Pericytes in Development and Pathology of Skeletal Muscle

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Abstract: Increasing attention is currently devoted to the multiple roles that pericytes (also defined as mural, Rouget, or perivascular cells) may play during angiogenesis, vascular homeostasis, and pathology. Many recent excellent reviews thoroughly address these topics (see below); hence, we will not discuss them in detail here. However, not much is known about origin, heterogeneity, gene expression, and developmental potential of pericytes during fetal and postnatal development. This is likely because of the paucity of markers expressed by pericytes and the absence of truly unique ones. Thus, in vivo identification and perspective isolation are challenging and explain the relative little data available in comparison with neighbors but far more characterized cells such as the endothelium. Despite this preliminary knowledge, we will propose that contribution to growing mesoderm tissues may be an important role for pericytes. Thus, their ability to contribute to tissue regeneration may be a consequence of their role in tissue growth. However, in a severely damaged or diseased tissue, acute or chronic inflammation likely results in the production of signaling molecules that are different from those present in developing tissues, thus explaining why pericytes are easily diverted from a regenerative to a fibrotic fate. (Circ Res. 2013;113:341-347.)

Key Words: angiogenesis ■ pericyte differentiation ■ smooth and skeletal muscle ■ tissue histogenesis and regeneration

Pericytes are mural cells that surround the endothelium of small vessels. They regulate numerous functions, such as vessel growth, stabilization, and permeability.1,2 Currently, there is no real distinction other than differential location across a basal lamina, with vascular smooth muscle cells, so that, we do not know whether pericytes are progenitors or neighbors of the latter cell types.3,4,5 In addition, pericytes have been causally linked to vascular calcification6 and atherosclerosis7 as well as tumor angiogenesis.8,9 Diseases that represent the most frequent cause of morbidity and mortality in the Western world. Despite their crucial role in physiological and pathological angiogenesis,10 not much is known about pericytes,11 possibly because of their paucity, anatomic distribution, alleged heterogeneity, and existence of very few, if any, truly specific markers.10 Some of these markers, such as smooth α actin (SMA), Desmin, and PDGF receptor β, are also expressed by skeletal myoblasts,12–14 myofibroblasts, and other cell types.15 Other markers, such as Alkaline Phosphatase (AP), are expressed by osteoblasts,16 liver,17 and kidney cells,18 and still others, such as Annexin V, are expressed also by apoptotic cells.19 To make the situation even more complex, there is no single pericyte that expresses all markers simultaneously and, currently, there is no evidence that expression of subsets of markers may identify a specific subset of pericytes. This is one of the reasons why very little is known about these cells; different methods to isolate and expand them in vitro have been published, but until now mainly from retinas, and it is unclear whether they maintain a bona fide pericyte phenotype after long-term culture.20–22

Origin of Pericytes

During embryogenesis, pericytes originate from both lateral and paraxial mesoderm, more specifically from the splanchnopleura and the somites, respectively (reviewed in References 11, 23, 24). A single vessel may contain pericytes originating from both layers.25 Pericytes of the head, the thymus, and of the outflow tract of the aorta originate from neural crest, whereas pericytes of vessels in the internal viscera originate from the mesothelium, a single layer of squamous epithelium that covers the coelomic cavities (the future peritoneum, pericardium, and pleura). Probably in all cases, pericytes are recruited by endothelial cells of the growing vessels through a PDGF-BB/PDGF receptor β loop.26 In addition, the newly emerging concept of endothelial-mesenchymal transition, mainly described for pathological conditions,27–29 implies that an endothelial cell undergoes a mesenchymal transition and remains, at least transiently associated with the vessel as a bona fide pericyte. However, endothelial-mesenchymal transition also occurs during embryogenesis and has been well studied in the heart.30,31 In the body wall of the mouse fetus, we occasionally observed CD34 expression, usually confined to endothelial cells, also in few cells located outside of the endothelium where SMA is expressed. These cells may represent endothelial cells in the

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process of undergoing an endothelial-mesenchymal transition. In addition, AP positive cells are located in the external layer of the vessel (Figure 1). Published lineage tracing experiments do not address this issue in details, so that the question is still unanswered.

