Revascularization and endothelial progenitor cells in stroke

Gema Esquiva, Alba Grayston, and Anna Rosell
Neurovascular Research Laboratory and Neurology Department, Vall d’Hebron Research Institute, Universitat Autònoma de Barcelona, Barcelona, Spain

Submitted 22 May 2018; accepted in final form 9 August 2018

Esquiva G, Grayston A, Rosell A. Revascularization and endothelial progenitor cells in stroke. Am J Physiol Cell Physiol 315: C664–C674, 2018. First published August 22, 2018; doi:10.1152/ajpcell.00200.2018.—Stroke is one of the leading causes of death and disability worldwide. Tremendous improvements have been achieved in the acute care of stroke patients with the implementation of stroke units, thrombolytic drugs, and endovascular trombectomies. However, stroke survivors with neurological deficits require long periods of neurorehabilitation, which is the only approved therapy for poststroke recovery. With this scenario, more treatments are urgently needed, and only the understanding of the mechanisms of brain recovery might contribute to identify new therapeutic agents. Fortunately, brain injury after stroke is counteracted by the birth and migration of several populations of progenitor cells towards the injured areas, where angiogenesis and vascular remodeling play a key role providing trophic support and guidance during neurorepair. Endothelial progenitor cells (EPCs) constitute a pool of circulating bone-marrow derived cells that mobilize after an ischemic injury with the potential to incorporate into the damaged endothelium, to form new vessels, or to secrete trophic factors stimulating vessel remodeling. The circulating levels of EPCs are altered after stroke, and several subpopulations have proved to boost brain neurorepair in preclinical models of cerebral ischemia. The goal of this review is to discuss the current state of the neuroreparative actions of EPCs, focusing on their paracrine signaling mechanisms thorough their secretome and released extracellular vesicles.

angiogenesis; endothelial progenitor cells; neurogenesis; secretome; stroke

INTRODUCTION

Stroke Disease and Brain Injury

Stroke is one of the leading causes of death and long-term disabilities worldwide. According to the World Health Organization (WHO), 15 million people suffer a stroke every year. Nearly 5 million of these people die and another 5 million are permanently disabled. Stroke occurs when a blood clot or thrombus blocks a brain artery (ischemic) or when an intracranial artery ruptures (hemorrhagic), leading to cell death in specific brain areas if blood flow is not rapidly restored. Therefore, stroke is considered a medical emergency that requires a rapid diagnosis and intervention to minimize brain injury because the sudden reduction of the physiological levels of oxygen and glucose is followed by cellular edema, inflammation, neurodegeneration, blood-brain barrier (BBB) injury, and the loss of neurological functions (45, 57). In the case of ischemic strokes, during reperfusion of the artery, additional damage can occur related to the arrival of new oxygen to the tissue. Such injury includes oxidative stress and BBB breakdown with subsequent intracerebral hemorrhages, and the initial ischemic lesion can expand in minutes to hours in parallel with the activation of spontaneous mechanisms of repair that will continue for weeks to months (55).

Fortunately, research in the stroke field over the last decades has provided enormous improvements for acute stroke. Together with stroke care in specialized stroke units, hyperacute thrombolytic treatments and the newest thrombectomy strategies are available, providing treatments for artery recanalization within the first 8 hours of symptom onset for ischemic stroke candidates (8, 28, 37, 89). However, despite all of medical efforts, stroke survivors with neurological deficits require long periods of neurorehabilitation, which is the only approved therapy for poststroke recovery, to achieve functional independence. Evidence-based stroke rehabilitation care includes several types of health interventions, such as early admission to specialized stroke rehabilitation units and intensive rehabilitation programs (46, 92), with the goal of helping stroke survivors become as independent as possible in the performance of living activities and achieving the best possible quality of life in the long term. Beyond the proven benefits of multidisciplinary rehabilitation programs, they do not guarantee complete recovery for all patients despite their wide therapeutic window and long-lasting rehabilitation programs.
Therefore, it is necessary to develop new stroke treatments that could be used to treat a large number of patients and provide treatments in the delayed phases of the disease to repair and rewire the injured tissue. This need is connected to the accepted concept that endogenous neurovascular plasticity and remodeling are also activated in the earliest phases of ischemic events and participate in functional recovery after stroke (55).

Stroke Neurorepair

The nature of stroke disease leads to a scenario in which severely damaged tissue is in very close proximity to functional peri-infarct tissue. The classical view of neuron rescue/repair has changed in the last decades in favor of a more global understanding of the brain as a whole and now includes other cell types (e.g., glia and inflammatory and progenitor cells), the extracellular matrix, and the communication between these components. Therefore, endogenous mechanisms of neurovascular repair include angio-vasculogenesis (the formation of new blood vessels), gliogenesis (the formation of new glia), neurogenesis (the formation of new neurons), remyelination (the construction of new myelin sheaths on demyelinated axons), and other mechanisms. Several of these endogenous mechanisms are activated in the minutes following the ischemic trigger in the peri-infarct areas (12, 54), and different populations of newborn progenitor cells have been identified in remodeling areas (12, 36, 69), such as neural progenitor cells (NPCs), endothelial progenitor cells (EPCs), and oligodendrocyte progenitor cells (OPCs). Moreover, trophic support has been described to occur in areas in which brain endothelial cells secrete soluble factors that maintain the CNS stem cell self-renewal and neurogenic potentials in vitro (84). The cross talk between the endothelium and oligodendrocytes/oligodendrocyte progenitors is nourished by trophic factors that are released by endothelial cells, and this cross talk has been described in the normal and ischemic brain in which brain-derived neurotrophic factor (BDNF), TGF-ß, VEGF, or matrix metalloproteinases (MMPs) could be responsible for maintaining the oligovascular niche (65, 76).

All of these facts demonstrate the plastic nature of the brain and oppose the more classical view of a passively dying brain. In this context, the scope of the present review will focus on the role of EPCs as cellular mediators of vascular remodeling and repair in the poststroke brain during recovery (Fig. 1).

ENDOTHELIAL PROGENITOR CELLS

Introduction to Endothelial Progenitor Cells

The formation of new blood vessels was formerly thought to be limited to embryogenic vasculogenesis and to be followed by the sprouting of endothelial cells from preexisting vessels during angiogenesis (75). Nonetheless, Asahara and colleagues (3) discovered the presence of endothelial progenitor cells (EPCs) in adult peripheral blood, being first identified as CD34 antigen-positive (CD34+/H11001) mononuclear cells (MNCs) with endothelial characteristics. The proportion of these cells generally ranges between 0.1 and 2% of the total MNC in the bone marrow (BM), peripheral blood, and cord blood. EPCs are mobilized from the BM into the peripheral blood as an endogenous response to the pathophysiological demands of neovascularization and can be differentiated into functional endothelial cells in vitro and ex vivo. EPCs are known to play an important role in adult vasculogenesis and angiogenesis by participating not only in the formation of vessels but also in vessel repair and remodeling (52). For this reason, EPCs have become a focus of study in the field of neurorepair strategies, including in ischemia-related diseases (24, 52, 67).
Classification

EPCs are defined as cells that express both stem cell markers and endothelial cell markers, although these cells continue to be controversial because no single marker has been identified for their unique identification (6, 19). However, the most widely accepted phenotypic definition is the coexpression of the cell-surface markers CD34 and VEGF receptor 2 (VEGFR-2; 51, 82). Because EPC subtypes have demonstrated different proliferative and angiogenic capabilities, it is of great importance to precisely define these subtypes to determine their specific natures and mechanisms of action. Such knowledge would enable a true and greater understanding of their angiogenic potential in vitro and in vivo and allow for the highest therapeutic revascularization efficacy in the setting of ischemic diseases.

