PDGFs and their receptors in vascular stem/progenitor cells: Functions and therapeutic potential in retinal vasculopathy

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Abstract

Vascular stem/progenitor cells (VSCs) include endothelial progenitor cells, smooth muscle progenitor cells, pericytes, and mesenchymal stem cells. VSCs can produce functional and mature vascular cells required to build blood vessels. VSCs therefore play critical roles in vascular repair and regeneration, particularly in various retinal vasculopathies, in which vascular defects are a devastating pathology. The platelet-derived growth factors (PDGFs) and their receptors (PDGFRs) are important regulators of numerous physiological events and diseases, and they play key roles in regulating the formation and function of blood vessels. A better understanding of the effects of PDGFs/PDGFRs on VSCs and a thorough elucidation of their therapeutic potential in the treatment of retinal vasculopathies are critical for both basic and translational research and may lead to better therapies for human vascular diseases.

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1. Introduction

Blood vessels, formed mainly through vasculogenesis and angiogenesis (Carmeliet, 2005), are vital for blood supply and the functions and structural properties of organs and tissues. Blood vessels are mainly composed of two cell types: endothelial cells (ECs) and mural cells (MCs). There are two types of MCs: vascular smooth muscle cells (SMCs) and pericytes. ECs line the vascular lumen to provide a barrier between circulating blood and tissues, whereas MCs are located peripherally and coat the EC tube. SMCs are associated with large blood vessels, such as arteries, arterioles, and veins. The contractile ability of SMCs is responsible for the regulation of blood flow and blood pressure. Pericytes are found in capillaries, small venules, and newly formed blood vessels. Pericytes stabilize the vessels and regulate blood flow too. The proper maintenance and reciprocal interaction between ECs and MCs are required for the normal formation, stabilization, and function of the vasculature (Armulik et al., 2005). It has been long thought that the formation of new blood vessels in adults only requires mature vascular cells (Parker Kerrigan et al., 2017). However, two decades ago, the discovery of CD34+VEGFR2+ endothelial progenitor cells (EPCs) in peripheral blood that are capable of generating new blood vessels changed the paradigm of vascular biology (Asahara et al., 1997). Since then, numerous studies have reported that cells residing in the vascular wall, bone marrow, circulation, or other extravascular tissues can differentiate into various types of mature vascular cells needed to build blood vessels. These cells have been named vascular stem/progenitor cells (VSCs) (Zhang and Xu, 2014).

The platelet-derived growth factors (PDGFs) are critical players in a variety of physiological and pathological processes (Ishii et al., 2017). In particular, PDGFs play important roles in blood vessel formation and retinal vasculopathies (Sadiq et al., 2016). Major retinal vasculopathies include diabetic retinopathy (DR), retinopathy of prematurity (ROP), the wet form of age-related macular degeneration (wAMD) and retinitis pigmentosa (RP), which are the major causes of vision loss or blindness. In this review, we summarize the functions of PDGFs/PDGFRs in the regulation of VSCs, and further discuss their therapeutic potentials in retinal vasculopathies in relationship to VSCs.

2. PDGFs and their receptors

The PDGFs belong to the cystine knot protein superfamily. They are encoded by four different genes PDGF-A, PDGF-B, PDGF-C, and PDGF-D, and exist as homodimers interconnected by disulfide bonds (PDGF-AA, PDGF-BB, PDGF-CC, and PDGF-DD) or as heterodimers (PDGF-AB) (Boor et al., 2014). Initially identified in 1970s as serum proteins that stimulate fibroblast and SMC growth and migration, PDGF-AA, -BB, and -AB have been studied intensively and are considered as classical PDGFs (Heldin and Westermark, 2014).
3. Vascular stem/progenitor cells (VSCs)

The integrity and functions of blood vessels may be impaired by various stresses or pathologies, thereby requiring the capacity of blood vessels to repair and regenerate after damage. Accumulating evidence suggests that there exist different types of vascular stem/progenitor cells, which can give rise to differentiated vascular cells (Psaltis and Simari, 2015). There are mainly four types of VSCs, namely vascular endothelial progenitor cells (EPCs), smooth muscle progenitor cells (SMPCs), mesenchymal stem cells (MSCs), and pericytes. EPCs are the first isolated adult vascular progenitor cells that are found to be able to differentiate into ECs (Asahara et al., 1997). The presence of human SMPCs in peripheral blood that can give rise to SMCs was first reported in 2002 (Simper et al., 2002). MSCs have the potential to differentiate into both ECs (Oswald et al., 2004) and SMCs (Kashiwakura et al., 2003). In addition, MSCs cocultured with EPCs can enhance the differentiation of MSCs to pericyte-like cells (Loibl et al., 2014). Pericytes have been reported to behave like MSCs that can generate different types of cells, such as chondrocytes, osteocytes, adipocytes, skeletal muscles and neurons (Armulk et al., 2011). Pericytes have been shown to be capable of differentiating into coronary artery SMCs in response to the activation of Notch pathway (Volz et al., 2015). However, a recent study using a lineage-tracing method to mark Tbx181 cells as pericytes reveals different results. It shows that these cells maintained their own identity and did not behave like progenitors for other lineages in several mouse disease models (Guimaraes-Camboa et al., 2017). This notion challenges the view that pericytes are multipotent cells. In this section, we mainly summarize current findings on the origins of VSCs and discuss the criteria to characterize VSCs. The effects of PDGFs/PDGFRs on VSCs are also discussed.

