Developmental Basis of Vascular Smooth Muscle Diversity

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Abstract—The origins of vascular smooth muscle are far more diverse than previously thought. Lineage mapping studies show that the segmental organization of early vertebrate embryos leaves footprints on the adult vascular system in the form of a mosaic pattern of different smooth muscle types. Moreover, evolutionarily conserved tissue forming pathways produce vascular smooth muscle from a variety of unanticipated sources. A closer look at the diversity of smooth muscle origins in vascular development provides new perspectives about how blood vessels differ from one another and why they respond in disparate ways to common risk factors associated with vascular disease. (Arterioscler Thromb Vasc Biol. 2007;27:000-000.)

Key Words: vascular development ■ embryo ■ lineage ■ serum response factor ■ heterogeneity

In most review articles on vascular development, one finds the statement that newly formed blood vessels recruit smooth muscle cells (SMCs) and pericytes from surrounding mesenchyme. This simple statement masks the complexity inherent in the recruitment process because mesenchyme is not uniform throughout the embryo, and it is not the only source of vascular SMCs in development. The first indication that vascular smooth muscle origins might be diverse came from neural crest fate mapping studies in avian embryos, where SMCs in the great vessels were traced back to a distant source of progenitors at the dorsal surface of the cranial neural tube. As more and more attention was focused on the question of how blood vessels develop, a new picture began to emerge, catalyzed by the appearance of novel genetic tools for lineage analysis including retroviral markers and Cre recombinase-activated reporter genes. As a result, we now realize that vascular smooth muscle is a mosaic tissue produced from at least seven unique and nonoverlapping origins in vertebrate embryos. The purpose of this article is to review the developmental origins of vascular smooth muscle diversity, and to discuss the implications of such diversity for blood vessel function and disease.

Vascular Smooth Muscle Heterogeneity: A Lineage Perspective

Most of what we have traditionally called SMC heterogeneity comes from studies of cytoskeletal and contractile protein expression in vascular development and disease. The common finding is that expression of smooth muscle marker genes is coordinately upregulated in quiescent, differentiated SMCs and downregulated in proliferating SMCs, an expression profile known as phenotypic switching. Other types of SMC heterogeneity that have been described include stable differences in cell morphology, organization at confluence, production of peptide growth factors, sensitivity to heparin-mediated growth inhibition, and ability to proliferate in serum-free medium. Some of these forms of phenotypic variation may be developmental stage-dependent, whereas others may be revealed by assay conditions in vitro. Regardless, it is not possible to discern whether the above results are attributable to phenotypic variants of a single type of SMC, or reflect the activity of a mixture of different SMC subtypes using the methods employed.

A conceptually different approach, one that relies on fate mapping techniques in developing embryos, produces a quite different view of the smooth muscle composition within the vascular system. With this approach, we see that the variations in SMC marker gene expression observed during phenotypic switching are common to all SMC types. More importantly, we also see that different vessels, or even different segments of the same vessel, are composed of SMC populations that arise from distinct sources of progenitors, each with its own unique lineage and developmental history (Figure 1). What appeared to be uniform and seamless, is now revealed to be discontinuous and mosaic inasmuch as distinct SMC subtypes are found within a common arterial tree (Figure 1). The boundaries between SMCs of different origins are often remarkably sharp with little or no intermixing.

Moreover, SMCs from different embryonic origins respond in lineage-specific ways to common stimuli, even when tested under identical conditions in vivo or in vitro. These findings suggest that a better understanding of the origins of vascular smooth muscle in development would provide important new insights into the heterogeneous patterns of adaptive or pathologic responses characteristic of the mature vascular tree.
The first lineage maps for vascular SMCs were produced from embryonic chick-quail chimeras. The goal of these early studies was to map developmental fates of different regions of the neural crest. For cranial neural crest, two important fates were identified with respect to the vascular system, namely an accumulation of these cells in the walls of pharyngeal arch arteries where they differentiate into vascular SMCs, and the continued migration of a subset of these cells into the cardiac outflow tract where they mediate septation of the aorta and pulmonary trunk. These results provided the first view of a mosaic pattern of smooth muscle in the vasculature, because neural crest-derived SMCs populated only a small segment of the aorta and other great arteries in close proximity to the heart itself (see Figure 1). These pioneering observations in avian embryos are now known to represent an evolutionarily conserved pathway for neural crest migration, differentiation, and vascular development in many different vertebrate species.

More recently, the role of neural crest cells in mammalian vascular development was examined using a genetic approach that used a Wnt1-Cre transgenic line crossed with a floxed stop Rosa26 reporter line (R26R). The resulting activation of a lacZ reporter gene in neural crest cells and their descendents served as a sensitive lineage marker. This analysis showed that murine neural crest contributes SMCs to the ascending and arch portions of the aorta, the ductus arteriosus, the innominate and right subclavian arteries, as well as the right and left common carotid arteries (Figure 1). Equally important, it also showed that SMCs in the descending thoracic aorta, abdominal aorta, coronary arteries, pulmonary arteries, left subclavian artery, and distal portions of the internal carotid arteries were not labeled by the Wnt1-Cre lineage marker, and were therefore of nonneural crest origin. Essentially identical results were reported using a different neural crest-specific transgenic line (P0-Cre) and a different reporter line that carried a floxed EGFP transgene. As in chick-quail chimeras, sharp boundaries between neural crest-derived SMCs and nonneural crest-derived SMCs were also seen in mouse arteries. Endothelial cells were not labeled by the neural crest lineage markers, and the adventitia also appeared to derive from an origin other than neural crest.