Inconsistent results have been obtained among EPC studies regarding the definitions and classifications of the subpopulations that emerge from peripheral blood MNC-derived EPCs at different times in culture, exhibiting phenotypes that are between the hematopoietic and the endothelial lineages. However, there is a consensus about the classification of these cells into two major types that emerge from MNC cultures, which were initially named as “early EPC” or circulating angiogenic cells (CACs) and “late EPC” or outgrowth endothelial cells (OECs). These types of cells have different origins and provide different contributions to angiogenesis (19, 61). The early EPC appear after 3–10 days of peripheral blood MNC culture, and the late EPCs appear after 2–3 weeks approximately. While the early EPCs present with a spindle shape within heterogeneous populations of cells, the late OECs or endothelial colony-forming cells (EFCFs) form cobblestone and palisading colonies and exhibit clonogenic capacity (27, 34). Minami et al. (63) redefined this classification by further classifying OECs into the following three subpopulations: “moderate”-outgrowth EPCs (MOCs), which emerge in culture at days 10–16; “late”-outgrowth EPCs (LOCs), which emerge at days 17–23; and “very late”-outgrowth EPCs (VOCs), which emerge at days 24–30. The recruitment of OECs is known to contribute to both angiogenesis and arteriogenesis in a paracrine manner and is directly involved in neovascularization and the incorporation of new blood vessels. Thus, OECs exhibit proliferative and tubulogenic potentials (27, 34, 80, 83), whereas early populations are thought to contribute to angiogenesis mainly through paracrine signals (19). Among the late EPCs, LOCs have been found to exhibit the highest levels of expression of angiogenic genes and are the only cells that significantly promote blood flow recovery and increase capillary collateral formation in a mouse model of hindlimb ischemia. LOCs mediate this process by incorporating into newly formed vessels and promoting the release of proangiogenic factors (63). More recently, Huizer et al. (33) improved the characterization of OECs using a FACs sorting protocol in which they defined homogeneously highly expressed and stable markers (i.e., CD146, CD144, CD105, CD31) on the one hand, and heterogeneous and unstable markers on the other (i.e., CD34, c-kit, CD133). The lack of consistency among studies in the identification and classification of EPC subsets has been attributed to the low frequencies of these cells in the bloodstream, the different methods used for their isolation, and differences in the immunophenotyping protocols that have been performed thus far. However, based on the perspective of the last 20 years of research in the field, it has also been proposed that this lack of consistency may indicate that endothelial progenitors exhibit a dynamic phenotype in space and time (19).

Endothelial Progenitor Cell Mobilization

Regarding the kinetics of EPCs, several factors have been found to promote EPC mobilization from the BM into the peripheral circulation from which they eventually incorporate into sites of neovascularization. These mobilizing factors include, among others, the following: granulocyte macrophage-colony stimulating factor (GM-CSF); vascular endothelial growth factor (VEGF) (3, 91); granulocyte-colony stimulating factor (G-CSF) (24); angiopoietin-1 (29); stromal-derived factor-1 (SDF-1), which interacts with the CXC receptor 4 (CXCR4) that is present in EPCs (74); and HMG-CoA reductase inhibitors (statins) (17, 53, 98). We would like to highlight the SDF-1/CXCR4 axis because it has been reported to play a key role in EPC mobilization in response to hypoxia or injury. The basal levels of SDF-1 in the circulation and BM are low, but after an ischemic event, hypoxia-inducible factor-1 (HIF-1) is upregulated, and it can activate both SDF-1 and VEGF. EPCs are then mobilized to the ischemic region because they follow SDF-1 gradients (Fig. 2). This axis is important for the homing or recruitment of circulating EPCs to the ischemic site where their angiogenic and repairing functions occur in the context of tissue remodeling (13, 15, 103).

Interestingly, HMG-CoA reductase inhibitors (statins), which were drugs originally used as inhibitors of cholesterol biosynthesis showing other pleiotropic effects, were found to stimulate EPCs in different ways. On one hand, statin therapy...
has been shown to stimulate the growth of new blood vessels in ischemic limbs of normocholesterolemic rabbits (44), and further investigations showed that this proangiogenic effect was due to an increase in the mobilization of EPCs by mediating the phosphatidylinositol 3-kinase (PI3K)/Akt pathway (62). Similar results have been described in a mouse model of hindlimb ischemia related to the SDF-1α/CXCR4 axis and nitric oxide regulation (14). Other examples of the effect of statins on EPC mobilization have been described in both rodent (98) and human studies (45) under statin therapy showing increased EPC levels, and more recently in the context of ischemic stroke (26).

Endothelial Progenitor Cells and Poststroke Neurorepair

EPCs are involved in the direct repair of damaged blood vessel and angiogenesis in ischemic tissues, but they are also indirectly involved through paracrine signaling (Fig. 1). These cells have become a focus of study in the field of neurorepair strategies in ischemia-related diseases and have gained increasing importance in cerebral ischemia treatments because they are known to play critical roles in both the physiopathology and tissue repair associated with ischemic stroke. There are several mechanisms by which EPCs participate in endothelial repair. Ischemia mobilizes bone marrow-derived EPCs that interact with endothelial cells, extravasate, and reach the ischemic site, where they can directly incorporate into the vascular wall (32, 111). Moreover, EPCs are known to release protective cytokines and growth factors that can induce the self-repair of injured endothelial cells (ECs) or promote the extension of normal ECs into the injured sites and thereby assume a repair function (50, 51). In this regard, it is believed that the regenerative effect of EPCs may be attributable not only to the addition of new cells to new vessels but also to the secretion of factors that influence pathways of paracrine communication (59, 81, 82). These factors can induce the aforementioned mechanisms of the repair of damaged blood vessels, participate in the formation of new blood vessels from preexisting vessels, and promote the de novo formation of blood vessels in ischemic sites (50, 51).