3.1. The origins of VSCs: many sources but low abundance

3.1.1. Sources of EPCs

Bone marrow (Murayama and Asahara, 2002) and peripheral blood (Asahara et al., 1997) are considered as the major sources for EPCs. In addition, EPCs can also be found in vascular walls (Zengin et al., 2006), liver, small intestine (Aicher et al., 2007) and perinatal tissues, such as umbilical cord blood (Ingram et al., 2004) and placenta (Fig. 1) (Rapp et al., 2012). Circulating EPCs have been considered as the most important source of cells for vessel regeneration because their isolation is relatively easy. Moreover, numerous studies have shown that the number of circulating EPCs may serve as a biomarker since the numbers have been shown to be correlated with many pathological conditions, such as tumor and peripheral artery diseases (Bitterli et al., 2016; Ge et al., 2015). On the other hand, the results of some other studies question the importance of circulating EPCs. Firstly, it has been shown that the ability of circulating EPCs to differentiate into ECs and to engraft into vessels after injury is less than that of resident EPCs (Hagensen et al., 2010; Kawasaki et al., 2015; Rapp et al., 2012). Secondly, it is reported that the contribution of circulating EPCs to neovascularure is more likely mediated by a paracrine effect, such as secretion of angiogenic factors (Baker et al., 2013), or by releasing microparticles or microvesicles (Deregiibus et al., 2007; Wang et al., 2013). Thirdly, it has been shown that the number of circulating EPCs is very limited as they comprise less than 0.01% of mononuclear cells in peripheral blood (Gross and Herbrig, 2004). Although it has been shown that cord blood contains more EPCs, their absolute number is still small (up to 0.64% of all mononuclear blood cells), which may hinder their therapeutic implication in cell therapy (Case et al., 2007). Attempts to increase the number of human EPCs via different in vitro expansion methods may offer potential ways to obtain sufficient EPCs needed. A comparison of these methods is listed in Table 1.

3.1.2. Origins of SMPCs

In adults, SMPCs can be found in the bone marrow, circulating blood, vascular wall, and extravascular matrix (Orlandi and Bennett, 2010). Other reservoirs for SMPCs include the skin (Steinbach and Husain, 2016), adipose tissue (Ma et al., 2017), and umbilical cord blood (Fig. 1) (Le Ricouesse-Roussanne et al., 2004). The importance of circulating SMPCs have been shown in both preclinical and clinical studies. For example, SMPCs are found to play an important role in preclinical models of arteriosclerosis (Sun et al., 2016). In patients with different pathologies, the number of functions of SMPCs are often compromised. For example, in patients with diabetes or coronary artery disease, the numbers of SMPCs are lower than those of healthy population (Sugiyama et al., 2006; van Ark et al., 2012). In patients with Moyamoya disease, SMPCs exhibit altered gene expression profiles during the progression of the disease (Kang et al., 2014). Many studies have investigated how to increase the number and functions of SMPCs and several regulating molecules have been identified, such as TGF-β (Wang et al., 2012a) and HB-EGF (Wang et al., 2016b). Still, due to the relatively scarce amount of SMPCs in vivo, knowledge on SMPCs are still poor and more studies are need to better characterized them.

3.1.3. Sources of MSCs

MSCs are one of the most intensively studied stem cells for regenerative medicine. MSCs display great potential for cell therapy in both preclinical models and clinical trials. Compared with other types of stem/progenitor cells, MSCs have unique advantages. Firstly, they are relatively easy to harvest and have a fair availability. Secondly, they have an immunosuppressive effect and can secret many beneficial factors, such as cytokines and exosomes (Kim and Park, 2017; Lou et al., 2017). Owing to their ability to differentiate into ECs and SMCs, MSCs are considered to be a promising source of cells for vascular regeneration. The niches for MSC include bone marrow, vascular wall, heart, adipose tissue, skeletal muscle, liver, umbilical cord blood and placenta (Fig. 1) (Hashemian et al., 2015). Notably, adipose tissue can yield around 500-fold more MSCs than bone marrow, representing an important source of MSCs (Fraser et al., 2006).
3.1.4. Sources of pericytes
Pericytes are located on the outside surface of ECs in the microvasculature and are endowed with essential roles in vascular homoeostasis. Pericytes can be found in different tissues, such as the central nervous system (CNS), retina, gut, lung, liver, heart, skeletal muscle, and bone marrow (Fig. 1) (Armulik et al., 2011; Birbrair et al., 2013; Lamagna and Bergers, 2006). However, the absolute numbers of pericytes are relatively low, and their density varies greatly depending on the specific organ and physiological or pathological condition. Pericytes are most abundant in the CNS and retina, with a pericyte/EC ratio of 1:1, whereas in the skeletal muscle, the pericyte/EC ratio is approximately 100:1 (Armulik et al., 2011). Noteworthy, in diabetic retina, the pericyte/EC ratio decreases from a normal level of 1:1 to 1:4 (Speiser et al., 1968).

