Blood vessels are composed of two primary cell types, endothelial cells and smooth muscle cells, each providing a unique contribution to vessel function. Signaling between these two cell types is essential for maintaining tone in mature vessels, and their communication is critical during development, and for repair and remodeling associated with blood vessel growth. This review will highlight the pathways that endothelial cells and smooth muscle cells utilize to communicate during vessel formation and discuss how disruptions in these pathways contribute to disease.

**Blood Vessel Structure and Function**

All blood vessels have the same basic structure (44, 72, 111). They are comprised of a single concentric layer of endothelial cells (the endothelium) that forms the inner tube or intimal layer of the vessel. Surrounding the intima is a secondary layer, termed the media, composed of smooth muscle cells (or smooth muscle cell-related pericytes). The final layer, or adventitia, is a mixture of extracellular matrix (ECM), fibroblasts, and nerve cells. Recently, evidence suggests that this layer may serve as a niche for stem cells (88, 123). Adaptations of this basic structure arise from the extremely diverse functional duties ascribed to the individual blood vessels. The vascular system is responsible for the extremes of fluid handling by being duly responsible for moving large volumes of blood throughout the body, while providing precise delivery of oxygen and nutrients to tissues and individual cells. The largest arteries like the aorta have multiple layers of smooth muscle cells intertwined with an elaborate elastin and collagen-based matrix that is required to sustain systemic pressure under pulsatile flow. Veins, on the other hand, serve as a low-pressure reservoir and facilitate the return of blood from the organs to be reoxygenated. Venous vessels are typically less muscular, with fewer layers of smooth muscle and less complex ECM component. The smallest vessels, the capillaries, have limited smooth muscle (or pericyte) coverage to allow for maximum diffusion, with endothelial cells that are highly permeable to permit gas exchange and nutrient delivery to cells via tiny pores or fenestrations. Despite these significant vascular bed-specific differences, endothelial cells and smooth muscle cells retain similar functions in all blood vessels.

The individual functions of endothelial cells and smooth muscle cells are dependent on proper communication between these cell types. This communication begins early on in embryogenesis as the blood vessels begin to form (90, 113). During development, the endothelial cells differentiate from vascular progenitor cells termed angioblasts. They migrate and proliferate throughout the developing embryo, and undergo a process of tubulogenesis to form nascent tubes consisting of the primitive vascular plexus. These structures are devoid of smooth muscle cells or pericytes, but the endothelial cells initiate the dialogue by sending a signal to recruit smooth muscle cells and pericytes from surrounding mesenchymal or neural crest-derived tissues. The smooth muscle cells in turn reciprocate with their own signals, and the relationship begins (38, 113). The vascular plexus continues to expand with the growth of the embryo and undergoes remodeling and refinements that include additional sprouting and pruning, and ultimately differentiation and maturation of the vessels. This process is regulated by extrinsic factors, such as growth factor gradients and tissue hypoxia, but the signaling between the endothelial cells and smooth muscle cells at these earliest stages is essential for vessel formation and ultimately proper function (7, 25, 38). In mature vessels, developmental signals continue to be required for maintenance of the blood vessel, but additional communication occurs related to vascular function to regulate tone and blood pressure (30, 125). These two cell types utilize an array of signaling tactics to convey information. Mechanistically, these signaling strategies can be divided into two categories, those that occur through a soluble or secreted molecule, and those that require direct physical contact between the two cell types (Table 1).

**Diffusible Signaling**

Diffusion of soluble factors and concentration gradients are fundamental themes utilized during...
embryogenesis, and blood vessel formation is no exception. During development, endothelial cells secrete the polypeptide platelet-derived growth factor-B (PDGF-B) (5, 38). As endothelial cells differentiate and undergo tubulogenesis, secreted PDGF-B is thought to form a concentration gradient, which is sensed by surrounding smooth muscle precursors via the tyrosine kinase receptor PDGFR-β to promote their migration and proliferation, resulting in recruitment and assembly of the vessel wall. Many excellent studies using both in vitro and in vivo models have cemented the role of PDGF signaling in endothelial smooth muscle cell interactions (5, 29). These findings have collectively shown their importance for smooth muscle recruitment and proliferation, and have demonstrated their requirement for blood vessel maintenance. Although the importance of PDGF signaling is clear, the complexities of its actions are somewhat cloudy. Evidence suggests that cross talk with other signaling pathways may alter how smooth muscle cells respond to endothelial cell-secreted PDGF-B (16, 22, 42, 53, 105). Of particular note, the ratio between PDGF-B and vascular endothelial growth factor (VEGF) appears to play a critical role in the fine balance required for blood vessel growth and maturation (11, 20, 42).