Additionally, these growth factors can explain the relationship between angiogenesis and neurogenesis that guides the migration of neuroblasts and increases their survival in the so-called neurovascular niche (69). Neurogenesis in the adult brain occurs in two areas, i.e., the hippocampal subgranular zone (SGZ) and subventricular zone (SVZ) (68, 69); these areas renew cells in the dentate gyrus and olfactory bulb, respectively. Neural stem cells (NSCs) reside in specific niches, such as the SVZ, and display partial differentiation and enhanced proliferation with specific fates, for example, neuroblasts or oligodendrocyte progenitor cells (OPCs) (69). After cerebral ischemia, these areas that are rich in neural progenitor niches have been demonstrated to exhibit cells that can proliferate, migrate, and graft into most perilesional brain areas where they can differentiate into new neurons or glial cells and renew the cell population (68). For this process to happen there must be signals that guide the migrating neuroblasts to areas in which cell renewal can occur, and EPCs could be the source of these signals (81).

Therapeutic Potential of the Use of Endothelial Progenitor Cells

Several preclinical studies have reported beneficial effects of the transplantation of EPCs after cerebral ischemia (Table 1). The first studies utilized a mouse model of transient middle cerebral artery occlusion (MCAO) to demonstrate that the administration of human early EPCs was associated with a reduction in infarct volume, reduced cortical atrophy, increased angiogenesis, and improved neurobehavioral outcomes (20, 70, 81). Furthermore, the administration of outgrowth populations of human EPCs improved the neurological function in a rat model of ischemia-reperfusion (66).

Despite all of the preclinical evidence suggesting the benefits of EPC treatments for stroke by enhancing neurorepair mechanisms, there is a lack of clinical trials testing the safety and/or efficacy of EPC therapy treatments. To our knowledge only one Phase I clinical trial has tested and proved the safety and feasibility of administering intra-arterially autologous, bone marrow-derived CD34+ cells (which includes the EPC population) in five stroke patients (5). Several reasons could influence the current scenario such as: 1) the relatively recent identification of EPCs in adult humans (3); 2) the existence of other accessible stem/progenitor cells used for regenerative purposes in stroke, including commercial genetically modified stem cells or whole bone-marrow aspirates/peripheral blood-derived mononuclear cells (101); 3) the use of other stem cells such as mesenchymal stem cells early after their identification in a large number of clinical trials showing safety (88); 4) the fact that EPCs were first thought to exclusively influence vascular repair when other repair/remodeling mechanisms have been described later to also benefit from the cross talk with EPCs (58); 5) the anatomical/structural barrier to reach the brain tissue which limits the delivery of any cell product with minimally invasive procedures and good delivery efficiencies (101) might have discouraged the design of cell-based stroke clinical trials; and 6) the lack of consensus for standardized isolation and cell culture methods to expand the low numbers of circulating EPCs in healthy subjects (9).

In this context, further efforts have been applied from the preclinical side to offer improved efficiencies of treatments based on the use of EPCs. For example, EPCs transfected with lentiviral vectors encoding the human adiponectin gene elicit improvements in behavioral function, infarct extension, microvessel density, and cell apoptosis rates (109). Another study has recently demonstrated that the application of CXCL12-engineered EPCs with a lentivirus (used to deliver the cxcl12 gene into human umbilical cord blood EPCs) in a preclinical model of stroke at 1 week after ischemia resulted in increased blood vessel density but also promoted myelin sheath integrity and the proliferation and migration of OPCs (49).

However, it is important to highlight that despite the lack of clinical trials using EPCs in ischemic diseases, several observational studies have been carried out to assess the levels of circulating EPCs in patients with ischemic diseases such as stroke or myocardial infarction (15, 79), thus suggesting their use as a biomarker of endothelial function, integrity, or repair response.

In stroke patients different studies have shown an increase in circulating EPCs in the acute and subacute phases of the
Related for the first time EPC levels with poststroke reha-
disease including a peak in EPCs after 7 days of ischemic stroke (55, 60, 90) that is associated with better outcomes (55, 108), a positive correlation with infarct volume (15), suggesting that the mobilization of EPC from the bone marrow into peripheral circulation occurs as a stress re-
sponse to an ischemic event, or a decrease in the EPCs counts at 3 months of the event (56). A recent study has also related for the first time EPC levels with poststroke reha-
ilitation therapy showing that the expected long-term decrease in circulating EPC does not occur in patients under intensive rehabilitation therapy (23). However, other inves-
tigations have reported lower baseline circulating EPC levels in acute ischemic stroke patients in comparison with the control group (15, 95), perhaps related to the presence of cardiovascular risk factors such as blood pressure and or hypercholesterolemia (15).
Endothelial Progenitor Cells as Boosters of Cell Communication

Cell communication is an essential process in living organisms. Intercellular communication was long thought to be mediated exclusively through direct cell-to-cell interactions and the secretion of soluble intercellular molecules (e.g., growth factors, cytokines, neurotransmitters, lipids, hormones, etc.) that transmit the signal by binding to specific receptors on the target cell and/or via uptake into that cell. Recently, extracellular vesicles (EVs), which were initially considered to be innercellular debris, have been proposed as a new frontier of cell-to-cell communication (48). The presence of these vesicles is commonly related to a large number of diseases and pathologic conditions, and it is believed that they exert an important role in pathologies because the dysregulation of intercellular communication leads the progress of the disease (10). As described in the previous section, although EPCs have demonstrated therapeutic effects when transplanted into several animal models of ischemic disease, it has been reported that very few cells incorporate into the newly formed vessels in the ischemic regions, which suggests that there must be a paracrine mechanism that accounts for their beneficial effects (66). In this regard, it is known that EPCs communicate and interact with neurons, cerebral endothelial cells, astrocytes, and the surrounding extracellular matrix in the so-called neurovascular unit, and all of these interactions contribute to neurovascular remodeling after stroke (47, 54) (Fig. 1).

Endothelial Progenitor Cell-Derived Secretome

The repair capacities of EPCs are not exclusively due to their homing and engraftment because circulating EPCs also contribute to reendothelization and tissue regeneration following ischemic events by releasing paracrine proangiogenic factors, such as stromal-derived factor-1 (SDF-1), insulin-like growth factor-1, hepatocyte growth factor (HGF), G-CSF, VEGF, endothelial nitric oxide synthase, inducible nitric oxide synthase, IL-8, and IL-9 among many others. These factors can promote endothelial cell proliferation and reduce cell apoptosis, but are also involved in the regulation of endogenous progenitor cell recruitment, vascular growth, and remodeling (34, 35, 77, 80, 82, 96). The paracrine factors released by EPCs not only promote the angiogenic activities of vascular cells but also preserve this capacity and protect differentiated endothelial cells from apoptosis under conditions of oxidative stress (106). Other studies supporting the therapeutic effect of EPC-secreted factors have reported that cultured cortical neurons that are exposed to oxygen-glucose deprivation exhibit less axon degeneration when they are treated with EPC-conditioned media (72). The release of these factors appears to be increased by hypoxia; for example, Di Santo and colleagues (82) found that angiogenin, HGF, IL-8, platelet-derived growth factor homodimer BB (PDGF-BB), SDF-1, and VEGF-A are increased in EPC-conditioned media in hypoxic conditions, which supports the protective role of EPCs in the context of cerebral ischemia.

