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Targeting Perciytes for Angiogenic Therapies

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Abstract

In pathological scenarios, such as tumor growth and diabetic retinopathy, blocking angiogenesis would be beneficial. In others, such as myocardial infarction and hypertension, promoting angiogenesis might be desirable. Due to their putative influence on endothelial cells, vascular pericytes have become a topic of growing interest and are increasingly being evaluated as a potential target for angioregulatory therapies. For example, the strategy of manipulating pericyte recruitment to capillaries could result in anti- or pro-angiogenic effects. However, our current understanding of pericytes is limited by knowledge gaps regarding pericyte identity and lineage. To use a music analogy, this review is a “mash-up” that attempts to integrate what we know about pericyte functionality and expression with what is beginning to be elucidated regarding their regenerative potential. We explore the lingering questions regarding pericyte phenotypic identity and lineage. The expression of different pericyte markers (e.g., SMA, Desmin, NG2 and PDGFR-β) varies for different subpopulations and tissues. Previous use of these markers to identify pericytes has suggested potential phenotypic overlaps and plasticity toward other cell phenotypes. Our review chronicles the state of the literature, identifies critical unanswered questions, and motivates future research aimed at understanding this intriguing cell type and harnessing its therapeutic potential.

Keywords

pericytes; stem cell; angiogenesis; therapy

INTRODUCTION

Pericytes are elongated mural support cells that extend along endothelial cells (41, 46). First identified by French physiologist and anatomist Charles–Marie–Benjamin Rouget in 1873 and termed “Rouget cells,” pericytes are present in every vascularized tissue in the body (32). Although it has been suggested that pericytes may reside in large vessels (5), pericytes are most commonly associated with the microvasculature. At the capillary level, pericytes

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regulate vessel permeability, vessel diameter, and endothelial cell proliferation through both paracrine signaling (43, 47, 64) and direct contact with endothelial cells (41), (101). Through cell-matrix and cell-cell interactions, pericytes play an essential role in contractile force transmission (59, 60, 63), vessel diameter regulation (81), vessel stabilization (41), and endothelial cell survival (38). Pericytes can even directly control capillary diameter, as their presence has been shown to be necessary for constriction (81). The functional effects of pericyte-endothelial cell signaling have previously been highlighted by comprehensive reviews (30, 112). Pericytes are considered “angioregulators” in that they can both stabilize and promote angiogenesis. Their importance in angiogenesis is evident when considering the cases of diabetic retinopathy and tumor growth, in which pericyte loosening from the endothelium is associated with uncontrollable angiogenesis (31, 117). But importantly, their function can be dependent on the type of stimulus. For example, angiogenesis in skeletal muscle has been correlated with both the withdrawal of pericytes and an increase in pericyte number (32, 33). In recent years, new and intriguing roles have emerged for the pericyte. These include mediating leukocyte trafficking (10, 100), contributing to fibrosis (87), and functioning as a tissue-resident stem or progenitor cell (107, 108). The multi-faceted role for pericytes emphasizes the need to both carefully identify their structural alterations during different pathological scenarios and better understand how their specific phenotypes relate to their functions. Meanwhile, pericytes have been convincingly established as critical players in angiogenesis and as potential therapeutic targets in inflammation and tissue regeneration. In addition to serving as putative drug targets, pericytes have been implicated as tools for cell-based therapies due to their angioregulatory capabilities.

As pericytes receive increasing attention from a wider array of research domains, it has become even more apparent that advancing our knowledge about pericytes and our ability to therapeutically manipulate their function will require answering fundamental questions about pericyte ancestry, progeny, and phenotypic differentiation capacity that have confounded studies since Rouget’s time. Even within the field of pericyte biology, varying terminology has created confusion (7). The perplexity of pericyte identity can be attributed to pericyte phenotypes being cell and tissue specific and is best exemplified by considering Ito cells in the liver. Ito cells, also known as hepatic stellate cells, are thought to be mesenchymally derived and display smooth muscle-like, pericyte-like characteristics (42). Ito cells, like pericytes, can exist in a quiescent or activated state depending on the local environment (42, 93). Ito cells interact with the sinusoid endothelium and control blood flow. They also can express multiple pericyte markers and participate in fibrogenesis. Based on the similarities to pericytes, Ito cells are, unsurprisingly, classified as the pericytes of the liver (83). In this review we further identify sources of potential confusion regarding pericyte phenotypes based on their overlaps with other cell types. Then, we focus on the plastic relationships between pericytes and mesenchymal stem cells and the potential for therapeutically using stem cells to play the roles of pericytes. Pericytes represent a scientifically intriguing and therapeutically exciting cell type, but there are far more questions than answers. We approach some of these questions by convolving some of the basic science understanding about pericyte form and function with emerging evidence that suggest multipotency and points to their application as a cell-based therapy.

PERICYTE DYNAMICS

Much of what constitutes our understanding about a cell population, and more importantly, the "identity" of a given cell population, are the behaviors and functionality that cells and their environment impart on one another. This is particularly true for pericytes, for which no cell type-specific expression markers exist. Even more informative than their behaviors during homeostasis is their behavior during dynamic processes. In the case of pericytes, the dynamic process of reference is angiogenesis, defined as the sprouting of new capillaries off of existing vessels. The role of pericytes in regulating angiogenesis has been reviewed extensively (12, 59), so we briefly summarize some of the landmark findings that have shaped the field's understanding about pericyte function during this dynamic process.

