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Contribution of pericyte paracrine regulation of the endothelium to angiogenesis

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ABSTRACT

During physiological development and after a stressor event, vascular cells communicate with each other to evoke new vessel formation—a process known as angiogenesis. This communication occurs *via* direct contact and *via* paracrine release of proteins and nucleic acids, both in a free form or encapsulated into micro-vesicles. In diseases with an altered angiogenic response, such as cancer and diabetic vascular complications, it becomes of paramount importance to tune the cell communication process. Endothelial cell growth and migration are essential processes for new vessel formation, and pericytes, together with some classes of circulating monocytes, are important endothelial regulators. The interaction between pericytes and the endothelium is facilitated by their anatomical apposition, which involves endothelial cells and pericytes sharing the same basement membrane. However, the role of pericytes is not fully understood. The characteristics and the function of tissue-specific pericytesis are the focus of this review. Factors involved in the cross-talk between these cell types and the opportunities afforded by micro-RNA and micro-vesicle techniques are discussed. Targeting these mechanisms in pathological conditions, in which the vessel response is altered, is considered in relation to identification of new therapies for restoring the blood flow.

Contents

Abbreviations: AGEs, Advanced glycation end-products; ALP, Alkaline phosphatise; α-SMA, Alpha smooth muscle actin; Ang, Angiopoietin; BM, Basement membrane; CNS, Central nervous system; ECs, Endothelial cells; ECM, Extracellular matrix; EVs, Extracellular vesicles; HSPG, Heparan sulfate proteoglycans; MMPs, Matrix metalloproteinases; MSCs, Mesenchymal stem cells; MPs, Microparticles; miR, microRNA; NG2, Neural/glial antigen 2; PPARγ, Peroxisome proliferator-activated receptor gamma; PDGF, Platelet-derived growth factor; PDGFR-β, Platelet-derived growth factor receptor-β; SMCs, Smooth muscle cells; S1P, Sphingosine-1-phosphate; TGF-β, Transforming growth factor-β; VEGF-A, Vascular endothelial growth factor-A.

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1. Introduction

Despite the large amount of data describing the important role of pericytes in controlling vascular homeostasis, the nature of these cells is still controversial. Lack of specific markers, the associated difficulty to unequivocally identify them, and vast organ-specific differences in number and morphology observed in the pericytes from different source, make pericytes a neglected cell type. Pericytes have been attracting the attention of scientists and clinicians due to increasing body of evidence showing their regenerative properties. Being of mesenchymal nature, pericytes not only finely tune the angiogenic process, but they also retain their plasticity and ability to differentiate into other cell type in a tissue specific manner.

The first description of pericytes dates back to the late 1800s when Eberth and Rouget described them as adventitial/mural cells juxtaposed to capillary endothelial cells (ECs). In 1923, Zimmerman coined the term pericyte referring to their location adjacent to capillaries and embedded within the same basement membrane (BM) (Diaz-Flores et al., 2009). The advent of electron microscopy has helped better define pericytes' anatomical position and morphology. They were described as the cells residing around the endothelial wall of capillaries, having a huge nucleus oriented towards the albuminal side of capillary vessel and with elongated structures that the cell uses to directly interact with underneath endothelium (Allt & Lawrenson, 2001). Depending on the vascular bed and their differentiation state, pericytes exhibit varying morphologies ranging from typical flat and stellate shape in the central nervous system (CNS), to a more rounded shape in kidneys (Armulik et al., 2011).

Most pericytes are identified by the expression of CD146, alpha smooth muscle actin (α-SMA), neural/glial antigen 2 (NG2) and platelet-derived growth factor receptor-β (PDGFR-β) markers, although some exceptions exist. For instance, α-SMA is not noticeably expressed in skin and CNS. The pericytes from capillaries are $NG2^{+}/\alpha\text{-}SMA^{-}$, whereas pericytes from arterioles and venules are respectively $NG2^{+}/\alpha\text{-}SMA^{+}$ and $NG2^{-}/\alpha\text{-}SMA^{+}$, but all equally express CD146 and the PDGFR-β (Crisan et al., 2012).

The pericyte density differs between organs and depends on the stringency of endothelial barrier function (van Dijk et al., 2015). The blood brain barrier or the blood retinal barrier shows 1:1 pericytes/ECs ratio in order to control para-cellular and transendothelial flow, avoiding perturbation of the delicate and highly specialized tissue. Also, higher coverage is observed in the capillaries of lower extremities, which have to counteract orthostatic blood pressure (Sims, 2000). Conversely, human lung and skin have an estimated 10:1 pericytes/ECs ratio. In skeletal muscle the pericytes/ECs ratio was estimated 100:1 even though this evidence is not robust.

Pericytes–ECs communication and vessel stabilization are crucial for the physiology of blood vessels and an impaired stabilization leads to aberrant (excessive or poor) vascularisation typical of vessel pathologies, e.g. diabetic complications, tumor growth and metastasis, kidney diseases or neurodegenerative disorders.

