Perivascular Ancestors of Adult Multipotent Stem Cells

Mirko Corselli, Chien-Wen Chen, Mihaela Crisan, Lorenza Lazzari, Bruno Péault

Abstract—Independent studies by numerous investigators have shown that it is possible to harvest multipotent progenitor cells from diverse dissociated and cultured fetal, perinatal, and principally adult developed tissues. Despite the increasingly recognized medical value of these progenitor cells, the archetype of which remains the mesenchymal stem cell, this indirect extraction method has precluded the understanding of their native identity, tissue distribution, and frequency. Consistent with other researchers, we have hypothesized that blood vessels in virtually all organs harbor ubiquitous stem cells. We have identified, marked, and sorted to homogeneity by flow cytometry endothelial and perivascular cells in a large selection of human fetal, perinatal, and adult organs. Perivascular cells, including pericytes in the smallest blood vessels and adventitial cells around larger ones, natively express mesenchymal stem cell markers and produce in culture a long-lasting progeny of multilineage mesodermal progenitor cells. Herein, we review results from our and other laboratories that suggest a perivascular origin for mesenchymal stem cells and other adult progenitor cells. Recent experiments illustrate the therapeutic potential of human pericytes to regenerate skeletal muscle and promote functional recovery in the diseased heart and kidney. (Arterioscler Thromb Vasc Biol. 2010;30:1104-1109.)

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Be fruitful and multiply.

—Genesis 1:22

It is only at early embryonic stages that massive stem cell recruitment, expansion, migration, and differentiation can be visualized and followed within emerging tissues. As development proceeds and adult maturity approaches, stem cells rarefy, and tissue renewal and repair usually become quantitatively marginal, which makes it difficult or impossible to document stem cell presence and activity in anatomic terms. Conformational traits of a given organ have permitted more precise allocation of stem/progenitor cells, facilitating experimental tracking of their regenerative role. This is the case for crypt cells in the intestine, satellite cells in skeletal muscle, and the bulge region of hair follicles, where multipotent skin stem cells reside. Even the best-known stem cells remain difficult to identify in situ within their natural environment: the adult hematopoietic stem cell has been thoroughly characterized in terms of phenotype and function, yet remains an elusive constituent of the bone marrow tissue, where the stromal elements responsible for the maintenance, retention, proliferation, and differentiation of blood-forming cells are controversial.1 Perhaps the most extreme example in terms of tissue elusiveness is adult multilineage stem cells, known as mesenchymal stem cells (MSCs),2 multipotent adult progenitor cells,3 muscle-derived stem cells,4 or fat tissue–derived stem cells,5 each of which has only been identified in cultures of its dissociated tissue of origin, giving no clue as to their native identity, frequency, and anatomic location.

Multipotent stem cells with grossly identical characteristics and developmental potentials have been indirectly extracted from multiple mouse and human organs, such as skeletal muscle, bone marrow, skin, pancreas, fat, dental pulp, placenta, and umbilical cord. Thus, we assumed that a structure common to all these organs is hosting these ubiquitous regenerative units and the candidate tissue that naturally stood out is the blood vessel. Blood vessels are present in virtually all organs and are already known to contribute nonvascular cells, such as hematopoietic cells6 and myogenic cells.7 Studies described in the current review support the contention that perivascular cells constitute a stock of multilineage progenitor cells. Combinations of immunohistochemistry and stringent cell sorting techniques have been used to identify and purify discrete populations of perivascular cells in human organs. These experiments have shown that pericytes (also known as mural cells) that surround endothelial cells in capillaries and microvessels8 and adventitial cells found around larger vessels natively express antigenic markers of MSCs and behave in the long-term in vitro culture like genuine MSCs. We conclude by postulating the existence...
of a ubiquitous reserve of multilineage progenitor cells in the immediate vicinity of capillaries, veins, and arteries of all sizes.

