Blood vessels exhibit a common structure composed of layers of cells encircling a central lumen. Arteries generally have more layers than veins have, and different arteries in the same individual can have different numbers of layers. Because all blood vessels are built around a monolayer of endothelial cells, the variation in wall structure is due to variable numbers of smooth muscle-containing layers in the tunica media. Indeed, Wolinsky and Glagov’s classic article showed that the relation between lumen diameter and wall thickness across a wide range of mammalian species is a function of the number of layers of smooth muscle and elastic fibers that are present in the arterial media. Yet precisely how layers of smooth muscle are formed during vascular development and what molecular mechanisms operate to produce different numbers of layers in different blood vessels is still poorly understood.

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Formation in Developing Arteries

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Patterning the Artery Wall by Lateral Induction of Notch Signaling

Virginia J. Hoglund, BS; Mark W. Majesky, PhD

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quency is set into motion that assembles SMC progenitors into the number of layers characteristic for a given artery.\(^1\) This process appears to involve physical contact between incoming cells and cells already present in the developing vessel wall.\(^8,9\) Notch signaling is initiated by 4 Notch receptors (Notch 1–4) interacting with 5 Notch ligands (Jagged 1, 2 and Delta-like 1, 3, and 4).\(^10–12\) Both the ligands and receptors are transmembrane proteins whose activation is promoted by cell-cell contact. Evidence that Notch signaling plays an important role in endothelial contact-dependent differentiation of smooth muscle progenitor cells in vivo was provided by the results of cell-specific deletion of Notch signaling activity in neural crest-derived progenitors\(^13\) and in experiments where the Notch ligand Jagged-1 (Jag1) was deleted specifically in endothelial cells.\(^14\)

**Positive Feedback Regulation of Jag1 Expression in Smooth Muscle Cells**

As a further test of the hypothesis that Notch mediates patterning of the artery wall, Manderfield et al report in this issue of *Circulation* that expression of dominant-negative mastermind-like protein (DN-MAML), an inhibitor of target gene activation by all forms of Notch receptor,\(^15\) produced significant reductions in Jag1 expression in neural crest-derived SMCs surrounding paired aortic arch arteries.\(^16\) Moreover, when E17.5 aortic SMCs were plated on a substrate that contained immobilized Jag1-Fc fragments that are known to activate Notch receptors, an increase in expression of Jag1 was observed, suggesting a positive feedback regulation of Jag1 expression by these cells. Evidence supporting the idea that Notch signaling directly regulates Jag1 expression in aortic SMCs was obtained by identification of an evolutionarily conserved region (ECR) in intron 2 of the Jag1 locus that bound both Rbp-j/CSL and Notch ICD and drove expression of a reporter gene in a Notch-responsive fashion in cultured SMCs. This genomic element, termed ECR6, was then examined in transient transgenic mice and found to be sufficient to direct LacZ reporter gene expression in a temporal and spatial pattern that closely matched that of the endogenous Jag1 gene.\(^16\) Interestingly, the ECR6 reporter transgene in stable transgenics exhibited robust activity in SMCs derived from the cardiac neural crest, but was not active in descending aortic SMCs that originate from paraxial mesoderm.\(^17–19\) Finally, loss of Jag1 expression in developing neural crest-derived aortic SMCs produced congenital heart defects consisting of abnormal aortic arch artery patterning, reduced expression of the canonical Notch target gene Hrt1, and reduced expression of SMC differentiation markers.\(^16\)

**Role of Lateral Induction in Assembly of the Artery Wall**

Therefore, the current evidence strongly suggests that endothelial cells of developing aortic arch arteries express Jag1 on their cell surface that then engages a Notch receptor on adjacent neural crest-derived SMC progenitor cells to promote differentiation and assembly of the first layer of the tunica media. Results presented by Manderfield et al,\(^16\) and by Feng et al\(^20\) and Liu et al,\(^21\) as well, argue that the initial signal is then propagated to the next layer of artery wall by lateral induction of Jag1 expression in newly differentiated smooth muscle cells, thus initiating a feed-forward pathway in the nascent medial layer (Layer 1). Jag1 expressed by cells in Layer 1 engages Notch receptors in surrounding mural cell progenitors leading to smooth muscle differentiation and upregulation of Jag1 expression in mural cells that will go on to form Layer 2. This positive feedback response of Jag1 induction by Notch signaling in the developing artery wall is called lateral induction. By this sequential lateral induction process, a multilayered artery wall is formed.