A reciprocal signaling pathway to PDGF is the Angiopoietin1-Tie2 pathway (34). Angiopoietin1 (Ang1) is a growth factor mainly secreted by smooth muscle cells and mesenchymal cell precursors. By binding to the Tie2 receptor, which is predominantly expressed on endothelial cells, Ang1 facilitates vessel assembly and stability (33). Studies on Ang1/Tie2 function indicate that it likely affects vessel assembly indirectly by influencing the stability of smooth muscle precursor cells. Deletion of either Ang1 or Tie2 in blood vessels results in a similar phenotype, characterized by a poor association between endothelial cells and smooth muscle cells (119). Additional experiments have suggested that Ang1/Tie2 may not be important for cell recruitment per se but is important for maintaining the physical interaction of endothelial and smooth muscle cells, and is critical for prevention of cell death (24, 57, 108). Thus Ang1/Tie2 signaling might be positioned downstream of PDGF signaling in the cascade of vessel assembly. Another receptor-ligand combination involved in this interaction is the sphingosine-1-phosphate (S1P) pathway (68, 69). S1P is a sphingolipid metabolite that signals through a family of G-protein-coupled receptors [S1P1-5]. Deletion of the S1P1 receptor on endothelial cells results in significant defects in smooth muscle coverage, suggesting importance in smooth muscle recruitment by endothelial cells. The mechanism of how activation of the S1P pathway influences this recruitment is not well understood. Data indicate that S1P promotes expression of TIMP-2 in smooth muscle cells (91), which could facilitate migration and regulate recruitment.

For the signaling pathways described above, receptor and ligand expression is cell-type enriched, making it easy to envision how the signal is passed from one cell type to the other. The transforming growth factor (TGF)-β signaling pathway, however, is more complex, and receptor-ligand combinations are expressed on both endothelial and smooth muscle cells (3, 56). Despite this complexity, there is clear evidence that this signaling is important for their communication during development and likely beyond (100). The details of the evidence are extensive, but some of the most compelling evidence comes from conditional knockout mouse models in which TGF-β signaling components have been deleted in endothelial cells, resulting in a failure of smooth muscle to become invested or to differentiate within the blood vessel wall (4, 10, 56, 67, 83, 89, 107). Additionally, the importance of TGF-β signaling is further demonstrated by its role in vascular pathogenesis and disease progression (3, 32, 40, 59, 92). As with most signaling pathways, there is extensive cross talk with other signaling mediators that facilitate the directionality of the actions that occur (81, 122).

In mature blood vessels, the close proximity of endothelial and smooth muscle cells makes signaling via secreted or diffusible factors an efficient mechanism of communication. In the adult vasculature, endothelial-derived factors, such as nitric oxide (NO), prostacyclin, and hyperpolarizing

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**Table 1. Primary signaling pathways in EC and SMC communication**

<table>
<thead>
<tr>
<th>Pathway</th>
<th>Mode of Action</th>
<th>Primary Role</th>
<th>Direction of Signal</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDGF-B/PDGFRβ</td>
<td>Secreted</td>
<td>Recruitment</td>
<td>EC → SMC</td>
</tr>
<tr>
<td>Ang1/Tie2</td>
<td>Secreted</td>
<td>Stability/survival</td>
<td>EC ← SMC</td>
</tr>
<tr>
<td>S1P</td>
<td>Secreted</td>
<td>Recruitment</td>
<td>EC → SMC</td>
</tr>
<tr>
<td>TGF-β</td>
<td>Secreted</td>
<td>Differentiation/maturation</td>
<td>EC → SMC</td>
</tr>
<tr>
<td>Notch</td>
<td>Cell contact</td>
<td>Differentiation/maturation</td>
<td>EC → SMC</td>
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<tr>
<td>Eph/ephrin</td>
<td>Cell contact</td>
<td>Differentiation/maturation</td>
<td>EC → SMC</td>
</tr>
<tr>
<td>Connexin</td>
<td>Cell contact</td>
<td>Differentiation/maturation</td>
<td>EC → SMC</td>
</tr>
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EC, endothelial cell; SMC, smooth muscle cell.
agents diffuse to smooth muscle cells to cause vascular relaxation (30, 125). Similarly, endothelial cell-released contracting agents like endothelin and angiotensin II are perceived by smooth muscle cells to increase vascular tone (30, 125). The literature pertaining to endothelial cell-dependent regulation of vascular reactivity is beyond the scope of this review but represents the best-described example of the importance of endothelial-smooth muscle cell interactions. What role these developmental secreted mediators have in the regulation of vascular function in the adult is not clear, although studies have demonstrated a role for them in modulation of vascular tone (9, 14, 67, 77, 79, 87).