Pericyte dynamics during angiogenesis have been shown to include alterations in cell-to-cell contacts with endothelial cells, migration, growth factor presentation, proliferation, and extracellular matrix modulation (Figure 1) (6, 41). Pericytes are present along capillary sprouts and have been shown to both lag and lead endothelial cells at the sprout tip (11, 84). In some cases, pericytes can even bridge the gaps between two sprouting endothelial cell segments (75). Subsequently, the integration of pericyte dynamics during capillary sprouting logically can be linked to endothelial cell guidance. While the full scope of communication between pericytes and endothelial cells remains to be elucidated, work in this area has established that the pericyte-endothelial cell interactions during angiogenesis are regulated in part by Ang-1/Tie2, TGF-β and PDGFB/PDGFR-β signaling (36).

Perhaps the most convincing evidence for a requirement of endothelial cell-pericyte dynamic interaction during angiogenesis has been shown through work with PDGFB and PDGFR-β (43, 44, 47, 64). Manipulating proper pericyte investment by altering recruitment to the endothelium through PDGFB-PDGFR-β signaling can cause lethal microvascular dysfunction, affect vascular patterning during development, and affect the formation of new vessels during physiological and pathological angiogenesis (41). Indeed, tremendous research efforts using transgenic mice and co-culture *in vitro* assays have generated a molecularly detailed picture of the proteins that regulate pericyte dynamics during angiogenesis (60).

These and other landmark studies are foundational to the field's current understanding of what pericytes are and the how they function during angiogenesis. Advancing our knowledge requires identifying how and when pericyte behaviors change during the transition from quiescent to angiogenic states. This entails not only a description of their spatial and temporal dynamics, but also a prospective method for identifying pericytes and potential specific subpopulation cell types. As we explore in the next section, this has proven to be a challenge that has created insights, as well as some degree of confusion, about pericyte lineage and plasticity. Indeed, the scope of pericyte dynamics broadens upon considering the potential of transdifferentiation of pericytes into or from other cell types (73, 74, 88). The putative links between interstitial cell populations (including macrophages, fibroblasts, and progenitor cells), pericytes, and SMCs necessitates discussion of how cell phenotypes, rather than cell nomenclature *per se*, relate to specific functions.

A CASE OF MISTAKEN IDENTITY?

As researchers recognize the importance and therapeutic potential for vascular pericytes, more questions than answers have been generated. Reflection on the literature reveals some historical confusion about pericytes – confusion propagated by a lack of cell type-specific markers, potential for transdifferentiation, and visualization in histological cross-sectional views that may obscure their true identity.

Pericyte Morphology

Cell types by are typically identified by their morphology and expression of certain genes and/or proteins. A quintessential hallmark of pericyte identity has long been the characteristic wrapping of cell processes, or filopodia, around capillary endothelium. Another morphological trait is the sharing of a common basal lamina with the endothelium (61, 95). Pericytes are most convincingly identified by their morphology when using electron microscopy of sectioned tissues (50) or confocal microscopy of whole-mounted tissues (70, 105). However, in most cases pericytes are identified using lower resolution techniques, which limits the classification to their general localization next to an endothelial cell. At low magnification (e.g., 20x or lower), it is impossible to determine where the ablumenal membrane of the endothelium ends and where the pericyte membrane starts, creating the possibility for one to mistake an extravasating monocyte, a proliferating endothelial cell, or any number of other cell types in the niche for a pericyte. Moreover, other cell types that can occupy space in the perivascular niche (e.g. macrophages, fibroblasts) may also express the same cell surface marker(s) being used to denote a pericyte phenotype. While morphology alone may be insufficient for ascribing a pericyte identity to an observed cell, marker expression is similarly inadequate for distinguishing pericytes from other cell types.

Pericyte Phenotypic Markers

Unlike some cell types, such as the endothelial cell, that are classically and specifically identified by their expression of cell type-specific genes or proteins (e.g. VE-cadherin and PECAM-1), a cell-type specific marker for pericytes has yet to be identified. Pericytes are known to express a battery of different proteins that can be visualized using off-the-shelf antibodies, but expression is shared by other cells, many of which occupy similar locations in tissues, further exacerbating the challenge of distinguishing pericytes from non-pericytes. Although this has the potential to create uncertainty, this challenge is an opportunity to gain new insights into pericyte lineage and identity.

Commonly used pericyte markers include SMA, Desmin, NG2 and PDGFR-β (41); however, their expression patterns are not pericyte-specific and differ depending on species, tissue, and even developmental stage (6, 41). The question remains: How do we unequivocally identify a pericyte? The use of the above mentioned markers and morphological characteristics provide a partial solution, but as Table 1 highlights, the “marker equals phenotype” approach can create confusion. Table 1, which lists the various pericyte markers that have been used by investigators interested in identifying pericytes, also raises the question whether the combination of different pericyte markers can be used to

identify different pericyte subpopulations. As seen in Figure 2, the expression of known markers (NG2, Desmin, SMA) can identify two neighboring cells. Expression differences highlight what we still do not know regarding how local cues might regulate pericyte phenotypes. We postulate that observations like these implicate a possibility of pericyte phenotype specialization and motivate a new area of research that might be analogous to tip-cell versus stalk-cell sub-classifications for endothelial cells along a capillary sprout.

Pericyte Marker Overlap with Other Cell Types

As mentioned above, a pericyte-specific marker has not been identified by current research. Consequently, we must resort to identifying pericytes using a combination of markers, each of which is expressed by a multitude of other cell types that can occupy the perivascular niche (Figure 3). Recognition of labeling overlaps offers a potential opportunity to gain fundamental insight into both the lineage and plasticity of pericytes. Although the shared expression of one or more markers does not confirm that two different cells share the same lineage, it may suggest the possibility of a common lineage and should not be ruled out without further investigation. The idea that pericytes and other cell types resident in the perivascular-niche share a common lineage, which is evidenced by overlapping marker expression, is provocative.

