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Development of the Endothelium: An Emphasis on Heterogeneity

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Abstract

The endothelium is composed of specialized epithelial cells that line the vasculature, the lymph vessels, and the heart. These endothelial cells are characterized by their stratification and are connected via intercellular junctions that confer specific permeability. Although all endothelium acts as a barrier, considerable heterogeneity exists among different organs and even within vessels. During development, the endothelial cells are specified before they migrate to their final destination, and then they commit to an arterial or venous fate. From the venous endothelial cell population, a subset of cells is further specified as lymphatic endothelium. The endothelium can be highly permeable, as in the lymph vessels, or impenetrable, as in the blood-brain barrier. These differences arise during development and are orchestrated through a series of signaling pathways. This review details how endothelial cells arise and are directed to their specific fate, specifically targeting what differentiates endothelial populations.

Keywords

Hemangioblast; arterial; venous; lymphatic; endothelium

THE ORIGIN OF ENDOTHELIAL CELLS

During the early stages of development, the embryo and extraembryonic tissue consist of two cell layers: the epiblast and the hypoblast. The epiblast expresses bone morphogenic protein (BMP) 4, which is downregulated when the primitive streak forms and the epiblast ingresses to form the mesoderm (Fig. 1A).[1] Although BMP4 is sufficient to induce mesodermal differentiation in embryonic stem cells,[2,3] epiblast-derived basic fibroblast growth factor (bFGF) and hypoblast-derived activin induce mesoderm formation in the embryo.[4–6] Interestingly, the hypoblast can be removed before gastrulation and endothelial precursor blood islands still form,[7] indicating that bFGF is sufficient for mesoderm induction. As the mesoderm ingresses, the epiblast re-expresses BMP4 in the non-neural ectoderm.[8] This BMP signal is necessary for patterning the mesoderm and setting aside a ventral mesodermal population that can give rise to the endothelium.[4,9]

From the mesoderm, bFGF and activin A induce specification of the hemangioblast, the common precursor of endothelial and hematopoietic cells (Fig. 1B). This induction is observed within 3 hours of mesodermal exposure to bFGF and activin,[3] and a capillary plexus can form within 7 hours.[10] BMP4 can also induce the expression of a hallmark endothelial cell marker, receptor tyrosine kinase Flk1 (fetal liver kinase 1),[2,3] suggesting

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that the results observed with bFGF and activin may be caused by the induction of mesoderm rather than the specification of the hemangioblast. In addition to Flk1, stem cell leukemia (SCL) and the adherens junction protein vascular endothelial (VE)-cadherin also mark these endothelial precursors.[11,12] Flk1/SCL-positive hemangioblasts within the mesoderm swell to form blood island clusters (Fig. 1C).[13,14] These Flk1/SCL-positive clusters are induced by vascular endothelial growth factor (VEGF)-A and Indian hedgehog signals from the extraembryonic endoderm[15–19] and express transcription factor GATA-1.[14] These clusters aggregate between the ectoderm (the former epiblast) and the endoderm, which also forms during gastrulation.[20] These blood island clusters will form both endothelial and hematopoietic cells.

Additional transcription factors can induce Flk1. Cloche is expressed in the zebrafish hemangioblast and is upstream of the homeobox gene hhex and SCL, and hhex can induce both Flk1 and another endothelial marker, Fli1; in chick, hhex is also expressed in blood islands.[21,22] In the zebrafish, the Ets transcription factor etsrp is restricted to endothelial precursors and differentiated endothelial cells.[23] Etsrp mRNA can rescue the cloche mutant zebrafish, and SCL expression is reduced in a subset of endothelial precursors in etsrp morphant zebrafish,[23] indicating that estrp falls between cloche and SCL in the pathway from mesodermal precursor to hemangioblast. Although these upstream transcription factors are important for inducing Flk1, Flk1-null mice lack blood islands and vessels and die between E8.5 and E9.5, highlighting the importance of this specific gene for vasculogenesis.[15]

The cells in these blood islands undergo one of two transitions: The outer cells flatten and become endothelium while the inner cells differentiate as hematopoietic cells.[13] The endothelial cells can now be identified by their expression of VE-cadherin, receptor tyrosine kinase Tie2, and PECAM (platelet endothelial cell adhesion molecule, also known as CD31). Although no markers yet distinguish different subsets of endothelial cells at this stage, cell-tracing experiments have shown that each endothelial cell will contribute only to arteries or veins.[24] However, further differentiation is required before the endothelial cells are fully committed to their particular fate.