3.2. Markers and characteristics of VSCs: markers are not always the unique ID

3.2.1. Markers and characteristics of EPCs
Numerous studies have identified and isolated EPCs using CD34, VEGFR2/KDR and CD133 as surface markers. However, such populations of cells also contain cells of hematopoietic lineage since these markers are also expressed by hematopoietic progenitors. To date, accumulating knowledge suggests that a subtype of EPCs, namely endothelial colony forming cells (ECFCs), behave more like true EPCs, since it has been shown that these cobblestone-like cells contribute to vessel formation in vivo (Richardson and Yoder, 2011). In general, the following criteria are commonly used to define EPCs. The cells express stem cell surface markers (CD34, CD133) and endothelial markers (CD31, CD105, CD144, CD146, VEGFR2/KDR), but not hematopoietic markers (CD45). The cells can attach to fibronectin-coated culture dishes and uptake UEA-1 and acetylated-low density lipoprotein. The cells display a high proliferative capacity for up to 6 months in culture (clonogenic assay) (Le Ricoussel-Rousanne et al., 2004). These cells also show functional properties of ECs, such as tube formation capacity in vitro or vessel-formation ability in vivo after transplantation (Bertagnolli et al., 2017; Richardson and Yoder, 2011).

Fig. 1. Major sources of vascular stem/progenitor cells (VSCs).
VSCs can be found in various tissues, such as bone marrow, heart, liver, adipose tissue, umbilical cord blood, circulating blood, and vascular wall. EPC: endothelial progenitor cell, SMPC: smooth muscle progenitor cell, MSC: mesenchymal stem cell, EC: endothelial cell, SMC: smooth muscle cell.
<table>
<thead>
<tr>
<th>Source</th>
<th>Markers used for EPC isolation</th>
<th>Amount of starting material</th>
<th>Expansion time, media supplement</th>
<th>Fold of enrichment, number of cells yield</th>
<th>Properties of expanded EPCs, markers and assays to verify EPC identity</th>
<th>Reference PMID</th>
</tr>
</thead>
<tbody>
<tr>
<td>Umbilical cord blood (UCB)</td>
<td>CD34⁺</td>
<td>70-100 ml UCB</td>
<td>21 days, SCF, TPO, Flt-3L, IL-3, GM-CSF, VEGF, IGF, EGF, etc</td>
<td>1400-fold, ~2 × 10⁶ cells</td>
<td>Express CD31, CD144, vWF, FVIII, clonogenic assay, tube formation, in vivo transplant and vessel formation ability</td>
<td>28173870</td>
</tr>
<tr>
<td>Lung</td>
<td>CD31⁺</td>
<td>The whole lung</td>
<td>5-6 weeks, cEGM-2 medium without hEGF, FBS and PSA</td>
<td>~10⁷ cells</td>
<td>Express CD31, CD146, CD144, CD105 but not CD45, uptake acLDL and UEA-1, clonogenic assay, tube formation, in vivo transplant and vessel formation ability</td>
<td>26448359</td>
</tr>
<tr>
<td>Peripheral blood</td>
<td>CD34⁺</td>
<td>6 × 10⁵ CD34⁺ cells</td>
<td>7 days, quality and quantity media with SCF, Flt-3L, TPO, IL-6, VEGF</td>
<td>7-fold, 4 × 10⁶ cells</td>
<td>Express CD34, CD133, tube formation</td>
<td>26475753</td>
</tr>
<tr>
<td>PSCs (ESCs or iPSCs)</td>
<td>NA</td>
<td>10⁴ PSCs</td>
<td>61 days, Activin-A, BMP-4, FGF-2 and VEGF</td>
<td>1.1 × 10¹² cells</td>
<td>Express CD31, CD144, NR1P1, clonogenic assay, tube formation, in vivo transplant and vessel formation ability</td>
<td>25306246</td>
</tr>
<tr>
<td>ESCs</td>
<td>NA</td>
<td>5 × 10⁴ ESCs</td>
<td>20 days, FGF-2, BMP4, activinA, VEGF, TGF-β inhibitor</td>
<td>7.4-fold, ~3.6 × 10⁸ cells</td>
<td>Express CD31, VEGFR2, VE-cadherin, clonogenic assay, in vivo transplant and vessel formation ability</td>
<td>20081865</td>
</tr>
<tr>
<td>Cord blood</td>
<td>CD133⁺</td>
<td>5.8–6.8 × 10⁹ CD133⁺ cells</td>
<td>30 days, SCF, Flt3 ligand, TPO</td>
<td>148-fold, ~9.3 × 10⁷ cells</td>
<td>Express CD31, VEGFR2, vWF, uptake acLDL, tube formation, in vitro and in vivo migration &gt;90% of the cells uptake acLDL and UEA-1, express VE-cadherin, VEGFR2, CD31, CD133</td>
<td>20161785</td>
</tr>
<tr>
<td>Circulating blood</td>
<td>CD34⁺</td>
<td>250 ml venous blood</td>
<td>5 days, VEGF</td>
<td>~10⁸ cells</td>
<td>Express VEGFR2, CD31, VE-cadherin, uptake acLDL and UEA-1, in vivo transplant and vessel formation ability</td>
<td>17418297</td>
</tr>
<tr>
<td>Peripheral blood mononuclear cells (PBMCs)</td>
<td>CD34⁺</td>
<td>1 × 10⁶ PBMCs</td>
<td>7-10 days, VEGF, bFGF, IGF, and EGF</td>
<td>80-90-fold increase, 4-5 × 10⁶ cells</td>
<td>Express VEGFR2, CD31, VE-cadherin, uptake acLDL and UEA-1, in vivo transplant and vessel formation ability</td>
<td>10725398</td>
</tr>
</tbody>
</table>