As explained above, several studies have suggested that early EPCs are capable of secreting large amounts of proteins, although this secretion is not the only role of this EPC population. Abe et al. (1) reported the contribution of EPC-conditioned medium (CM), in which EPCs secrete VEGF, on three-dimensional (3D) microvessel formation using an in vitro model. These authors demonstrated that EPC induced 3D network invasion into gels by creating a local VEGF gradient. In another study, Hur and colleagues (34) found higher levels of VEGF in the supernatant of early EPCs compared with late EPCs. VEGF produces nitric oxide (NO) in endothelial cells through the kinase insert domain receptor (KDR), and it is known that the upregulation of KDR on endothelial cells causes an increase in VEGF-mediated tube formation on Matrigel matrices. These authors also describe a greater expression of KDR by late EPCs, which might explain the higher tubulogenic potential of this population. Based on these findings, it has been proposed that early EPCs contribute to neovascularogenesis mainly by secreting proangiogenic factors that promote endothelial cell recruitment and induce endothelial cell proliferation and survival. In contrast, late EPCs contribute to neovascularogenesis also through their high proliferation rate that provides a source of endothelial cells (34, 66, 86).

Interestingly, EPC-derived CM has aroused interest as an alternative to cell therapy in the last years. Many studies have reported therapeutic effects in animal models of different ischemic diseases following a cell-free but cell-based therapeutic approach. The first study demonstrated that EPC-CM transplantation stimulated neovascularization and vascular maturation and thereby improved hindlimb perfusion and muscle function (82). More recently, intravenously administered EPC-CM appeared to enhance vascular remodeling in a cortical model of stroke and led to better functional outcomes (81). Moreover, for the first time, Maki et al. (59) observed recovery from white matter injury after EPC-CM administration in a prolonged hypoperfusion model that was mediated by increased vessel density, increased numbers of proliferating oligodendrocyte-lineage cells, and enhanced myelination in the corpus callosum accompanied by an improved cognitive function.

Several growth factors that are present in EPC-derived CM appear to be involved not only in angiogenesis but also in neurogenic processes and have been linked to stroke-induced neurogenesis by different authors (31, 38, 66). Some of the EPC-secreted factors reported in these investigations include the following: growth-regulated oncogene-a (GRO-a), interleukin 8 (IL-8), tissue inhibitors of metalloproteinases (TIMP-1 and TIMP-2), metalloproteinases 2 and 9 (MMP-2 and MMP-9), epidermal growth factor (EGF), monocyte chemotactic protein-1 (MCP-1), PDGF-BB, angiopoietin 2 (Ang-2), erythropoietin (EPO), VEGF, G-CSF, fibroblast growth factor-2 (FGF-2), brain-derived neurotrophic factor (BDNF), insulin-like growth factor (IGF-1), and stem cell factor (SCF), among many others.

This neurovascular niche supports adult neurogenesis from neural progenitor cells by signaling mechanisms that include contact-mediated and vascular-derived soluble factors (47, 69) (Fig. 1). Direct contact between NPCs and vascular endothelial cells in neurogenic sites maintains NPC quiescence through contact-dependent signaling (71), while soluble endothelial factors have been demonstrated to promote the proliferation of NPCs and to stimulate brain remodeling processes (30). These findings are in accordance with a study in which cocultured human EPCs and NPCs obtained from induced pluripotent stem cells synergistically protected ECs from hypoxia/reoxygenation-induced apoptosis and dysfunction, and these benefits...
resulted from paracrine activation of the PI3K/Akt signaling pathway mediated by VEGF and BDNF (99). G-CSF, which is among the factors present in the EPC-derived secretome, also plays a role in neurogenesis through VEGF. When G-CSF binds to its receptor, cytoplasmic tyrosine kinases are recruited, and these kinases activate STAT proteins. Activated STAT translocates to the nucleus, where it regulates gene expression and thereby promotes cell proliferation and mobilization. STAT3 has been reported to directly upregulate VEGF expression, and its kinase receptors VEGFR-1 (Flt-1) and VEGFR-2 (Flk-1 and KDR) are also expressed in nonvascular cells, such as neurons and NPCs (38). In a study performed by Zhu et al. (112) that utilized a mouse model of permanent focal ischemia, IGF-1 was demonstrated to enhance vascular density in the peri-infarct region and to increase neurogenesis at 7 days poststroke. However, whether IGF-1 directly promoted neurogenesis or this effect resulted from increased vascular density was not determined. Others have found that Ang-2 promotes an increase in the number of proliferating NSCs in culture (2). Ang-2 activates a pathway downstream of the Notch receptor that is known to induce the expansion of NSC populations, and NSCs in the subventricular zone (SVZ) and throughout the brain express the angiopeptin receptor Tie-2; furthermore, these cells appear to be in close proximity to blood vessels (2).

Other examples of the neurotrophic actions of factors secreted by EPCs relate to NPC migration from the SVZ neurogenic niche to the injured region of the brain that is guided by several receptor-ligand signaling pathways and molecular factors such as SDF-1/CXCR4 and MMP (41, 69, 100). These studies demonstrated how, after ischemic stroke, SDF-1 is released from endothelial cells, and its receptor CXCR4 is expressed in NSC and migrating neuroblasts.

**Endothelial Progenitor Cell-Derived Exosomes**

The secretion of extracellular vesicles (EVs) is a phenomena that is evolutionarily conserved from simple to complex multicellular organisms. EVs are considered communicosomes, i.e., nanosized extracellular organelles that are limited by lipid bilayers, have an average diameter of 30–500 nm, and play diverse pathophysiological roles in intercellular communication (7, 78, 97). EVs are normally found in all environments and in all body fluids, including the blood, saliva, breast milk, urine, and cerebrospinal fluid, as well as in stool. EVs have physiological functions, such as coagulation, regulation of immune responses, communication in the brain, and functions during pregnancy. EVs also have deleterious functions, e.g., they can suppress immune responses in a manner that leads to pathological conditions such as premetastatic niche formation and tumor angiogenesis (94).

Among other methods, EVs can be isolated by the centrifugation of either biofluids or cell-cultured supernatants (102, 104). There is no consensus in the nomenclature of the different EVs because of their heterogeneity, but some authors have named EVs according to the cell type from which they originate, for example, oncosomes (tumor-derived microvesicles), prostasomes (secreted by the prostate gland epithelial cells into the seminal fluid), and many others. However, we can classify EVs into three subtypes based on their biogenesis: exosomes, ectosomes, (shedding microvesicles), and apoptotic bodies. EVs are known to be secreted by a cell into the extracellular space. Exosomes are released by exocytosis, ectosomes are secreted by outward budding of the plasma membrane, and apoptotic bodies are released by dying cells during the later stages of apoptosis (39). EVs contain and deliver cargo that reflects their cellular origins and can include DNA, mRNA, microRNA (miRNA), proteins, and lipids (42). Moreover, the different intracellular origins of EVs are probably related to their different functions (73). However, the subtypes of EVs are not well characterized. Despite separate biogenesis pathways, definitive markers of EV subtypes are nonexistent (40, 42, 64).

