-6 and VEGF following exposure of pericytes to unconjugated bilirubin (unpublished data). In turn, these mediators can contribute to BBB disruption [4] as observed when bilirubin was used.

In malignant tumors, pericytes are recruited to confer stability to the vasculature, depending on the degree of recruitment on the functional state of the tumor vascular bed [169]. This is an important observation since it supports the possibility of a pericyte-targeted therapy against the progression of tumors, especially when they are still immature.

Pericyte dysfunction or their loss plays an important role in the pathogenesis of some diseases. Indeed, reduction of pericytes has been observed after stroke [170], diabetic retinopathy [171], and in a variety of angiopathies [172]. After brain ischemia, pericytes contribute to capillary constrictions and consequently to blood flow disruption [133]. In traumatic brain injury, the migration of approximately 40% pericytes from their microvascular location to the parenchyma was observed, with a consequent drop in the pericyte to endothelial ratio from 1:5 in normal animals to 1:10–12 in injured animals. According to the authors of this study, this loss of vascular coverage parallels angiogenesis and neovascularization [89], which is in line with the role played by pericytes in blood vessel formation previously discussed.

Concluding Remarks

A proper function of the NVU is essential for brain homeostasis and its dysfunction has increasingly been recognized to precede or be involved in neurodegeneration [9]. Pericytes are central cells in the NVU, interacting with the other components by pathways that are presently being unraveled. A better understanding of the cross-talk between pericytes and the other brain cells, as well as of the signaling mechanisms involved in such communication in health conditions, will allow the identification of targets to modulate in pathological states. This is an important issue considering the vault increase in neurodegenerative diseases associated with the aging of the population, the incidence of pathologies involving vascular disorders, such as diabetes, and the diversity and frequency of brain tumors among others. Thus, the modulation of appropriate players will provide new opportunities to prevent or treat neurovascular disorders. Pericytes appear as potential cellular candidates and the pathways where they intervene constitute potential targets for therapeutic intervention. Therefore, further studies are necessary for a better understanding of the biopathology of the NVU in general and of pericytes in particular.

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