Finally and equally importantly, pericytes are able to proliferate and, therefore, new pericytes can be derived from in vivo expansion of preexisting pericytes. In conclusion, it seems that multiple roads from multiple sources lead to the formation of a pericyte. During the rapid growth of a blood vessel that occurs either in fetal or tumor growth, endothelial cells need pericytes and may recruit to this fate surrounding mesoderm cells,32 which may only need the widely expressed PDGF-Rβ to respond and be recruited.

Promiscuity in Fetal and Postnatal Developing Mesoderm

It is generally assumed that mammalian embryonic cells are instructed towards one or another fate choice by signals emanating from neighboring tissues (eg, notochord released Sonic Hedgehog specifies the ventral neural tube). This model accommodates the immense amount of data that accumulated during the past 20 years (see for example33). The border of 2 adjacent tissue anlagen may be exposed to subthreshold levels of signals dictating different developmental choices and, thus, maintain a sort of bi or tripotent state.34 A second problem with this model relates to fetal development. At this stage, all the primordia of organs and tissues have been laid down, and subsequent development is essentially growth in size of structures that maintain the same shape, although relative proportion (eg, head versus body) may vary. Growth occurs by hypertrophy (growth in size of single cells), hyperplasia (proliferation of pre-existing cells), accumulation of extracellular matrix, and finally, addition of new cells migrating into the developing tissue. Besides migratory events that occur during embryogenesis (neural crest, myoblasts to the limb, germ cells, reviewed in Reference 35) another possible way for cells to enter a tissue would be in association with growing vessels awaiting to be instructed by signals emanating by already present differentiating/differentiated tissues. For example, vessels entering into developing skeletal muscle would be embedded in a loose connective tissue filled with proliferating skeletal myoblasts, differentiated primary myofibers,36 and possibly less committed mesoderm cells, generally and improperly termed fibroblasts. Thus, cells associated with vessels could be recruited by differentiating cells. Intriguingly, these cells, once explanted in culture37 have a hybrid phenotype, coexpressing some early endothelial markers, SMA, and in some case also myogenic determination gene (MyoD). Once established as clonally derived, proliferating mesoderm cells (termed mesoangioblasts) mainly express only 1 family of markers, depending on the stage at which they are derived. It is important to underline that mesoangioblasts do not exist as such in vivo but represent the in vitro expanded counterpart of vascular cells, just like embryonic stem cells represent the in vitro expanded counterpart of the blastocyst inner cell mass. When isolated from the embryonic aorta, mesoangioblasts express early endothelial markers (ie, CD34, vascular-endothelial cadherin, fetal liver kinase 1) and SMA.38 However, when isolated from postnatal tissues, mesoangioblasts express mainly pericyte markers (ie, AP, SMA, desmin, neural glial proteoglycan 2, PDGF receptor β).39 Although this fact may simply reflect the use of the same name for different cell types, it is possible that, as mentioned above, an endothelial cell undergoes EndoMT in the fetus and turns into a pericyte whose remains associated with the growing vessel.

Pericytes Can Be Converted to Skeletal Myoblasts and Vice Versa

When pericytes are isolated and cultured from postnatal skeletal muscle, they show the ability to undergo skeletal myogenesis, on proper culture conditions.39 This ability varies among different mouse strains and even among individual clones from the same mouse. Skeletal muscle differentiation is also variable among pericytes from different human donors and, in some cases, it is indistinguishable from differentiation of myoblasts derived from satellite cells, the canonical resident stem cells of skeletal muscle.40,41 In contrast, embryonic/fetal mesoangioblasts are unable to differentiate spontaneously and require coculture with bona fide skeletal myoblasts. This fact is open to different interpretations: 1 is that fetal cells follow the growing vessels awaiting to be instructed by signals...
released by the tissue where the vessel enters. In contrast, adult cells have already been instructed and maintain in vitro the memory of this commitment. This is why pericytes from bone and from skeletal muscle have many similar features but are committed to give rise to bone, cartilage and fat in one case and to skeletal muscle in the other, besides smooth muscle that may be considered a default pathway.

