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Review Article

Brain mesenchymal stem cells: physiology and pathological implications

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Mesenchymal stem cells (MSCs) are defined as progenitor cells that give rise to a number of unique, differentiated mesenchymal cell types. This concept has progressively evolved towards an all-encompassing concept including multipotent perivascular cells of almost any tissue. In central nervous system, pericytes are involved in blood–brain barrier, and angiogenesis and vascular tone regulation. They form the neurovascular unit (NVU) together with endothelial cells, astrocytes and neurons. This functional structure provides an optimal microenvironment for neural proliferation in the adult brain. Neurovascular niche include both diffusible signals and direct contact with endothelial and pericytes, which are a source of diffusible neurotrophic signals that affect neural precursors. Therefore, MSCs/pericyte properties such as differentiation capability, as well as immunoregulatory and paracrine effects make them a potential resource in regenerative medicine.

Key words: blood–brain barrier, cancer, mesenchymal cells, neurovascular unit, pericyte.

Brain mesenchymal stem cells

Mesenchymal stem cells (MSCs; Caplan 1991) were described using classical experiments of bone marrow transplantation to heterotopic anatomical sites obtaining ectopic bone and marrow cells (Friedenstein *et al.* 1970). MSCs are characterized by their rapid adherence to tissue culture vessels and by their fibroblastic appearance in culture. They are defined as progenitor cells that give rise to a number of unique, differentiated mesenchymal cell types (Caplan 1991). In bone marrow, MSCs are forming the hematopoietic stem cells niche and producing osteoblast and fibroblast (Mitsiadis *et al.* 2007; Wilson *et al.* 2007). Importantly, there is not an exclusive and universal marker for immunophenotyping MSCs. The Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy proposed a combination of different markers to identify MSCs for these cells (Dominici *et al.* 2006). To define human MSCs, cells must be positive for CD105, CD73, and CD90 and negative for CD45, CD34, CD14 or CD11b, CD79a or CD19, and
HLA-DR.

MSCs have been mainly studied in culture and have been described based on of their capability of self-renew and differentiation into mesodermal cell lineages (Baksh *et al.* 2004; Javazon *et al.* 2004). Nevertheless, MSCs have been poorly studied *in vivo* and their behavior under physiological conditions remains unknown. An important question is if these ubiquitous cells behave *in vivo* as stem cells or if their stem cell potential is a cell culture artifact (Bianco *et al.* 2013). Moreover, the concept of mesenchymal stem cell, initially well-defined and restricted to a multipotent progenitor for skeletal tissues and residing within the bone marrow has progressively evolved towards an all-encompassing concept including multipotent perivascular cells of almost any tissue (Bianco *et al.* 2013).

Many studies have been focused in the use of MSCs for cell therapy due to MSCs ability to home to injured sites. Transplanted MSCs exert beneficial effects by their differentiation capability (Zhao *et al.* 2002; Dai *et al.* 2005; Fazel *et al.* 2005) and by secreting trophic factors (Kinnaird *et al.* 2004; Gnecchi *et al.* 2005;

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Jones *et al.* 2010, 2013, 2015; Pastor *et al.* 2012, 2013; Jaramillo-Merchan *et al.* 2013; Cruz-Martinez *et al.* 2014Shim *et al.* 2016). MSCs, isolated from different adult tissues, differentiate into neural cells in culture (Zhao *et al.* 2002) and in grafting assays (Kopen *et al.* 1999). Experiments of transplants in brain lesion models showed therapeutic effects of MSCs (Li *et al.* 2000; Gutierrez-Fernandez *et al.* 2011; Jones *et al.* 2015). On the other hand, several studies suggest that functional improvements are due to a paracrine effect or cell–cell interactions rather than by the successful transdifferentiation of implanted MSCs into neural cells (Caplan, 2009; Wilkins *et al.* 2009; Jaramillo-Merchan *et al.* 2013; Jones *et al.* 2015).