DIFFERENTIATION INTO HETEROGENEOUS POPULATIONS

Once specified, endothelial cells from the blood islands coalesce to form interconnected tubes that create a capillary plexus. Interestingly, endothelial cells express PlexinD1, which sets up migratory patterns and provides guidance cues.[25] One of its ligands, Semaphorin3A, is present in the somites of zebrafish, and PlexinD1 specifically guides the intersomitic vessels in zebrafish, with no effect on specification or differentiation.[25] After the heart begins contracting, the vascular plexus undergoes dramatic remodeling, and the endothelial tubes are exposed to environmental cues that influence their differentiation. The apical side of the endothelial cells senses mechanical forces such as transmural pressure, shear stress, and pulsatile flow, and the basolateral side senses mechanical forces through integrin receptors.[26] Shear stress can induce PECAM in embryoid bodies.[27] Healthy shear stress can induce transcription factors like Kruppel-like factor-2, which regulates peripheral vascular resistance.[28,29] In addition, laminar shear stress, which is present in straight arterial vessels, induces VE-cadherin; the strongly expressing VE-cadherin-positive endothelial cells elongate compared with their cuboidal counterparts in more torturous venous vessels, which are exposed to slower flow rates and less shear stress.[30] In addition, laminar shear stress also induces gap junction proteins connexins 37 and 40, which maintain intercellular communication, and organizes F-actin, which is essential for a functional endothelial barrier.[29] The tight junction protein occludin is also induced by shear stress, and it inhibits

permeability.[31] These biochemical and biomechanical forces trigger distinct signaling pathways, including those pathways initiated by PECAM, VE-cadherin, and VEGF-R.[30]

In addition to environmental cues, endothelial cells are also exposed to signaling pathways that affect their differentiation. A striking example of how a signaling pathway can affect both arterial and venous differentiation is observed in the dorsal aorta and cardinal vein. The endothelial cells that will comprise the dorsal aorta are close to the notochord, a Sonic hedgehog source.[32] Sonic hedgehog induces high levels of VEGF in the somites, which in turn induces Notch in the adjacent dorsal aorta, and Notch is upstream of arterial marker ephrin B2, Notch receptor delta-like protein 4, neuropilin-1, and phosphorylated Erk.[32,33] Transcription factors Foxc1 and Foxc2 also induce neuropilin-1, which is a VEGF co-receptor, and Notch, eph, PE-CAM, Flk, Tie2, and delta-like protein 4.[34,35] In addition, Sonic hedgehog also inhibits the lymphatic endothelial marker and Tie2 ligand angiopoietin-2, thus reinforcing the arterial fate of the endothelial cells closest to the notochord.[36]

To achieve a venous fate, presumably lower Sonic hedgehog levels act on the cardinal vein to induce low levels of VEGF, which induces chicken ovalbumin upstream promoter transcription factor II (COUP-TFII). COUP-TFII inhibits Notch signaling and thus blocks arterial differentiation.[37,38] The Hox genes also program venous fate; HoxA13 co-localizes with PECAM and induces FoxF1 and Tie2, which is initially expressed in all endothelial cells but eventually restricts to the venous lineage.[39] Furthermore, venous endothelium expresses the ephrin receptor ephB4 (rtk5 in zebrafish),[40] which allows signaling between the arterial and venous endothelium and may be responsible for segregating arterial and venous endothelium.[41]