Contact-Dependent Signaling

Physical communication relies on membrane-bound proteins “hooking up” on adjacent cells, and, in developing blood vessels, heterotypic interactions occur through a limited number of known proteins. Mammalian Notch signaling is an evolutionarily conserved pathway that utilizes membrane-bound receptors (Notch1–4) activated by membrane-bound ligands (Jagged1, 2, and Delta-like 1, 3, and 4) on adjacent cells (8). Its actions in vascular development are well established and has been shown to have a prominent role in arterial-venous identity, tip cell specification and sprouting, and smooth muscle cell differentiation (15, 43). Many of these functions are thought to be primarily homotypic cell interactions, but strong evidence exists for a role of Notch signaling in endothelial cell-smooth muscle cell cross talk. Deletion of Notch ligand Jagged1 in endothelial cells leads to vascular defects associated with smooth muscle differentiation. Although the endothelial cells in these deficient mouse embryos are specified properly and form an initial vascular network, the smooth muscle cells fail to differentiate around them (50). In vitro studies substantiate the importance of this interaction by showing that endothelial cell-expressed Jagged1 can induce the expression of Notch3 in cocultured smooth muscle cells, and this induction is critical for smooth muscle differentiation (80). More recent findings also show that Notch signaling between endothelial and smooth muscle cells facilitates vessel maturation by regulating integrin adhesion and can be inhibited by Von Willebrand Factor (93, 112).

EPH receptor tyrosine kinases (RTKs) form a large family of transmembrane proteins that are activated by binding to ephrin ligands, which are linked to the cell membrane via glycosylphosphatidylinositol anchor (ephrin class A) or via a transmembrane domain (ephrin class B) (71). The receptor-ligand pair EphB4 and ephrin-B2 are the best described in the vasculature. They are reciprocally expressed on venous and arterial endothelial cells and provide identity to these unique vascular beds in yet undefined ways. Although ephrin-B2 functions downstream of Notch, data also indicate that the Eph-ephrin axis independently specifies vascular identity (70). Whether Eph-ephrin signaling conveys arterial-venous identity from endothelial cells to smooth muscle cells is not yet known. Additional evidence indicates that the Eph-ephrin axis also contributes to endothelial-smooth muscle cell communication during vessel formation. Inactivation of the ephrinB2 gene specifically in smooth muscle cells of mice causes perinatal lethality (36). Examination of the vasculature in these mice showed smooth muscle cells and pericytes were loosely associated around the vessel wall leading to compromised vessels and hemorrhaging. A recent paper further revealed that Ephrin-B2 regulates the PDGFβ receptor endocytosis, suggesting a unique role on smooth muscle cells that controls endothelial-smooth muscle cell cross talk (97).

Connexins are transmembrane proteins that make up gap junctions and have been widely studied for their role in direct cell-cell communication (19, 46). Gap junctions are formed from six connexin subunits that create channels allowing for exchange of ions and metabolites between coupled cells. Of the 21 connexins in mammals, only 4 (Cx37, Cx40, Cx43, Cx45) are expressed in the vasculature (19, 46), where they are noted for enabling changes in membrane potential mostly in homotypic interactions of endothelial cells or smooth muscle cells. In development, Cx43 and Cx45 have both been shown to be important for endothelial cell-dependent smooth muscle differentiation (35, 39, 52). Interestingly, Cx43 was shown to drive differentiation of smooth muscle cells by controlling TGF-β activation (52), indicating cross talk with the secreted family of mediators to regulate heterotypic interactions.