MSCs immunoregulatory properties have been well studied as it has been reviewed in Da Silva Meirelles *et al.*, 2008, Le Blanc & Ringden 2007; Uccelli *et al.* 2007; Glenn & Whartenby 2014 and more recently in Mattar & Bieback 2015. These cells seem be able to suppress T lymphocyte activation and proliferation (Di Nicola *et al.* 2002), T helper cell activation (Aggarwal & Pittenger 2005) and antigen-primed cytotoxic T cells and natural killer cells proliferation (Potian *et al.* 2003; Spaggiari *et al.* 2006). Finally, MSCs also inhibit the differentiation and maturation of antigen-presenting cells (Jiang *et al.* 2005). MSCs also have antiinflammatory function by reducing the production of tumor necrosis factor (TNF)-α and interleukin (IL)-12 and by increasing the synthesis of IL-10 by macrophages (Kaplan *et al.* 2011). For all these properties, MSCs have been proposed as a potential resource in regenerative medicine.

Mesenchymal stem cells were first isolated from the bone marrow but they have been found in many organs and tissues, including adipose, tonsils, umbilical cord, skin, and dental pulp (Huang *et al.* 2009; Bueno *et al.* 2013; Lai *et al.* 2014; Li *et al.* 2014; Ryu *et al.* 2014). Experiments using brain derived MSCs demonstrated that brain MSCs can transdifferentiate into neuronal cells (Paul *et al.* 2012). Considering this, several authors suggested a relationship between MSCs and blood vessels to explain that MSCs can be isolated from all tissues (Brighton *et al.* 1992; Doherty *et al.* 1998; Bianco & Cossu 1999; Farrington-Rock *et al.* 2004; Da Silva Meirelles *et al.* 2006, 2008; Bianco *et al.* 2013). A perivascular location could explain the rapid recruitment of MSCs to injured sites where they could regulate tissue regeneration (Crisan *et al.* 2008a,b, 2009; Paquet-Fifield *et al.* 2009; Appaix *et al.* 2014). In addition to that, some authors have suggested that MSCs are perivascular cells such as pericytes (Fig. 1; Caplan & Dennis 2006; Crisan *et al.* 2008a,b). Therefore, brain pericytes could constitute another stem cell population in the central nervous system distinct to the neural stem cell pool.

Brain MSCs and pericytes

Pericytes are defined as peri-endothelial vascular mural cells (Zimmerman, 1923). They are forming a layer on the abluminal surface of endothelial cells and they act as supportive vasculature and contribute to blood–brain barrier (BBB). At present, pericytes functions also include angiogenesis, bioactive molecules synthesis, vascular tone regulation and may form a stem progenitor cells pool in the adult tissue (Fig. 2).

The existence of similarities between MSCs and pericytes have been well documented (Schwab & Gargett 2007; Covas *et al.* 2008; Zannettino *et al.* 2008). Two interesting studies published in 2008 (Covas *et al.* 2008; Crisan *et al.* 2008a,b) provided evidence that MSCs are perivascular cells such as pericytes. They demonstrated that a subset of perivascular cells from

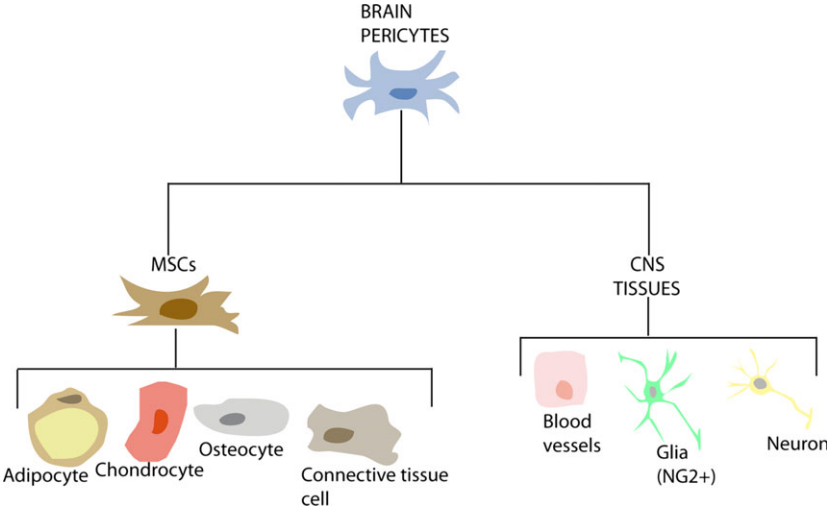

Fig. 1. Differentiation potentiality of brain pericytes *in vitro*. Pericytes are perivascular mural cells. These cells are defined by self-renewal and the ability to differentiate into the mesodermal cells (adipocytes, chondrocytes, osteocytes, and connective tissue cells) and of the central nervous tissues (neurons, glia, blood vessels). CNS, central nervous system; MSCs, mesenchymal stem cells. Adapted from Glenn & Whartenby 2014.