The venous endothelium gives rise to the lymphatic endothelium. In mouse, a subset of the venous endothelial cells express Sox18 at E9.5,[42] which subsequently induces Prox1, the master regulator of lymphatic vascular development, by E10.5.[43–45] Prox1-positive cells bud off the cardinal vein and move dorsoanteriorly to form the lymphatic network.[43] Prox1 upregulates FGF receptor 3,[46] which is the main receptor for FGF-2 (bFGF) and promotes proliferation. FGF-2 induces VEGF-C,[47] whose main receptor, VEGFR3, is restricted to lymphatic endothelial cells.[48] Another factor, collagen and calcium-binding EGF domain 1 (CCBE1), is required in the adjacent mesenchyme during lymphatic sprouting and is thought to provide migratory cues, as the CCBE1-null zebrafish (full of fluid) lacks budding lymphatic vessels.[49] In addition to these positive regulators, HoxA13, which promotes the venous endothelial fate, must be turned off to allow lye-1 (lymphatic endothelial gene) to be expressed.[39] Because the lymphatic endothelium is not under the influence of Sonic hedgehog, it expresses angiopoietin-2,[39] which destabilizes the adhesion of mural cells to the endothelium and is required for lymphatic activity.[50] An additional factor that is essential for lymphatic development is the transmembrane protein podoplanin,[51] an adhesive protein that binds to the secreted protein galectin-8.[52] Because galetcin-8 binds extracellular matrix proteins such as integrins,[53] podoplanin and galetcin-8 may be required to anchor the lymphatic endothelial cells once they have completed their migration.

VASCULAR ENDOTHELIUM CHARACTERISTICS

The vascular endothelium consists of a single layer of cells that line the luminal surface of the vascular system and regulate the transport of macromolecules and blood components from the interstitium to the lumen of the vessel. The vascular endothelium is divided into arterial and venous endothelia, with additional differences between larger and smaller vessels. These specific vascular regions have distinctive differences that support their varied

roles, ranging from changes in the vessels themselves to differences in the surrounding milieu.

Morphological Differences

As the primitive vascular plexus begins to remodel, the first observable differences are between arterial and venous vessels. The arterial vessels undergo more retraction to yield vessels with few branches and a large surrounding avascular area,[54] whereas the veins are more irregular in shape, with more branches (Fig. 2). Proliferation is also higher in venous endothelial cells, which may further contribute to these morphological differences.[55]

Endothelial Support Differences

Arteries are surrounded by vascular smooth muscle cells. The endothelium secretes extracellular matrix proteins, so the smooth muscle cells have no direct contact with the endothelial cells (Fig. 2). The vascular smooth muscle provides mechanical support and elasticity, mediated by contractile proteins such as smoothelin-B.[56] These vascular smooth muscle cells migrate toward the larger primary arteries and subsequently cover the branches.[54] Because the arterial endothelial cells are under such high shear stress, it is unsurprising that they would need this additional support. In contrast, venous endothelial cells have few supporting vascular smooth muscle cells, have thinner walls, and experience less shear stress.

Interestingly, some arterial endothelial cells, such as the coronary arteries, are athero-susceptible, whereas other arterial endothelial cells, such as the iliac arteries, are athero-resistant.[57] The coronary arteries preferentially express atherosclerosis-related genes, such as transcription factor early growth factor-1, proinflammatory transcription factor c-FOS, and genes that are related to inflammation, oxidation, and lipid metabolism.[57] Iliac arteries, in contrast, express genes that mediate vascular remodeling and angiogenesis as well as cytoprotective genes.[57] Although the cause of these differences is currently unknown, Zhang et al hypothesize that the changes may be caused by the local mechanical environment, such as differences in shear stress and circumferential strain.[57]