NA: not available.
using PMA, carbachol, angiotension II or by physiological stimulus using ET-1. SMPCs also display an ability to form capillary-like structures in vitro and vascular engraftment in vivo (Biel et al., 2015; Merkulova-Rainon et al., 2012). In addition, SMPCs have been shown to display a high proliferation capacity for up to three months (Le Ricousses-Roussanne et al., 2004).

3.2.3. Markers and characteristics of MSCs

MSCs display a spindle-shaped morphology with an in vitro clonogenic potential to form colony-forming fibroblasts (Cordeiro-Spini et al., 2014). Given the significant therapeutic potential of MSCs in tissue repair and regeneration, the International Society for Cellular Therapy (ISCT) has proposed the minimum criteria to define human MSCs. These include the ability to adhere to plastic when cultured under standard conditions, tri-lineage (osteogenic, chondrogenic, and adipogenic) differentiation capacity in vitro, expression of CD73, CD90 and CD105, and the lack of expression of CD45, CD34, CD14, CD11b, CD79a, CD19 or HLA-DR (Dominici et al., 2006). In many studies, putative MSCs met the former two ISCT criteria but not the third one in terms of the expression of surface markers. Other markers, such as CD106, CD271, Stro-1, SSEA-4 have also been reported in human MSCs (Mabuchi et al., 2013), whereas PDGFR-α and Sca-1 have been shown to be expressed in murine MSCs (Houlihan et al., 2012). Some commonly used markers to distinguish MSCs from other types of cells, or to specify the different stages, sources or vasculature-related functions of MSCs are described in Table 2.

3.2.4. Markers and characteristics of pericytes

Pericytes express a variety of markers, such as PDGFR-β, α-SMA, NG2, CD146, CD13, CD133, and RGS5 (Geevarghes and Herman, 2014; Wong et al., 2015). However, these markers are also shared by other cell types, such as MSCs or SMCs. Moreover, pericytes display dynamic changes in gene expression patterns depending on the developmental stage, physiological or pathological conditions and their locations. Consequently, identification of pericytes requires the combination with other exclusion markers depending on their specific location (Wong et al., 2015). Given the high similarity in the sources, markers and multipotent capacities between pericytes and other cell types, particularly MSCs, difficulties in distinguishing pericytes from other cell types have been shown (de Souza et al., 2016; Wong et al., 2015). Moreover, a recent lineage tracing study on Tbx18+ cells suggested a view that pericytes do not appear to be progenitor cells in vivo (Guimaraes-Camboa et al., 2017). Thus, more studies are needed to better understand the dynamics and multipotency of pericytes.

3.3. Effects of PDGFs/PDGFRs on VSCs

3.3.1. Effects of PDGFs/PDGFRs on EPCs

Numerous studies have demonstrated the effects of PDGFs/ PDGFRs on EPCs (Fig. 2). Particularly, PDGF-CC has been intensively studied regarding its roles in EPC regulation. PDGF-CC overexpression increases the proliferation, migration and adhesion of rat EPCs (Tang et al., 2013). It has also been shown that stimulation with PDGF-CC promotes mouse EPC mobilization in a mouse model of limb ischemia (Li et al., 2005). Moreover, PDGF-CC activates PDGFR-α in human bone marrow-derived AC133+CD34+ VSCs and enhances their differentiation into both ECs and SMCs (Li et al., 2005). VEGF, however, only induces the differentiation of VSCs into ECs (Li et al., 2005). Thus, PDGF-CC may play an important role in both the formation and stabilization of blood vessels due to its ability to induce both ECs and SMCs (Li et al., 2005). PDGFR-β has been shown to be essential in promoting the proliferation, migration and survival of EPCs (Guo et al., 2012; Lu et al., 2017; Wang et al., 2012b). In a mouse model of carotid artery injury, transplantation of PDGFR-β-overexpressing EPCs increased vascular reendothelialization and suppressed neointima formation (Wang et al., 2012a).