For example, SMA is expressed by pericytes, immature SMCs, which express SMA but not smMHC, and mature SMCs, which express both contractile proteins (73, 85, 110, 115). Myofibroblasts, which can also reside in the perivascular niche, also can express SMA. For example, SMA expression identifies myofibroblasts in scenarios of wound healing and fibrosis (89). Recent studies have further suggested that SMA-positive myofibroblasts in the kidney originate from pericytes (49), lending support to the idea that coincident expression of the same marker in pericytes and other cell types may, in fact, signify a common lineage. Desmin and PDGFR-β are also expressed by mature SMCs and interstitial fibroblasts (72).

Perhaps the most common marker used to identify pericytes in recent years has been neuron-glia antigen 2, or, more commonly, NG2 (75, 76). We have demonstrated that NG2 is dramatically upregulated along venules during capillary sprouting, implicating its involvement and potential as an angiogenic specific marker (69). But like other pericyte markers, NG2 is not pericyte specific. During development, NG2 is expressed by oligodendrocyte progenitor cells, immature chondroblasts, skeletal myoblasts, and cardiomyocytes (97). NG2-positive cells in the CNS have been referred to as O2A cells because they can be differentiated into either oligodendrocytes or another type of glial cell *in vitro*. In the adult, NG2-positive cell populations increase following CNS injury, in part due to upregulation of NG2 by glial cells and macrophages (97). NG2 expressing cells also include Schwann cells, at least in mouse, and can be found near the nodes of Ranvier in the peripheral nervous system. This suggests a role for NG2-positive cells in regulation of myelination (97). NG2 was also found to be the same as HMP, previously associated with tumor cells, further confirming the concept that NG2 is expressed by highly active cell types (18). As mentioned above, NG2 is expressed by pericytes throughout the microvasculature in most, if not all, quiescent tissues. This above evidence suggests that pericyte NG2 expression pattern in the microvasculature mimics that in the nervous system, wherein NG2

expression contributes to both homeostasis and regeneration. Indeed, NG2-positive pericytes acquired from the central nervous system and exposed to bFGF in culture can acquire phenotypes that overlap with glial cell lineages (29). The common use of NG2 as a pericyte marker and its analogous expression pattern – and possibly function -- in neural and vascular support cells raises the question of whether other neural phenotypic markers also identify pericytes during angiogenesis.

Class III β-tubulin, which like NG2 has been identified as a marker of neural progenitor cells in the CNS and peripheral nerves in the adult, is another candidate pericyte marker. In contrast to other markers, class III β-tubulin might offer a temporal and spatial marker of angiogenic pericytes *in vivo* (99). In unstimulated adult rat mesenteric networks, class III β-tubulin is nerve specific and absent along arterioles, venules, and capillaries. After the networks are stimulated to undergo angiogenesis, class III β-tubulin is upregulated by pericytes along these vessel types and is subsequently down regulated to unstimulated levels after capillary sprouting (99). Class III β-tubulin is one of seven β-tubulin isotypes that forms α/β-tubulin heterodimers with six α-tubulin isotypes during microtubule assembly and is most commonly used as a marker of neural phenotypes (54). Much like NG2, during development in the central nervous system class III β-tubulin is transiently expressed by glial precursor cells; in the adult it is expressed by peripheral nerves (54). Outside the nervous system, class III β-tubulin expression by tumor cells correlates with increased metastasis and resistance to tubulin binding agents (40, 55). The positive expression of class III β-tubulin by tumor cells and human pericytes *in vitro* (unpublished data) highlights a potential issue with using class III β-tubulin expression to indicate a neural phenotype. For example, stem cell differentiation into nerves has been confirmed, in part, based on class III β-tubulin expression (13, 37). However, data from our laboratories, suggests that class III β-tubulin can be expressed by pericytes and is not nerve specific. We have also confirmed that human placenta-derived pericytes, human mesenchymal stem cells (Figure 3), and mouse embryonic stem cells also express class III β-tubulin *in vitro* (data not shown). Since mesenchymal stem cells might be a source of vascular pericytes in adult tissues (9) and, vice versa, vascular pericytes can be induced to exhibit multipotent stem cell activity (29), we speculate that the transient class III β-tubulin *in vivo* identifies a precursor cell population.

Both NG2 and class III β-tubulin expression by pericytes and neural cells highlight an emerging area of microvascular research focused on the link between neural and vascular patterning (34, 35, 113). This link is supported at the molecular level when considering growth inhibitors in the CNS such as ephrins, semaphorins, NG2, and Nogo (91), and is commonly presented in the context of either endothelial cell tip cells or arterial/venous identity. The overlap between neural and vascular patterning offers an exciting new perspective on the study of adult microvascular remodeling. The shared expression of NG2 and class III β-tubulin by pericytes and neural cells motivates the need to understand if and how perineural cells and pericytes are related.

Indeed, these findings motivate an even broader question: are pericytes able to differentiate into other cell types? Emerging evidence suggests the answer is “yes,” and a putative mechanism is via a bridging cell type that has well known multipotent differentiation capabilities: the MSC. For example, G. Paul et al. (80), recently isolated, purified and

characterized a progenitor cell population from the ventricular wall and the neocortex in the adult human brain. They confirmed that these cells co-express markers for MSCs and pericytes *in vivo* and *in vitro* and have multilineage potential towards both mesodermal and neuroectodermal phenotypes. As discussed above, it is intriguing that the brain harbors a cell population with the ability to both modulate angiogenesis as pericytes and regenerate neural tissues as neural progenitor cells. Evidence from other tissues corroborates this interesting finding in the brain, and in the following section we use recent reports to conceptualize the putative linkage between pericytes and MSCs (Figure 4).

PERICYTES OR MESECHYMAL STEM CELLS (OR BOTH)?

