Endothelial Cells Heterogeneity

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Abstract: The vascular endothelium constitutes approximately 1% of body mass (1kg) and has a surface area of approximately 5000m (2). The endothelium is a multifunctional endocrine organ strategically placed between the vessel wall and the circulating blood, and has a key role in vascular homeostasis. The endothelium forms the inner cellular lining of blood vessels. It is now well established that endothelial cells are highly metabolically active, and play a critical role in many physiological processes, including the control of vasomotor tone, the trafficking of blood cells between blood and underlying tissue, the maintenance of blood fluidity, permeability, angiogenesis, and both innate and adaptive immunity. It is also recognized that the endothelium is involved in most if not all disease states, either as a primary determinant of pathophysiology or as a victim of collateral damage. This study highlights the molecular and the physiological aspects of endothelial cells heterogeneity and its clinical implications.
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Introduction

The endothelium is an expansive spatially distributed organ. Endothelial cells participate in a large number of physiological processes including the control of vasomotor tone, the trafficking of cells and nutrients, the regulation of permeability, and the maintenance of blood fluidity. In addition, the endothelium mediates new blood vessel formation, contributes to the balance of pro- and anti-inflammatory mediators, and may play a role in antigen presentation. In accomplishing these tasks, the endothelium exhibits a remarkable "division of labor". For example, arteriolar endothelium is primarily responsible for mediating vasomotor tone; endothelium in postcapillaryvenules regulates leukocyte trafficking; capillary endothelial cells display organ-specific barrier properties (eg, blood brain barrier versus fenestrated, discontinuous endothelium in hepatic sinusoids); and endothelial cells from different vascular beds balance local hemostasis via the expression of site-specific patterns of anticoagulants and procoagulants. In recent years, in vivo phage display and direct proteome mapping of the intact vasculature have revealed a rich diversity in endothelial cell surface markers.***(Aird, 2007).***

The formation of new blood vessels in the adult organism not only contributes to the progression of diseases such as cancer and diabetic retinopathy but also can be promoted in therapeutic approaches to various ischemic pathologies. Because many of the signals important to blood vessel development during embryogenesis are recapitulated during adult blood vessel formation, much work has been performed to better-understand the molecular control of endothelial differentiation in the developing embryo.***(Ferguson et al., 2005).***

Microvascular heterogeneity extends to properties of endothelial cells thought to be involved in tumour angiogenesis and metastasis, such as growth factor responsiveness and expression of cell adhesion molecules. These findings are not only of relevance to the unambiguous identification and characterization of cultured endothelial cells, but it may explain the phenomenon of preferential organ tumour metastasis and provide novel opportunities for antitumour therapy. ***(Plummer et al., 2013).***

Aim of the Study

The aim of this work is to highlight the molecular and the physiological aspects of endothelial cells heterogeneity and its clinical implications.

Endothelial ontogeny:

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.**(*Laura & Cam, 2010).***

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 Although BMP4, is sufficient to induce mesodermal differentiation in embryonic stem cell**. *(Pearson et al., 2008).***

Epiblast-derived basic fibroblast growth factor (bFGF) and hypoblast-derived activin induce mesoderm formation in the embryo. Interestingly, the hypoblast can be removed before gastrulation and endothelial precursor blood islands still form, indicating that bFGF is sufficient for mesoderm induction. As the mesoderm ingresses, the epiblast re-expresses BMP4 in the non-neural ectoderm. This BMP signal is necessary for patterning the mesoderm and setting aside a ventral mesodermal population that can give rise to the endothelium. ***(Laura & Cam., 2010).***

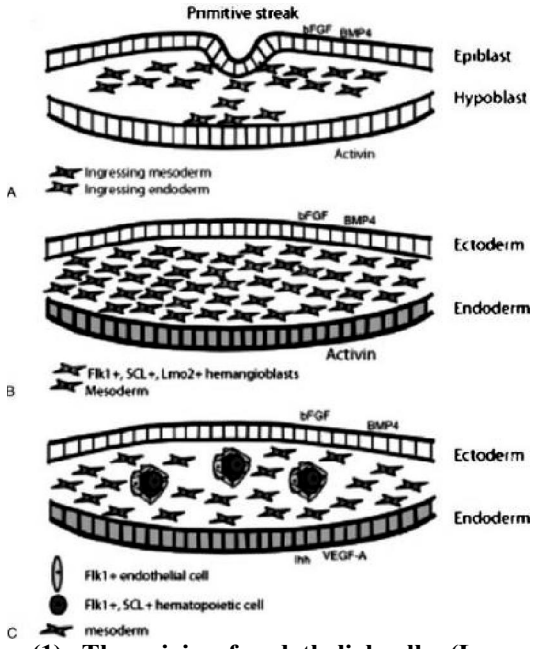

Fig. (1): The origin of endothelial cells. (Laura & Cam, 2010).

