The bone marrow constitutes a reservoir of pericyte progenitors

Chrystelle Lamagna and Gabriele Bergers

Department of Neurological Surgery, Brain Tumor Research Center and UCSF Comprehensive Cancer Center, University of California San Francisco

Abstract: Adult bone marrow is a rich reservoir of hematopoietic and mesenchymal stem and progenitor cells. Mobilization and recruitment of bone marrow-derived cells to injured or ischemic tissue or tumors endorse the initiation and maintenance of angiogenic processes in the adult by incorporating endothelial progenitor cells (EPC) into the developing vasculature and by recruiting accessory hematopoietic cells. Recent data have now revealed that the origin of bone marrow-derived vascular cells is not restricted to endothelial cells but also includes pericytes—the perivascular support cells. Several laboratories have now reported the existence of pericyte progenitor cells, and these cells, like EPC, can be mobilized and recruited to the remodeling vasculature under ischemic conditions and in tumors. This review focuses on pericytes in vessel formation and on recent discoveries about their bone marrow origin in the adult. J. Leukoc. Biol. 80: 000–000; 2006.

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INTERACTION OF ENDOTHELIAL CELLS AND PERICYTES IN BLOOD VESSEL FORMATION

Blood vessels consist of endothelial cells, which form the inner lining of blood vessels and pericytes, also referred to as vascular smooth muscle cells or mural cells, and are embedded within the vascular basement membrane and envelop the surface of the vascular tube. When blood vessels develop in the embryo, mesodermal precursors (angioblasts) or hematopoietic precursors (hemangioblasts) first assemble into a primitive, vascular plexus in a process termed vasculogenesis [1]. This primitive endothelial network then undergoes extensive remodeling, referred to as angiogenesis, in which endothelial sprouting, intussusception, and pruning extend the network to ensure appropriate vascularization of growing organs. In vessel sprouting, endothelial cells invade the surrounding extracellular matrix and form a column of migrating and proliferating endothelial cells. These sprouts then cease proliferation and form a new lumen-containing vessel. Next, vessels become enveloped by pericytes, when activated endothelial cells secrete platelet-derived growth factor (PDGF)-B, which signals through its receptor PDGFR-β, expressed by pericytes, resulting in proliferation and recruitment of pericytes to the newly formed vessels [2, 3]. Pericytes promote vascular maturation and terminate the remodeling process. As a result of their contractile capabilities and their multiple cytoplasmic processes, pericytes have mainly been associated with stabilization and hemodynamic processes of blood vessels. They express myofilaments such as α-smooth muscle actin (α-SMA) or desmin and also PDGFR-β, NG2-proteoglycan/HMW-MMA, and the regulator G-protein signaling RGS5. These markers, however, are not solely pericyte-specific, and their expression is stage- and tissue-specific [4, 5]. In contrast to endothelial cells, pericytes have a more complex ontogeny, as they can develop from various cells, as a function of their location in the embryo. They can arise from the neurocrest [6] to develop into the CNS or from the cardiac tract to envelop coronary vessels [7]. Most commonly, however, pericytes appear to develop from mesenchymal stem cells [8]. It also has been reported that embryonic endothelial cells can transdifferentiate into perivascular cells, although this process appears to be rather rare in normal development [9]. In addition, Flk1+ cells derived from mesodermal tissue can differentiate into endothelial cells or pericytes upon stimulation by vascular endothelial growth factor (VEGF) or PDGF-B, respectively, and organize into vessel-like structures in vitro. This suggests that endothelial cells and pericytes share a common progenitor cell during the development—the Flk1+ angioblast [10]. As soon as homogenous vessels are formed, they undergo arteriovenous differentiation and regional specialization to support the specific needs of the different tissues that they supply. For example, endothelial cells in the brain form a continuous endothelium with tight barriers to prevent the entry of neurotoxic blood-derived factors, whereas in the kidney glomerulus, capillaries are highly permeable to facilitate the exchange of salts and metabolites [11]. Similarly, the dense population of pericytes in the brain, with their elongated and multiple cytoplasmic processes that wrap around the capillaries, is essential to maintaining the blood-brain barrier, whereas pericytes of the glomerular capillaries in the kidney are more compact and rounded, making minimal focal contacts with vessels to ensure sufficient blood ultrafiltration [4, 5].