Within the past ten years, a number of studies have demonstrated that MSCs and pericytes co-localize within the perivascular niche and express many of the same cell surface markers. Implying that the perivascular niche may serve as a systemic reservoir of tissue-resident stem cells (20, 52), these findings have generated a number of provocative questions around the topic of MSC and pericyte identity and lineage, and their possible overlap (Figure 4). In this section we will briefly review the literature in this area with respect to two fundamental questions that have yet to be completely answered: 1) are pericytes MSCs? and 2) do MSCs give rise to pericytes?

Are pericytes MSCs?

Initially isolated from the bone marrow, MSCs have been defined by the International Society for Cellular Therapy as a population of cells that, upon removal from tissue depots, is adherent to plastic, expresses a panel of defined surface markers (CD73, CD90, and CD105, and lack of CD11b or CD14, CD19 or CD79, CD45, and HLA-DR), and have the ability to differentiate into adipocytes, chondrocytes, and osteoblasts (28). Emerging evidence confirms the presence of MSCs within the perivascular niche in a wide array of tissues throughout the body, including fat, muscle, bone, and brain (65, 90, 96, 102, 103). Indeed, several studies have suggested that blood vessels throughout the circulation contain multi-lineage precursors and contribute to tissue repair/regeneration (25, 39). Dr. Bruno Péault and colleagues (26) were the first to identify, purify, and characterize distinct populations of MSCs from the vasculature of multiple human organs (19, 104). MSCs in the perivascular niche have been classified into subsets that include pericytes (26), adventitial cells (24), and myogenic endothelial cells (119). Each subset is able undergo adipogenesis, osteogenesis, and chondrogenesis in culture, and can elicit substantial regenerative capabilities when injected back *in vivo*. The regenerative capacity of multipotent pericytes, isolated by flow cytometry selection of CD34–/CD146+/CD45–/CD56– cells from adipose and other tissues, has been evaluated in skeletal muscle (79), lung (68), dermal (118), and nervous tissues (17). The lack of CD34 expression (CD34–) is thought to be a hallmark of multipotent pericytes in most tissues (26), but a rare CD34+/CD146+/CD45–/CD31– population of adipose-derived pericytes has also been identified in adipose tissue (109, 114, 120–123) using stringent rare-event strategies for its detection and isolation by flow cytometry (116, 121). So in many ways, detection of a multipotent subpopulation of pericytes, as is the case for pericyte identification in general, is still invariably elusive using the currently available techniques, such as flow cytometry and immunohistochemistry (65).

The extent to which pericytes share a common identity or lineage with MSCs may differ from tissue to tissue and depend on how quiescent or stimulated the tissue may be (67). There are likely a number of different mechanisms that control the endogenous phenotype of a pericyte at any given point in time (Figure 4). An alteration in chemical cues—either diffusible or matrix bound, is likely a key contributor to pericyte-MSC differentiation *in situ*. The source of these chemical cues is likely variable but attributable to cells in and around the perivascular niche, like endothelial cells, fibroblasts, and macrophages. Lee et al. (62) found that IL-1β –activated macrophages promote differentiation of perivascular-resident MSCs in adipose tissue to vascular SMCs through a prostaglandin F2α –mediated paracrine mechanism. It is also likely that changes in the extracellular matrix composition itself are candidates for regulators of MSC and pericyte differentiation (71). Mechanical stiffness of the underlying substrate has also been shown to influence de-activation of stem cells and myofibroblasts (57), so there are likely mechanical cues, in addition to chemical cues, that guide pericyte differentiation in situ. A disturbance in the endothelial-pericyte interaction has also been proposed as a driver for osteogenic and adipogenic differentiation of pericytes. In settings where endothelial-pericyte interactions are disrupted, like heterotopic ossification and atherosclerosis, pathological bone mineralization and adipogenesis has been observed, suggesting that disruption of endothelial-pericyte interactions may be another key driver of pericyte/MSC differentiation (75, 78).

Do MSCs differentiate into pericytes?

The relevance of this perivascular stem cell niche to tissue homeostasis and regeneration is only beginning to be explored, but one of its more obvious implications is as a sustainable source for new pericytes *in vivo*. As an example, MSCs derived from excised bone marrow and co-cultured with endothelial cells have been shown to differentiate into pericytes that can support engineered vessels in three-dimensional collagen gels implanted *in vivo* for more than 130 days (58). In another study where MSCs derived from the bone marrow of GFP transgenic mice were intravenously injected into mice that had received cutaneous wounds, GFP-positive bone marrow cells in the wound expressed pericyte markers (92).

Support for MSC differentiation into vascular pericytes is also provided by bone marrow lineage studies. Ozerdem et al. showed that NG2-expressing pericytes were recruited from the bone marrow when the mouse cornea was treated with bFGF to induce angiogenesis (77). Over ninety percent of these pericytes expressed CD45 or CD11b, indicating their hematopoietic origin and mesenchymal lineage overlap. Similar findings were presented by Rajnate et al. during tumor and VEGF induced angiogenesis. Song et al., also demonstrated that PDGFR-β expressing pericyte progenitors from the bone marrow reside in the tumor interstitium and are capable of differentiating into NG2, Desmin, and SMA-positive pericytes. Jung et al. identified what they termed “multipotent pericyte-like cells” (51) in the circulating blood based on fluorescence or magnetic activated cell sorting with anti-PDGFR-β antibody.

While compelling evidence seems to support that pericytes can be derived from MSCs and contribute to vascular growth and remodeling, the dynamics of this process remain an open avenue for discovery. Whether or not MSCs are derived from the bone marrow and circulate

systemically to tissues throughout the body, or if they self-renew within tissue-resident perivascular niches, or both, have yet to be determined and will likely require some of the tools presented in the final section of this review.