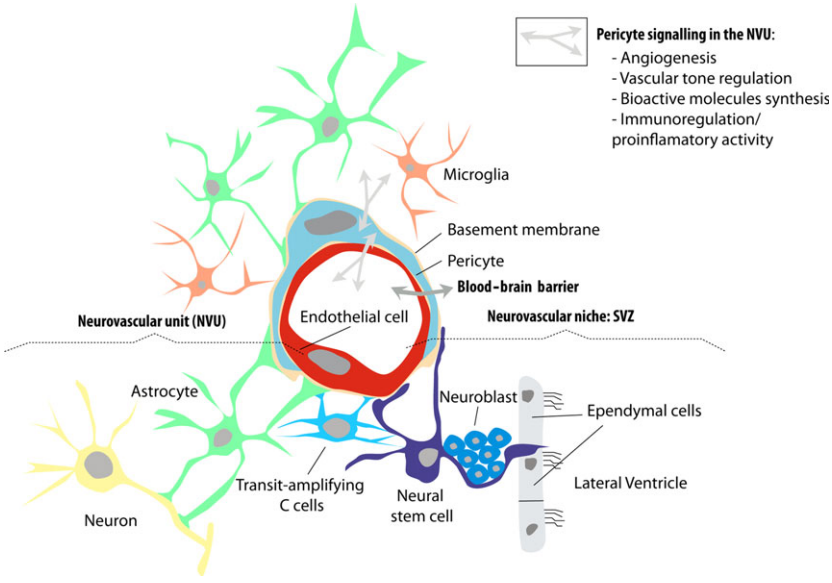

Fig. 2. Schema of physiological functions of pericytes in the brain. The schema shown a capillary cross-section where the pericytes is located on the capillary wall and share a common basement membrane with endothelial cells. The endothelial cells and pericytes constitutes the blood–brain barrier (BBB) that with glial cells and neurons form the neurovascular unit (NVU). In addition, this functional structure (NVU) provides an optimal microenvironment for neural proliferation in the adult brain forming the neurovascular niches in the subventricular zone (SVZ). Adapted from Winkler *et al.* 2011 and Tavazoie *et al.* 2008.

adult tissues, identified on CD146, neuronal/glia 2 (NG2) and platelet derived growth factor receptor beta (PDGFR-β) expression, exhibited in culture the same osteogenic, chondrogenic, adipogenic and myogenic potentials than MSCs. Pericytes in culture also exhibit surface antigens of MSCs (e.g, CD44, CD73, CD90, and CD105). These authors identify pericytes as potential progenitor cells to non-bone marrow derived MSCs (Fig. 1). In other experiments, inoculation of bovine retina pericytes intraperitoneally implanted into athymic mice showed osteogenic potential of pericytes *in vivo* and *in vitro* (Farrington-Rock *et al.* 2004; Doherty *et al.*, 1998).

The possibility that MSCs *in vivo* are pericytes would adequately explain why MSCs can be cultured from all tissues and why they could function as a source of stem cells for the regeneration of local lesions (Da Silva Meirelles *et al.* 2008). Moreover, pericytes have been suggested to play a role in skin regeneration (Desmouliere *et al.* 2005). Collectively, these results suggest multilineage stem cells derived from primary cultures of multiple fetal and adult organs is descended from vascular pericytes (Corselli *et al.* 2010). In a recent review, Caplan (2016) suggests that adult pericytes become activated MSCs when the vessel is damaged or inflamed. These activated MSCs respond secreting a cascade of bioactive molecules that control the local immune cells and the MSCs' trophically established zone of regeneration.