Reports of ventral hindbrain neural tube (vent) cells that contribute SMCs to craniofacial arteries and the great vessels have also appeared. Vent SMCs are reported to emerge only after dorsal neural crest cells have ceased migrating into the pharyngeal arches. Ventral neural tube cells migrate in association with developing cranial nerves and populate their target tissues with multipotential cells that can differentiate into various types of mesenchymal cells, including vascular SMCs. However, the existence of vent SMCs has been called into question by Boot et al who used retroviral cell tagging and chick-quail chimeras, and could not observe a population of ventral neural tube-derived cells contributing to the developing heart and outflow vessel system.

A second, clearly distinct type of vascular SMC is found in the walls of coronary arteries (for reviews on coronary development, see). Fate mapping studies have shown that progenitors for coronary vessels are found in the proepicardium, a transient primordium that arises from septum transversum mesenchyme in the area of the sinoatrial junction (Figure 1). Although the coronary lumen is continuous with the
aortic lumen, coronary vessels do not develop as outgrowths of the aorta.21 Rather, they arise separately by vasculogenesis within the subepicardium and then invade the aorta to make contact with the aortic endothelium.17 Neural crest cells make little or no contribution to the formation of coronary smooth muscle beyond the immediate origins of coronary stems from the aorta.

The proepicardium appears at the looped heart stage in mouse (E9.5) and chick (E2.5) embryos.22,23,24 It reaches the heart either by direct contact of proepicardial villi with the myocardium, or by release of mesothelial cell aggregates by vesicle formation.24,25 The adherent proepicardial cells then migrate over the surface of the heart to form the epicardial layer.20,24 In response to signals from the myocardium, some epicardial cells undergo epithelial to mesenchymal transformation (EMT) and migrate into the heart where they participate in formation of early coronary vessels and provide precursors for coronary SMCs.26,27,28 Using retroviral vectors that express a LacZ reporter gene, Mikawa and Fishman18 and Mikawa and Gourdie29 showed that precursor cells in the proepicardium give rise to the epicardium, coronary endothelium and coronary SMCs. Thus development of coronary vessels is separate and distinct from that of the systemic vasculature.

Genetic evidence confirms the unique origins of coronary and systemic arteries. Friend of GATA protein (FOG)-2 physically associates with the N-terminal zinc finger of GATA-4 and can either activate or repress transcription, depending on target gene context. FOG-2-deficient embryos die at midgestation with a complete absence of coronary vasculature.30,31 The epicardium forms normally, and the expression of epicardial marker genes, including Wilms' Tumor-1, capsulin, and retinaldehyde dehydrogenase, does not differ from wild-type embryos. Yet epicardial cells fail to undergo EMT, and coronary endothelial cells do not appear in FOG-2/−/− embryos.30 Failure to form coronary vessels is a non–cell-autonomous phenotype for epicardial-derived cells, as coronary vasculature is produced normally when FOG-2 expression is restored only in cardiac myocytes.32 Significantly, no other vascular bed is defective in formation or maturation in FOG-2 null mice. Therefore, genetic analysis also supports the idea that coronary vessels are under developmental controls that are unique to this vascular bed. These results may begin to explain why coronary arteries respond differently than systemic arteries to identical stimuli. For example, Badimon et al reported that balloon angioplasty injury to pig coronary artery resulted in greater platelet-thrombus formation and neointimal thickening than did the identical injury to common carotid arteries in the same animals.33 As discussed in more detail below, the progression of atherosclerotic disease and its recurrence after surgical intervention proceeds at different rates in coronary vessels compared with other disease-prone arteries within the same patients, whose systemic risk factors might be expected to affect all vessels to similar extents.

### Origin From Mesothelium

The evidence that proepicardial mesothelium produces coronary SMCs raises the obvious question of whether or not smooth muscle formation is a property of other mesothelia. The significance of this question is underscored by the fact that most internal organs are covered by a mesothelial lining. Thus, the lungs are enclosed within a pleural mesothelium, the liver, stomach, and intestines within a peritoneal mesothelium, the heart within a pericardial mesothelium, and the brain within a subdural mesothelium. It is also important to recognize that mesothelium is an evolutionarily ancient tissue type that is intimately associated with production of mesenchymal cells and phagocytic macrophage-like cells in many lower vertebrates and some invertebrates.34,35 It has even been suggested that the endothelium-lined system of blood vessels found in vertebrates evolved as a specialization of the phylogenetically more primitive mesothelial lining of coelomic cavities.36 The essential property of primitive mesothelia may be the ability to generate multipotential mesenchymal cells with migratory and invasive properties.