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.

From the mesoderm, bFGF and activin A induce specification of the hemangioblast, the common precursor of endothelial and hematopoietic cells. This induction is observed within 3 hours of mesodermal exposure to bFGF and activin, and a capillary plexus can form within 7 hours.***(Pearson et al., 2008).***

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. 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. However, further differentiation is required before the endothelial cells are fully committed to their particular fate.***(Zhong et al., 2001).***

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. 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.***(Torres et al., 2004).***

In addition to environmental cues, endothelial cells are also exposed to signaling pathways that affect their differentiation.***(Lawson et al., 2001).***

The molecular regulation of arteriovenous specification:

The formation of a hierarchical vascular network, composed of arteries, veins, and capillaries, is essential for embryogenesis and is required for the production of new functional vasculature in the adult. Elucidating the molecular mechanisms that orchestrate the differentiation of vascular endothelial cells into arterial and venous cell fates is requisite for regenerative medicine, as the directed formation of perfused vessels is desirable in a myriad of pathological settings, such as in diabetes and following myocardial infarction. Additionally, this knowledge will enhance our understanding and treatment of vascular anomalies, such as arteriovenous malformations (AVMs). From studies in vertebrate model organisms, such as mouse, zebrafish, and chick, a number of key signaling pathways have been elucidated that are required for the establishment and maintenance of arterial and venous fates. These include the Hedgehog, Vascular Endothelial Growth

Factor (VEGF), Transforming Growth Factor-β (TGF-β), Wnt, and Notch signaling pathways. In addition, a variety of transcription factor families acting downstream of, or in concert with, these signaling networks play vital roles in arteriovenous (AV) specification. These include Notch and Notch-regulated transcription factors (e.g., HEY and HES), SOX factors, Forkhead factors, β-Catenin, ETS factors, and COUP-TFII. It is becoming apparent that AV specification is a highly coordinated process that involves the intersection and carefully orchestrated activity of multiple signaling cascades and transcriptional networks***.(Jason and Joshua, 2015).***

Specification of arterial, venous, and lymphatic endothelial cells during embryonic development:

Table (1): Transcription factors regulating arterial, venous lymphatic specification.*(Swift & We instein,2009).*

Arterial Specification	
Factor	Function
Shh	Loss of Shh results in lack of arterial identity in zebrafish. Shh acts upstream of VEGF.
VEGF	VEGF acts downstream of Shh signaling to activate Notch via the PLCγ/ERK pathway in zebrafish. Mutant mice expressing only VEGF188 lack arterial differentiation.
Nrp1	Null mice display impaired arterial differentiation. Nrp1 is involved in a positive feedback loop of VEGF signaling.
Notch	Notch acts downstream of Shh and VEGF signaling in zebrafish. Notch1; Notch4 mutant mice have abnormal vascular development.
Dll4	Null mice lack arterial specification.
Dll1	Null mice fail to maintain arterial identity.
Hey1/2 (Grl)	Null mice lack arterial specification. Lack of grl in zebrafish results in loss of arterial specification.
Foxc1/c2	Foxc1; Foxc2 mutant mice lack arterial specification. Foxc1 and Foxc2 directly regulate Dll4 and Hey2 expression. Foxc1 and Foxc2 are also involved in lymphatic vessel development.
Sox7/18	Lack of Sox7/18 results in loss of arterial identity in zebrafish.
Snrk-1	Snrk-1 acts downstream or parallel to Notch signaling inzebra fish
Dep1	Dep1 acts upstream of PI3K in arterial specification in zebrafish.
Crlr	Shh regulates VEGF activity by controlling crlr expression in zebrafish.
EphrinB2	Null mice lack boundaries between arteries and veins. EphrinB2 is involved in lymphatic vascular remodeling and maturation.
Venous Specification	
COUP-TFII	COUP-TFII suppresses arterial cell fate by inhibiting Nrp1 and Notch. COUP-TFII also interacts with Prox1 to regulate lymphatic gene expression.
EphB4	Null mice lack boundaries between arteries and veins.
Lymphatic Specification	
Sox18	Null mice fail to specify lymphatic endothelial cells. Sox18 induces Prox1 expression.
Prox1	Prox1 induces lymphatic markers and maintains lymphatic cell identity.

