Nerve Cell Signposts in the Blood Vessel Roadmap
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The vasculature and the nervous system both form extensively branched networks in the vertebrate body, incorporate into very different tissue environments, and establish connections over long distances. Although there are many obvious morphological and functional differences between blood vessels and nerve fibers, one should not ignore striking conceptual similarities in the assembly of these networks through guided growth and branching processes. There is accumulating evidence that this resemblance is not just coincidental and instead reflects that the morphogenesis of both systems use similar mechanistic principles and molecular tool kits.1–3 During development of the nervous system, neurons extend a long axonal process that carries a highly motile sensory structure, termed growth cone, at its distal tip. Growth cones dynamically form filopodial extensions to explore the spatial environment and recognize repulsive or attractive guidance cues acting as molecular signposts. Growth cones have the ability to interpret a multitude of these signals and decide whether it is appropriate to carry on, stall, retract, or change direction. A series of correct decisions will direct the growing axon to its proper target where it forms synaptic connections. Unlike neurons, endothelial cells do not extend long processes and instead line the inner surface of blood vessels with their cell bodies. During growth and remodeling processes, a specialized endothelial cell, termed tip cell, covers the distal end of vascular sprouts. Tip cells dynamically extend long filopodia and explore signals presented by surrounding cells and the matrix environment.4 Live imaging data obtained in zebrafish embryos and phenotypes of mutant mice indicate that tip cells steer the growth of blood vessels into certain directions, control the branching or fusion of vascular sprouts, and thereby ensure that tissues are adequately connected to the blood circulation. Thus, the role of endothelial tip cells as highly motile sensors and decision makers is remarkably reminiscent of axonal growth cones. Other endothelial cells, termed stalk cells, exhibit fewer filopodia and form capillary tubes right behind the tip cell front. The lumen enclosed by the endothelial cells of the stalk contains blood and is connected to the rest of the network.4,5 In this respect, the stalk is somewhat analogous to axons and nerve fiber bundles.

There are now several examples of gene families with established roles in axon pathfinding that control endothelial tip cell guidance, filopodia formation, and angiogenic sprouting of blood vessels. Semaphorins, ligands for Neuropilin and Plexin receptors, are well known as repulsive guidance cues in the nervous system but also regulate vessel branching.3 Netrins are secreted matrix-binding molecules that can either act as attractants, by interacting with DCC receptors, or as repellents, which is mediated by binding to Unc5 family receptors or unc5-DCC complexes.1 In the vascular system of fish and mice, Netrin binding to the receptor Unc5b suppresses the formation of endothelial tip cell filopodia and branching of blood vessels.6 Eph receptor tyrosine kinases and their ephrin ligands regulate a wide range of patterning and guidance processes. Their roles include axon guidance, angiogenic remodeling of the endothelium, as well as blood vessel wall assembly.7,8 Roundabout (Robo) receptors, named after characteristic defects in the nervous system of mutant flies, and their Slit ligands act as repellents for some neuronal growth cones but stimulate the growth and branching of other axon populations.1 Previous publications have shown that some of these molecules are also involved in angiogenic remodelling, presumably through signaling in endothelial cells.9–11 In this issue of Circulation Research, De Leon and coworkers show that several Slit/Robo molecules are expressed by blood vessels and that their interaction controls the migration of vascular smooth muscle cells.12

Slits are secreted proteins found in a wide range of invertebrate and vertebrate species. They can function as diffusible long-range cues or as cell membrane-associated short-range cues controlling axon pathfinding, branching, and cell migration.13 Three mammalian Slit family proteins (Slit1, 2, and 3) are known. They all contain an N-terminal signal peptide, 4 tandem leucine-rich repeats (LRRs), 9 epithelial growth factor-like (EGF) repeats, 6 and 7 (Figure, A). Laminin G domain because of sequence homologies, separate LRRs, Netrin binding to the receptor Unc5b suppresses the formation of endothelial tip cell filopodia and branching of blood vessels. Semaphorins, ligands for Neuropilin and Plexin receptors, are well known as repulsive guidance cues in the nervous system but also regulate vessel branching.3 Netrins are secreted matrix-binding molecules that can either act as attractants, by interacting with DCC receptors, or as repellents, which is mediated by binding to Unc5 family receptors or unc5-DCC complexes.1 In the vascular system of fish and mice, Netrin binding to the receptor Unc5b suppresses the formation of endothelial tip cell filopodia and branching of blood vessels.6 Eph receptor tyrosine kinases and their ephrin ligands regulate a wide range of patterning and guidance processes. Their roles include axon guidance, angiogenic remodeling of the endothelium, as well as blood vessel wall assembly.7,8 Roundabout (Robo) receptors, named after characteristic defects in the nervous system of mutant flies, and their Slit ligands act as repellents for some neuronal growth cones but stimulate the growth and branching of other axon populations.1 Previous publications have shown that some of these molecules are also involved in angiogenic remodelling, presumably through signaling in endothelial cells.9–11 In this issue of Circulation Research, De Leon and coworkers show that several Slit/Robo molecules are expressed by blood vessels and that their interaction controls the migration of vascular smooth muscle cells.12