STEM CELL APPLICATIONS FOR PERICYTE TARGETED ANGIOGENIC THERAPIES

Clearly, confusion remains: do pericytes and MSCs belong in the same pool, or are they distinct populations that share no such common lineage? Regardless of the answer, the fields of tissue engineering and regenerative medicine have continued to move forward, focusing on isolating and injecting these cells, despite their uncertain lineage, for therapeutic gain. While the mechanisms influencing their recruitment out of the circulation, differentiation, and dominant function are undefined, their application and our developing ability to manipulate their multifaceted role during angiogenesis offers promising potential for future therapies. This section will overview just a small sampling of the many studies (pre-clinical and clinical) that have explored cell-based angioregulation therapies where a pericyte-like role has either been explicitly mentioned or indirectly implicated (Figure 5; Table 2).

As mentioned above, it has been suggested that MSCs can be derived from multiple tissue sources, including circulating blood. Our group was among the first to attribute a pericyte-like role to MSCs harvested from adipose tissue (Figure 6). Passaged MSCs from adipose tissue (also known as hASCs) were injected I.P. into Nude mice that had been stimulated by Compound 48/80 to invoke angiogenesis in the mesenteric tissues (2). Ten days later, the injected cells exhibited pericyte-like morphologies, expressed NG2 and SMA, and significantly increased vascular density. These effects persisted out to sixty days, suggesting that the injected MSCs may have differentiated into vessel-stabilizing pericytes. In the setting of diabetic retinopathy, Mendel el al. (66) also very recently demonstrated that MSCs obtained from human and mouse adipose tissue, operating in a pericyte-like capacity, stabilized the compromised vasculature in different murine models of retinal vasculopathies, including oxygen-induced retinopathy and Akimba diabetic mice. The authors showed that injected MSCs integrated alongside host endothelial cells in a vasculoprotective mechanism that was strengthened with TGF-β1 treatment, across all three murine models of disease.

Cardiac and peripheral ischemic diseases also represent potential uses for MSC-pericytes. Katare et al. (53) evaluated the effects of BMSC pericyte progenitor cells in an infarcted heart model and concluded that the delivered cells worked through a paracrine mechanism to reduce myocardial scaring, apoptosis, and fibrosis, while increasing vascular stability and attenuating permeability. In the setting of peripheral ischemic disease, Rehman et al. (86) reported that MSCs from adipose tissue increased endothelial cell growth and reduced endothelial apoptosis in a pericyte-like manner. In a model of hindlimb ischemia, perfusion recovery was accelerated when MSCs from adipose tissue were injected.

Another promising application of MSCs is in cutaneous wound healing. Kim et al. (56) demonstrated that topical transplantation of allogeneic MSCs in canine cutaneous wounds increased the rate of wound closure and degree of collagen production, cell proliferation, and angiogenesis primarily through paracrine effects on the local cell population. Using a

model of delayed diabetic wound healing, Amos et al. (3) demonstrated that topically applied MSCs from adipose tissue also increased rate of wound closure through the production of extracellular matrix proteins and soluble factors. Similar results were obtained in a clinical trial by Vojtassak et al. (111) using autologous MSCs derived from bone marrow. In this study, a chronic non-healing diabetic ulcer was treated with BMSCs and autologous skin fibroblasts delivered in a collagen membrane. The ulcer experienced increased vascularity and dermis thickness, as well as significant wound closure over the 29 days of treatment. While pericytes were not explicitly monitored in any of these wound healing studies, each reported increases in vascularity, which is consistent with pericyte contributions.

Future studies like the examples detailed above will serve to transcend our understanding of MSC and pericyte functionality, especially in a therapeutic context. Whether or not MSCs and pericytes share a common lineage, the ease of culture expansion and transplantation make MSCs an attractive cell source for therapeutic angioregulation in different disease settings.

FUTURE PERSPECTIVE

Pericytes have recently attracted a lot of attention from the research community at large. Moving forward, new model systems and imaging approaches will greatly advance our understanding of their dynamics, functionality, and phenotypic flexibility. The ability to monitor and track endogenously-labeled pericytes *in vivo* using confocal imaging and other high-resolution intravital imaging approaches enables us to observe the dynamic behaviors of cells in tissues, such as the ear (100). *Ex vivo* explant systems where pericytes can be labeled and dynamically visualized will also help us learn how they interact with the microvasculature and other cell types throughout the tissue space and over time (98). Using these and other new tools will help us learn more about pericyte identity and lineage, which in turn will help us leverage our understanding of this unique cell population for therapeutic means. Meanwhile, fundamental questions remain to be answered regarding where pericytes come from, where they go, what they do, and what they can become.

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ABBREVIATIONS

Ang1	Angiopoietin 1
Ang2	Angiopoietin 2
Tie2	Angiopoietin receptor tyrosine kinase receptor
TGF-β	Transforming growth factor-β
PDGFB	Platelet-derived growth factor B

PDGFR-β	Platelet-derived growth factor receptor-β
SMA	Smooth muscle alpha-actin
SMCs	Smooth muscle cells
NG2	Neuron-glia antigen 2
HMP	Human melanoma proteoglycan
PECAM-1	Platelet endothelial cell adhesion molecule-1
VE-Cadherin	Vascular endothelial cadherin
VEGF	Vascular endothelial growth factor
CNS	Central nervous system
O2A	Oligodendryocyte-type 2 astrocyte
hASCs	Human adipose-derived stem cells
bFGF	Basic fibroblast growth factor
MSC	Mesenchymal stem cell
GFP	Green fluorescent protein
IL-1β	Interleukin-1β
STZ	Streptozotocin
RT-PCR	Reverse transcription polymerase chain reaction
BDNF	Brain-derived neurotrophic factor
CNTF	Ciliary neurotrophic factor
GDNF	Glial cell line-derived neurotrophic factor
NGF	Nerve growth factor
TLR2	Toll-like receptor-2
TLR4	Toll-like receptor-4
FPR2	Formyl peptide receptor 2
TNFR1	Tumor necrosis factor receptor-1
NLRP3	NOD-like receptor protein-1
NOD	Nucleotide-binding oligomerization domain-containing protein
ICAM-1	Intercellular adhesion molecule-1
smMHC	Smooth muscle Myosin heavy chain
RGS5	Regulator of G-protein signaling 5
FACS	Fluorescence-activated cell sorting
BMSC	Bone marrow-derived stem cell