**Exosomes and Stroke**

After stroke, exosomes secreted by cerebral endothelial cells and NPCs regulate intercellular communication between components of the neurovascular unit and thus contribute to the coupling of neurogenesis and angiogenesis during brain repair. These exosomes exhibit a bidirectional exchange of genetic and molecular information between stem cells and injured cells and reprogram the latter cells to repair damaged tissues (10). In this manner, increased levels of exosomes of mainly endothelial origin have been observed in cardiovascular pathology (87). These vesicles have been well documented to play an important role in angiogenesis, and it has been demonstrated that miRNAs are crucial in vascular endothelial cell angiogenesis as well as in stroke pathogenesis (107). Furthermore, it has been reported that cargo proteins are altered in the exosomes of plasma endothelial cells after stroke (25).

In vitro and in vivo experiments have demonstrated that exosomes from EPCs transfer miRNAs, such as miR-126 and miR-296, which activate the PI3K/Akt signaling pathway and lead to angiogenesis (11, 16). Moreover, the upregulation of miR-27b (43) and miR-181d (93) enhance angiogenesis, whereas the upregulation of miR-328 enhances cell migration (105) and promotes good clinical outcomes after ischemic stroke. In contrast, the downregulation of miRNA-15a in a mouse model of focal cerebral ischemia also promotes stroke-induced angiogenesis in the cerebral vessels of the peri-infarct region by increasing FGF-2 and VEGF levels (107). Beyond exosome vascular stimulation, NSCs also exchange molecular signals with blood vessels, neighboring cells, cerebrospinal fluid, and astrocytes through exosomes to mediate synaptic and axonal plasticity (18), and these signals could be involved in brain remodeling following ischemia. Furthermore, these signals may also communicate with the immune system after stroke (110). Regarding glial cells, it has been reported that cultured oligodendrocytes secrete exosomes that contribute to the neuronal-mediated coordination of myelination (22), and exosomes from oligodendrocytes can also improve neuron
viability under cell stress conditions and reduce ischemic neuronal death through the transfer of superoxide dismutase, catalase, and other antioxidant enzymes (21). Moreover, microglia exosomes can regulate oligodendrocytogenesis and neurogenesis under physiological and pathological conditions (85), including ischemic stroke.

CONCLUSIONS

EPCs have been documented to be confident candidates for preclinical regenerative medicine approaches for ischemic stroke, contributing to the recovery of neurological functions and activating neurorepair mechanisms after EPC transplantation. EPCs contribute to tissue repair by both direct incorporation into remodeling vessels or by indirect secretion of proteins or molecule-containing vesicles supporting cell communication, serving as blood biomarkers of injury, cardiovascular risk, or poststroke recovery. However, despite the well-known neuroreparative potential of these cells in the context of an ischemic event, this therapeutic approach has not reached the clinical setting yet and to date, only two clinical trials using EPCs are being registered (https://www.clinicaltrials.gov). Several limitations for this scenario have been discussed in this review including the extensive use of other stem cells for tissue regeneration such as mesenchymal stem cells, the complex administration and delivery of cell products in the brain, and the lack of a standardized protocol for the isolation, culture, and expansion of EPCs. It is in this light that cell-free (but cell-based) therapies are now also considered as an alternative to standard cell therapies, taking advantage of the capacity of EPCs to release soluble growth and communicating molecules (106). Special interest should also be focused on future treatments increasing EPC mobilization from the bone marrow to peripheral circulation to increase the pool of EPCs that would eventually reach the ischemic site to promote neurorepair; available drugs such as HMG-CoA reductase inhibitors have already shown this ability, but additional investigations on new pharmacotherapies to increase the pool or function of EPC will also be focused on these future cell-based (but cell-free) strategies. These approaches would avoid the disadvantages related to the direct administration of cells in more classical approaches, but could trigger a wider neurorestorative response by activating different mature and precursors cell types at once. For this reason, in the coming years it would also be important to investigate the exosomes derived from EPCs as future therapeutic agents or therapeutic targets to regulate as communication agents during stroke repair.

ACKNOWLEDGMENTS

A. Grayston holds a predoctoral fellowship from Instituto de Salud Carlos III (PI17/00073). A. Rosell is supported by the Miguel Servet program (CPII15/00003), and G. Esquiva holds a postdoctoral fellowship from the Catalan Research and Innovation Department (PERIS SLT002/16/00352).

GRANTS

This work was supported by Instituto de Salud Carlos III, Spain Grants PI16/00981, AC17/00004, and RETICS INVICTUS RD16/0019/0021, co-funded by the European Regional Development Fund. AC17/00004 is part of the MAGBBRIS project, funded under the Euronanomed III 8th joint call on innovative nanomedicine.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

G.E., A.G., and A.R. prepared figures; G.E., A.G., and A.R. drafted manuscript; G.E., A.G., and A.R. edited and revised manuscript; G.E., A.G., and A.R. approved final version of manuscript.