In central nervous system, pericytes are involved in blood brain barrier, and angiogenesis and vascular tone regulation. They form the neurovascular unit (NVU) together with endothelial cells, astrocytes and neurons (Fig. 2) This functional structure provides an optimal microenvironment for neural proliferation in the adult brain. Neurovascular niches have been described in subgranular zone (SGZ) of dentate gyrus of hippocampus (Palmer *et al.* 2000), in the subventricular zone (SVZ; Tavazoie *et al.* 2008) and in cerebral cortex during development (Stubbs *et al.* 2009). In the adult hippocampus angiogenesis occurs together with neurogenesis (Palmer *et al.* 2000), whereas blood vessels in the adult SVZ seem to contribute to neurogenesis but not to angiogenic sprouting of endothelial cells (Tavazoie *et al.* 2008). Neurovascular niche include both diffusible signals and direct contact with endothelial and perivascular cells such as pericytes. Endothelial and perivascular cells are a source of diffusible signals, such as VEGF, FGF2, IGF1, PEDF, BDNF that affect neural precursors (Biro *et al.* 1994; Leventhal *et al.* 1999; Jin *et al.* 2002; Shen *et al.* 2004; Ramirez-Castillejo *et al.* 2006). A recent study demonstrated that bFGF (FGF2) upregulates the expression of PDGFR-β in cultured central nervous system (CNS) pericytes in response to acidification *in vitro* and in peri-infarct areas after ischemic stroke *in vivo*. Authors suggest that bFGF may activate pericytes functions via the interaction with platelet-derived growth factor beta polypeptide-receptor beta (PDGF-BB) through the upregulation of PDGFR-β in CNS pericytes, which might contribute to neuroprotective and angiogenic actions (Nakamura *et al.* 2016). On the other hand, other authors observed that pericytes release the soluble form of PDGFR-β after brain microvascular damage in patients with dementia and Alzheimer Disease (AD; Sagare *et al.* 2015). These finding support the potential neuroprotective role of CNS pericytes.

Different studies have tried to demonstrate that MSCs originate at least in part from pericytes analyzing

their stem cell potential of brain pericytes. These cells are able to differentiate into neuronal phenotypes *in vitro* (Fig. 1; Dore-Duffy *et al.* 2006; Paul *et al.* 2012). These observations suggest the idea that central nervous system perivascular cells such as pericytes might contribute to brain repair either directly by generating new neurons (Dore-Duffy *et al.* 2000; Nakagomi *et al.* 2011;) or indirectly via their immunomodulatory properties or the secretion of neurotrophins (Ishitsuka *et al.* 2012).

Identity and origin of pericytes

Pericytes are the mural cells located at the surface of capillary blood vessels. They were originally named as "Rouget" cells by the French scientist Charles-Marie Benjamin Rouget in 1873 as a population of contractile cells that surround the endothelial cells of small blood vessels. However, because of their anatomical location at perivascular regions, Rouget cells were renamed as "pericytes" by Zimmerman in 1923 (i.e., surrounding [peri] brain endothelial cells [cytes]). Both pericytes and vascular smooth muscle cells (vSMCs) have been deemed as mural cells (Armulik *et al.* 2005; Gaengel *et al.* 2009). Anatomically, pericytes are located directly on the capillary wall and share a common basement membrane with endothelial cells where they extend long cell processes across the surface of endothelial cells (ECs) (Allt & Lawrenson 2001), establishing cell-to-cell contacts with endothelial cells via gap junctions (Armulik *et al.* 2005) In the brain, pericytes are located in pre-capillary arterioles, capillaries, and post-capillary venules.

Pericytes are encased within the endothelial basement membrane where they are thought to contribute to and regulate basement membrane assembly via interactions with ECs (Winkler *et al.* 2011). However, in areas lacking a basement membrane, pericytes make direct contact with endothelial cells through peg-socket connections, gap junctions and adherent junctions (Li *et al.* 2011; Winkler *et al.* 2011).

