Merfeld-Clauss et al⁴ describe an activin A–mediated, transforming growth factor (TGF-β)–independent pathway that regulates endothelial-induced mural cell differentiation from mesenchymal progenitors derived from adult human adipose tissue. These studies provide insights that should prove beneficial for the efficient generation of autologous smooth muscle cells (SMCs) for vascular tissue engineering and reparative medicine and may also shed light on the pro-assembly, as discussed herein.

Blood Vessel Assembly: Mural Cells Follow the Endothelial Cell Leader

Embryonic blood vessel formation begins with the de novo emergence of primordial endothelial cells shortly after gastrulation, when their parent mesoderm progenitors are formed. The differentiation of endothelial cells from multipotent mesodermal progenitors, and their coalescence into vascular plexi, is a complex process involving multiple signaling pathways and transcriptional regulators, which has been recently reviewed.⁶

Newly formed endothelial tubes then govern the subsequent acquisition of mural cells (pericytes or SMCs) that make up the surrounding vessel wall. Proliferating endothelial cells secrete platelet-derived growth factor-B that acts as a chemoattractant and mitogen for mural cell precursors, derived from the mesenchyme surrounding the endothelial tubes.⁸ On contact with endothelial cells, newly recruited mesenchymal progenitors are induced toward a mural cell fate, in a process known to involve heterocellular gap junction channel formation, which is necessary for the subsequent activation of TGF-β.³,⁷

Activated TGF-β is thought to induce mural cell–specific gene expression via TGF-β-control elements in the promoter region of genes such as SM-α-actin. TGF-β also induces mural cell differentiation via the upregulation of the transcription factor, serum response factor. Serum response factor binds to a DNA sequence referred to as a CArG box, and recruits myocardin, a coactivator that is necessary and sufficient for mural cell–specific gene expression.⁴,⁶

Mural Cell Differentiation: TGF-β Not the Only Director

The study by Merfeld-Clauss et al⁴ established an in vitro model of human blood vessel assembly, and used it to demonstrate an alternative mechanism by which endothelial cells induce mural cell differentiation during this process, via activin A in a TGF-β–independent process (Figure). They found that direct contact between adipose stromal cells (ASC) and endothelial cells lead to release of activin A, which then promoted the expression of mural cell–specific genes in ASC. In contrast, when endothelial cells and ASC were cocultured in close proximity but without direct contact, activin A was not upregulated or released, and ASC were not induced to differentiate toward a mural cell phenotype. Endothelial-induced differentiation of ASC was blocked by neutralizing antibodies against activin A or small molecular inhibitors of ALK4/5/7 signaling. Interestingly, although the investigators found that TGF-β could upregulate activin A in ASC solo cultures, inhibition of TGF-β signaling in endothelial cell–ASC cocultures did not prevent endothelial-induced ASC differentiation, suggesting an alternate, as yet undefined mechanism.

Of note, differentiated SMCs express a characteristic repertoire of contractile proteins. Many, but not all of these, were induced in ASC by endothelial cell contact, conditioned media, or activin A treatment. For example, smooth muscle myosin heavy chain, the most definitive marker of SMC differentiation, was not induced in this model system. One possibility is that endothelial cell contact and activin A induce partial SMC differentiation in ASC, whereas other factors may be required for full mural cell differentiation.

Endothelial cell–ASC cocultures or conditioned medium induced Smad2 activation in ASC, which was suggested as a potential signaling pathway underlying the induction of mural cell gene expression. Although recent work has shown an inhibitory role for Smad3 in myocardin expression and SMC differentiation, other studies demonstrate an integral role for Smad2 and myocardin-related transcription factor-B in promoting TGF-β–induced SMC differentiation of neural crest progenitors. It is possible that activin A promotes a complex interplay among Smad signaling, myocardin, and myocardin-related transcription factors that results in the partially differentiated SMC phenotype exhibited by the ASC, and perhaps other mesenchymal cell types.