Table 2

<table>
<thead>
<tr>
<th>Potential markers for human MSCs.</th>
<th>Source</th>
<th>Major findings</th>
<th>Reference PMID</th>
</tr>
</thead>
<tbody>
<tr>
<td>Markers specific for MSCs</td>
<td>Vascular wall</td>
<td>HOX87, HOX6C, and HOX8C are highly expressed in MSCs compared to SMCs, ECs or ESCs. Silence of these genes induces the differentiation of MSCs into SMCs. Linear poly-N-acetyllactosamine is a specific marker for MSCs compared to differentiated adipogenic or osteogenic cells. TM4SF1 is a specific marker for MSCs compared to endothelial cells or fibroblasts.</td>
<td>24145756</td>
</tr>
<tr>
<td></td>
<td>Umbilical cord blood</td>
<td>CD45, CD34, CD11b, CD146, CD13, CD133, and RGS5 are highly expressed in MSCs. CD45 is a specific marker for MSCs compared to other cell types, such as umbilical cord blood.</td>
<td>21933024</td>
</tr>
<tr>
<td></td>
<td>Bone marrow or adipose</td>
<td>CD271 is a suitable marker for MSCs derived from bone marrow and adipose, but not those of other sources, such as umbilical cord blood.</td>
<td>20486778</td>
</tr>
<tr>
<td>Markers for different stages of MSCs</td>
<td>Adipose</td>
<td>Single cell analysis indicates a developmental hierarchy of MSCs. ALDH expression combined with other surface markers determine the premature state of MSCs.</td>
<td>28233376</td>
</tr>
<tr>
<td></td>
<td>Bone marrow or adipose</td>
<td>CD271 is a suitable marker for MSCs derived from bone marrow and adipose, but not those of other sources, such as umbilical cord blood.</td>
<td>19929314, 23689142</td>
</tr>
<tr>
<td>Markers for MSCs of different sources</td>
<td>Bone marrow or adipose</td>
<td>CD271 is a suitable marker for MSCs derived from bone marrow and adipose, but not those of other sources, such as umbilical cord blood.</td>
<td>26184084</td>
</tr>
<tr>
<td>Markers for vascular related functions of MSCs</td>
<td>Bone marrow or adipose</td>
<td>CD271 is a suitable marker for MSCs derived from bone marrow and adipose, but not those of other sources, such as umbilical cord blood.</td>
<td>27888602</td>
</tr>
<tr>
<td></td>
<td>Umbilical cord blood</td>
<td>N-cadherin enrichment determines the therapeutic efficacy of MSCs in a myocardial infarction rat model.</td>
<td>22068423</td>
</tr>
<tr>
<td></td>
<td>Vascular wall</td>
<td>CD44+CD90+CD73+CD34+CD45+ cells behave like MSCs and are capable of differentiation into SMCs and pericytes, thus contributing to vessel formation.</td>
<td>21637782</td>
</tr>
</tbody>
</table>
3.3.2. Effects of PDGFs/PDGFRs on SMPCs

Although the PDGFs are major inducers of SMC proliferation, migration and survival, their roles in SMPC regulation, however, have not been well studied (Ricci and Ferri, 2015). Nonetheless, the effects of PDGFs/PDGFRs on SMPCs have been demonstrated by several studies. For example, PDGF-AA was found to be highly expressed in SMPCs in a pulmonary hypertension model (Jones et al., 2006). Furthermore, it has been shown that during development, PDGF-BB is essential for the differentiation of stem cells into SMCs (Zhang et al., 2010). Moreover, it has been shown that PDGF-BB can induce the differentiation of bone marrow- or vascular wall-derived SMPCs into SMCs (Shen et al., 2016; Yu et al., 2010).

3.3.3. Effects of PDGFs/PDGFRs on MSCs

It has been shown that PDGF-AA and PDGF-BB regulate the proliferation and migration of human bone marrow-derived MSCs via PDGFR-α and PDGFR-β respectively (Fig. 2) (Ball et al., 2010). PDGF-AA promotes the migration of mouse bone marrow-derived MSCs by modulating PDGFR-α activity and by activating the BMP-Smad1/5/8 pathway (Li et al., 2014). PDGF-BB is required to protect MSCs from apoptosis and senescence and to maintain their immunosuppressive effect (Zhang et al., 2016). PDGF-CC and PDGF-DD have been shown to enhance the proliferation of human MSCs and to maintain their osteogenic, adipogenic and chondrogenic differentiation capabilities (Sotoca et al., 2013). However, the roles of PDGF-AA and PDGF-BB in the differentiation of MSCs into vascular cells remain to be better studied (Sotoca et al., 2013). PDGF-BB is not detectable in human adipose-derived MSCs (Hye Kim et al., 2015). PDGF-DD has been shown to display a robust effect on MSC proliferation, migration and the production of other growth factors, such as VEGF-A (Hye Kim et al., 2015). It has been reported that PDGFR-α is abundantly expressed in human MSCs and a high ratio of PDGFR-α versus PDGFR-β has been shown in MSCs (Ball et al., 2007a). Interestingly, it has been reported that in concert with VEGF-A, PDGFR-α and PDGFR-β can promote the proliferation and migration of human MSCs (Ball et al., 2007b). It has also been shown that VEGF-A-induced activation of PDGFR-α is responsible for MSC differentiation into ECs triggered by nitric oxide (NO) deficiency (Gomes et al., 2013). Moreover, PDGFR-α has been used as a marker of VSCs in both human fetal and adult diseased hearts. Such PDGFR-α+ cells have been shown to be able to give rise to both SMCs and ECs, but not cardiomyocytes (Chong et al., 2013).