Identity of pericytes

Pericytes comprise a heterogeneous cell population that can be identified using various markers (Armulik *et al.* 2005, 2011; Diaz-Flores *et al.* 2009; Krueger & Bechmann 2010). A variety of molecular markers for pericytes have been proposed (reviewed in Trost *et al.* 2016; Armulik *et al.* 2010) including PDGFR-β (Lindahl *et al.* 1997; Winkler *et al.* 2010), NG2 (Ozerdem *et al.* 2001; Trost *et al.* 2013), CD13 (Kunz *et al.* 1994), desmin (Nehls *et al.* 1992), vimentin (Bandopadhyay *et al.* 2001), G-protein signaling-5 (RGS5; Bondjers *et al.* 2003). On the other hand, the potassium channel complex Kir 6.1 has been used as a marker as a marker particular for CNS pericytes (Bondjers *et al.* 2006).

However, it is important to remember that pericytes may alter their expression in combination with developmental states, pathological reactions, *in vitro* conditions, etc. For example, previous work used smooth muscle actin (aSMA) as marker to pericytes *in vitro* and in vivo (Armulik *et al.* 2011), however recently aSMA has been identified as marker to identify pericytes *in vitro* but do not *in vivo* (Trost *et al.* 2013; Hill *et al.* 2015).

In conclusion, the specific identification of pericytes needs a combination of well-preserved tissue morphology and at least two pericyte markers.

Origin of pericytes

Pericytes are generated during embryonic and postnatal life (Armulik *et al.* 2011; Winkler *et al.* 2011; Trost *et al.* 2016). The origin of the mural cells in CNS have been mapped using quail-chick experiments technique where quail neural crest or mesoderm were transplanted into developing chick embryos. These studies demonstrated that during developmental stages, transplanted neural crest initiated pericyte populations in the forebrain (telencephalon and diencephalon) whereas mesodermal (no neural crest) transplantation initiated pericyte populations in the mid-brain, brainstem, spinal cord, heart, lung, liver, and gut (Couly *et al.* 1992, 1993; Etchevers *et al.* 2001; Korn *et al.* 2002; Kurz 2009). In addition, during embryonic development and the early postnatal period, the pericytes relies on the proliferation expansion of pre-existing pericytes (Hellstrom *et al.* 1999; Ozerdem & Stallcup 2003 Abramsson *et al.* 2007; Stenzel *et al.* 2009). At the site of angiogenesis, endothelial cells stimulate the proliferation, migration and attachment of pericytes along the adjacent endothelial tube (Hellstrom *et al.* 1999; Ozerdem & Stallcup 2003, 2004; Abramsson *et al.* 2007; Stenzel *et al.* 2009). The relative contributions of pre-existing CNS pericyte pools to renewal of pericytes in the adult CNS under normal conditions and after CNS acute and chronic injury remain to be determined. Recently, studies have proposed that circulating mesoderm-derived bone marrow progenitor cells have given rise to adult CNS pericytes after ischemic conditions and in tumors (Hess *et al.* 2004; Ozerdem & Stallcup 2004; Rajantie *et al.* 2004; Ziegelhoeffer *et al.* 2004; Song *et al.* 2005; Kokovay *et al.* 2006; Lamagna & Bergers 2006; Piquer-Gil *et al.* 2009; Winkler *et al.* 2011; Trost *et al.* 2016).

Physiological functions of pericytes

Blood–brain barrier

The BBB regulate the communication between the blood-stream and the parenchyme tissue, which is important to maintain its stability, regulation of CNS blood flow, transport of molecules and nutrients into the CNS as well as protect the CNS from injury and disease (Zlokovic 2008; Armulik *et al.* 2010; Daneman *et al.* 2010; Sengillo *et al.* 2013). The constituents of the BBB (Fig. 2) are the endothelial cells and pericytes that with vSMCs, glial cells, neurons and perivascular macrophages forms the NVU (Del Zoppo 2010). The pericyte density around ECs varies between different organs and vascular beds. The central nervous system vasculature is the tissue with the highest abundance of pericytes with a 1:1–3:1 ratio between endothelial cells and pericytes, and an approximately 30% coverage of the abluminal surface (Sims 1986; Mathiisen *et al.* 2010; Sa-Pereira *et al.* 2012). A high degree of pericyte coverage in the central nervous system has been thought to correlate with CNS-specific vascular features (Shepro & Morel 1993). Initially, pericytes were described as contractile cells involved in controlling neurovascular tone. However, the role of pericytes in the BBB function is still not fully elucidated. Recent reports suggest the role of pericyte in the regulation of neurovascular parameters including capillary diameter (Hamilton *et al.* 2010; Elali *et al.* 2014), blood flow (Balabanov & Dore-Duffy 1998; Fisher 2009; Kutcher & Herman 2009; Fernandez-Klett *et al.* 2010; Elali *et al.* 2014), and vascular barrier formation (Lindahl *et al.* 1997; Elali *et al.* 2014).