Most mesothelial cells express the zinc finger-containing transcription factor Wilms’ Tumor-1 (WT1).36 Using WT1-Cre mice crossed with a floxed R26R reporter strain, Wilm et al found that β-galactosidase-positive cells marked all blood vessels of the mesenteric vasculature as well as their branches that penetrate and perfuse the gut wall.37 Close inspection of lacZ-positive cells in mesenteric arteries revealed the characteristic perpendicular arrangement of SMCs in the tunica media. LacZ-positive staining was also seen in fibroblast-like cells throughout the gut, highly reminiscent of the cardiac fibroblast fate of proepicardial mesothelial cells in heart development.38 The longitudinal and circumferential layers of enteric SMCs in the gut wall itself are negative for lacZ activity, and therefore do not arise from mesothelium. Most of the endothelial cells in blood vessels in the gut are not derived from WT1-positive mesothelium, and probably arise from an early vascular plexus that develops before the gut wall itself. When examined in adult mice, most mesenteric vascular SMCs (78%) expressed the lacZ reporter gene and thus comprised a stable cohort of SMCs derived from serosal mesothelium. An origin for vascular SMCs from mesothelium is not likely to be limited to epicardium and peritoneum. For example, cells that express the mesothelial marker cytokeratin can be traced from the mesothelial lining of the pleural-peritoneal coelom into the wall of the dorsal aorta where they coexpress SM α-actin and the 1E12 antigen (a smooth muscle-specific α-actinin isoform).38 These important findings greatly expand our view of the potential sources of vascular SMCs in development. They also raise the question of whether or not the mesothelium serves as a reservoir of SMC precursors in adult tissues that may be activated in response to injury, infection, or disease.

### Origin From Secondary Heart Field

Cardiac neural crest-derived SMCs are found in the ascending aorta, but they do not extend into the base portions of the aorta or pulmonary trunk. Moreover, although proepicardial cells contribute SMCs to the coronary vessels, they do not populate the aortic root.19 To identify the origins of SMCs at the base of the aorta and pulmonary trunk, Kirby and colleagues tested the idea that these SMCs arise from the secondary heart field, a population of progenitor cells lying beneath the floor of the foregut that provides myocardium to
the arterial pole of the heart as the outflow tract elongates during embryonic development.\textsuperscript{39} Using microinjection of lineage tracer dyes, secondary heart field cells were labeled at stages 14 to 15 or stages 18 to 20, and the fate of labeled cells was determined.\textsuperscript{40} Two distinct waves of cell migration from the secondary heart field into the arterial pole of the heart were observed. The first group marked at stages 14 to 15, migrated to the outflow tract and differentiated into cardiac myocytes. The second group, labeled at stages 18 to 20, also migrated to the outflow tract, but then entered the aortic sac and differentiated into SMCs that formed the base of the aorta and pulmonary trunk. Secondary heart field-derived SMCs expressed SM \( \alpha \)-actin, SM22\( \alpha \), and SM-myosin light chain kinase.\textsuperscript{40} Similar findings were reported by Maeda et al using transgenic mice expressing Cre recombinase under the control of a 1.1-kb Tbx1 enhancer.\textsuperscript{41} Expression of Tbx1 in the secondary heart field is controlled by forkhead factors interacting with two conserved Fox binding sites in the 5' regulatory region of the Tbx1 locus. Cells expressing Tbx1-cre were traced into the walls of the main pulmonary trunk and ascending aorta, where they differentiated into vascular SMCs.\textsuperscript{41} By contrast, the ductus arteriosus and coronary arteries were negative for Tbx1-cre-mediated reporter expression. Ablation of the secondary heart field produced cardiac defects, pulmonary atresia, overriding aorta, and coronary artery anomalies.\textsuperscript{42} Thus the need to provide myocardial cells for elongation of the arterial pole of the heart is met by a contribution of secondary heart field-derived cardiac progenitors. At the same time, the aorta and pulmonary trunk also elongate, and these vessels recruit additional SMCs from the same secondary heart field. As pointed out by Waldo et al, this segmental growth of the outflow vessels results in two seams in the arterial pole, namely the myocardial junction with secondary heart field-derived SMCs, and secondary heart field SMCs with neural crest-derived SMCs.\textsuperscript{40} Both of these seams are sites of aortic dissections that occur in Marfan’s syndrome.

