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The “Magic Touch”: Endothelial Cells Muscle-up Adipose

Kathleen A. Martin, PhD^{1,2} and Karen K. Hirschi, PhD^{1,3}

¹Department of Internal Medicine, Yale Cardiovascular Research Center and Vascular Biology and Therapeutics Program, Yale University, New Haven, CT 06511, USA

²Department of Pharmacology, Yale Cardiovascular Research Center and Vascular Biology and Therapeutics Program, Yale University, New Haven, CT 06511, USA

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Introduction

Merfeld-Clauss and colleagues (1) describe an Activin A-mediated, 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 (SMC) for vascular tissue engineering and reparative medicine, and may also shed light on the process of SMC differentiation during embryonic blood vessel 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 (2, 3). The differentiation of endothelial cells from multi-potent mesodermal progenitors, and their coalescence into vascular plexi, is a complex process involving multiple signaling pathways and transcriptional regulators, which has been recently reviewed (4).

Newly formed endothelial tubes then govern the subsequent acquisition of mural cells (pericytes or smooth muscle cells) that make up the surrounding vessel wall. Proliferating endothelial cells secrete platelet-derived growth factor-B (PDGF-B) that acts as a chemoattractant and mitogen for mural cell precursors (5, 6, 7), derived from the mesenchyme surrounding the endothelial tubes (8, 9). Upon 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 (10, 11), which is necessary for the subsequent activation of transforming growth factor-beta (TGF- β) (6, 12).

³Corresponding author: Karen K. Hirschi, PhD, karen.hirschi@yale.edu, (203)737-4533.

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Activated TGF- β is thought to directly induce mural cell-specific gene expression via TGF- β -control elements (TCE) in the promoter region of genes such as SM- α -actin (13). TGF- β also induces mural cell differentiation via the upregulation of the transcription factor, serum response factor (SRF) (14). SRF 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 (15, 16).

Mural Cell Differentiation: TGF- β not the only director

The study by Merfeld-Clauss and coworkers established an in vitro model of human blood vessel assembly, and used it to demonstrate an alternate mechanism by which endothelial cells induce mural cell differentiation during this process, via Activin A in a TGF- β -independent process (depicted in Figure 1). 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 co-cultured 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-ASC co-cultures did not prevent endothelial-induced ASC differentiation, suggesting an alternate, as yet undefined mechanism.

Of note, differentiated SMC 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 a partial SMC differentiation program in ASC, while other factors may be required for full mural cell differentiation.

Endothelial cell-ASC co-cultures or conditioned medium induced Smad2 activation in ASC, which was suggested as a potential signaling pathway underlying the induction of mural cell gene expression. While recent work has shown an inhibitory role for Smad3 in myocardin expression and SMC differentiation (17), other studies demonstrate an integral role for Smad2 and myocardin-related transcription factor (MRTF)-B in promoting TGF- β -induced SMC differentiation of neural crest progenitors (18). It is possible that Activin A promotes a complex interplay between Smad signaling, myocardin and MRTFs that results in the partially differentiated SMC phenotype exhibited by the ASC, and perhaps other mesenchymal cell types.

Although a key finding of the Merfeld-Clauss et al. study was that direct contact between the ASC and 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 upon 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 smooth muscle cell wall during blood vessel assembly (depicted in Figure 1). 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 hr due to palate-malformation-induced feeding problems (19); 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 and coworkers 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 multi-potentiality (20), with the ability to differentiate toward osteogenic, adipogenic, chondrogenic, as well as myogenic, lineages (21). The mechanistic insights gained from the Merfeld-Clauss study 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 (22), and ASC can support the survival and function of endothelial cells (23). 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.

Smooth Muscle Cell Manipulation: Relax and be flexible

Once differentiated, mature SMC retain plasticity to de-differentiate, proliferate, and migrate in response to stimuli including injury, inflammation, and hypoxia. While this property allows for repair of vessel injury, or vascular remodeling in response to changes in tissue demand, SMC de-differentiation also contributes to vascular pathophysiology, including atherosclerosis and restenosis (24). Interestingly, two prior studies found that viral overexpression of Activin A inhibited intimal hyperplasia in vivo (25, 26). 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 non-fibrotic phenotype (27). The Merfeld-Clauss study 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 (HUVEC) into a SMC-like phenotype in vitro (28). While this did not occur under the conditions employed by the current study, the findings of Merfeld-Clauss and coworkers 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 fulfil its promise in the clinic. Merfeld-Clauss and coworkers now demonstrate that the heterogeneous stromal vascular fraction from human adipose tissue that exhibits multi-potentiality, 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. While the findings are predominantly in vitro, they identify a novel TGF β -independent mechanism of endothelial-induced mural cell differentiation that employs 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 de-differentiation.

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Figure 1.

In vitro model of human blood vessel assembly. Merfeld-Clauss and coworkers demonstrate an alternate mechanism by which endothelial cells induce mural cell differentiation during this process, via Activin A in a TGF- β -independent process.