In this article we review the current knowledge on pericytes paracrine interaction with the endothelial cells and discuss the potential strategies to improve it.

2. The multifaceted nature of pericytes: tissue-specific differences

Tissue-specific marker expression of pericytes has been better characterized when researchers attempted to isolate them from different organs. Being that pericytes are crucial modulator of EC function, it was clear that a better understanding of pericytes biology could help develop new therapeutic strategies for pathological conditions in which angiogenesis was impaired. Moreover, pericytes represent a class of cells that could be exploited in regenerative medicine due to their mesenchymal nature that confers their plasticity and ability to differentiate in other cell types. From these isolation/expansion/characterization studies we have learned that pericytes can be subdivided in specialized subclasses that generally resembled the tissue of origin in terms of markers expression and differentiation capacities. For instance, pericytes from the retina were extensively studied, obtained from the retinal surgical leftovers from patients affected by diabetic retinopathy. This is a condition in which the vascular tree is aberrantly increased, and retinal pericytes highly express α-SMA and NG2 (Miller et al., 2006). In subjects with diabetic retinopathy, NG2 pericytes also highly express connective tissue growth factor (CTGF) compared with non-diabetic subjects. CTGF expression is induced by transforming growth factor-β (TGF-β), vascular endothelial growth factor-A (VEGF-A), advanced glycation end-products (AGEs) and plays a role in extracellular matrix (ECM) production and thickening of capillary basement membrane in the retina (Kuiper et al., 2004).

In the CNS, pericytes contribute to the formation of the blood brain barrier, whereby they express PDGFR-β, NG2, and nestin, and locate in between endothelial cells and astrocytes. This is a strategic location since they can differentiate into both of these cell lines and astrocytes tightly envelope the pericyte–endothelial unit with long processes that enter the basal membrane (Hawkins & Davis, 2005). Recently Nakagomi and co-workers demonstrated that brain pericytes can be reprogrammed by hypoxia in vitro and can differentiate both into vasculogenic and neurogenic cells when cultured in proper differentiation media. The different fate is evidenced by the expression of different markers like CDH5, endoglin, thrombomodulin and other markers typical of vascular cells or alternatively Tuj1 or MAP2 when they enter the neuronal path (Nakagomi et al., 2015).

Dermis pericytes from foreskin were isolated and selected for the expression of 3G5 antigen, a useful marker of pericytes in normal human skin because it is not shared by fibroblasts, the most abundant cell type in the skin. Those pericytes also expressed α-SMA and vimentin while being negative for the expression of endothelial markers (Helmbold et al., 2001). Others isolated dermis pericytes using a combination of 3G5 antigen, PDGFR-β and/or high molecular weight melanoma associated marker (HMW-MAA-marker of pericyte activation). Those pericytes were shown to express ANGIOPOIETIN (Ang)-1 but not Ang-2 or Tie2 which were expressed by ECs from the same tissue (Sundberg et al., 2002).

A landmark paper in the field of pericytes control of angiogenesis was published by Campagnolo et al. who demonstrated that the *adventitia* of saphenous vein holds a population of $CD34^{+}/CD31^{-}$ cells expressing also the typical pericyte markers PDGFR-β and NG2. Those pericyte-like cells are located in the close vicinity to *adventitia* of *vasa vasorum* and once isolated they lost the CD34 positivity but retained pericyte markers that rather increase upon differentiation. Isolated saphenous vein pericytes (SVPs) were able to promote ECs tubulization when co-cultured on matrigel in vitro. Upon contact with ECs, SVPs were also able to reorganize N-cadherin, which was polarized on pseudopodia. Saphenous vein pericytes were also able to shed the pro angiogenic Ang-1 in the culture medium, thus fostering ECs tubulisation in a paracrine way (Campagnolo et al., 2010).

Cossu and Dellavalle demonstrated, for the first time, the existence of pericytes in the vascular compartment of skeletal muscle, which show myogenic differentiation ability and can differentiate spontaneously in myotubes, thus contributing to muscle regeneration. These cells express annexin V, alkaline phosphatase (ALP), desmin, vimentin and PDGFR-β at high levels together with α-SMA and NG2 but they do not express ECs (CD31) or myotube (CD56) and myogenic (MyoD, Myf5 and myogenin) characteristic markers. Indeed, when injected in the muscle of mouse dystrophic models, skeletal muscle pericytes formed efficiently dystrophin expressing myotubes (Dellavalle et al., 2007). The hypothesis of myogenic potential of muscle pericytes was further consolidated by the observation in mouse models that following a tissue loss, pericytes fuse with the developing myofibres and enter the satellite cell compartment (Dellavalle et al., 2011). Conversely, Meng recently demonstrated that human $CD133^{+}$ progenitors but not pericytes give rise to $Pax7^{+}$ satellites when transplanted into murine models of