### Origin From Somites

The dorsal aorta develops in close proximity to the somites\textsuperscript{43} (see Figure 1). Within 24 hours after grafting quail somites into chick embryos, a contribution of somite-derived cells to aortic endothelium was observed,\textsuperscript{44} consistent with earlier studies.\textsuperscript{45,46} When examined 3.5 days after grafting, numerous quail cells were found surrounding the aorta, many of which had incorporated into the aortic tunica media and were expressing SM \( \alpha \)-actin.\textsuperscript{44} The region of the aorta thus labeled was caudal to the pharyngeal arches and therefore outside the domain of cardiac neural crest-derived SMCs. Moreover, trunk neural crest cells in the region of the somite graft do not normally make vascular SMCs, and thus differ from cranial neural crest cells in that regard.\textsuperscript{47} Somite-derived SMCs most likely arise from progenitors in the sclerotome.\textsuperscript{48} Evidence that some aortic SMCs may also arise from myotome was reported by Esner et al who analyzed clones of Pax3-expressing cells in the developing mouse somite.\textsuperscript{48} Pax3 is a transcription factor expressed in presomitic paraxial mesoderm, and later in dermomyotome where it is required for the survival, migration, and differentiation of skeletal muscle myoblasts. To trace the fate of Pax3-positive cells during somite development, EGFP was introduced into the murine Pax3 locus.\textsuperscript{48} At E10.5, cells that were both EGFP-positive and SM \( \alpha \)-actin-0positive were found in the ventral wall of the dorsal aorta,\textsuperscript{48} consistent with reports that the first aortic SMCs appear on the ventral aspect of the dorsal aorta in chick embryos.\textsuperscript{49} Labeled aortic SMCs are found adjacent to a labeled somite, suggesting very little dispersion of somite-derived SMCs along the anterior-posterior axis. Thus, smooth muscle of the descending thoracic aorta appears to be “segmental” insofar as the aortic wall is built up of SMCs contributed by individual somites, each somite producing SMC progenitors within locally restricted spatial domains (Figure 1). An important contribution of Pax3-expressing somite-derived cells to aortic smooth muscle is suggested by the observation that the ventral wall of the aorta in Pax3-deficient embryos at E10.5 is thinner than in wild-type embryos.\textsuperscript{46} In Pax3 mutants, rates of cell death in hypaxial dermomyotome are increased, thus reducing the number of SMC progenitors available to colonize the aortic wall.\textsuperscript{40} By contrast, aortic endothelial cells at E8.5 and E10.5 were Pax3-EGFP negative.

### Origin From Mesoangioblasts

Cossu and colleagues isolated a multipotential cell type from explants of E9.5 murine dorsal aorta that could differentiate into skeletal muscle, smooth muscle, and other mesenchymal cell types in vitro.\textsuperscript{51,52,53} These aorta-derived satellite-like cells were called mesoangioblasts because they express both myogenic and endothelial cell markers.\textsuperscript{51} The name mesoangioblast draws distinction from another class of multipotential cell, the hemangioblast, which gives rise to both hematopoietic and endothelial cell lineages.\textsuperscript{54,55,56} Unlike hemangioblasts, however, mesoangioblasts do not express \( \alpha \)-SMA, and do not form hematopoietic cells after transplantation in vivo. When genetically-tagged mesoangioblasts were transplanted into the tibialis anterior (TA) muscle of newborn SCID mice, they were found to contribute to normal postnatal fiber growth and regeneration, and to fuse with resident satellite cells in vivo.\textsuperscript{51}

To test the full range of differentiation potential of mesoangioblasts in vivo, Minasi et al grafted segments of either quail or mouse embryonic aorta into chick embryos.\textsuperscript{52} They found that grafted mesoangioblasts first incorporated into host blood vessels and were dispersed by the circulation, and then later they were found integrated into a wide range of mesodermal tissues including blood, cartilage, bone, smooth muscle, cardiac muscle and skeletal muscle. In blood vessels, they were found in the medial layers of arteries where they expressed desmin and SM \( \alpha \)-actin. Mesoangioblasts can be cloned in vitro, passaged indefinitely (>60 passages), and undergo self-renewal as indicated by the ability of serially passaged cells to generate a wide range of mesodermal cell types when transplanted into chick or mouse embryos in vivo.\textsuperscript{52,53} The ability to travel within the bloodstream distinguishes mesoangioblasts from true satellite cells that have lost this property. Given the close physical proximity of somites and the dorsal aorta\textsuperscript{43} (Figure 1, inset), it is reasonable to suggest that mesoangioblasts may correspond to...
progenitor cells from the hypaxial dermomyotome that migrated to the developing dorsal aorta and failed to differentiate thereby retaining multipotential properties.

**Origin From Stem/Progenitor Cells**

A variety of different types of self-renewing progenitor cells have been reported to form SMCs in vitro and in vivo. Mouse embryonic stem (ES) cells cultured on type IV collagen and then sorted for expression of flk1 (VEGF receptor-2), were found to differentiate into either SMCs or endothelial cells depending on the type of growth factor to which they were exposed. In the presence of 10 ng/mL PDGF-BB, more than 95% of the flk1-positive cells expressed SM α-actin, acquired a spindle shape characteristic of vascular SMCs, and lost expression of flk1. Combined growth factor stimulation of clones of flk1-positive ES cells produced colonies consistent of both endothelial and SMCs, consistent with the presence of a bipotential progenitor. Finally, genetically tagged, flk1-expressing ES cells injected into chick embryos were found to differentiate into SMCs and incorporate into yolk sac blood vessels.