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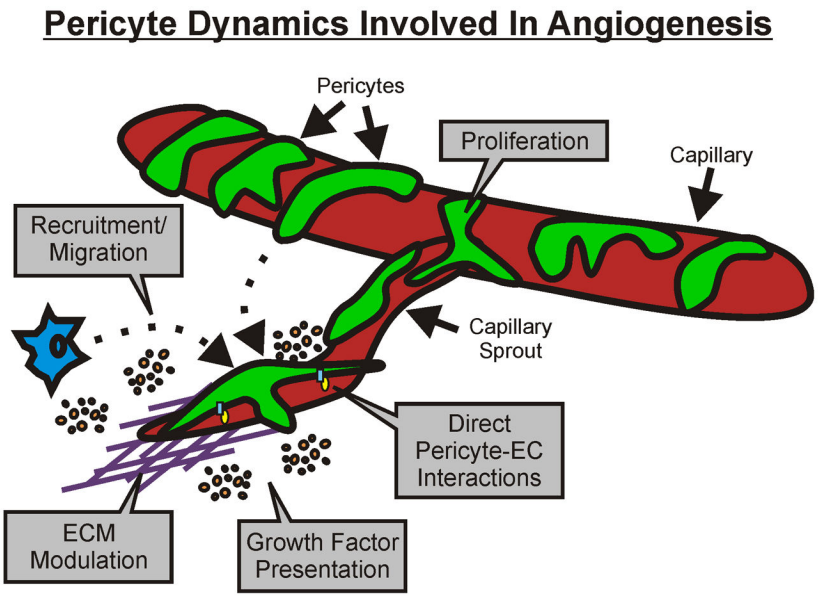

Figure 1.

Pericyte dynamics involved in angiogenesis. The main dynamics include cell-to-cell contacts with endothelial cells, migration, growth factor presentation, and extracellular matrix modulation. In some cases, pericyte proliferation has also been implicated. Each cellular interaction represents a target to manipulate capillary sprouting.

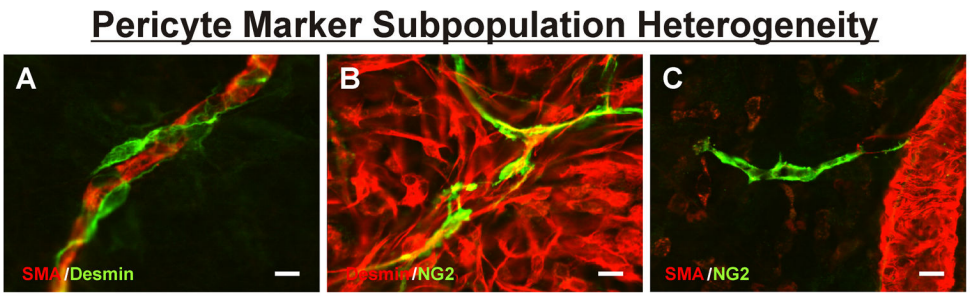

Figure 2.

Pericyte marker subpopulation heterogeneity between neighboring cells in adult rat mesenteric microvascular networks. (A) Example of Desmin-positive cells wrapping around a SMA covered capillary in a quiescent scenario. (B) Example of NG2-positive pericytes along capillaries that co-localize to a sub-population of Desmin-positive cells during a wound healing response. (C) Example of an NG2-positive pericyte apparently along a capillary sprout off a SMA covered venule. Scale bars = 10 μm.

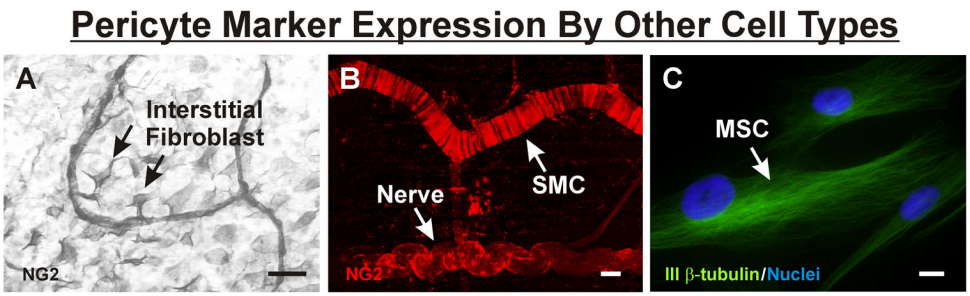

Figure 3.

Pericyte marker expression by other cell types. (A) Example of NG2 identification of interstitial fibroblasts in remodeling adult rat mesenteric networks. The fibroblasts apparently interact with NG2-positive pericytes along capillaries. (B) Example of NG2 identification of tightly wrapped SMCs along an arteriole and a nerve in unstimulated mouse spinotrapezius muscle. (C) Example of class III β-tubulin identification of MSCs *in vitro*. Scale bars = 50 μm (A), 20 μm (B), and 10 μm (C).