The first evidence that pericytes play a functional role in the vascular barrier was provided by *in vitro* studies in PDGFR-β-deficient mice (Bernstein *et al.* 1982; D'amore & Smith 1993). In addition, *in vivo* studies using a pericyte-deficient mouse model (pdgfr$\beta-/-$), which have defects in pericyte generation, supported the results obtained *in vitro*. These animals show increased vascular permeability, hemorrhagic alterations and embryonic lethality (Armulik *et al.* 2010; Daneman *et al.* 2010; Shimizu *et al.* 2011). The existence of pericytes is not required for the expression of the vascular barrier genes, but loss of pericyte increases the expression of genes related to vascular permeability, including angiopoietin 2 (Ang-2), Plvap, and leukocyte adhesion molecules (LAMs) (Daneman *et al.* 2010). These experiments establish the necessity of pericyte coverage for proper BBB function during embryogenesis as well as a complex signaling network between the cells of the BBB for homeostatic vascular permeability.

Immune function

The immunoactive properties of pericytes are still a matter of debate. However, recent studies suggest that brain pericytes may act as immune cells (Caspani *et al.* 2014). *In vitro* and *in vivo* studies revealed that pericytes secrete chemokines and cytokines into media in response to pro-inflamatory cues (Daneman *et al.* 2010; Kovac *et al.* 2011; Elali *et al.* 2014; Jansson *et al.* 2014). Under inflammatory conditions, pericytes respond to the immune challenge by increasing the expression of several pro-inflammatory cytokines of typical inflammatory molecules (Kovac *et al.* 2011; Pieper *et al.* 2014), co-stimulator molecules and major histocompatibility complex (MHC) (Verbeek *et al.* 1995; Balabanov *et al.* 1999; Proebstl *et al.* 2012; Stark *et al.* 2013).

Pathological implications of pericytes

Abnormal pericyte function has been observed in many diseases. The pericyte is relatively quiescent and is essential for vascular stability under normal conditions. In this way, pericytes are key players in other aspects of brain homeostasis and disease.

Cancer biology

As described above, several studies have shown that pericytes in the human brain look like perivascular multipotent mesenchymal stromal cells that share characteristics of both pericytes and mesenchymal stromal cells (Crisan *et al.* 2008a,b; Paul *et al.* 2012). Recent observations in several tissues indicate that pericytes are versatile and have the ability to respond to environmental stimuli such as stroke by means of PDGF-BB interaction (Ozen *et al.* 2014; Nakamura *et al.* 2016). Moreover, MSCs/pericytes grafted into malignant brain tumors, associate closely and integrate to tumor vessels (Bexell *et al.* 2009). In addition, brain pericytes seem to contribute to the immune defense in response to cytokines (Pieper *et al.* 2014) and to immunosuppression in human malignant glioma (Ochs *et al.* 2013). Pericytes are highly sensitive to TNF-α and act through the release of proinflammatory factors, suggesting a role in inducing brain inflammation (Matsumoto *et al.* 2014).