Progenitor cells with a capacity for SMC differentiation were also identified in adult arteries. Using flow cytometry to sort cells from adult mouse aorta, Sainz et al reported isolation of “side population” (SP) cells from intima–media digests, which were not present in adventitial tissues. In these studies, the SP fraction was reported to make up 6% of the total cell population of the aortic media and ~ 15% of the carotid media. These tunica media–derived SP cells expressed a Sca-1+, c-kit-low, Lin–, CD34-low marker profile. When isolated and tested for differentiation potential in vitro, adult aortic SP cells expressed an endothelial phenotype when cultured in the presence of VEGF, and a SMC phenotype when exposed to either transforming growth factor (TGF)-β1 or PDGF-BB. When plated in Matrigel, aortic SP cells formed vessel-like structures composed of both endothelial cells and SMCs in vitro. Adult aortic SP cells lacked erythroid, lymphoid, or myeloid potential, and were thus different from marrow–derived SP cells identified in skeletal muscle.

Adult mouse aortas contain a second, distinct population of SMC progenitors that reside in the adventitia. These cells express the Sca-1 marker, and are particularly abundant in the adventitial layer surrounding the aortic root. Unlike aortic medial SP cells, adventitial Sca-1+ cells do not arise from bone marrow. When freshly isolated and tested in vitro, adventitial Sca-1+ cells differentiated into SMCs when exposed to PDGF-BB, and into endothelial cells when exposed to VEGF-A. Moreover, when adventitial Sca-1+ cells from Rosa26 mice were transplanted to the adventitial side of vein grafts in apoE-deficient mice, β-gal-positive cells were found in graft neointima that were also SMC-marker positive. Therefore, there may be several distinct types of SMC progenitor cells normally resident in the adult artery wall with the ability to respond to injury or disease-promoting stimuli and differentiate into SMC-like cells in vivo.

Some progenitor cells in the adult vessel wall may be specified to form pericytes. Mesenchymal cells isolated from mature rat aortas and grown in serum-free stem cell media supplemented with basic FGF led to the appearance of slowly-proliferating, nonadherent cells that formed spheroid colonies in vitro. At the outset, these colonies failed to express endothelial or SMC markers, but were positive for CD34, tie2, nestin, and PDGF receptors. When exposed to 10% fetal calf serum, spheroidal cells lost expression of CD34, and acquired expression of SMC marker proteins. When spheroid–forming cells were cocultured with angiogenic outgrowths of rat aorta, or with endothelial cells, they differentiated into pericytes. Given the stabilizing role of pericytes as a source of survival factors for newly formed angiogenic sprouts, these pericyte progenitors may provide an important source of support cells for angiogenesis during vascular wound repair.

Other sources of multipotential cells that have been reported to differentiate into SMCs include human adipose tissue, multipotential cardiac progenitor cells, amniotic fluid–derived mesenchymal stem cells, bone marrow–derived stromal cells, and follicular dendritic cells. For more in-depth coverage of this topic, the interested reader is directed to several excellent recent reviews. The general conclusion from this work is that the formation of new vascular SMCs from undifferentiated progenitors is not limited to embryogenesis, and that the quiescent adult artery wall contains resident progenitor cells that can differentiate into SMCs and/or pericytes in vivo.

**Origin of Microvascular SMCs and Pericytes**

The few developmental fate maps that have been analyzed at the microvascular level suggest that pericytes and microvascular SMCs are recruited from the same lineage that supplies SMCs to neighboring large arteries. For example, the cephalic neural crest is known to supply mesenchymal cells to the ventral part of the head and neck. Etcheverry et al used chick-quail chimeras and a panel of cell type–specific antibodies to show that both large vessel SMCs and microvascular pericytes in the forebrain and face are cranial neural crest–derived. Whether or not microvascular SMCs and pericytes at other locations will be found to share the same embryonic origins as SMCs in nearby large vessels remains to be determined.

**Molecular Controls for SMC Differentiation**

As lineage mapping studies further define the cellular origins of vascular SMCs, one of the next important goals is to identify the molecular pathways that enable diverse types of progenitor cell, each arising from unique embryonic backgrounds, to produce the same cell type (SMC) on differentiation. How embryonic mesodermal cells of any origin become specified to express a SMC fate is not known. Yet the ultimate readout of that fate, transcription of SMC marker genes, has been the subject of considerable work. We know that most SMC markers require serum response factor (SRF) for gene transcription, however SRF itself is a relatively weak transcriptional activator that relies on interactions with coactivators and corepressors to regulate gene expression (Figure 2). Therefore, it is necessary to look more closely at SRF–cofactor interactions to understand how lineage-specific pathways produce a common SMC fate.
SRF is a highly versatile DNA-binding protein that effectively links different signaling inputs to common DNA targets. In this way, environmental cues that are unique to different smooth muscle origins can activate a common set of SMC target genes through combinatorial interactions of SRF with one or more transcriptional coregulators. A partial list of known activators and repressors of SRF-dependent transcription is shown (top). Depending on the composition of complexes formed between SRF and its various coregulators, the resulting outcome would enable SMC differentiation in response to environmental cues that are unique to individual lineage backgrounds (eg, neural crest versus proepicardium) (bottom).