REFERENCES

1. Abe Y, Ozaki Y, Kasuya J, Yamamoto K, Ando J, Sudo R, Ikeda M, Tanishita K. Endothelial progenitor cells promote directional three-dimensional endothelial network formation by secreting vascular endothelial growth factor. PLoS One 8: e82085, 2013. doi:10.1371/journal.pone.0082085.
2. Androuet-Théotokis A, Rueger MA, Park DM, Boyd JD, Pandamanaban R, Campanati L, Stewart CV, LeFranc Y, Plenz D, Walbridge S, Lonser RR, McKay RD. Angiogenic factors stimulate growth of adult neural stem cells. PLoS One 5: e9414, 2010. doi:10.1371/journal.pone.0009414.
3. Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, Witztumlicher B, Schattelman G, Isner JM. Isolation of putative progenitor endothelial cells for angiogenesis. Science 275: 964–966, 1997. doi:10.1126/science.275.5302.964.
4. Bai YY, Peng XG, Wang LS, Li ZH, Wang YC, Lu CQ, Ding J, Li PC, Zhao Z, Ju SH. Bone marrow endothelial progenitor cell transplantation after ischemic stroke: an investigation into its possible mechanism. CNS Neurosci Ther 21: 877–885, 2015. doi:10.1111/cns.12447.
5. Banerjee S, Bentley P, Hamady M, Marley S, Davis J, Shlebak A, Nicholls J, Williamson DA, Jensen SL, Gordon M, Habib N, Chat-away J. Intra-arterial immunoselected CD34+ stem cells for acute ischemic stroke. Stem Cells Transl Med 3: 1322–1330, 2014. doi:10.1002/scit.2013-0178.
6. Basile DP, Yoder MC. Circulating and tissue resident endothelial progenitor cells. J Cell Physiol 229: 10–16, 2014. doi:10.1002/jcp.24423.
7. Belting M, Wittrup A. Nanotubes, exosomes, and nucleic acid-binding peptides provide novel mechanisms of intercellular communication in eukaryotic cells: implications in health and disease. J Cell Biol 183: 1187–1191, 2008. doi:10.1083/jcb.200810038.
8. Berkhemer OA, Fransen PSS, Beumer D, van den Berg LA, Lingsma HF, Yoo AJ, Schonewille WJ, Vos JA, Nederkoorn PJ, Wernter MJH, van Walderveen MAA, Staals J, Hofmeijer J, van Oostaven JA, Lycklama à Nijeholt GJ, Boiten J, Brouwer PA, Emmer BJ, van der Bruijn SF, van Dijk LC, Kappel PJ, Lo RH, van Dijk EJ, de Vries J, de Kort PL, van Roij JWI, van den Berg JS, van Hasselt BA, Aerden LA, Dallinga RJ, Visser MC, Bot JC, Vroomen PC, Esghii O, Schreuder TH, Heijboer R, Keizer K, Tiebeek AV, den Hertog HM, Gerits DG, van den Berg-Vos RM, Karas GB, Steyerberg EW, Flach HZ, Marquering HA, Sprengers ME, Jenniskens SF, Beenen LF, van den Berg R, Koudstaal PJ, van Zwam WH, Roos YB, van der Lugt A, van Oostenbrugge RJ, Majoe CB, Dippel DW; MR CLEAN Investigators. A randomized trial of intraarterial treatment for acute ischemic stroke. N Engl J Med 372: 11–20, 2015. doi:10.1056/NEJMoa1411587.
9. Bueno-Beti C, Novella S, Lázaro-Franco M, Pérez-Cremades D, Heras M, Sanchis J, Herrnengelido C. An affordable method to obtain cultured endothelial cells from peripheral blood. J Cell Mol Med 17: 1475–1483, 2013. doi:10.1111/jcm.12133.
10. Camussi G, Deregibus MC, Bruno S, Cantaluppi V, Biancone L. Exosomes/microvesicles as a mechanism of cell-to-cell communication. Kidney Int 78: 838–848, 2010. doi:10.1038/kid.2010.278.
11. Cantaluppi V, Biancone L, Figliolini F, Beltramo S, Medica D, Deregibus MC, Galimi F, Romagnoli R, Salizioni M, Tetta C, Segoloni GP, Camussi G. Microvesicles derived from endothelial progenitor cells enhance neangiogenesis of human pancreatic islets. Cell Transplant 21: 1305–1320, 2012. doi:10.3727/096368911X627534.
12. Carmichael ST. Themes and strategies for studying the biology of stroke recovery in the poststroke epoch. Stroke 39: 1380–1388, 2008. doi:10.1161/STROKEAHA.107.499962.
13. Ceradini DJ, Kulkarni AR, Callaghan MJ, Tepper OM, Bastidas N, Kleinman ME, Capla JM, Galiano RD, Levine JP, Gurtner GC. Progenitor cell trafficking is regulated by hypoxic gradients through HIF-1 induction of SDF-1. Nat Med 10: 855–864, 2004. doi:10.1038/nm1075.
14. Chen C, Liu X, Wang J, Tang G, Mu Z, Chen X, Xu J, Wang Y, Zhang Z, Yang GY. Effect of HMGBl on the paracrine action of EPCs promotes post-ischemic neurovasculogenesis in mice. Stem Cells 32: 2679–2689, 2014. doi:10.1002/stem.1754.
13b. Chen J, Sanberg PR, Li Y, Wang L, Lu M, Willing AE, Sanchez-Ramos J, Chopp M. Intravenous administration of human umbilical cord blood reduces behavioral deficits after stroke in rats. Stroke 32: 2682–2688, 2001. doi:10.1161/hs1101.109367.

14. Chiang KH, Cheng WL, Shih CM, Lin YW, Tsao NW, Kao YT, Lin CT, Wu SC, Huang CY, Lin FY. Statins, HMGCoA reductase inhibitors, improve neovascularization by increasing the expression density of CXC-R4 in endothelial progenitor cells. PLoS One 10: e0136405, 2015. doi:10.1371/journal.pone.0136405.

15. Deng Y, Wang J, He G, Qu F, Zheng M. Mobilization of endothelial progenitor cell in patients with acute ischemic stroke. Neurosci 39: 437–443, 2018. doi:10.1016/S1474-7327(17)31434-3.

16. Deregbus MC, Cantaluppi V, Calogero R, Lo Iacono M, Tetta C, Biancone L, Bruno S, Bussolati B, Canussi G. Endothelial progenitor cell derived microvesicles activate an angiogenic program in endothelial cells by a horizontal transfer of mRNA. Blood 110: 2440–2448, 2007. doi:10.1182/blood-2007-03-078709.

17. Dimmel S, Aicher A, Vasa M, Mildner-Rihm C, Adler K, Tiemann M, Rütt H, Fichtlshrecher S, Martin H, Zeiher AM. HMGCoA reductase inhibitors (statins) increase endothelial progenitor cells via the PI 3-kinase/Akt pathway. J Clin Invest 108: 391–397, 2001. doi:10.1172/JCI131152.

18. Edelstein L, Smythies J. The role of epigenetic-related codes in neuro-computation: dynamic hardware in the brain. Philos Trans R Soc Lond B Biol Sci 369: 20135019, 2014. doi:10.1098/rstb.2013.0519.

19. Fadini GP, Losordo D, Dimmel S. Critical reevaluation of endothelial progenitor cell phenotypes for therapeutic and diagnostic use. Circ Res 110: 624–637, 2012. doi:10.1161/CIRCRESAHA.111.243386.

20. Fan Y, Shen F, Frenzel T, Zhu W, Ye J, Liu J, Chen Y, Su H, Young WL, Yang GY. Endothelial progenitor cell transplantation improves long-term stroke outcome in mice. Ann Neurol 67: 488–497, 2010. doi:10.1002/ana.21919.

21. Fröhlich D, Kuo WP, Frühbeis C, Sun JJ, Zehendner CM, Luhmann HJ, Pinto S, Toedling J, Trotter J, Krämer-Albers EM. Multifaceted role of epigenetic-related codes in neuro-computation: dynamic hardware in the brain. Philos Trans R Soc Lond B Biol Sci 369: 20135019, 2014. doi:10.1098/rstb.2013.0519.

22. Frühbeis C, Fröhlich D, Kuo WP, Krämer-Albers EM. Extracellular vesicles as mediators of neuron-glia communication. Front Cell Neurosci 7: 182, 2013. doi:10.3389/fncel.2013.00182.

23. Gabriel-Salazar M, Moranchao A, Rodriguez S, Buxó X, García-Rodriguez N, Colell G, Fernandez A, Giralt D, Bustamante A, Muñoz JC, Rovira CA. Importance of angiogenin and endothelial progenitor cells after rehabilitation both in ischemic stroke patients and in a mouse model of cerebral ischemia. Front Neurol 9: 508, 2018. doi:10.3389/fneur.2018.00508.