These properties make pericytes important players in cancer progression (reviewed in Ribeiro & Okamoto 2015). Tumor-driven angiogenesis is a critical step for tumor growth. During tumor angiogenesis pericytes secrete growth factors to stimulate sprouting of endothelial cells and then pericyte recruitment occurs (Gerhardt & Betsholtz 2003). Tumor vessels are highly

disorganized, irregularly shaped, tortuous, excessively branched, and leaky (Ruoslahti 2002). In tumor vessels, pericytes are loosely attached to the endothelium, present differential expression of typical markers and aberrant cytoplasmic projections that invade the tumor parenchyma (Morikawa *et al.* 2002; Raza *et al.* 2010; Barlow *et al.* 2013). Moreover, the amount of pericyte coverage on tumor vessels is also abnormal. Clinical studies have correlated pericyte coverage on tumor microvessels with cancer prognosis (Yonenaga *et al.* 2005; Zhang *et al.* 2012; Cao *et al.* 2013). Increasement of pericyte coverage improves vascular stability and perfusion favoring tumor growth. In contrast, low pericyte coverage compromises vessel structure integrity, which becomes leaky, facilitating tumor cell invasion/extravasation (Cooke *et al.* 2012).

The perivascular niche is critical to the maintenance of a stem cell-like state in tumor cells in brain cancer. Endothelial and periendothelial cells interact closely with self-renewing brain tumor cells and secrete factors that maintain these cells in a stem cell-like state (Calabrese *et al.* 2007). In addition, in glioblastoma, the most frequent and aggressive type of primary brain tumor, malignancy proceeds via specific and interactions of tumor cells with brain pericytes (Caspani *et al.* 2014).

Pericytes are also involved in vessel co-option, an important alternative pathway by which tumors obtain blood supply using pre-existent vessels (Caspani *et al.* 2014). Tumor cells interact with brain pericytes by mean of extensions called flectopodia and modify the normal contractile activity of pericytes. Interestingly, when authors inhibited flectopodia activity impaired vessel cooption and converted pericytes to phagocytic/macrophage-like cell. Tumor vessel pericytes origin remains controversial, Cheng *et al.* (2013) performed xenografts using green fluorescent protein (GFP)-labeled glioblastoma stem cells (GSCs) and observed pericytes derived from the tumor. They also demonstrated that GSCs were able to assume a pericyte lineage *in vitro*. On the other hand Svensson *et al.* (2015) infiltrated GFP positive pericytes co-expressing pericyte markers as PDGFR-β and observed that more than half of all PDGFR-β positive pericytes within the tumor were contributed by the host brain. More studies are necessary to elucidate tumor vessel pericytes origin.

Another important role of pericytes in cancer biology is their implication in tumor cell spreading. Pericytes have been proposed as negative regulators of metastasis (Xian *et al.* 2006). Studies in mice, later corroborated in clinical analysis, have shown that detachment of tumor vessels pericytes enhances the metastatic potential of tumor. Low pericyte coverage showed a significant correlation with metastasis (Taniguchi *et al.* 2001; Welen *et al.* 2009; Agrawal *et al.* 2014). Despite these results, the underlying cellular and molecular mechanisms through which pericytes reduce tumor metastasis remain unknown. On the other hand, other authors propose the idea that pericytes may be indirectly involved in tumor cell escape. They argue that local pressure increases due to leaky vessels as a result of pericyte depletion. The higher pressure produces tumor hypoxia which may generate metastasis through a hypoxia-induced epithelial mesenchymal transition mechanism (Cooke *et al.* 2012).

A recent study propose a high pericyte score as the best means to date of identifying patients with ovarian cancer at high risk of rapid relapse and mortality (Sinha *et al.* 2016). Other studies in serous ovarian cancer analyzed the stroma of primary tumors and metastasis and detected that high intensity of perivascular PDGFR-β staining and abundant PDGFR-β positive stroma were associated with shorter overall survival (Andrae *et al.* 2008; Hagglof *et al.* 2010; Corvigno *et al.* 2016). Alternatively, Corvigno *et al.* found that low perivascular expression of PDGFR-β was associated with shorter survival in metastatic colorectal cancer. These results suggest that the effect of pericyte expression of PDGFR-β may vary in different tumor types.

Pericytes play an important role in the immune response against tumor cells, producing cytokines, chemokines, growth factors, and adhesion molecules (Verbeek *et al.* 1995; Edelman *et al.* 2007; Stark *et al.* 2013). Indeed, pericytes are characterized by coexpression of CD90, PDGFR-β, and CD248. In this way they were also suggested to have immunosuppressive properties in human malignant glioma (Ochs *et al.* 2013).