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Proteolytic processing of Slit proteins gives rise to N-terminal (Slit-N) and C-terminal fragments (Slit-C). The former contains all 4 LRRs and the 5 N-terminal EGF repeats (amino acid residues 1 to 1117) whereas the Slit-C fragment includes the rest of the protein (Figure, A). Full-length Slit and its proteolytic products are equally secreted into the extracellular space but Slit-N appears to be more tightly associated with the cell membrane.13 The LRRs in Slit or Slit-N proteins are sufficient for binding to the single-pass transmembrane Robo receptors.14 Like the Slit ligands, Robos have been found in many

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organisms ranging from flies and worms to humans. Four Robo family members (Robo1, 2, 3, and 4) are known in mammals, most of which contain a series of 5 immunoglobulin (Ig) domains mediating Slit binding and 3 fibronectin type III (FN III) repeats in their extracellular region (Figure, A). Several conserved motifs (designated CC0, CC1, CC2, and CC3) in the cytoplasmic region of Robos are involved in signal transduction through association with the Abelson tyrosine kinase (Abl) and the cytoskeletal regulator Enabled (Ena).15 Furthermore, it has been shown that several small guanine triphosphatase (GTPase) activating proteins (GAPs) can bind the CC3 motif.16 GTPases are versatile signaling molecules that are only active in their GTP-bound forms. GAPs enhance GTP hydrolysis and thereby the formation of GDP-GTPase complexes lacking signal transduction activity. This important connection links Slit-Robo signaling to the Rho subfamily GTPases Rho, Rac and Cdc42, which control cytoskeletal reorganization, the formation of cellular protrusions and filopodia, and cell migration. In a nutshell, Robo-Slit interactions trigger GAPs. GAPs terminate signaling by small GTPases, which, in turn, leads to alterations in fundamental properties of cells such as their motility (Figure, A).

This model is also consistent with the new data presented by Liu et al.13 Treatment with engineered recombinant Slit2-N protein inhibits the migration of vascular smooth muscle cells (VSMCs) in response to PDGF, a potent chemoattractive factor controlling VSMC motility and recruitment.17,18 In contrast, mitogenic signals triggered by PDGF are not affected by Slit2-N.12

At which level could Slit interfere with the normal cellular response to PDGF? As mentioned above, Slit2-N does not affect PDGF-mediated mitogenesis, and, consistently, Liu et al found no changes in PDGFR tyrosine phosphorylation. As it was well know that PDGF signaling leads to activation of the small GTPase Rac1, the authors investigated whether Slit2-N can interfere with this pathway. Indeed, the transient increase in cellular GTP-Rac1 levels normally seen in response to PDGF stimulation is abolished by Slit2-N. Similarly, exposure of VSMCs to Slit2-N blocks effects of PDGF on the cytoskeletal level: lamellipodial protrusions no longer
form and the actin cytoskeleton fails to reorganize. It is thought that these PDGF-mediated cellular responses largely depend on Rac1 activation, which is further supported by the observation that a chemical Rac1 inhibitor and Slit2-N have similar effects.12

In conclusion, Liu et al show the expression of Slit2 and Robo receptors in blood vessels and vascular cells (Figure, B) and provide strong evidence that the N-terminal Slit2 fragment can inhibit VSMC migration. These findings raise several interesting questions: Do Slit2 and its Robo receptors control physiological blood vessel morphogenesis? Slit2 and Robo2 knockout mice die at birth and display kidney defects but it remains to be addressed whether the vasculature of these animals is normal.10 Functional redundancy among the 3 Slit ligands might complicate this issue and could make it necessary to analyze double or triple knockout mice. There is already good evidence that the receptor Robo4 (Magic Roundabout) controls blood vessel sprouting and branching in zebrafish.10 However, Robo4 lacks several of the N-terminal Ig domains and its physical interaction with Slit2 is controversial.11,11

Irrespective of these questions, it should be worthwhile to investigate whether Slit2 and Robo expression is altered in pathological processes. The known roles of PDGF signaling in experimental models of intimal hyperplasia, atherosclerosis, and fibrosis raise the question whether Slit2 may modulate VSMC behavior in these settings. It has also been reported that Slit-Robo signaling promotes tumor angiogenesis and cancer growth.20 Functional studies in animal models could address these issues but such experiments are complicated by the lethality of Slit2 knockout mice. Alternative approaches, such as the development of specific small molecular weight inhibitors, could help to overcome these limitations and permit preclinical studies. The example of the Slit/Robo family emphasizes that nerve fibers and blood vessels use similar strategies of guided growth and controlled branching to ensure the proper formation of complex hierarchical networks. The new findings reported by Liu et al indicate that Slits also regulate VSMC migration and raise the interesting question whether VSMC recruitment to blood vessels uses similar signals and principles as the guidance of neuronal growth cones and endothelial tip cells.12

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References


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