Figure 2. Activators and repressors of SRF-dependent transcription. SMC-specific gene transcription depends on complexes of SRF homodimers (purple) bound to cis-acting CArG elements (gold) in promoter/enhancer sites on SMC target genes. Blue represents DNA sequences flanking CARG boxes that are important for SMC subtype-selective gene expression (see text). SRF activity and/or binding affinity for smooth muscle CARG elements is largely determined by the combinatorial interactions of SRF with one or more transcriptional coregulators. A partial list of known activators and repressors of SRF-dependent transcription is shown (top). Depending on the composition of complexes formed between SRF and its various coregulators, the resulting outcome would enable SMC differentiation in response to environmental cues that are unique to individual lineage backgrounds (eg, neural crest versus proepicardium) (bottom).

The crystal structure of the MADS domain bound to DNA revealed a coiled-coil region of the α2 helix that is oriented away from DNA and serves as a docking site for accessory factors. These accessory factors modify the binding affinity or activity of SRF at degenerate CARG boxes found in most SMC marker genes, and thus couple transcription to upstream signaling pathways (Figure 2). For example, the myocardin family are a family of SAP domain proteins that are important SRF coactivators for SMC differentiation. Although all myocardin family members can stimulate transcription of CARG-dependent SMC promoter constructs in vitro, each family member has a unique expression pattern in vivo, and their individual loss of function phenotypes are distinct and non-overlapping. Thus, myocardin-null embryos die around E10.5 with yolk sac and vascular defects, whereas embryos deficient in myocardin-related transcription factor-B (MRTF-B) die at E13.5 with vascular defects limited to neural crest-derived SMCs. Of particular interest, the SRF-dependent SMα-actin gene is expressed normally in dorsal aorta, yolk sac and heart of MRTF-B-deficient embryos, but is down regulated in neural crest-derived SMCs. Mice lacking MRTF-A reach adulthood, but myoepithelial cells in the mammary gland fail to differentiate, thus resulting in the inability of those cells to provide the contractile force necessary to secrete milk for nursing pups. MRTF-A–deficient mice are otherwise normal with no apparent defects in yolk sac vessels, neural crest-derived SMCs, or any other smooth muscle tissue. Therefore, SMCs that originate from cardiac neural crest require MRTF-B for differentiation, whereas dorsal aorta SMCs use myocardin, and myoepithelial cells require MRTF-A. Given the long list of accessory factors known to interact with SRF (Figure 2), one can readily envision how a common set of SMC marker genes could be activated by unique combinatorial interactions involving SRF and one or another cofactor responding to lineage-specific signaling inputs in a wide range of different SMC progenitors.

In addition, many SMC marker genes use distinct cis-regulatory elements for transcription in different SMC subtypes. For example, a 16-kb fragment of the SM-myosin heavy chain (SM-MHC) gene directs expression in virtually all smooth muscle tissues in vivo, including large and small arteries and veins. The same construct with a 200-bp deletion of a DNase hypersensitive site positioned 8 kb downstream of the transcription start site lost expression in the aorta and pulmonary arteries, but retained expression in the coronary and mesenteric arteries (both mesothelium-derived). Similarly, a construct that retained only 6.7 kb of 5' sequence lost expression in coronary arteries, but maintained expression in the aorta. In fact, mutations in each of the three conserved CARG elements in the SM-MHC promoter-enhancer produced distinct patterns of reporter gene expression in different smooth muscle tissues in vivo. Therefore, individual SMC subtype-specific regulatory modules do not function in isolation, but interact with other regulatory modules in unique combinations to direct SM-MHC expression in vivo. These results suggest that the mosaic patterns of SMC origins in vascular development may, at least in part, be mirrored by the activity of distinct cis-acting regulatory modules in SMC marker genes such as SM-MHC.

What sequences constitute a SMC subtype-specific regulatory module? Olson and colleagues systematically swapped core and flanking domains of the CARG elements from SM22α (muscle-specific) and c-fos (constitutive) promoters in transgenic mice. Muscle-specific expression required sequences immediately flanking the CARG box core elements. Likewise, Parmacek and coworkers found that a nuclear factor-binding sequence devoid of a recognizable CARG element (SME-3) was required for full SM22α promoter specificity in vascular SMCs in vivo. Therefore, combina-
tiorial interactions between CaR-G elements and adjacent non-CaR-G-containing sequences are necessary to drive arter-
ial SMC-specific expression of SM22α in vivo. Moreover, Hoggatt and Herring made chimeric constructs between
SM22α (arterial SMC specific) and telokin (visceral SMC
specific) promoters. They found addition of a 45-bp telokin
module to the SM22α promoter directed SM22α promoter activity to visceral SMCs in the bladder. Similarly, a 172-bp
SM22α module fused to the telokin promoter directed telokin
promoter activity to arterial SMCs.