The important role of pericytes in cancer progression such as tumor growth, metastasis or immune response, suggests pericytes as cellular targets for new cancer therapies.

Alzheimer's disease

Pericytes are cells in the blood–brain barrier that degenerate in Alzheimer's disease (AD), a neurodegenerative disorder associated with neurovascular dysfunction, abnormal elevation of Aβ, tau pathology and neuronal loss. As described above, pericytes are crucial for maintaining BBB integrity. Whether pericyte degeneration can influence AD-like neurodegeneration and contribute to disease pathogenesis remains, however, unknown (review Winkler *et al.* 2014). Recent studies in humans and animal models have shown that brain pericyte dysfunction and/or degeneration correlates and/or results in BBB breakdown, leads to tissue accumulation of potentially neurotoxic blood-derived

products that normally do not enter into neural parenchyma, which contribute to neurovascular dysfunction and neurological disorders in AD (Farkas & Luiten 2001; Fiala *et al.* 2002; Zipser *et al.* 2007; Farrall & Wardlaw 2009; Hultman *et al.* 2013; Sengillo *et al.* 2013; Halliday *et al.* 2015; Montagne *et al.* 2015).

A recent study using crossed transgenic mice overexpressing the Swedish mutation of human Aβ precursor protein ($APP^{sw}/^{o}$) with PDGFR-β+/− mice revealed that loss of pericytes accelerates AD-like pathology (Sagare *et al.* 2013). In addition, as described above PDGFR-β is expressed in the brain by vascular mural cells–brain capillary pericytes and arterial vSMCs during development (Hellstrom *et al.* 1999; Armulik *et al.* 2011). Recent evidence shows that BBB disruption and increased permeability, positively correlates with elevated levels of soluble PDGFR-β (sPDGFR-β) in cerebrospinal fluid (CSF) in patients with mild dementia (Montagne *et al.* 2015). In addition, *in vitro* study show that human brain pericytes are more susceptible than arterial vSMCs to shedding of sPDGFR-β following exposure to divergent inducers of cell injury such as hypoxia and Aβ (Sagare *et al.* 2015).

HVI

Human immunodeficiency virus type 1 (HIV-1) infection is associated with impaired BBB stability and alteration of tight junctions (Avison *et al.* 2004; Persidsky *et al.* 2006). The effect that HIV-1 has on neurocognition might be related to persistence of inflammation (Kusao *et al.* 2012). A recent study has shown that brain pericyte coverage of BBB was diminished in HIV-1-infected patients even without HIV-1 encephalitis. In addition, exposure of pericytes to TNFα, IL-1β, or HIV-1 infection diminished pericyte support of endothelial barrier function. They also observed a downregulation of PDGFR-β expression resulting in attenuated pericyte coverage and a "leaky" BBB. Their results indicate that CNS inflammation can diminish BBB supportive functions of pericytes and promote their pro-inflammatory phenotype (Persidsky *et al.* 2016).

Microglia and macrophages are the main cells that support productive HIV-1 replication in the CNS. However, Nakagawa *et al.* (2012) reported that HIV-1 can infect, although at a low level, brain pericytes *in vitro*. In addition, infected pericytes negatively influence the barrier function of brain endothelial monolayers. In this way, BBB integrity is disrupted in HIV-1 brain infection, allowing the entry of virotoxins and HIV-1 virions into the CNS (Toborek *et al.* 2005; Banks *et al.* 2006).

Other studies have shown a cellular crosstalk in which lipopolysaccharide acts at the luminal surface of the brain endothelial cell, inducing abluminal secretions that stimulate pericytes to release substances that enhance the permeability of the brain microvascular endothelial cells monolayer to HIV (Dohgu & Banks 2013).

As a summary, chronic BBB impairment occurs in HIV-1 infection and pericyte are involved in BBB biology during neuroinflammation. The key role of pericytes in BBB function and brain immunological response, together with its accessibility to viral infection suggest that these cells may represent a new cellular target in virus-mediated gene therapy.

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Author contributions

The review was conceived and designed by SM and performed by AP, RG and SM. AP, RG and SM prepared the manuscript.

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