24. Gehling UM, Ergün S, Schumacher U, Wagener C, Miettinen M, Jousilahti P, Ristori P, Roselli E, Kaste M. Importance of endothelial progenitor cells after rehabilitation both in ischemic stroke patients and in a mouse model of cerebral ischemia. Front Neurol 9: 508, 2018. doi:10.3389/fneur.2018.00508.

25. Gotzel EI, Schwartz JB, Mustapic M, Lobach IV, Daneman R, Abner EL, Jicha GA. Altered cargo proteins of human plasma endothelial-cell-derived exosomes in atherosclerotic cerebrovascular disease. FASEB J 31: 3689–3694, 2017. doi:10.1096/fj.201700149.

26. Golab-Janowska M, Pawczewska E, Machalinski B, Meller A, Kotlega D, Safranow K, Wankowicz P, Nowacki P. Statins therapy is associated with increased populations of early endothelial progenitor cell (CD133+/VEGFR2+) and endothelial (CD34−/CD133−/VEGFR2+) cells in patients with acute stroke. Curr Neurovasc Res 15: 120–128, 2018. doi:10.2174/156602471666618061120546.

27. Gulati R, Jevremovic D, Peterson TE, Chatterjee S, Shah V, Vile RG, Simari RD. Diverse origin and function of cells with endothelial phenotype obtained from adult human blood. Circ Res 93: 1023–1025, 2003. doi:10.1161/01.RES.0000085541.75393.21.

28. Hacke W, Kaste M, Bluhmki E, Brozman M, Dávalos A, Guildetti D, Larrue V, Lees KR, Medeghri Z, Machnig T, Schneider D, von Kummer R, Wahlgren N, Torni EI; ECASS Investigators. Thrombolysis with alteplase 3 to 4.5 hours after acute ischemic stroke. N Engl J Med 359: 1317–1329, 2008. doi:10.1056/NEJMoa0804656.

29. Hattori K, Dias S, Heissig B, Hackett NR, Lyden D, Tateno M, Belin TR, Wang J, Zhu Z, Witte L, Crystal RG, Moore MAS, Rafii S. Vascular endothelial growth factor and angiopoietin-1 stimulate postnatal hematopoiesis by recruitment of vasculogenic and hematopoietic stem cells. J Exp Med 193: 1005–1014, 2001. doi:10.1084/jem.193.10.1005.
ENDOTHELIAL PROGENITOR CELLS IN STROKE

Endothelial progenitor cells (EPCs) are multipotent cells that can differentiate into mature endothelial cells in vitro and in vivo. They play a critical role in angiogenesis and have been implicated in various physiological and pathological processes, including wound healing, tissue repair, and cancer progression. Evidence suggests that EPCs can act as mediators of cell-to-cell communication and contribute to the repair of damaged tissues.

Miyamoto N, Pham LD, Seo JH, Kim KW, Lo EH, Arai K. Crossstalk between cerebral endothelium and oligodendrocytes. Cell Mol Life Sci 71: 1055–1066, 2014. doi:10.1007/s00018-013-1488-9.

Moubairik C, Guillet B, Youssef B, Codacccioni JL, Pierccemdi MC, Sabatier F, Lionel P, Dou L, Foucault-Bertaud A, Velly L, Dignat-George F, Pisano P. Transplanted late outgrowth endothelial progenitor cells as cell therapy product for stroke. Stem Cell Rev 7: 208–220, 2011. doi:10.1007/s12015-010-9157-y.

Navarro-Sobrino M, Rosell A, Hernandez-Guillamon M, Penalba A, Ribó M, Alvarez-Sabín J, Montaner J. Mobilization, endothelial differentiation and functional capacity of endothelial progenitor cells after ischemic stroke. Microvasc Res 80: 317–323, 2010. doi:10.1016/j.mvr.2010.05.008.

Ohab JJ, Carmichael ST. Poststroke neurogenesis: emerging principles of migration and localization of immature neurons. Neuroscientist 14: 369–380, 2008. doi:10.1177/1073858407309945.

Ohab JJ, Fleming S, Blesch A, Carmichael ST. A vascular niche for neurogenesis after stroke. J Neurosci 26: 13007–13016, 2006. doi:10.1523/JNEUROSCI.4323-06.2006.

Ohta T, Kikutaka I, Imamura H, Takagi Y, Nishimura M, Arakaya Y, Hashimoto N, Nozaki K. Administration of ex vivo-expanded bone marrow-derived endothelial progenitor cells attenuates focal cerebral ischemia-reperfusion injury in rats. Neuroscience 59: 679–686, 2006. doi:10.1016/j.neuroscience.2005.09.030.

Peichert M, Nayer AJ, Pereira D, Zhu Z, Lane WJ, Williams MC, Hicklin DJ, Witte L, Moore MA, Rafii S. Expression of VEGFR-2 and AC133 by circulating human CD34+ cells identifies a population of functional endothelial precursors [Online]. Blood 95: 952–958, 2000. https://www.ncbi.nlm.nih.gov/pubmed/10648408.

Pelegrini L, Bennis Y, Guillet B, Velly L, Garrigue P, Sabatier F, Dignat-George F, Brueder N, Pisano P. Therapeutic benefit of a combined strategy using erythropoietin and endothelial progenitor cells after transient focal cerebral ischemia in rats. Nat Cell Biol 16: 1045–1056, 2014. doi:10.1038/ncb3045.

Pepper MS. Manipulating angiogenesis. From basic science to the bedside. Arterioscler Thromb Vasc Biol 36: 2005–2016, 2016. doi:10.1016/1050-1738(78)90545-4.

Pola G, Mayr U, Evans C, Prokopi M, Vera DS, Yin X, Astroulakis Z, Xiao Q, Hill J, Xu Q, Mayr M. Proteomics identifies thymidine phosphorylase as a key regulator of the angiogenic potential of colony-forming units and endothelial progenitor cell cultures. Circ Res 104: 32–40, 2009. doi:10.1161/CIRCRESAHA.108.182261.

Ratajczak J, Wysoczynski M, Hayek F, Janowska-Wieczorek A, Ratajczak MZ. Membrane-derived microvesicles: important and underappreciated mediators of cell-to-cell communication. Leukemia 20: 1487–1495, 2006. doi:10.1038/sj.leu.2404296.

Regueiro A, Cuadrado-Godia E, Bueno-Betí C, Diaz-Ricart M, Oli-crete angiogenic growth factors. Circulation 107: 1164–1169, 2003. doi:10.1161/01.CIR.0000058702.69484-A0.

Rosell A, Morranco A, Navarro-Sobrino M, Martinez-Saez E, Hernández-Guillamon M, Lode-Piedrafita S, Barceló V, Borràs F, Penalba A,
García-Bonilla L, Montaner J. Factors secreted by endothelial progenitor cells enhance neurorepair responses after cerebral ischemia in mice. PLoS One 8: e73244, 2013. doi:10.1371/journal.pone.0073244.