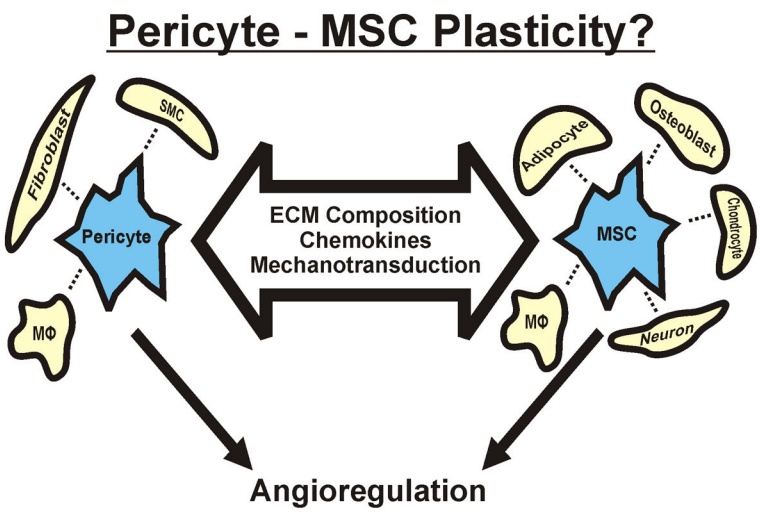

Figure 4.

Pericyte and MSC phenotypic plasticity. Recent studies have suggested that pericytes (or a subset therein) and MSCs share a common lineage and possess phenotypic plasticity that can be modulated by different environmental factors (large arrow). A functional intersection between the different phenotypes is their potential to participate in angioregulation by either stimulating angiogenesis or stabilizing new vessel formation.

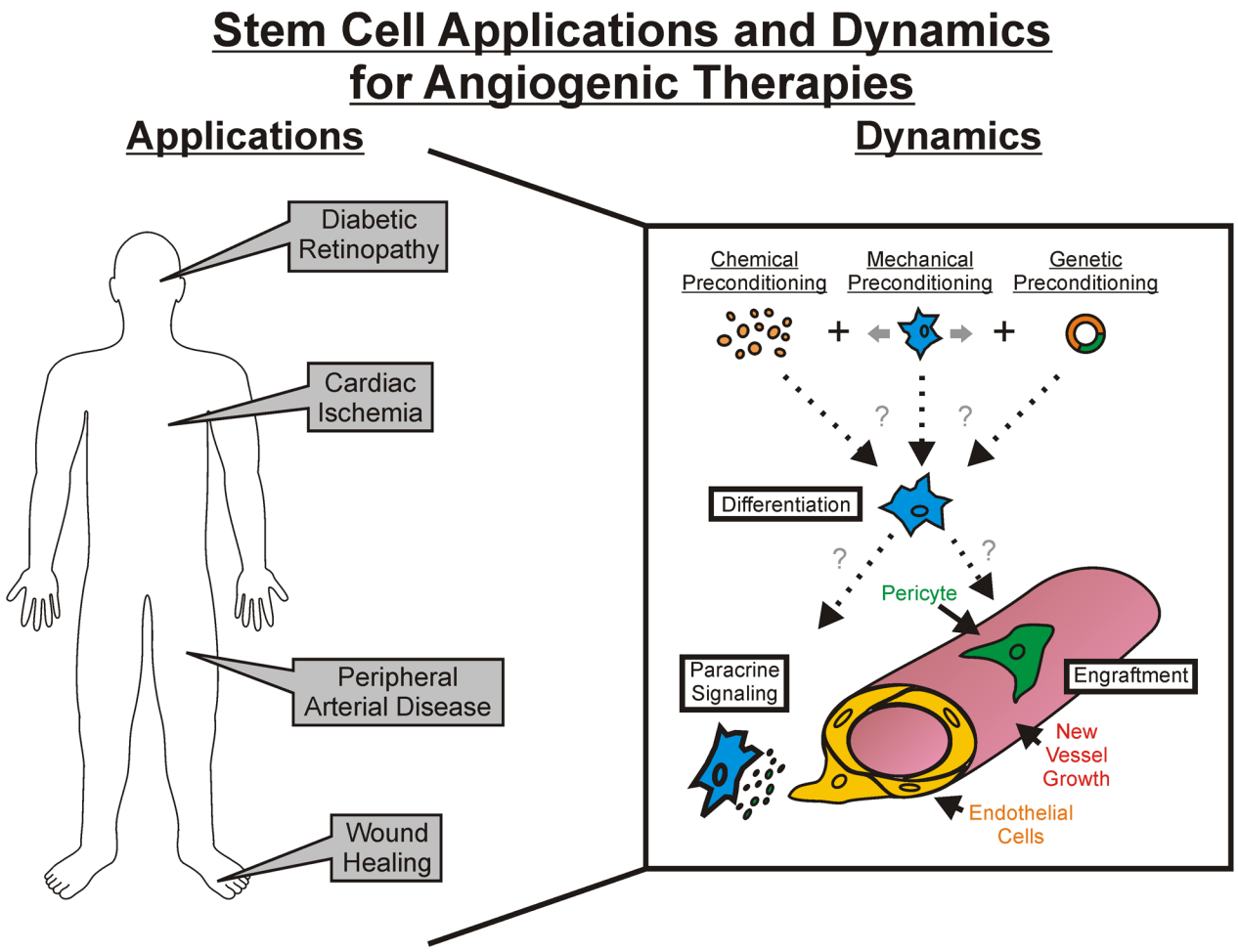

Figure 5.

The application of MSCs in cell based therapies to manipulate angiogenesis. The integrated schematic highlights the wide range of disease scenarios that can be targeted and the approaches to influence MSC fate and function for capillary sprouting. We hypothesize that optimal therapies will result from a combination of chemical, mechanical, and genetic preconditioning modalities. The embedded Table 2 details the pathology and delivery method for recent examples of stem cell therapeutic use.