Endothelial Plasticity

Despite evidence that arterial/venous fate is determined before endothelial tubes form, this distinction is plastic. In chick, the early endothelial cells have an anterior/venous and posterior/arterial association. However, as the vitelline artery forms, formerly artery-associated endothelial cells in the posterior capillaries receive less blood flow, retract from the artery, and become incorporated into the vitelline veins.[58] Quail-chick chimeras that have transplanted arterial and venous vessel grafts showed that these endothelial cells can change identity, incorporate into the other vessel, and switch the gene expression profile to match the host vessel.[59,60]

Large Vessel versus Microvessel Endothelial Cells

Because large vessels (arteries and veins) primarily serve to transport blood, whereas the smaller arterioles regulate vascular tone and venules are the primary site of permeability during inflammation,[61] it is unsurprising that large and small vessels express different sets of genes. Through microarray analysis of a series of endothelial cell lines, Chi and colleagues characterized the hallmark genes of endothelial cells from large and small arteries and veins.[62] Large vessel endothelial cells are surrounded by a thick vascular wall and express a set of genes involved in the biosynthesis and remodeling of the extracellular matrix. In addition to extracellular matrix proteins, they also express cell-cell adhesion proteins such as N-cadherin and activated leukocyte cell adhesion molecule, which help

support barrier function.[63] They also express several genes associated with neuronal migration, which suggests that these endothelial cells can respond to the same cues that guide neuronal migration.[62]

Microvessel endothelial cells, in contrast, express genes that encode basement membrane proteins and are also tightly associated with the basement membrane.[62] Although both macro- and microvessels have the same level of actin, the actin filaments are more stable in microvessels.[63] In addition, they express genes that traffic circulating blood cells and pathogens as well as genes that support lipid transport and metabolism.[62]

Lymphatic Endothelial Characteristics

The lymphatic endothelium is composed of a single layer of endothelial cells that line the lymph vessels and is more permeable than the vascular endothelium (Fig. 2). Its main functions are to remove plasma proteins that have filtered through the capillaries into the tissue spaces, transport immune cells, and take up dietary fat. These processes occur, not through tight or adherens junctions, but through overlapping button-like intercellular junctions that open and close in response to interstitial pressure.[64,65] In addition, the major lymphatic vessels are surrounded by smooth muscle cells that generate the pulsatile force needed to move lymph.[66]

SPECIALIZED ENDOTHELIUM

Endocardium

Although the endocardium consists of endothelial cells that line the heart, it is distinct from the endothelium. Like the endothelium, it expresses Flk1, VE-cadherin, and Tie2 during development.[67] However, its origin is controversial; studies in zebrafish indicate that it shares its origin with the hemangioblast, whereas labeling studies in chick indicate that the myocardium and endocardium are distinguished in the primitive streak.[68] However, lineage-marking studies in mouse have indicated that the mesoderm gives rise to both myocardium and endocardium.[69,70] Misfeldt and colleagues recently confirmed that the endocardium shares its origin with the myocardium as opposed to the endothelium, but these studies were performed in embryoid bodies and lack the spatiotemporal constraints of the embryo.[71] Thus it is unclear how to resolve the single-cell chick tracings with the lineage-marking mouse studies. Regardless of origin, the endocardium serves a major role in forming the cushions that will eventually remodel to form the valve leaflets[72] and also in inducing trabeculation.[73]

Fenestrated Endothelium

Fenestrated endothelium is characterized by the presence of circular windows (or *fenêtres*) in the cell body. These fenestrations allow transport of water and small hydrophilic molecules, and fenestrated endothelium is present in organs that resorb water and small molecules or hormones, such as the kidney and liver.[74,75]

Blood-Brain Barrier

The blood-brain barrier is unique in that it is a non-fenestrated endothelium. Specific tight junctions result in impermeable connections between endothelial cells, separating the cerebral capillaries and the brain. These tight junctions allow the selective transport of nutrients and consist of claudin 3 and occludins.[76] If blood-brain barrier endothelial cells are placed in culture, they rapidly lose their impermeability, indicating that they receive a constant supply of inductive factors, which may include bFGF, angiopoietin-1, and transforming growth factor β.[77,78] In addition to transport through the tight junctions, small gaseous molecules and lipophilic agents can diffuse through the endothelium.[78]