Although a key finding of the study of Merfeld-Clauss et al⁴ was that direct contact between the ASC and the endothelial cells was required to initiate mural cell gene expression in ASC, it was noted that activin A is a diffusible factor. Thus,
the generation of activin A on endothelial–mesenchymal cell contact, and its subsequent diffusion to neighboring cells, may represent a possible mechanism by which endothelial cells promote a mural cell phenotype in mesenchymal precursors that are not in direct contact, yet contribute to the formation of a SMC wall during blood vessel assembly (Figure). However, the role of activin A in the process of endothelial-induced mural cell differentiation in vivo requires additional investigation, given that activin A knockout mice survive until birth and then die within 24 hours because of palate-malformation–induced feeding problems; there are no reported defects in blood vessel formation or function in the activin A–deficient mice.

Recapitulation of Vascular Morphogenesis:
Making Adipose Clinically Useful
Another important aspect of the studies by Merfeld-Clauss et al is the fact that mural cell gene expression was promoted in mesenchymal cells derived from human adipose. Human adipose tissue, like bone marrow, contains a stroma that is easily isolated, and the stromal cells therein have been shown to exhibit multipotentiality, with the ability to differentiate toward osteogenic, adipogenic, chondrogenic, and myogenic lineages. The mechanistic insights gained from the study of Merfeld-Clauss et al should improve their directed differentiation specifically toward a SMC fate. Such information, combined with the accessibility and availability of human adipose, makes the ASC therein an excellent source of autologous progenitors for vascular tissue engineering and repair.

Interestingly, the phenotype of ASC overlaps to some extent with SMC and pericytes, and ASC can support the survival and function of endothelial cells. Thus, providing both cell types together for clinical therapies may prove useful for the optimization of vascular regeneration. In addition, ASC may be useful for stabilizing remodeling vessel structures in vivo, as an adjunct for pharmacological therapies designed to promote endothelial cell proliferation.

SMC Manipulation: Relax and Be Flexible
Once differentiated, mature SMCs retain plasticity to dedifferentiate, proliferate, and migrate in response to stimuli, including injury, inflammation, and hypoxia. Although this property allows for repair of vessel injury, or vascular remodeling in response to changes in tissue demand, SMC dedifferentiation also contributes to vascular pathophysiology, including atherosclerosis and restenosis. Interestingly, 2 previous studies found that viral overexpression of activin A inhibited intimal hyperplasia in vivo. A transcriptome analysis in cultured human venous SMC revealed that activin A and TGF-β induced similar patterns of gene expression, including contractile proteins, but that activin A was uniquely associated with a nonfibrotic phenotype. The study of Merfeld-Clauss et al provides further support for activin A promotion of a differentiated SMC program of gene expression. It also raises the possibility that activin A differentiation of other cell types could potentially contribute to the beneficial injury response. Others have reported that activin A can even promote the differentiation of human umbilical vein endothelial cells into a SMC-like phenotype in vitro. Although this did not occur under the conditions used by the current study, the findings of Merfeld-Clauss et al support the possibility that activin A signaling might influence vascular remodeling in vivo and may even have potential for in situ reprogramming.

Summary
Injection of autologous stem cells into ischemic tissues to promote revascularization is an attractive idea that has yet to fulfill its promise in the clinic. Merfeld-Clauss et al now demonstrate that the heterogeneous stromal vascular fraction from human adipose tissue that exhibits multipotentiality can generate endothelial cell tubes that then induce the migration and differentiation of ASC toward a mural cell phenotype, leading to a more mature and stable vasculature. Although the findings are predominantly in vitro, they identify a novel TGF-β–independent mechanism of endothelial-induced mural cell differentiation that uses paracrine actions of activin A. This work has important implications for revascularization of ischemic tissue and potentially for vascular remodeling in diseases of inappropriate SMC dedifferentiation.

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References

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