3.3.4. Effects of PDGFs/PDGFRs on pericytes

Plenty of studies have shown that the PDGFs are essential regulatory factors for pericytes under both physiological and pathological conditions. Genetic deletion of Pdgf-b in mice impairs pericyte coverage of blood vessels (Lindahl et al., 1997). It is reported that PDGF-BB enhances pericyte recruitment and vessel stabilization via the sonic hedgehog pathway (Yao et al., 2014). PDGF-BB has also been shown to promote pericyte recruitment to tumor vasculature (Furuhashi et al., 2004; McCarty et al., 2007; Wang et al., 2015). In addition, PDGF-BB has been reported to promote the transition of pericytes into fibroblasts via PDGFR-β (Hosaka et al., 2016). Moreover, numerous studies have reported the effects of PDGF-CC and PDGF-DD on pericytes. For example, PDGF-CC promotes pericyte recruitment to tumor vessels in osteosarcoma (Kuzmanov et al., 2012). PDGF-D deficiency has been shown to cause vascular defects in Pdgf-d−/− mice leading to abnormal pericyte morphology and disorganized blood vessels in the heart (Gladh et al., 2016). Moreover, in an ex vivo model of sprouting angiogenesis, stimulation with PDGF-DD, but not PDGF-
BB, enhanced pericyte recruitment to endothelial sprouts via PDGFR-β signaling and the NRP1 co-receptor (Muhl et al., 2017). Indeed, PDGFR-β has been shown to be a critical regulator of pericyte proliferation and migration in mouse brain and retina (Fig. 2) (Armulik et al., 2010; Olson and Soriano, 2011).

4. Therapeutic potential of VSCs in retinal vasculopathy

Different types of retinal vasculopathies are the major reasons of vision loss or blindness worldwide. Important retinal vasculopathies include diabetic retinopathy (DR), retinopathy of prematurity (ROP), wet age-related macular degeneration (wAMD), and retinitis pigmentosa (RP). DR is a microvascular complication of diabetes characterized by pericyte and EC apoptosis, increased vascular permeability, inflammation, vascular degeneration, and retinal neovascularization (Beltramo and Porta, 2013). ROP is a devastating retinal neovascular disorder of preterm neonates caused by oxygen toxicity and subsequent hypoxia, leading to retinal neovascularization, retinal detachment and scar formation (Luo et al., 2015). wAMD occurs in aged individuals and is characterized by the damage of retinal pigment epithelium (RPE), choroidal neovascularization and subsequent vision loss (Ma et al., 2015). RP is an inherited retinal dystrophy due to progressive loss of photoreceptors, alterations in RPE, and blood vessel degeneration, which subsequently cause severe vision impairment or blindness (Sorrentino et al., 2015). There are very limited therapies for these blinding diseases. The current available therapies focus mostly on the final stages of the diseases and generally have not given satisfactory outcomes. It is plausible that cell therapies using VSCs aiming at ameliorating the earlier stages of the pathologies may be a promising solution for the treatment of retinal vascular diseases.

4.1. Therapeutic potential of EPCs and MSCs: direct engraftment or paracrine effect?

The therapeutic potential of EPCs and MSCs has been shown in both preclinical and clinical studies. For example, in a murine retinal ischemia reperfusion injury model and a RP model, intravitreally injected CD34+ cells increased the survival and function of photoreceptors and was well tolerated (Moisseev et al., 2016; Park et al., 2012). Importantly, in patients with AMD in a clinical study, treatment with CD34+ cells improved visual acuity without obvious side effect ( Cotrim et al., 2017), even though CD34+ cells are a mixed population. The endothelial colony forming cells (ECFCs) are considered to be more like EPCs and their therapeutic potential have also been demonstrated. In a mouse retinal ischemia model, intravitreally injected ECFCs have been shown to incorporate into the damaged retinal vasculature and reduced the avascular zone (Medina et al., 2010). However, in another study, it is reported that the intravitreally injected ECFCs did not home to the ischemic retinal vasculature but remained in the vitreous in an oxygen-induced retinopathy (OIR) model (Sakimoto et al., 2017). It has been shown that ECFCs can produce various angiogenic factors, such as IGBP2B, which may be responsible for the beneficial effect (Sakimoto et al., 2017). These observations suggest that EPCs may function in a paracrine manner, apart from their direct engraftment into the vasculature in vascular repair (Sakimoto et al., 2017).