Affiliation Between Pericytes and MSCs
Recent studies have documented the existence of similarities between MSCs and pericytes; however, neither cell sorting nor stringent cell characterization was performed to further support a relationship between these cell types. A positive correlation between MSC frequency and vascular density in horse fat tissue also suggests an association between stem cells and blood vessels. We have used multicolor immunohistochemistry and confocal microscopy to mark pericytes in multiple human tissues (ie, skeletal muscle, pancreas, adipose tissues, skin, placenta, umbilical cord, bone marrow, heart, brain, and dental pulp) as expressing surface markers NG2, CD146, and platelet-derived growth factor β, at the exception of any known hematopoietic, endothelial, and myogenic cell marker. Human pericytes also express alkaline phosphatase and the human dexam 1 antigen. Fluorescence-activated cell sorter–purified pericytes were readily myogenic in culture and after injection into cardiotoxin-injured or genetically dystrophic severe combined immunodeficient mouse skeletal muscles. Pericytes grow proficiently in culture, exhibiting the morphology, mitotic activity, and surface antigens of MSCs (eg, CD44, CD73, CD90, and CD105). Full identity between MSCs and cultured pericytes was also observed in terms of developmental potential because the latter differentiate clonally into bone, cartilage, and fat cells when cultured under relevant inductive conditions. Moreover, human pericytes sorted from diverse sources regenerate muscle, bone, and even skin in vivo and in organ culture. Pericytes have been suggested to play a role in skin regeneration. Collectively, these results suggest that a fraction, at least, of the multilineage stem cells that emerge in primary cultures of multiple fetal and adult organs is descended from vascular pericytes. In further support of this concept, intact pericytes in their tissue of origin natively express the MSC markers CD44, CD90, CD73, and CD105. This physical association between blood vessels and multilineage progenitors may have been acquired early during evolution because a population of vascular mural cells has been described around the lateral dorsal aortae and anterior mesenteric arteries of the developing zebrafish. These cells share many of the morphological, molecular, and functional characteristics of vascular smooth muscle cells and pericytes found in higher vertebrates.

As an important caveat, pericytes have only been referred to in the strictest etymologic sense (ie, as periendothelial cells surrounding capillaries and microvessels, in all previously described investigations). As a consequence, the heterogeneity of this cell compartment may have been overlooked. Pericytes are functionally defined as contractile cells around the endothelium of microvessels, which serve to control the blood flow. However, we have identified a minor subset of pericapillary cells that express all pericyte markers (ie, NG2, CD146, and platelet-derived growth factor β), but do not contain α-smooth muscle actin. These cells may represent more primitive elements in the pericyte hierarchy, endowed with the described progenitor cell potential. Therefore, whether all bona fide contractile pericytes are MSC founders is unknown. The term pericyte has been used in its anatomic literal sense, without any functional connotation.

Nonpericyte Perivascular Cells as Alternate Originators of MSCs
All blood vessels apart from capillaries are composed of 3 layers (ie, tunica intima, tunica media, and tunica adventitia). The tunica intima consists of a single layer of endothelial cells, whereas the tunica media contains smooth muscle cells and represents the muscular component of blood vessels. The tunica adventitia is the outermost layer of blood vessels and contains fibroblasts and connective tissue. For a long time, the tunica adventitia has been considered an inactive component of blood vessels involved in structural support of the tunica media. Only recently has it been demonstrated that the adventitia plays a role in vascular remodeling and the development of vascular diseases, such as arteriosclerosis and restenosis. Indeed, adventitial cells can be activated in response to injury. The activation of adventitial progenitors results in proliferation, differentiation into myofibroblasts that migrate into the inner layers of the vascular wall, alteration of extracellular matrix deposition, and release of paracrine factors regulating vascular remodeling. For example, Hu et al identified and isolated in apoE−/− mice adventitial progenitor cells that are able to differentiate in vitro and in vivo into smooth muscle cells. These cells carry the LacZ gene under the control of the smooth muscle–specific promoter SM22 and are transplanted in the adventitial side of vein grafts in mice, where they differentiated into myofibroblasts and migrated into the inner layer of the vessel, as demonstrated by the presence of β-gal− smooth muscle cells in the neointima up to 4 weeks after grafting. Furthermore, most smooth muscle cells in the vascular lesion were derived from the adventitia, thus demonstrating the active contribution of adventitial progenitors in the establishment of the vascular disease.

The differentiation potential of adventitial progenitors is not restricted to myofibroblasts. Ingram et al have shown that the vessel wall, as with bone marrow and peripheral blood, contains endothelial progenitor cells involved in angiogenesis. CD34−CD31− progenitors able to differentiate into mature endothelial cells and form capillary sprouts were then localized in the “vasculogenic zone,” between the tunicae media and adventitia of the human thoracic aorta. As previously described, it was demonstrated that MSC–like cells originate from pericytes surrounding capillaries and microvessels. Multipotent progenitors displaying MSC phenotypic and developmental properties have also been described in the bovine artery wall and have recently been isolated from the tunica adventitia of the human pulmonary artery. These observations suggest indirectly that pericytes, exclusively present around capillaries and microvessels, are not the only ancestors of MSCs, as hypothesized previously. However, neither the phenotype of these adventitial progenitors nor their progeny of MSCs in culture has been described. We recently organized a sorting strategy to simultaneously...
isolate pericytes and other cell populations from the stromal vascular fraction of human adipose tissue, based on CD34, CD31, and CD146 differential expression. Apart from pericytes, only CD34<sup>hi</sup>CD31<sup>−</sup>CD146<sup>−</sup> cultured cells were able to yield a progeny of cells exhibiting the morphology, phenotype, and developmental potential of MSCs. Thorough immunolocalization in multiple human organs revealed that CD34<sup>hi</sup>CD31<sup>−</sup>CD146<sup>−</sup> cells reside in the tunica adventitia of blood vessels and, similar to pericytes, natively express MSC surface markers. These results strongly suggest that the tunica adventitia hosts multipotent progenitors that could be recruited from the “outside in,” in the case of vascular remodelling, and also be mobilized to the surrounding environment to participate in postnatal vasculogenesis and tissue repair (Corselli M, PhD, unpublished data, 2009). It will be of particular interest to document the role of these adventitial progenitors in the emergence and expansion of the vasa vasorum. Fluctuations in the size, cellularity, and properties of this network of small blood vessels, which irrigate the walls of large arteries, have been clearly correlated with the development of atheromatous plaques and atherosclerosis; however, the possible role of endogenous progenitor cells in this process remains unknown.