ENDOTHELIAL DYSFUNCTION

Arterial Dysfunction

The arterial endothelium releases nitric oxide, endothelin, and prostacyclin, which regulate vascular tone, platelet activity, and blood coagulation. Any event that disrupts endothelial integrity thus disrupts these processes as well. Coronary artery disease is one example of a disease that targets the arterial endothelium. Some risk factors cannot be changed, such as age and male gender, but other risk factors can be treated, such as hyper-cholesterolemia. In response to these risk factors, the endothelium increases the expression of adhesion molecules, which leads to intimal thickening and plaque formation.[79] In addition, hypertension and aging also decrease gap junction expression, thereby decreasing cell-cell communication.[80]

Venous Dysfunction

The venous circulation returns blood to the heart, which facilitates cardiovascular homeostasis. Chronic venous disease is characterized by obstructed and/or incompetent venous flow and varies based on clinical, etiological, anatomical, and pathophysiology signs and symptoms.[81] Although disease severity may vary, most cases are caused by vascular hypertension, which results in reflux in the superficial and/or deep venous system and higher-than-normal venous pressures.[81] Incompetent venous flow is caused by changes in the vessel wall, such as decreased smooth muscle and elastin and increased collagen; these changes result in a weakened, dilated vessel wall.[82] In addition, high shear stress activates leukocytes, thus activating the inflammatory response pathway.[81]

Lymphatic Dysfunction

Impaired lymphatic function results in overall swelling, known as lymphedema. This dysfunction can be caused either by decreased transport of lymph through the lymphatic network, as is observed in parasitic infections,[83] by increased fluid,[84] or by having fewer capillaries, which is seen in primary congenital lymphedema. Mutations in VEGFR3, FoxC2, and Sox18 have all been linked to lymphedema.[85]

Blood-Brain Barrier Dysfunction

Epidemiology studies have linked vascular defects in the blood-brain barrier with Alzheimer's disease.[86] In patients with Alzheimer's disease, the basement membrane is thickened and vacuous, and the microvessels thin and exhibit increased tortuosity compared with control subjects.[87] The vascular changes are due to both amyloid β–induced vascular dysfunction and reduced neuronal processing, and the degree of microvessel degeneration is related to amyloid plaque formation.[88,89] One potential connection between the vascular and neuronal biology is apolipoprotein E, which is involved in both atherosclerosis and mobilizing cholesterol in the brain after ischemia.[90] In addition, apolipoprotein E may destabilize the supporting smooth muscle cells and disrupt tight junctions.[89]

CONCLUSIONS

The endothelial cell may have humble origins during gastrulation, but the endothelium is an essential component of all organs. Understanding how a basic endothelial cell can differentiate into such specialized cells is essential for appreciating the complexity of endothelial disorders and providing better treatments. Different organs have different plumbing requirements, as it were, that are met by these heterogeneous endothelial populations. By appreciating the different functions and machinery that these populations possess, it should be easier to harness the cells' plasticity, resulting in more effective vessel grafts and the ability to reprogram endothelial cells that have become dysfunctional.