MSCs are capable of multipotent differentiation. They can also exert anti-apoptotic and immunomodulatory actions and secrete various neurotrophic factors (Exquer et al., 2014). MSCs thus display a great therapeutic value for the treatment of retinal vascular diseases. Indeed, in a rat model of diabetic retinopathy, intravitreally injected MSCs have been shown to incorporate into the damaged retinal vasculature, alleviated vascular leakage, reduced the death of ECs and ganglion cells, and suppressed the expression of pro-inflammatory molecules. Noteworthy and importantly, the proliferative property of MSCs and their ability to promote EC survival and vessel formation are not compromised by a high glucose level since transplantation of MSCs improved the integrity of blood-retinal barrier in a DR model (Rajashekhar et al., 2014). However, few injected MSCs have been observed to differentiate into photoreceptors or astrocytes (Yang et al., 2010). Yet, even though most of the injected MSCs do not integrate into the retinal vasculature in diabetic mice, they nonetheless demonstrate a protective effect in the retina by secreting neurotrophic factors and reducing oxidative damage (Exquer et al., 2016). In addition, it has been shown that exosomes derived from human MSCs can ameliorate retinal thinning and vascular damage in a ROP mouse model, likely by secreting pro-survival factors (Moisseev et al., 2017). However, on the other hand, under pathological conditions where neovascularization is a severe pathology, the use of MSCs requires caution, since the angiogenic factors secreted by MSCs may exacerbate ocular neovascularization, which is a major reason for vision loss in many ocular diseases (Exquer et al., 2014). Indeed, it has been shown that extracellular vesicles derived from MSCs can increase pericyte detachment, vascular permeability and angiogenesis, all of which can exacerbate DR (Beltramo et al., 2014). Thus, the use of MSCs for the treatment of retinal vascular diseases requires careful evaluation.

4.2. Therapeutic potential of pericytes and SMPCs: restoring the number and function of mural cells is vital

Pericyte loss or dysfunction is one of the early and key pathologies of DR. Therefore, finding ways to maintain normal pericyte number and function is crucial. It has been shown to be possible to replenish damaged pericytes with VSCs. For example, in a rat DR model, transplantation of VSCs restored the integrity of retinal vasculature and decreased vessel permeability (Kim et al., 2016). Also, it is reported that in a mouse ROP model, intravitreally injected adipose-derived pericyte-like cells can integrate into the retinal vasculature and protect the blood vessels from damage in a mouse ROP model (Mendel et al., 2013). Importantly, it is found that pericytes derived from healthy mice display a greater ability of protecting retinal blood vessels from vascular dropout than those derived from diabetic mice (Cronk et al., 2015). Therefore, enhancing the functionality and number of autologous pericytes is vital for the treatment of retinal vascular diseases.

SMPCs have important therapeutic potential for the treatment of ocular vascular diseases. It has been shown that the numbers of SMCs in the retinal vasculature decrease significantly in diabetic retinopathy (vom Hagen et al., 2005). Also, when α-SMA, a marker for SMCs, is depleted, the blood-retinal barrier impairs (Tomasek et al., 2006). Thus, maintaining a normal function and number of SMCs are critical for the retinal vasculature. Thus far, there have not been many studies on the therapeutic usage of SMPC in the treatment of retinal vascular diseases. However, one study has shown that autologous iPSCs can be used to generate SMCs or retinal pigment epithelial (RPE) cells (Zhao et al., 2015). However, SMCs derived from iPSCs can induce T cell response and are highly immunogenic, likely due to the abnormal expression of immunogenic antigens. On the other hand, RPE cells derived from iPSCs are well tolerated when transplanted in vivo. These findings suggest that the in vitro procedures to induce iPSC differentiation into SMPCs or SMCs may trigger the expression of immunogenic antigens. As such, SMCs produced this way may not be ideal for cell therapy. Interestingly, a recent study using an in vivo fate-mapping strategy shows that differentiated SMCs can become a subset of VSCs in the adventitia, which can subsequently differentiate into mature SMCs, ECs and macrophages, suggesting that SMCs might
have properties of stem/progenitor cells (Majesky et al., 2017).