**Percytes: Applications in Tissue Repair and Regeneration**

Stem cell–based tissue repair and regeneration has been deemed promising as a result of multiple advantages of stem/progenitor cells, including differentiation into desired cell lineages, paracrine secretions of growth factors, immunomodulation, regulation of postinjury tissue remodeling, and activation of endogenous repair/regeneration mechanisms. As previously described, researchers recently unraveled the multipotency of purified perivascular cells in vitro and in vivo. With the wide distribution of pericytes throughout the microvasculature of the human body, these cells may serve as a source of autologous stem cells for clinical applications. Furthermore, given the vascular affiliation of multipotent stem/progenitor cells, it was hypothesized that these cells are able to assist vascular restoration in damaged human organs more efficiently. A few of the most striking progresses in pericyte-based regenerative medicine are documented in the next section.

The ability of postnatal skeletal muscle to repair and regenerate itself on daily physical activity or injury is well documented. However, severe pathological conditions, such as compartment syndrome and muscular dystrophy, impede structural and functional recovery mediated by myogenic progenitors and require exogenous interventions to ameliorate the progression. Transplanted pericytes, purified from human skeletal muscle, fat, pancreas, and placenta, regenerate human myofibers in cardiotoxin-treated and dystrophic mouse muscles more efficiently than do myoblasts, total unfractionated tissue-derived cells, or endothelial cells. In addition to structural regeneration, functional recovery was demonstrated in dystrophic mouse tissue treated with pericytes isolated from muscle biopsy specimens from not only healthy adults but also, surprisingly, patients with Duchenne muscular dystrophy. Collectively, these results highlight the potential for pericytes to be used in the treatment of muscular injury and dystrophy in the clinical setting.

Coronary heart disease, caused by conditions that interfere with the coronary blood supply, may result in prolonged ischemia leading to ischemic cardiomyopathy and myocardial infarction. The limited capacity of the adult human heart to repair/regenerate after myocardial infarction results in short-term loss of cardiac function, dynamic ventricular remodeling, and progressive cardiac dysfunction, frequently leading to heart failure or death. A number of stem/progenitor cell therapies have been investigated as alternatives to heart transplantation in clinical trials, including skeletal myoblasts and bone marrow–derived cells, with variable success. There is a linear relationship between the outcome of treatment and the type of cells applied. Thus, stem cell populations that harbor high efficiency to restore impaired cardiac function and the ability to integrate into host cardiac tissue, as well as the desirable properties previously described, would be ideal for cardiac cell therapy. Furthermore, given the vascular nature of the pathology of coronary heart disease, stem/progenitor cells capable of repairing/regenerating host vascular networks may further increase the chance of success.

The existence in human skeletal muscle of a rare subset of myoendothelial cells was recently reported. These cells exhibit a superior potential in sustaining cardiac function after acute myocardial infarction than myoblasts and endothelial cells. The injection of myoendothelial cells significantly induced neoangiogenesis and reduced fibrosis in infarcted mouse hearts. Pericytes, with their inherent actions on the vasculature and recently documented multipotency, seem to match the scope of ideal cells for cardiac repair. By using purified skeletal muscle pericytes, we designed a series of experiments to determine the potential of these cells for cardiac repair/regeneration. Functional analyses showed that improved cardiac function was sustained by transplantation of cultured CD146<sup>hi</sup>CD34<sup>−</sup>CD45<sup>−</sup>CD56<sup>−</sup> pericytes into acutely infarcted hearts of nonobese diabetic mice with severe combined immunodeficiency. Muscle-derived pericytes also exhibited cardioprotective effects, such as induction of angiogenesis and reduction of scar formation (Chen CW, MD, unpublished data, 2009). Collectively, the results underline the clinical potential of purified pericytes as an effective donor cell population for cardiac therapy.