Fetal skeletal muscle can be seen as a mesoderm tissue composed of primary muscle fibers, aligned in parallel within a bundle that is the anlagen of the future individual muscle. The remaining is a loose connective tissue, rich in hyaluronic acid, and filled with undifferentiated mesoderm cells. These include fetal or secondary myoblasts (bound to form secondary fibers, initially disposed around the primary fibers), fibroblasts, mainly responsible for the production of the extracellular matrix that will form the endomysium and the perimysium and possibly pericyte progenitors. Vessels and nerves progressively penetrate into this tissue to established close and well-patterned interactions with the muscle fibers.

Several years ago, we proposed that cells from the vessel wall may receive signals instructing them to adopt the fate of the specific tissue, skeletal muscle in this case. This can be demonstrated by transplanting entire embryonic vessels or cells isolated from them inside a developing host embryo. This experiment, performed initially with quail cells in a chick host, proved that the cells have the potential to contribute to the formation of skeletal muscle; however, it did not prove that skeletal myogenesis is a natural fate of vascular associated cells during unperturbed tissue development. To address this issue it was necessary to lineage trace pericytes during pre- and postnatal development. This turned out to be a difficult task because, as mentioned above, there are no known genes exclusively expressed in pericytes, and many are also expressed in neighbor myoblasts. After considering different possibilities, we generated a transgenic mouse expressing the inducible Cre recombinase (Cre recombinase-estrogen receptor) under the transcriptional control of the Tissue Nonspecific AP (TNAP) gene. Despite this frightening name, the enzyme, after birth, is exclusively expressed in pericytes of striated muscle and many are also expressed in fetal or secondary myoblasts (bound to form secondary muscle fibers). Clonal analysis indicated that ≈70% of clones derived from TNAP expressing cells homogeneously express smooth muscle myosin, and the remaining 30% expresses homogeneously express skeletal muscle myosin. Mixed clones were rare, suggesting that at P10 TNAP expressing pericytes have already chosen a smooth or a skeletal muscle fate. Pericytes, and not endothelial cells, contribute to growing skeletal muscle because similar experiments repeated with mice expressing the Cre recombinase-estrogen receptor under transcriptional control of the endothelial-specific vascular endothelial cadherin gene, showed that endothelial cells did not contribute to skeletal muscle. Pericyte contribution varied among different muscles, ranging from <1% (tibialis anterior muscle) to ≈7% of the fibers (diaphragm) and was enhanced by acute or chronic muscle regeneration. In addition, pericytes also contribute to the satellite cell pool, during unperturbed, postnatal growth of the mouse. However, in adult dystrophic muscle, pericytes predominantly contribute to fibrosis (A. Dellavalle et al, unpublished data) as described below for other organs. Interestingly AP expressing pericytes are increased in biopsies from patients affected by different forms of muscular dystrophy, suggesting an attempt to regenerate the tissue that may rather end up in fibrosis.

Until now, only MyoD and myogenic factor 5 (Myf5) expressing cells have been lineage traced and, as expected, they have been shown to generate virtually all the skeletal muscles of the body. Some controversy exists on whether adult satellite cells, that in the quiescent state express Pax7 but not MyoD or Myf5, have in their previous developmental history expressed or not one of these genes. However, our description of TNAP expressing pericytes contributing to skeletal muscle development was the first demonstration that nonmyogenic cells are recruited to myogenesis in vivo during normal postnatal development. Almost at the same time, 3 articles were published showing that selective ablation of Pax7-expressing satellite cells during adult life prevents subsequent muscle regeneration, suggesting that no other endogenous cell types may substitute for satellite cells. As a matter of fact, the ablation of Pax7+ satellite cells was performed in adult mice, at an age when the contribution of AP+ pericytes to muscle regeneration becomes negligible; moreover, satellite cells may be required to induce other cell types to adopt a myogenic fate.