4.3. Clinical trials using VSCs for the treatment of retinal vasculopathy

Several clinical trials using VSCs for the treatment of retinal vasculopathies, such as AMD, DR, RP and vein occlusion (VO) have been conducted and the outcomes are mixed (Table 3). The results of one clinical trial using bone marrow derived CD34+ cells for the treatment of AMD, DR, RP and VO in six patient eyes shows that VSCs are safe and well tolerated (Park et al., 2014). The results of two clinical trials using bone marrow derived CD34+ cells for the treatment of RP show that the CD34+ cells are beneficial and partially restored vision (Siqueira et al., 2013, 2015). Moreover, the outcomes of two clinical trials show that transplantation of CD34+ cells into the eyes of one DR patient and ten AMD patients is safe and improved vision (Cotrim et al., 2017; Jonas et al., 2008). However, despite of these encouraging results, there are also clinical studies showing worrisome outcomes. For example, a recent case report on three AMD patients shows that intravitreal injection of adipose-derived MSCs led to severe adverse events, including vision loss (Kuriyan et al., 2017). Moreover, another clinical study shows that although intravitreal injection of bone marrow-derived MSCs improved vision in two RP patients, it failed to do so in the third patient and this patient developed fibrosis and tractional retinal detachment (Satarian et al., 2017). It is interesting to note that the MSCs derived from the third patient led to retinal fibrosis when injected into mouse vitreous, whereas the MSCs from the other two patients did not (Salarian et al., 2017) (Table 3), suggesting that patient-specific factors may affect the outcome of the therapy. In summary, both promising and dismaying results have been shown from the limited numbers of clinical trials. Future clinical investigations engaging more patients and longer follow-up times are needed to better define the safety and efficacy of VSCs for the treatment of ocular diseases.

5. Conclusions and perspectives

VSCs are capable of generating different types of vascular cells needed for blood vessel formation, repair and regeneration. VSCs therefore play important roles in the vascular system in both normal physiology and diseases. There are four major types of VSCs: EPCs, SMPCs, MSCs and pericytes. Numerous preclinical and clinical studies have shown that VSCs are promising candidates for cell therapy, and their significant therapeutic potential for the treatment of various vascular diseases, including retinal vasculopathy, has been amply demonstrated. On the other hand, many studies have also demonstrated various adverse effects of VSCs that may hinder their clinical usage. Therefore, more in-depth studies are still needed and answers to many important questions are still awaited before satisfying clinical outcomes can be seen. For example, better surface markers that can more accurately define VSCs and their functions remain to be identified. How disease- and individual-specific condition affects the outcomes of VSC therapy needs to be addressed. What are the best ways to expand endogenous VSCs since they are relatively scarce in vivo? How to improve the functions of endogenous VSCs as their functions are often compromised under pathological conditions? Can the PDGFs and their receptors be used to regulate VSCs for therapeutic purposes? Answers to these questions may shed new light into both basic VSC biology and new therapeutic possibilities to the treatment of human diseases.

Conflict of interest

The authors declare no conflict of interest.

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References


Table 3

<table>
<thead>
<tr>
<th>VSC type</th>
<th>Number of subject</th>
<th>Disease</th>
<th>Follow-up time</th>
<th>Result</th>
<th>Clinical trial number/case report PMID</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone marrow-derived CD34+ cells</td>
<td>6 eyes</td>
<td>AMD, DR, VO, RP</td>
<td>6 months</td>
<td>Feasible and well tolerated</td>
<td>NCT01736059</td>
</tr>
<tr>
<td>Bone marrow-derived CD34+ cells</td>
<td>20 patients</td>
<td>RP</td>
<td>3 or 12 months</td>
<td>Vision-related quality of life improved at 3 months, but no difference at 12 months.</td>
<td>NCT01560715</td>
</tr>
<tr>
<td>Bone marrow-derived CD34+ cells</td>
<td>1 patient</td>
<td>RP</td>
<td>1 month</td>
<td>Complete resolution of macular oedema</td>
<td>NCT01068561</td>
</tr>
<tr>
<td>Bone marrow-derived CD34+ cells</td>
<td>1 patient</td>
<td>DR</td>
<td>4 weeks</td>
<td>Feasible and safe</td>
<td>NCT01518127</td>
</tr>
<tr>
<td>Bone marrow-derived CD34+ cells</td>
<td>10 patients</td>
<td>AMD</td>
<td>6 or 12 months</td>
<td>Safe and improved visual acuity</td>
<td>NCT01518127</td>
</tr>
<tr>
<td>Bone marrow-derived CD34+ cells</td>
<td>3 patients</td>
<td>AMD</td>
<td>1 year</td>
<td>Severe vision loss</td>
<td>28579742 Case report</td>
</tr>
<tr>
<td>Bone marrow-derived MSCs</td>
<td>3 patients</td>
<td>RP</td>
<td>1 year</td>
<td>Safe and improved light perception in 2 patients, severe side effects in the third patient</td>
<td>28299008 Case report</td>
</tr>
</tbody>
</table>

multipotent mesenchymal stromal cell stroma prevent or delay the onset of diabetic retinopathy. Acta Ophthalmol 92 (2), e86–95.


Kim, J.M., Hong, K.S., Song, W.K., Bae, D., Hwang, I.K., Kim, J.S., Chung, H.M., 2016. Perivascular progenitor cells derived from human embryonic stem cells exhibit functional characteristics of pericytes and improve the retinal vasculature in a...
Siqueira, R.C., Messias, A., Voltarelli, J.C., Messias, K., Arcieri, R.S., Jorge, R., 2013. Ex vivo differentiated endothelial and smooth muscle cells generate a subpopulation of resident vascular progenitor cells in muscle cells. Stem Cells Int. 2013, 507301.