Osteogenic, odontoblastic, and adipogenic progenitors have also recently been shown to originate from perivascular niches in vivo, in agreement with the robust osteogenic and adipogenic properties found in purified pericytes. These discoveries imply that pericytes can potentially be applied to bone regeneration, dental repair, and adipose reconstruction.

Novel stem cell supplies have been explored in the past few years within fetal, perinatal, and adult organs. A promising stem cell source is represented by umbilical cord blood and the umbilical cord itself, not only in the treatment of hematologic diseases but also in the field of nonhematologic regenerative medicine. In 2000, MSCs were extracted for the first time from cord blood
(CBMSCs), however, the detailed study of these cells has taken place more recently. The morphology, division characteristics, and surface markers of CBMSCs are similar to those of bone marrow–derived MSCs, except for some differences in the expression of CD105 and CD44. More important, CBMSCs have also been identified as perivascular cells expressing CD146, platelet-derived growth factor, alkaline phosphatase, and NG2, but not CD34 or CD45. Owing to the noninvasive nature of cord blood collection, the unlimited availability of this tissue and its stem cell potential, CBMSCs could exhibit a significant advantage over other types of adult and embryonic stem cells for several clinical uses. For instance, scenarios for renal regeneration are urgently needed because acute and chronic kidney disease is a leading cause of morbidity and mortality worldwide, with overall mortality rates between 50% and 80%. Human CBMSCs/pericytes have been transplanted into an animal model of chemotherapy-induced acute renal failure. Higher therapeutic efficacy, including complete restoration of kidney function, was observed after infusion of CBMSCs/pericytes compared with regular bone marrow–derived MSCs. However, few donor cells were found in the restored area; also, it was shown in culture and in vivo that the observed renoprotective effects are mediated mainly by angiogenic and antiapoptotic factors secreted by the CBMSCs/pericytes.

Another promising source of stem cells is the umbilical cord itself, within which Wharton jelly (a gelatinous substance that insulates umbilical blood vessels) is a recognized source of MSCs. Perivascular cells have also been identified and characterized in full-term umbilical cords and at a higher frequency in fetal/preterm umbilical cords. Umbilical cord pericytes express the same surface markers as in other tissues; they also express SSEA-4, a glycolipid antigen that is commonly used as a marker of human embryonic stem cells and embryos at cleavage to blastocyst stages was also recently identified in bone marrow MSCs. Umbilical cord pericytes also express Oct-4, an embryonic cell marker arguably considered an indicator of stemness, underlining the multipotency of these cells. In the perspective of cell therapies, the migratory potential of human perivascular cells is an important parameter in seeking to ensure homing to injured tissues. A migration assay was devised in transwell cultures, in which a lung alveolar type II cell line was damaged with bleomycin and then exposed to human fetal umbilical cord pericytes. In this setting, the pericytes were mobilized and migrated toward the damaged cells, secreting high levels of antiapoptotic and angiogenic factors, such as vascular endothelial growth factor and keratinocyte growth factor. These findings suggest that pericytes can efficiently move to damaged sites and secrete growth factors that can play beneficial autocrine or paracrine roles in tissue repair.

Conclusions

The existence of a perivascular native ancestor of the cultured MSC is supported by the previously reported observations. Vascular adventitial cells were recently added to the pericytes initially placed at the top of the MSC hierarchy; despite a different antigenic profile, these adventitial cells possess the same ability to generate a progeny of multipotent cells in culture (Figure). Therefore, blood vessels of all diameters appear to be surrounded by cells endowed with broad developmental potential. We have almost no information regarding the role that perivascular progenitors may naturally play in organ development, renewal, and repair. Two exceptions to this are the ability of pericytes to regenerate chemona-blated Leydig cells in the rat testis and white adipocyte progenitors belonging to the mural cell compartment in mouse adipose tissue vasculature. These observations must be confirmed within multiple other cell lineages before a generalized role of perivascular cells in tissue homeostasis can be concluded. Nevertheless, as previously illustrated, perivascular cells are strong candidates for medical tissue regeneration, even before expansion in culture into MSC-like
progenitors. Although pericytes are able to regenerate the damaged skeletal muscle by direct contribution to the stock of myofibers, chimera was modest or absent in pericyte-treated hearts and kidneys, despite significant functional improvement (Chen DW, MD, unpublished data, 2009).

This suggests that pericytes, like their MSC progeny, also act as paracrine/juxtacrine cells, possibly recruiting host stem cells to the site of injury. Indeed, purified human pericytes secrete diverse growth factors, some abundantly, and are, therefore, candidate trophic “drugstore” cells.

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Disclosures

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References

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