Choosing Between Skeletal and Smooth Muscle Fates

Data described above show that pericytes from skeletal muscle can choose to activate either smooth or skeletal myogenesis. How is this decision regulated? In vivo studies would not address this question easily because crosses between different mutant and lineage traced mice would be required. We took an in vitro approach, adapted from previous work on somites. Briefly, we isolated embryonic dorsal aorta from E11.5 myosin light chain 1/3 fast transgenic embryos. Expression of nuclear β galactosidase was restricted to the striated muscle. We cocultured dorsal aorta from these transgenic embryos with differentiating C2C12 or primary, wild-type myoblasts. Under these conditions, muscle differentiation from cells originating from the vessel can be quantified. We found a substantial number (≈50 on C212 myoblasts) of LacZ+ nuclei, from a single aorta in coculture, but none when the aorta was cultured alone or cocultured with fibroblasts. Thanks to this simple and fast assay, we could investigate signals that may recruit these progenitors to a skeletal myogenic fate. Results indicated that Noggin (but not Follistatin, Chordin, or Gremlin) stimulates, whereas bone morphogenetic protein 2/4 inhibits skeletal myogenesis from dorsal aorta progenitors; neutralizing antibodies and shRNA greatly reduce these effects. In contrast, transforming growth factor-β1, vascular endothelial growth factor, Wnt7A, Wnt3A, basic fibroblast growth factor, platelet-derived growth factor BB (PDGF-BB), and insulin-like growth factor 1 had no effect.
Sorting experiments indicated that the majority of these skeletal myogenic progenitors express the pericyte marker neural glial proteoglycan 2. Moreover, they are abundant in the thoracic segment at embryonic day (E)10.5 and in the iliac bifurcation at E12.5, suggesting the occurrence of a cranio-caudal wave of competent cells, which parallels the cranio-caudal succession of skeletal muscle formation in the embryo. Bone morphogenetic protein 2 is expressed in the dorsal aorta and Noggin in newly formed muscle fibers, suggesting that these 2 tissues compete to recruit mesoderm cells to a myogenic or to a perivascular fate in the developing fetal muscle. Moreover, a schematic representation of this process is shown in Figure 2.

Together, these data suggested that mesoderm progenitors undergo a unidirectional shift from a perivascular to a skeletal myogenic fate. However, it is well known that growing vessels recruit surrounding mesoderm progenitors to a perivascular fate. It is conceivable that some of these surrounding cells may be skeletal myoblasts, at different steps of progression toward terminal differentiation (ie, Pax3+/Myf5-MyoD-, Pax3+/Myf5+MyoD+, Pax3-/Myogenin+/MyHC+) and their fate may be diverted in the opposite direction.

Skeletal muscle progenitors originate in the dermomyotome where fate choice is dictated by signaling molecules, such as bone morphogenetic protein, transforming growth factor-β, and Notch. Moreover, Pax3-expressing myogenic precursors are also able to migrate ventrally and contribute to the smooth muscle of the aorta.

We postulated that a similar signaling may operate also during fetal development and, thus, pericytes may derive from direct conversion of skeletal myoblasts. When exposed to DLL4 and PDGF-BB, skeletal myoblasts downregulate myogenic genes, except Myf5, and upregulate pericyte markers. Moreover, when co-cultured with endothelial cells, skeletal myoblasts, previously treated with DLL4 and PDGF-BB, adopt a perivascular position stabilizing newly formed vessel-like networks in vitro and in vivo. In a transgenic mouse model in which cells expressing MyoD activate Notch, skeletal myogenesis is abolished and pericyte genes are activated. Even if overexpressed, Myf5 does not trigger myogenesis because Notch induces Id3, partially sequestering Myf5 and also inhibiting myocyte enhancer factor 2 expression. Myf5-expressing cells adopt a perivascular position, as infrequently observed also in wild-type embryos.

These data indicate that endothelium, via DLL4 and PDGF-BB, induces a fate switch in adjacent skeletal myoblasts. Hence, it seems that during embryonic and fetal development, cells choose a specific fate depending on the timely exposure to the right concentration of signaling molecules emanating from the surrounding cells, the inducing tissue in the embryo and the differentiating neighboring tissues in the fetus. However, these fate choices are not irreversibly fixed but are reinforced and stabilized by the microenvironment. In the case of developing skeletal muscle, developing fibers and endothelium are likely to compete for surrounding mesoderm cells, whose final fate is irreversibly fixed only at the onset of terminal differentiation. A developmental logic may be based on timing: when muscle fibers grow in size they recruit myoblasts, but also other uncommitted mesoderm cells that, once fused and exposed to MyoD, activate myogenesis. The increase in mass induces hypoxia, and vascular-endothelial growth factor release and angiogenesis occur: vessels growing inside the muscle recruit pericytes from the surrounding mesoderm cells, including committed skeletal myoblasts. This would result in a distribution of progenitors consequent to the specific need of the growing structure.