Although future studies will produce a detailed understand-
ing of SMC subtype-specific cis-regulatory modules, it
should be pointed out that there is as yet no direct evidence
for a SMC-specific regulatory module that responds to trans-
acting factors in a strictly lineage-specific manner. It
should also be pointed out that SRF can be modified by
phosphorylation, acetylation, and sumoylation, and posttrans-
lational modifications of SRF may well exhibit lineage-specific
interactions with transcriptional coactivators and corepres-
sors. It is also apparent that SMC differentiation in any
lineage requires epigenetic modifications of intact chromatin
to enable SRF and its cofactors to gain access to important
cis-regulatory sites on SMC target genes. Whether or not
lineage-specific chromatin remodeling complexes are formed
during vascular SMC development is an important direction
for future study. As fate mapping studies further define
vascular SMC origins, it will be interesting to learn which
signaling pathways, chromatin remodeling factors, and SRF
cofactors mediate SMC differentiation in different lineage backgrounds (Figure 1). The weight of current evidence
suggests that multiple molecular entry points into the SMC
differentiation pathway exist that are used by different SMC
progenitors during vascular development.

Relation of SMC Diversity to
Morphogenetic Cues

Recognition that vascular SMCs arise from multiple sources
(Figure 1) raises the important question of whether or not
SMCs from different lineage backgrounds are functionally
distinct. To address this question, SMC lineage maps were
used to isolate neural crest-derived SMCs from the aortic arch and
splanchnic mesoderm-derived SMCs from the abdominal
aorta of E14 chick embryos. When compared under identical
conditions in vitro these two types of aortic SMCs exhibited
striking differences in growth and transcriptional responses to
TGF-β1. DNA synthesis and cell proliferation were in-
creased by TGF-β1 (0.4 to 400 pmol/L) in neural crest-
derived SMCs, whereas mesoderm-derived SMCs isolated
from the same vessels were growth inhibited by the same
concentrations of TGF-β1. Moreover, neural crest derived
SMCs displayed strong autoinduction of TGF-β gene expres-
sion, and greatly increased transcription of a plasminogen
activator inhibitor (PAI) promoter construct (12- to 16-fold)
in response to TGF-β1. By contrast, mesoderm-derived
SMCs displayed no autoinduction responses and only minor
increases in PAI-1 promoter activity (2- to 3-fold) after
identical treatment in vitro.9 Rosenquist and coworkers
showed that collagen gel contraction responses to TGF-β1
were much greater in mesoderm-derived SMCs compared
with neural crest–derived SMCs. Although both types of
SMC required β1-integrins to produce gel contraction,
TGF-β1 stimulated much greater increases in α5β1 integrin
expression in mesoderm-derived SMCs.95 These results sug-
gest that different types of SMC found within a common
vessel wall could respond in lineage-specific ways to impor-
tant soluble factors that control development, growth and
remodeling of the vessel wall.

These intriguing results for cultured SMCs still leave us
with the question of whether or not lineage-dependent SMC
responses to morphogenetic cues occur in vivo. An experi-
mental test of this question used surgical ablation to remove
one source of SMC progenitors in early chick embryos, and
monitored SMC recruitment responses of the developing
vascular network. For example, the fourth pharyngeal arch
artery becomes the ascending aorta, and its media is normally
made up entirely of cardiac neural crest–derived SMCs.96
When the cardiac neural crest is surgically ablated, SMCs in
the ascending aorta are supplied instead by the nodose placoade, a neural ectoderm-derived primordium that normally
produces sensory nerves. Placode-derived SMCs, however,
cannot fully rescue the loss of cardiac neural crest because
defects remain in septation of the truncus arteriosus and in
organization of elastic fibers in the great vessel walls. If both
the cardiac neural crest and nodose placode are ablated, then
severe malformations of the aortic arch arteries result.8
Lateral plate mesoderm-derived SMCs are recruited in place
of the missing SMC types, and they form a multilayered
(visceral SMC specific) and telokin (visceral SMC
speciﬁc) promoters. Although both types of
SMC require β1-integrins to produce gel contraction,
TGF-β1 stimulated much greater increases in α5β1 integrin
expression in mesoderm-derived SMCs. These results sug-
gest that different types of SMC found within a common
vessel wall could respond in lineage-specific ways to impor-
tant soluble factors that control development, growth and
remodeling of the vessel wall.

The important conclusion from these studies is that although
three different lineages can produce SMCs that occupy the
same position within the developing aortic arch complex,
they do not respond equivalently to the morphogenetic cues
that operate at this site. Therefore, the major determinants of
the manner in which SMCs from different embryonic origins
respond to important signals for vascular development in the
pharyngeal arch complex are lineage-dependent and not
environment-dependent.