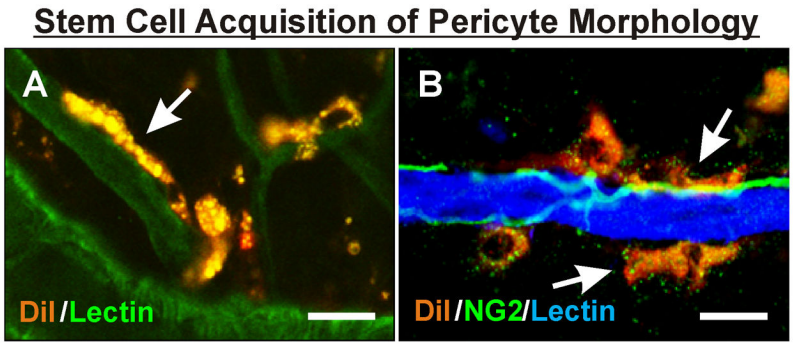

Figure 6.

MSCs from adipose tissue (hASCs) adopt a putative pericyte fate *in vivo* following therapeutic delivery. DiI-labeled MSCs from human adipose tissue were injected intraperitoneally and identified in the inflamed Nude rat mesentery (A; (4)) or injected intravenously and identified in the ischemic spinotrapezius muscle of NOD-SCID mice (B). In both tissues, injected MSCs (arrows) were observed aligning along BSI-Lectin-positive capillaries in a manner similar to native pericytes and exhibited morphologies similar to that of native pericytes. NG2 labeling in (B) also identifies nerves. Scale bar = 10 μm.

Table 1

Expression Markers Used for Pericyte Identification

Cell Surface Markers	Physiological Location	Detection Method	Citation
Aminopeptidace N, Aminopeptidase A, Nestin	Adult Mouse Brain	IHC	Alliot, et al. J. Neurosci Res. 1999. (1)
Endosialin, Mayer's Hemalaun	Human Primary Pericytes	IHC, RT-PCR, Western Blot	Christian, et al. Am J Pathol. 2008. (21)
NG2	Rat Mesentery	IHC	Murfee, et al. Microcirculation. 2006. (69)
NG2	Mouse Retina	IHC	Taylor, et al. Microvasc Res. 2010. (106)
NG2, Alkaline Phosphatase	Human Skeletal Muscle	IHC, RT-PCR	Dellavalle, et al. Nat Cell Bio. 2007. (27)
NG2, Class III β-Tubulin	Rat Mesentery	IHC	Stapor, Murfee. Microvasc Res. 2012. (99)
NG2, PDGFRβ, CD29, CD90, CD146	Mouse Femoral Artery	IHC	Tigges, Stallcup. J Vasc Res. 2012. (107)
NG2, PDGFRβ, RGS5	Mouse Brain, Lung, Gut, Kidney, Artery, Vein	IHC, RNA Microarray, *in situ* Hybridization	Bondjers, et al. Am J Pathol. 2003. (15)
NG2, SMA	Rat Mesentery, Spinotrapezius, Dorsal Subcutaneous Tissue	IHC	Taylor, et al. Microciruation. 2007. (105)
NG2, SMA 3G5	Bovine Retina	IHC	Kutcher, et al. Am J Pathol. 2007. (60)
NG2, SMA, Desmin	Mouse Choroid	IHC	Condren, et al. PLOS ONE. 2013. (23)
NG2, SMA, PDGFRβ	Mouse Kidney Interstitium	IHC	Schrimpf, Duffield. Curr Opin Nephrol Hypertens. 2011. (94)
NG2, SMA, PDGFRβ, Calponin I, smMHC	Murine Infantile Hemangioma	IHC, qRT-PCR, Western Blot	Boscolo, et al. Arterioscler Thromb Vasc Biol. 2011.(16)
NG2, SMA, PDGFRβ, Nestin	Rat Aorta	IHC, RT-PCR	Howson, el al. Am J Physiol Cell Physiol. 2005. (48)
NG2, SMA, TLR2, TLR4, FPR2, TNFR1, NLRP3, ICAM-1	Human Placental Pericytes	IHC, RT-PCR	Stark, et al. Nat Immunol. 2012. (100)
SMA, 3G5	Human Neonatal Foreskin	IHC, Phase-Contrast Microscopy, Magnetic Bead Isolation, FACS	Helmbold, et al. Microvasc Res. 2001.(45)

Table 2

MSC/Pericyte Therapeutic Uses

Pathology	Delivery Method	Fate/Function	Citation
Infantile Hemangioma	Subcutaneous Matrigel	Pervascular Niche/Endothelial Cell Contact Mediated Vessel Formation	Boscolo, et al. Arterioscler Thromb Vasc Biol. 2011.(16)
Diabetic Retinopathy	Intravitreal Injection	Perivascular Niche/Vessel Stabilization	Mendel, et al. PLOS ONE. 2013. (66)
Myocardial Infarction	Intracardiac Injection	Perivascular Niche/Paracrine Signaling	Katare, et al. Circ Res. 2011. (53)
Bone Defects	Ectopic Intramusclar Implant	Osteoblast/Vascularized Bone Regeneration	Askarinam, et al. Tissue Eng Part A. 2013. (8)
Multiple Sclerosis	Intravenous Injection	Undetermined/CNS Inflammation Reduction	Cohen. Neurology. 2012. (22)
Malignant Gliomas	Intravenous Injection	Perivascular Niche/Tumor-Directed Migration	Bexell. Mol Ther. 2009. (14)
Bronchopulmonary Dysplasia	Intratracheal Injection	Perivascular Niche/Paracrine Signaling	Pierro, et al. Thorax. 2013. (82)