Cell-cell association between endothelial and smooth muscle cells during development is different than in adults, with the cells undergoing a dynamic array of proliferative and migratory behaviors that promote vessel assembly. In mature blood vessels, the basement membrane and the internal elastic lamina (IEL) are considered significant barriers to physical interactions of endothelial-smooth muscle cells. Despite this notion, evidence of myoendothelial communication has existed for years via myoendothelial junctions (MEJs) that link the plasma membranes of juxtaposed endothelial and smooth muscle cells (48, 110). Many of the MEJ studies have focused on the role of MEJs in regulating calcium dynamics and membrane potential for the control of blood flow in small resistance arteries. Evidence that cell contact-dependent developmental mediators play a
role in adult blood vessel function does exist. Notch3 acts to regulate vascular tone in small arteries, suggesting that, in adult blood vessels, endothelial-smooth muscle cell interactions are also controlled by Notch signaling (12, 17). Similarly, the Eph ligand Efnb1 and the Eph receptor Ephb6 also modulate blood pressure (124, 127); but, since both proteins are expressed in smooth muscle cells, it is unclear whether their function is dependent on endothelial cell signaling. It is well established that connexins regulate vascular reactivity in adult blood vessels (45, 55, 117, 118). Thus, as with the secreted developmental factors, cell-cell contact and cell-to-muscle recruitment appear to function in adults to control blood vessel function.

**Aberrant Communication in Vascular Disease**

In vascular disease, alterations in heterotypic cell communication can produce a host of problems leading to vascular insufficiency. This is best illustrated by endothelial dysfunction, a defined pathological state of the endothelium leading to disruptions in vascular function (51, 94). Although there is not always a clear line between cause and effect, endothelial dysfunction is associated with hypertension, hypercholesterolaemia, atherosclerosis, and diabetes (51, 94). What is consistent in all of these diseases is a change in the vascular reactivity and composition of the vascular wall, most notably the smooth muscle cells. The literature describing endothelial dysfunction and its effects on smooth muscle cells is immense, much of which is focused on diminished NO bioavailability or variations in the release of vasoactive compounds from the endothelium (51, 94). Despite their importance, most of these studies do not address how endothelial dysfunction directly modifies the conversation between the two cell types. Evidence that the dialogue has changed is sparse but seems to suggest that the developmental signaling pathways are affected. One study showed that mechanical strain promotes expression of heparan sulfate proteoglycans in endothelial cells, which in turn alters the response of smooth muscle cells to TGF-β (10). Another report showed that endothelium-derived NO negatively regulates the PDGF pathway during flow-dependent vascular remodeling (129). In spontaneously hypertensive rats, there is a greater incidence of myoendothelial junctions present in caudal arteries, which may promote greater interaction (109). Consistent with this, GAP-junction inhibitors targeting the myoendothelial junction blocked endothelial cell-induced contraction in a hypertensive rat model (121).

Vascular injury arising from atherosclerosis is also thought to be manifested by disruptions in endothelial cell-smooth muscle cell signaling. Lesion formation is a complex process, with the initiation and progression being dependent on a localized inflammatory response that facilitates changes in the vessel wall (31, 85). The disruptions in signaling between endothelial cells and smooth muscle cells is not easily defined due to the contribution of inflammatory cells, monocytes, and lymphocytes. A primary feature of atherosclerotic plaques is the transition of smooth muscle cells into a synthetic state, becoming proliferative and secreting excess ECM that largely contributes to plaque buildup (31). It is widely thought that endothelial cell injury and reactivation of the PDGF signaling pathway is the trigger for smooth muscle proliferation and ECM synthesis, although this has been difficult to directly prove. A few reports have shown that modifications to endothelial cells promote excessive smooth muscle proliferation in plaque formation (23, 105, 128, 131). Intervention by angioplasty to remove diseased plaques causes endothelial cell denudation, damage, and further dysfunction, which seemingly result in the complete loss of the suppressive effects on smooth muscle cell proliferation, causing restenosis (130). As a consequence, extensive effort has gone toward strategies to re-endothelialize the blood vessel as a means to halt smooth muscle proliferation (18, 37, 61, 65).

Diabetic retinopathy is characterized by microaneurysms and hemorrhages of the retinal blood vessels, and the first morphological indicator of the disease is loss of pericytes, referred to as pericyte dropout (95, 102). Although the actual cause of pericyte dropout is not understood, experiments with animal models suggest that it involves disruptions in PDGF/PDGFβR signaling between endothelial cells and pericytes. Once pericytes are lost, endothelial cells lose stability, resulting in vascular disruptions and collapse. Recently, Apelin, an endothelium-expressed ligand for the G-protein-coupled receptor APJ, was shown to decrease pericyte recruitment, and promote endothelial cell growth and pathological retinal angiogenesis (63). Interestingly, in development, loss of endothelial-expressed Apelin causes defects in vascular maturation and smooth muscle recruitment (62), indicating a possible overlapping function with the PDGF pathway.