The groundbreaking discovery about arterial and venous expression of ephrinB2 and EphB4, respectively, in early embryonic development has led to a new paradigm for vascular research, providing compelling evidence that arterial and venous endothelial cells are established by genetic mechanisms before circulation begins. For arterial specification, vascular endothelial growth factor (VEGF) induces expression of Notch signaling genes, including Notch1 and its ligand, Delta-like 4 (Dll4), and Foxc1 and Foxc2 transcription factors directly regulate Dll4 expression. Upon activation of Notch signaling, the Notch downstream genes, Hey1/2 in mice or gridlock in zebrafish, further promote arterial differentiation. On the other hand, the orphan nuclear receptor COUP-TFII is a determinant factor for venous specification by inhibiting expression of arterial specific genes, including Nrp1 and Notch. After arterial and venous endothelial cells differentiate, a subpopulation of venous endothelial cells is thought to become competent to acquire lymphatic endothelial cell fate by progressively expressing the transcription factors Sox18 and Prox1 to differentiate into lymphatic endothelial cells ***(Tsutomu, 2010).***

Heterogeneity of the endothelial cell:

The first hint of endothelial cell heterogeneity, a structural heterogeneity, was obtained following electron microscopy observations where differences in intercellularjunctions led to the classification of continuous endothelium, fenestrated endothelium and discontinuous endothelium. ***(Félétou 2011).***

Structural heterogeneity of the endothelial cells also includes various cellular shapes, various amounts of structural component of the endocytic pathway, such as clathrin-coated pits or the transcytosis pathway such as of caveolae, various levels of expression of the predominant types of intercellular junctions, tight junctions, adherens junctions or gap junctions, various compositions of the glycocalyx and the associated endothelial surface layer (a stationery layer much thicker than the glycolalix that excludes red blood cells), etc. this heterogeneity in endothelial cells is linked to both intrinsic, i.e., genetic factor, and extrinsic factors, i.e., environmental causes such as location, soluble mediators, cell to cell contact, cell–matrix interactions, pH, pO_2, mechanical forces (shear stress, physical constraints), etc. epigenetic-induced heritable changes, histone methylation and acetylation, occur in the early phase of differentiation, while micro-environmental changes, which are not transmitted during mitosis, occur predominantly in the late phase of differentiation, i.e., organ-specific differentiation of the endothelial cells***. (Aird., 2006).***

Continuous Nonfenestrated endothelium:

Continuous nonfenestrated endothelium is found predominantly in arteries, veins, and capillaries of the brain, skin, muscle, heart, and lung. Tight junctions and adherens junctions are the 2 main types of barrier forming intracellular junctions found in this type of endothelium***. (Bazzoni & Dejana 2004).***

The expression of Continuous nonfenestrated endothelium is variable across the endothelial tree, with higher expression in the continuous endothelium of arterioles compared with capillaries and venules. Molecules cross this endothelium by the active process of transcytosis, which is mediated by specialized structures including caveolae and vesiculo-vacuolar organelles. Caveolae, flask-shaped membrane bound vesicles (≈70 nm in diameter) that usually open to the luminal or abluminal side.***(Parton & Simons 2007).***

Caveolae, are present in all types of endothelium but are highest in capillaries that contain continuous nonfenestrated endothelium.***(Bendayan 2002).***

Continuous Fenestrated endothelium:

Fenestrae are transcellular pores (≈70 nm in diameter) that extend through the full thickness of the EC and are thought to allow rapid exchange of molecules between the circulation and the surrounding tissue. ***(Atkins et al., 2013).***

The majority of fenestrae contain a thin diaphragm across their opening that acts as a molecular filter. The type II membrane glycoprotein plasmalemmal vesicle–associated protein-1 is currently the only molecular protein localized to the diaphragm, and it has been discovered to be both necessary and sufficient for diaphragm formation in cultured EC.***(Ioannidou et al., 2008).***

Compared with nonfenestrated endothelium, continuous fenestrated endothelium is more permeable to water and small solutes. This endothelium typically occurs in locations that are characterized by increased filtration or increased transendothelial transport and is found in capillaries of all exocrine and endocrine glands, digestive tract mucosa, and kidney (eg, glomeruli and a subpopulation of renal tubules).***(Stan 2009).***

Discontinuous Fenestrated endothelium:

Discontinuous fenestrated endothelium is characterized by large heterogeneous fenestrae (100 to 200 nm in diampeter) without diaphragms. It has few caveolae and contains clathrin-coated pits and vesicles, which play an important role in receptor-mediated endocytosis. This endothelium is found in certain sinusoidal vascular beds, most notably the liver and bone marrow, which lack a well-formed basement membrane***. (Braet & Wisse 2002).***