Relation of SMC Diversity to
Vascular Disease

What is the significance of SMC lineage diversity for adult
vascular disease? One approach to this question used aortic
homograft transplantation to examine the responses of differ-
ent aortic segments to a high fat atherogenic diet. Conven-
tional wisdom holds that the differences in atherosclerotic
lesion formation between abdominal (atherosclerosis-prone)
and thoracic (atherosclerosis-resistant) aorta are attributable
to turbulent hemodynamic flow patterns in the abdominal
aorta. To test this idea, Haimovici and colleagues made a
series of aortic homograft transplantsations in which athero-
sclerosis-prone abdominal aortic segments were relocated to
atherosclerosis-resistant positions in either the thoracic aorta
(a high pressure site) or jugular vein (a low pressure site), and
The displaced thoracic aorta or jugular vein segments were repositioned to the abdominal aorta (Figure 3). After surgery, the animals were fed an atherogenic diet for a year, and then the distribution and severity of atherosclerosis in the transplanted segments was examined. Contrary to expectations, the atherosclerosis-prone abdominal aortic segments developed severe atherosclerotic lesions in all locations into which they were transplanted, even though these alternate locations generally do not develop atherosclerosis. Likewise, atherosclerosis-resistant segments failed to develop intimal lesions when transplanted into the atherosclerosis-prone environment of the abdominal aorta. In related experiments carried out by another group, segments of abdominal aorta were transplanted into the disease-resistant pulmonary artery, and very similar results were obtained (ie, the abdominal aortic segment developed atherosclerosis even when transplanted into the pulmonary circulation). These reports suggest that patterns of atherosclerosis susceptibility are strongly influenced by intrinsic differences in the artery wall cells found at different locations with the vascular system. They are compatible with a role for SMC lineage diversity as one important determinant of the unique properties of artery wall cells found at different locations in the vascular tree.

Results of experimental aortic homograft transplantation experiments are suggestive, but the relation of these data to complex human disease remains uncertain. Of particular interest in this regard is a report by DeBakey and Gleaser describing an analysis of 11,890 patients admitted for surgical treatment of occlusive atherosclerotic vascular disease at The Methodist Hospital in Houston, Texas. These authors divided the arterial tree into four segments for statistical analysis, (1) coronary arteries, (2) ascending aorta and its major arteries, (3) abdominal aorta at the level of the renal arteries, and (4) terminal abdominal aorta and femoral arteries involved in peripheral vascular disease. Their analysis of this large patient population revealed that each vascular bed exhibits its own distinctive response to the atherogenic process, even though the risk factors are systemic and therefore would be expected a priori to have similar effects in all vascular beds. Moreover, the rates of recurrence and survival after surgical intervention were distinctly different for each arterial bed within the same individual. The authors conclude that these arterial bed-specific differences in disease progression are attributable to "genetic differences in the composition of the vessel wall" in each of the four different vascular segments. It is reasonable to suggest that lineage-dependent differences in vascular smooth muscle properties may play a role in the heterogeneous patterns of disease progression and recurrence among different human arterial beds.

Another important feature of human atherosclerosis that is better understood when viewed in the context of vascular development is the monoclonal character of human lesions. In 1973, Benditt reported that human atherosclerotic plaques expressed either one or another of the two major isoenzyme markers for glucose-6-phosphate dehydrogenase (G6PD), a gene that undergoes X-chromosome inactivation early in development, whereas normal artery wall expressed an equal mixture of both isoenzymes. The results suggested that human plaques were clonal masses, and one way such masses could be produced was by somatic mutation followed by selective expansion of a mutant clone within the arterial intima. Alternatively, clonal masses in the intima might arise by expansion of a preexisting clonal patch that formed during development of the artery wall, particularly if such patch sizes were large. To test this question, Schwartz and colleagues used a sensitive polymerase chain reaction (PCR) assay targeting the androgen receptor locus, a gene like G6PD that is subject to X-inactivation. This analysis confirmed the original reports of clonality in human plaques, and also produced the novel finding that patch sizes in normal human aorta are surprisingly large, often exceeding 4 mm in length. The intima overlying the patch was typically skewed to the same allele, suggesting focal expansion of the original medial patch into the intima. These results suggest that normal human arteries grow by expansion of smooth muscle clones with little or no intermixing, so that clonal patch sizes become comparatively large. They are reminiscent of the report by Mikawa and Fishman showing that the coronary artery wall develops by forming smooth muscle clones.
muscle polyclones that also show little or no intermixing.\textsuperscript{18} Taken together, it is likely that human plaques are clonal masses because they arise from preexisting clonal patches of SMCs produced during development of the artery wall.\textsuperscript{107,108} In the future, it will be of interest to map clonal patch sizes in different arteries, and determine whether different SMC lineages produce clones of different patch sizes in vivo.

Summary and Challenges Ahead

The observation that human atherosclerotic plaques arise from preexisting clonal patches of vascular smooth muscle brings us full circle back to the developing artery wall, and the realization that we know very little about how SMCs from different embryological origins differ from one another. Indeed, a better understanding of these differences may be important for advances in vascular development, as well as for revealing new approaches to risk factor assessment based on the recognition that atherogenic stimuli elicit different responses in different arteries. If that is true, then the patterns of SMC lineage diversity within the developing vascular system may point the way toward a better understanding of the vascular bed–specific patterns of atherosclerosis in the adult vascular tree.

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Disclosures

None.

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