Genetic diseases manifested by disruptions in endothelial cell-smooth muscle cell interactions are also known. Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is an inherited disease caused by mutations in the NOTCH3 gene (60), leading to stroke and vascular dementia, and characterized by the degeneration of smooth muscle cells of small arteries. The hallmark of the disease
is the accumulation of granular osmiophilic material (GOM) in the basement membrane of blood vessels, which creates excess space between the endothelial cells and smooth muscle cells (114). Whether disruption of communication between the two cell types contributes to disease progression is not known. In vitro data have demonstrated that endothelial cells activate Notch signaling in neighboring smooth muscle cells through increased expression of NOTCH3 (80), and CADASIL patients exhibit signs of endothelial dysfunction (101). Furthermore, animal models of CADASIL exhibit disruptions in vascular tone and have increased incidence of ischemic stroke (6, 12, 17, 73), all of which point to deficiencies in cell-cell communication. Hereditary hemorrhagic telangiectasia (HHT) is an autosomal-dominant disorder caused by mutations in the Endoglin gene, a type III receptor within the TGF-β signaling pathway (92). Subsequent HHT-causing mutations were later identified in other TGF-β signaling mediators (40, 59). HHT is characterized by dilations of the vascular lumen and thinning of blood vessel wall, leading to arteriovenous malformations and hemorrhage (13, 76, 126). Endoglin is highly expressed in endothelial cells, and utilization of mouse models deficient in endoglin demonstrate its importance for smooth muscle investment in an endothelial cell-dependent manner (89). Endoglin may serve an unrelated function in mature vessels to maintain vascular tone, since mice that are heterozygous for the endoglin mutation display defects in NO-dependent reactivity (58, 124). Adams-Oliver syndrome (AOS) arises from a congenital defect of unknown etiology that manifests various cardiovascular anomalies, including arteriovenous malformations and pulmonary hypertension (116). Analysis of pulmonary blood vessels in a group of patients indicated abnormal recruitment of pericytes, suggesting this might contribute to the disease (99). Interestingly, the Notch signaling pathway mediator RPBJ was discovered to be mutated in two AOS families, indicating that Notch signaling contributes to this disease (47).

**Perspectives**

Communication between the two major cell types in blood vessels is fundamental to the formation and function of the vasculature. Although we understand a considerable amount about how endothelial cells convey signals to smooth muscle cells for the regulation of vascular tone, and we have defined the basic communication that occurs during vessel assembly, there is still much to learn. The vascular-bed-specific characteristics of endothelial cells and smooth muscle cells undoubtedly have a substantial impact on how the two cell types communicate in distinct vessels in normal and disease states (1, 2). For example, microvascular resistance arteries respond differentially than large conduit arteries to vasoactive compounds (74, 82, 117). Although some of these differences can be attributed to bed-specific gene expression patterns or flow-dependent effects that alter how the cells communicate, the details are still not well understood. Additionally, in development, both Notch and Ephrin signaling have roles in arterial-venous specificity, which is established in the early stages of endothelial cell-dependent plexus formation (66, 75). How this specificity is conferred to recruited smooth muscle cells during development is not known. In fact, vascular smooth muscle cells of Notch3 mutant mice lack arterial identity (27), suggesting that the two cell types may be specified independently. Alternatively, Notch3 might be required to convey arterial identity from endothelial cells. Both endothelial cells and smooth muscle cells synthesize and secrete ECM, which likely influences neighboring cells function (26). Experiments have shown that these cells can regulate ECM gene expression in one another, resulting in a matrix that is a complex mixture of endothelial and smooth muscle cell-derived components (78, 86, 96). Although traditionally thought to serve as a cellular scaffold and foundation for mechanical properties of blood vessels, it is now known as a rich source of signaling mediators (54, 64). Therefore, alterations in ECM not only have structural implications but can lead to signaling changes that disrupt endothelial-smooth muscle cell interactions. In Marfan syndrome, defects in the gene encoding the ECM protein fibrillin-1 cause structural abnormalities in the vessel wall and is associated with an increase in TGF-β signaling (32, 98). Endothelium-dependent vasomotor dysfunction was observed in the small arteries of a mouse model of Marfan syndrome, suggesting defects in heterotypic cell communication (120). Finally, current findings suggest that microRNAs are secreted from cells and can be picked up by other cells (115). MicroRNAs miR-143/145 were shown to be transported from endothelial cells to smooth muscle cells, and this vesicle-mediated transfer exhibited protection against atherogenesis (49). More recently, direct transmission of miR-126 from endothelial cells to smooth muscle cells, and this vesicle-mediated transfer exhibited protection against atherogenesis (49).
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References