Mechanisms of endothelial heterogeneity:

The shape of cells varies across the vascular tree. Although ECs are typically flat, they are plump or cuboidal in high endothelial venules***. (Girard et al., 1999).***

Each EC is analogous to a miniature adaptive nonlinear input/output device. Input arises from the extracellular environment and consists of biomechanical (eg, shear stress and cyclical strain) and biochemical forces (eg, growth factors, cytokines, chemokines, hormones, complement, nitric oxide, oxygen, and reactive oxygen species). Output represents the cellular phenotype and may be measured as cell shape, calcium flux, protein expression, mRNA expression, migration, proliferation, survival/apoptosis, vasomotor tone, hemostatic balance, release of inflammatory mediators, and leukocyte adhesion/transmigration. Input is coupled to output by signaling pathways that typically begin at the cell surface and end at the level of transcription or posttranscriptional modification. at any point in time, the net input of biomechanical and biochemical signals varies across the vasculature. For example, ECs in the brain are exposed to myriad astroglial-derived paracrine factors that are essential for maintenance of the blood brain barrier. In contrast, ECs lining capillaries in the heart are exposed to regional forces generated from the contracting heart, and paracrine factors derived from neighboring cardiomyocytes. At any single site of the vasculature, net signal input varies from one moment to the next. For example, liver sinusoidal endothelium is exposed to portal venous blood of vastly different composition in the pre- and postprandial period. because signal input varies in space and time, and because ECs are

capable of sensing and responding to the micro environment, EC phenotypes display marked spatial and temporal heterogeneity.***(Aird 2006).***

The wide range of signal inputs from one organ to the next is sufficient to generate phenotypic heterogeneity across the vascular tree. When endothelial cells are removed from their native tissue and grown in tissue culture, they become uncoupled from critical extracellular cues and undergo phenotypic drift. For this reason, studies of cultured endothelial cells are fraught with limitations. Second, certain site-specific properties are epigenetically "fixed" and impervious to changes in the extracellular environment. Such properties are mitotically stable, and are thus retained under in vitro culture conditions. The relative roles of epigenetic and nonepigenetic forces in mediating phenotypic heterogeneity are not fully understood***.(Aird 2012).***

In the final analysis, there is strong evidence to support a role for both mechanisms—environment and epigenetics—in mediating endothelial cell heterogeneity, thus creating a balance between stability and plasticity in gene expression and phenotype. For example, the study of human tonsillar endothelial cells referred to above demonstrated that some, but not all, site-specific transcripts were altered after 2 days of culture.***(Lacorre et al., 2013).***

Conclusion:

Endothelial cells, which form the inner cellular lining of blood vessels and lymphatics, display remarkable heterogeneity in structure and function.

The endothelium provides a broad menu of functions that are adapted to the diverse needs of the underlying tissues.

Endothelial dysfunction appears to play a critical role in a variety of human disorders, including peripheral vascular disease, stroke, heart disease, diabetes, insulin resistance, chronic kidney failure, tumor growth, metastasis, venous thrombosis, SLE, rheumatoid arthritis & vasculitis.

Because the endothelium is spatially distributed throughout the body, because it communicates with each and every tissue, and because it is involved in most disease states, it represents a powerful organizing principle in human health and disease.

Expanding our understanding of endothelial function further will lead to targeted therapies for a myriad of diseases, including cancer, cardiovascular disease, and inflammation.

Many pharmacological interventions have been targeted to the endothelium with the intent of restoring it to its quiescent state. Various pharmacological interventions such as angiotensin-converting enzyme (ACE) inhibitors, statins, insulin sensitizers, and L-arginine, as well as agents that target endothelial nitric oxide synthase (eNOS) "coupling" such as folates or tetrahydrobiopterin (BH4) have been noted to improve the function of the endothelium.

Recommendations*:*

- More effort should be done for understanding endothelium-dependent regulation of vascular Tone and its clinical applications.
- Further investigations are crucial to get a better understanding of the complex epigenetic interactions and to provide new ways for the treatment of vascular disease.
- Future and intensive studies on the EPC biology will be needed for improving or inducing vascular neoformation and angiogenesis in different CVD conditions. This might likely permit inducing and improving vascular regeneration under ischemic or other CVDevents or provide a good substrate for vascular grafting, that is, bypass surgery and vascular reconstruction following aneurisms or traumatic injuries.

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