**Remaining Questions**

1. We know that individual layers in the arterial media are composed of more than SMCs alone. Indeed the term elastic lamellae is often used to describe these layers, a reference to the abundance of cross-linked elastin, fibrillin, lysyl oxidase, and elastic fiber proteins that also alternate in layers across the artery wall.\(^7\) Does Notch signaling by lateral induction also drive expression of genes encoding tropoelastin and the multiple elastic fiber proteins that are required to
make a mature layer of artery wall? If so, is this a direct effect of Notch signaling on individual matrix protein gene regulatory elements, or is it an indirect effect of triggering SMC differentiation in appropriate progenitor cells?

2. How is lateral induction of Notch signaling coordinated with other signals that are important for artery wall formation, such as transforming growth factor-β, bone morphogenetic protein, and myoxygenin-related transcription factor B?22

3. The genomic regulatory element identified here, ECR6, mediates lateral induction of Jag1 expression in neural crest-derived progenitors during SMC differentiation. Yet ECR6 does not seem to have this activity for SMCs that differentiate from non–neural crest progenitors.16 Because the structure of the artery wall with respect to layers of SMCs and elastic fibers is not obviously different in arterial segments of non–neural crest origin, does lateral induction of Notch signaling also explain layer formation in these non–neural crest arterial segments? If so, what genomic elements are responsive to lateral induction in the Jag1 locus in non–neural crest-derived SMC progenitor cells? If not, then what signaling pathway(s) mediate smooth muscle layer formation in non–neural crest-derived arteries?

4. What molecular mechanisms become active to produce more elastic layers in aortas of Eln+/- mice?5,6,23 Is Notch signaling by lateral induction reactivated late in artery wall development (~E18.5) to mediate formation of an ectopic series of elastic lamellae in Eln+/- aorta?5-7

5. Given the consistently observed correlation between the physical forces producing wall tension and the structural component of wall thickness in many different arteries, is lateral induction of Notch signaling responsive to changes in wall tension during vascular development? If so, what is the biochemical nature of the coupling mechanism?

6. If all arteries begin wall formation in the same way—endothelial expression of Jag1 activates Notch signaling in SMC progenitors leading to Jag1 expression, etc.—what mechanisms terminate the iterative layer sequence to produce arteries with different numbers of SMC layers in their tunica media? Do morphogenic pathways associated with development of the tunica adventitia, such as sonic hedgehog signaling,24 act to terminate smooth muscle layer formation, or does the lateral induction signal decay at some point, thus ending the repeating sequence?

7. Although mammals produce artery walls with SMCs as essentially the only cell type within elastic lamellae, other species including birds and reptiles produce alternating layers of SMCs and non-SMCs (called interlaminar cells) within the media of large elastic arteries.25,26 Does lateral induction of Notch signaling also operate to build a multilayered artery wall in these phylogenetically distinct cases as well?

Summary
Lateral induction of Notch signaling is an attractive mechanism to play an important role in building a multilayered artery wall. Such a role is consistent with Jag1 loss-of-function phenotypes in mice14,20 and in patients with Alagille syndrome where haploinsufficiency for Jag1 leads to congenital heart disease and mispatterning of the great vessels.27,28 Although many questions remain to be answered about morphogenesis of the tunica media, pursuit of Notch signaling through Jag1 in SMC progenitors promises to be a very fruitful way forward.

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Disclosures
None.

References

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