In summary, endothelial cells and pericytes regulate vessel formation, maturation, and specification, all of which requires the orchestration of tightly regulated molecules. They communicate with each other by direct cell contact and by paracrine signaling pathways. It is therefore conceivable that pericytes

1 Correspondence: University of California San Francisco, Department of Neurological Surgery, 513 Parnassus Avenue, San Francisco, CA 94143-0520. E-mail: gabriele.bergers@ucsf.edu

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and endothelial cells are interdependent and that defects in either can affect the vascular system. For example, defects in PDGF-B/PDGFR-β signaling affect pericytes and endothelial cells, leading to an abnormal vasculature. During angiogenesis in the embryo and in the adult, PDGF-B is expressed by the sprouting endothelial cells, whereas its receptor, PDGFR-β, is localized on pericytes, indicating a paracrine signaling circuit between the two cell types [12, 13]. Indeed, genetic deletions of PDGF-B or PDGFR-β in mice produce identical phenotypes. Mice deficient in PDGF-B or PDGFR-β, both die during late gestation from cardiovascular complications, which endorse widespread microvascular leakage and edema. Blood vessel dilation and microaneurysms in mutant mice correlate with a severe reduction or even a total loss of pericytes on the affected vessels [12, 14–16]. Furthermore, mice bearing specific mutations in the Src homology 2, binding phosphorylated tyrosines of PDGFR-β, and thus, harboring an impaired PDGFR-β signaling pathway have been shown to exhibit a reduction in pericytes in various tissues, such as brain, heart, and retina [17, 18].

It is interesting that deficiency in the PDGF-B/PDGFR-β pathway also affects the functionality of tumor vessels, which are poorly organized, appear tortuous and irregularly shaped, and are often unable to support efficient blood flow [19, 20]. This can be explained by the fact that tumors appear to be in a constant active state of vascular remodeling as a result of the imbalanced expression pattern of angiogenic factors, which involves simultaneous formation and regression of vascular tubes [2]. This phenotype is seen in endothelial cells and pericytes found in tumors. In general, pericytes are less abundant in tumors than in respective normal tissues; they often are more loosely attached to the vascular tubes, and their cytoplasmic processes can extend into the interstitial tumor tissues [4, 21]. For all of these reasons, pericytes were thought to be rather abnormal and dysfunctional in tumors, but recent data from several laboratories point to the opposite conclusion: Tumor pericytes are still functional and important for vessel stability and survival. For example, the administration of a neutralizing antibody against PDGFR-β or pharmacological inhibition of PDGFR-β signaling in a transgenic mouse model of pancreatic tumorogenesis (rat insulin promoter 1-T-antigen 2) leads to the dissociation and depletion of tumor pericytes but not of normal quiescent pericytes and consequently, to enlarged and hyperdilated tumor vessels [22–25]. It is most important that pericyte depletion induced high levels of apoptotic endothelial cells in tumors, as pericytes provide essential survival factors for endothelial cells, such as VEGF [22]. In agreement, immature blood vessels without pericytes were much more vulnerable to anti-VEGF therapy [26], and combinatorial inhibition of PDGF and VEGFR signaling to target pericytes and endothelial cells in tumors had synergistic efficacy in late-stage disease, disrupting the established tumor vasculature and affecting tumor regression [25, 27]. These data further strengthen the knowledge that tumor pericytes function in protecting endothelial cells, partly by expressing the survival factor VEGF [22]. Recent data demonstrated that dysfunctions in pericyte-endothelial communication can even lead to metastatic spread of tumor cells, further supporting the notion that endothelial-pericyte interactions are critical to maintaining a healthy vasculature [28].

**THE BONE MARROW CONNECTION**

Historically, the formation of new vessels has been associated with activation of existing endothelial cells within the injured tissue or tumor. However, several reports describe that angiogenesis in the adult is also supported by the mobilization and functional incorporation of bone marrow-derived circulating endothelial progenitor cells and by the recruitment of accessory hematopoietic cells [29–31]. The percentage of endothelial progenitor cells and other bone marrow-derived cells, which are recruited to the tumor vasculature, can vary dramatically between tumor types and sometimes even between different stages of one tumor type [32, 33]. Therefore, the functional significance of adult vasculogenesis remains an area of intense debate but also of high interest. At least two major pathways have been described for the attraction of bone marrow-derived cells. In the first pathway, chemokines such as VEGF and PDGF, which are released by the tumor or injured tissue, induce and activate matrix metalloproteinase-9 in the bone marrow to release soluble Kit ligand, thereby promoting stem cell cycling and motility [34, 35]. In the second pathway, CXCR4 ligand stromal-derived factor 1 ([SDF-1]/CXCL12), which can be induced by hypoxia and VEGF and is expressed by myofibroblasts or endothelial cells, is involved in the recruitment of CXCR4+ hematopoietic progenitors to ischemic tissues and tumors. It has been proposed that SDF-1 retains CXCR4+ bone marrow-derived cells around angiogenic blood vessels [29, 36, 37].