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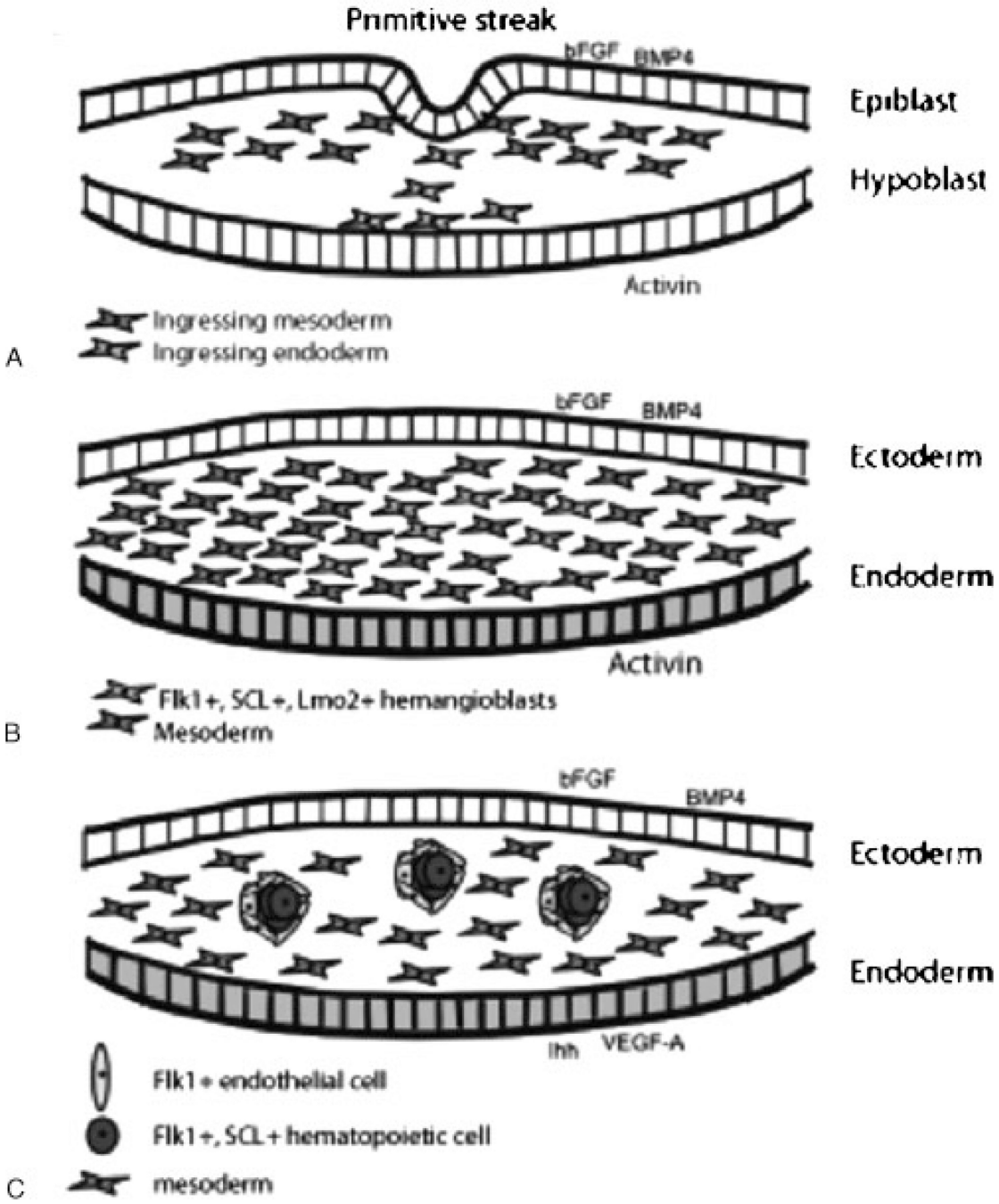

Figure 1.

The endothelium is derived from ingressing mesoderm. (A) Signals from the epiblast and hypoblast induce mesoderm formation. (B) Additional signals from the ectoderm then induce a subset of mesoderm to become hemangioblasts. (C) These hemangioblasts will swell and form clusters. These clusters show the first distinction between the outer endothelial cells and the inner hematopoietic cells.