82. Di Santo S, Yang Z, Wyler von Ballmoos M, Voelzmann J, Diehn N, Baumgartner I, Kalka C. Novel cell-free strategy for therapeutic angiogenesis: in vitro generated conditioned medium can replace progenitor cell transplantation. PLoS One 4: e5649, 2009. doi:10.1371/journal.pone.0005649.

83. Schneider-Lucke C, Fichtlscherer S, Aicher A, Tschibe C, Schultheiss H-P, Zeiher AM, Dimmeler S. Quantification of circulating endothelial progenitor cells using the modified ISHAGE protocol. PLoS One 5: e13790, 2010. doi:10.1371/journal.pone.0013790.

84. Shen Q, Goderie SK, Jin L, Karanth N, Sun Y, Abramova N, Vincent P, Pumiglia K, Temple S. Endothelial cells stimulate self-renewal and expand neurogenesis of neural stem cells. Science 304: 1338–1340, 2004. doi:10.1126/science.1095505.

85. Shimogamo-Mogami Y, Hoshikawa K, Goldman JE, Sekino Y, Sató K. Microglia enhance neurogenesis and oligodendrogenesis in the early postnatal subventricular zone. J Neurosci 34: 2231–2243, 2014. doi:10.1523/JNEUROSCI.1619-13.2014.

86. Aicher A, Hennig M, Harnisch F, Kostic G, Fölsch U, Aicher G, Le Deist F, von Delius F, Saasin D, Simon D, Sordat T, Sato N, Thiele H, Stoeck A, Wernig M, Riedel M, Zipp F, Werdan K, Müller MF, Appleby L, Finlayson C, Martinou J, Rozek K, Kusche-Gunther B, Kinkelin J, Grabenwöger F, Müller-Giersbach C, Haase A, Neumann T, Bode LC, Schaper J, V Implements miR-214 to secrete exosomes that suppress senescence and induce angiogenesis in human and mouse endothelial cells. Blood 121: 3997–4006, 2013. doi:10.1182/blood-2013-02-478925.

87. Walter DH, Rittig K, Bahlmann FH, Kirchmar R, Silver M, Murayama T, Nishimura H, Losordo DW, Asahara T, Issner JM. Statin therapy accelerates reendothelialization: a novel effect involving mobilization and incorporation of bone marrow-derived endothelial progenitor cells. Circulation 105: 3017–3024, 2002. doi:10.1161/01.CIR.0000101866.84319.55.

88. Wang J, Chen Y, Yang Y, Xiao X, Chen S, Zhang C, Jacobs B, Zhao B, Bibl J, Chen Y. Endothelial progenitor cells and peripheral progenitor cells synergistically protect cerebral endothelial cells from hypoxia/reoxygenation-induced injury via activating the PI3K/Akt pathway. Mol Brain 9: 12, 2016. doi:10.1186/s13041-016-0193-7.

89. Wang L, Zhang ZG, Zhang RL, Gregg SR, Hozeska-Solotog A, Le Tourneau Y, Yang Y, Chopp M. Matrix metalloproteinase 2 (MMP-2) and MMP9 secreted by erythropoietin-activated cells promote endothelial cell progenitor cell migration. J Neurosci 26: 5996–6003, 2006. doi:10.1523/JNEUROSCI.5380-05.2006.

90. Wechsler LR, Bates D, Strooer P, Amsen-Zwilling YS, Aizman I. Cell therapy for chronic stroke. Stroke 49: 1066–1074, 2018. doi:10.1161/STROKEAHA.117.018290.

91. Zhang R, Kusano KF, Masuo O, Kawamoto A, Silver M, Murasawa S, Bossh-Marece M, Masuda H, Losordo DW, Issner JM, Asahara T. Stromal cell-derived factor-1 effects on ex vivo expanded endothelial progenitor cell recruitment for ischemic neovascularization. Circulation 107: 1322–1328, 2002. doi:10.1161/01.CIR.0000053131.77510.22.

92. Yamashita T, Takahashi Y, Nishikawa M, Takakura Y. Effect of exosome isolation methods on physicochemical properties of exosomes and clearance of exosomes from the blood circulation. Eur J Pharm Biopharm 98: 1–8, 2016. doi:10.1016/j.ejpb.2015.10.017.

93. Zhang SF, Lee WJ, Tan P, Tang CH, Hsiao M, Hsieh FK, Chien MH. Upregulation of miR-328 and inhibition of CREB-DNA-binding activity are critical for resveratrol-mediated suppression of matrix metalloproteinase-2 and subsequent matrixolytic ability in human osteosarcoma. Oncotarget 6: 2736–2753, 2015. doi:10.18632/oncotarget.3088.

94. Yang Z, Di Santo S, Kalka C. Current developments in the use of stem cell therapy for therapeutic neovascularisation: is the future therapy “cell-free”? Swiss Med Wkly 140: w13130, 2010. doi:10.4414/smw.2010.13130.

95. Yin KJ, Hamblin M, Chen YE. Angiogenesis-regulating microRNAs and ischemic stroke. Curr Drug Discov Technol 15: 352–365, 2015. doi:10.2174/15701511576664823.

96. Yip HK, Chang LT, Chang WN, Lu CH, Liou CW, Lan MY, Liu JS, Zhang ZG, Chopp M. Increased proangiogenic activity of mobilized CD34+ progenitor cells in patients with acute ST-segment-elevation myocardial infarction: role of differential microRNA-378 expression. Arterioscler Thromb Vasc Biol 37: 341–349, 2017. doi:10.1161/ATVBAHA.116.308695.

97. Théri C, Ostrowski M, Seguera E. Exosome and exosome vesicles as conveyors of immune responses. Nat Rev Immunol 9: 581–593, 2009. doi:10.1038/nri2567.

98. Tsai NW, Hung SH, Huang CR, Chang HW, Chang WN, Lee LH, Wang HC, Lin YJ, Lin WC, Cheng BC, Chiang YF, Su YJ, Tsai TR, Lu CH. The association between circulating endothelial progenitor cells and outcome in different subtypes of acute ischemic stroke. Clin Chim Acta 427: 1–10, 2014. doi:10.1016/j.cca.2014.02.019.

99. Théri C, Ostrowski M, Seguera E. Exosome vesicles and miR-214 induce macrophage polarization. J Mol Cell Cardiol 39: 733–742, 2005. doi:10.1016/j.yjmcc.2005.07.003.

100. Valadi H, Ekström K, Bossios A, Jómland M, Lee JJ, Lővblad JO. Exosome-mediated transfer of miRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol 9: 654–659, 2007. doi:10.1038/ncb1596.

101. van Balkom BW, de Jong OG, Smits M, Brummelmann J, den Ouden K, de Bree PM, van Eijndhoven MAJ, Peetel DM, Stoorvogel W, Würdinger T, Verhaar MC. Endothelial cells require miR-214 to secrete exosomes that suppress senescence and induce angiogenesis in human and mouse endothelial cells. Blood 121: 3997–4006, 2013. doi:10.1182/blood-2013-02-478925.