**Pericyte Contribution to Fibrosis**

Recent work has highlighted the role of pericytes and smooth muscle cells in fibrosis. For an extensive review of fibrosis in muscular dystrophy, refer to Serrano et al, where the role of pericytes is discussed. Chronic injury triggers pericyte proliferation and differentiation into a kind of collagen-secreting, myofibroblasts, or fibrocytes with the ability to migrate away from vessels, causing also damage and rarefaction of the microvasculature. There is a major focus on pericyte to myofibroblast transition in different types of diseases, such as type 1 diabetic nephropathy, which implies regulation of pericyte differentiation into myofibroblasts as a possible therapeutic target.
Moreover, adult kidney pericytes undergo differentiation into SMA + myofibroblasts in a chronic condition of disease. After this process, the phenomenon proceeds with intimal thickening and fibrosis, hypertrophy, and fibro-proliferative changes in the adventitia. It is also possible to observe an extension of the smooth muscle layer into vessels previously devoid of a thick muscle layer. Most of these changes are associated with the accumulation of α-SMA positive cells (pericyte like) and inflammatory cells. Up to now, neither the origin of these cells (vessel wall versus parenchyma versus bone marrow), nor the molecular mechanisms responsible for their accumulation, proliferation, and migration in any of the compartments of the vessel wall have been fully elucidated or understood.

Bone marrow–derived, circulating progenitor cells have also been shown to be recruited directly to sites of vascular injury and to easily assume both endothelial and smooth muscle like phenotypes. What is described for the kidney applies also to fibrosis occurring in other organs, such as the liver, the heart, and the brain as described in detail elsewhere.

Conclusions

Until now, relatively few examples of pericyte contribution to nonvascular tissues have been reported. Contribution to osteochondrogenic precursors was reported for SM22 expressing vascular smooth muscle68 in agreement with the observation that perivascular cells (identified by the expression of CD146) are able to form an ectopic osseous able to recreate the hematopoietic microenvironment.69 On the same lines, Feng et al70 reported that pericytes give rise to odontoblasts, although not to all of them, suggesting a possible multiple origins for this cell type. Besides mineralized tissues, it is still largely unknown whether pericytes may be able to contribute to the genesis of other mesoderm tissues. Recently, we observed that heart pericytes also contribute to cardiomyocytes during postnatal development of the heart (A. Dellavalle et al, unpublished data). However, whether they contribute also to nonmesoderm tissue is completely unknown and possibly unlikely, given the ability of epithelial cells to maintain proliferation throughout life.

Some time ago, 2 intriguing reports showed the contribution of Wnt1 expressing neural crest cells to the development of tooth and mandibular71 and the aorticopulmonary septum and conotruncal cushion in the development of heart.72 It was reported that although contribution of neural crest to the embryonic structures was massive, with further development, labeled cells were outnumbered by other cells that could not have derived from neural crest. Authors concluded that during postembryonic development the organs would continue to grow by contribution of cells, which were different from the founder cells, although their origin was and remains undefined. However, it is tempting to speculate that these cells may also derive from pericytes. Once again, paucity of specific and reliable pericyte-specific genes is hampering these kinds of studies. We think that current studies on pericytes will soon lead to the identification of novel transcription factors or surface markers, truly specific for pericytes, and that the availability of these reagents will allow a thorough investigation of their real contribute to tissue growth during perinatal and postnatal growth as well as during tissue repair. With a complementary approach, it will also be possible to lineage trace resident progenitor cells that are recruited to a vascular fate during developmental and pathological angiogenesis because recent data support the hypothesis that pericytes may represent the resident progenitor of mesoderm in different tissues.

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None.

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