Recently, bone marrow-derived cells, which developed into pericytes, were identified, enveloping the growing vasculature in tumors and injured tissues. One of the first studies, which proposed the existence of bone marrow-derived pericyte progenitors, showed that CD11b and CD45 hematopoietic cells, expressing the pericyte marker NG2, are detected in close proximity to blood vessels in a subcutaneous Bl6-F1 melanoma model [33]. The existence of bone marrow-derived pericyte progenitor cells (PPCs) was further substantiated by the identification of PDGFR-β+ pericyte progenitors (PPPs) in an endogenous mouse model of pancreatic tumorogenesis [22]. It is important to note that in these tumors, only perivascular cells express PDGFR-β, whereas endothelial cells secrete the ligand PDGF-B, resembling the same situation as during embryonic vessel formation. PPPs in these tumors were able to differentiate into mature pericytes, expressing the markers NG2, α-SMA, and desmin, as well as regulate vessel stability and vascular survival of tumors [22]. It is interesting that whereas PDGFR-β+ PPCs induced NG2 and α-SMA when cultured alone, coculture of PDGFR-β+ PPCs with endothelial cells was essential to promote development of desmin+ pericytes. This suggests the necessity of paracrine signaling pathways between endothelial cells and pericytes to enable the induction of desmin. It is intriguing that a subset of PDGFR-β+ PPCs was found to be recruited from the bone marrow to perivascular sites within tumors. These bone marrow-derived cells expressed hematopoietic markers such as
stem cell antigen-1 (Sca-1) and CD11b, although it is still unclear whether PPPs are of hematopoietic or mesenchymal origin. Bone marrow transplant experiments and cultures of bone marrow-derived or tumor-derived Sca-1+ cells with endothelial cells revealed that it is the Sca-1+ cell population from the bone marrow that is recruited to angiogenic sites of the tumor and then matures into pericytes [22]. In agreement with these findings, pericyte progenitors also appear to be recruited from the bone marrow in an orthotopic model of neuroblastoma (Fig. 1) [39].

There are several indications that recruitment of pericyte progenitors is not limited to tumors. Bone marrow-derived pericyte progenitors were also found to infiltrate the brain after middle cerebral artery occlusion in an experimental mouse model stroke. PPPs were observed around growing blood vessels in ischemic areas and developed into desmin+ pericytes, which express TGF-β and VEGF [40]. Furthermore, bone marrow-derived cells were observed in angiogenic vessels of the cornea after basic fibroblast growth factor-induced neovascularization and differentiated into NG2+ /PDGFR-β+ perivascular cells [41]. It is interesting that only 50% of the NG2+/PDGFR-β+ PPP population was derived from the bone marrow and expressed the hematopoietic markers CD45 and CD11b.

Given that only subsets of PPPs are recruited from the bone marrow [22, 41], it is conceivable that PPPs can also be attracted to angiogenic vessels from the local environment or become activated within the injured tissue or tumor. For example, it has been shown that stroma-derived mesenchymal progenitor cells found in tumors, expressing the markers Sca-1+/Tie2+/CD13+, can differentiate into α-SMA+ pericytes, which then become negative for Tie-2 [30].

It is important to note that there are several reports describing the presence of bone marrow-derived cells that display perivascular localization in tumors but are not pericytes. Among these cells, hematopoietic and monomyelocytic cells probably represent the largest population of bone marrow-derived cells [35]. De Palma et al. [42] described a population

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**Fig. 1.** Pericyte progenitors are recruited from the bone marrow and the tumor microenvironment. Just as demonstrated for endothelial progenitors, it is likely that tumors secrete cytokines, which induce a cascade of events leading to the mobilization of bone marrow-derived pericyte progenitors to angiogenic sites of tumors or injured tissue (Steps 1–5). At least two populations of bone marrow-derived cells give rise to tumor pericytes (Step 6). The PDGFR-β+/ Sca-1+/CD11b+ population differentiates into NG2+/α-SMA+/Desmin+ mature pericytes [22], whereas the CD45+/CD11b+ cell population differentiates into pericytes, which only express NG2 [38]. In addition, pericyte progenitors can originate from the local environment. These cells were shown to express the markers Sca-1, Tie2, and CD13 and develop into α-SMA+ cells [30].
of Sca-1+/CD45+/CD11b+ myeloid tumor-infiltrating cells that express the Tie2 angiopoietin receptor (Tie2-expressing monocytes (TEMs)), which promotes tumor angiogenesis. When co-injected with mammary tumor cells, TEMs increase the number of CD45+ infiltrating leukocytes and NG2+ pericytes. However, even when TEMs displayed a perivascular location similar to that of pericytes, they did not express the pericyte markers NG2 or α-SMA [30]. Similarly, Grunewald et al. [29] described the recruitment of CXCR4+ bone marrow-derived cells, which localize in close proximity to blood vessels but do not appear to contribute to the pericyte population. Finally, myofibroblasts, which resemble fibroblasts but share mechanical properties and cytoskeletal proteins such as α-SMA with smooth muscle cells, were also reported to partly originate from the bone marrow in tumors [43], but in contrast to pericytes, myofibroblasts display a stromal localization.

In summary, there is emerging evidence that bone marrow-derived cells play a crucial role in the formation of blood vessels in the adult by incorporating into the growing vasculature, localizing adjacent to it, or enveloping the vasculature to serve as permissive support cells.

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