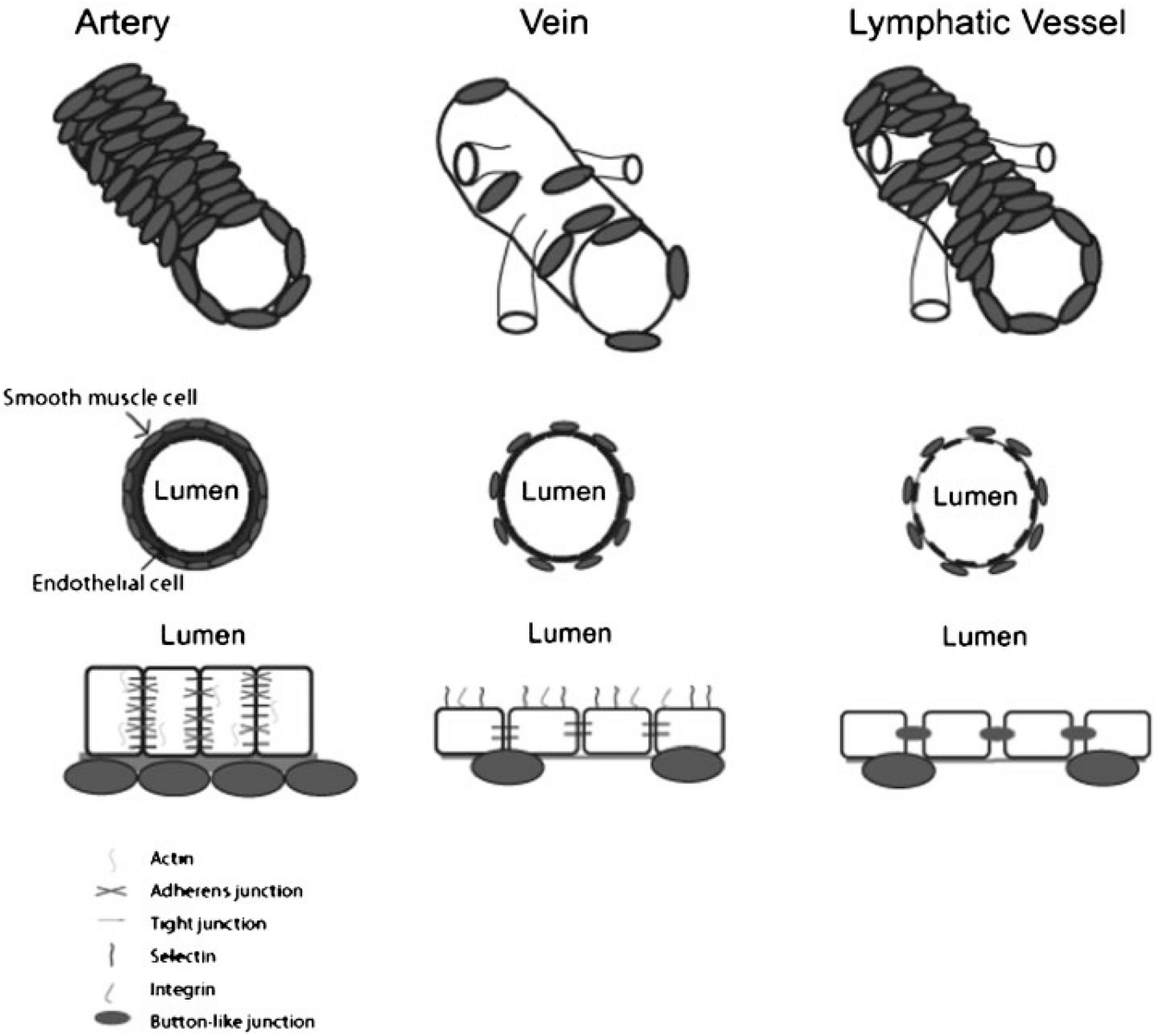

Figure 2.

Arterial, venous, and lymphatic vessels have different characteristics, which stem from the endothelial cell lining. Arterial endothelial cells are tightly connected via tight and adherens junctions, express high levels of actin, deposit a thick layer of extracellular matrix, and are supported by smooth muscle cells. Venous endothelial cells are more permeable with less extracellular matrix, and venous vessels are more flexible than their arterial counterparts. In addition, they display integrins and selectins on their apical surface. Lymphatic endothelial cells are highly permeable, and the major lymphatic vessels are supported by smooth muscle cells; they are connected via large button-like junctions.