Chapter 3
Endothelial Cell Activation

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Abstract: The initiation of the angiogenic cascade from a pre-existent vascular network requires the selective departure of individual endothelial cells from differentiated capillaries. The process entails the activation of specific signaling pathways that enable endothelial cells to exit their vessel of origin, invade the underlying stroma and initiate a new vascular sprout. Two major signaling pathways: VEGF and Notch, coordinate this process to select a subset of leading endothelial cells, referred to as tip cells. These cells display long filopodia and are highly migratory, but remain linked to their followers, the stalk cells. The stalk cells constitute the body of the sprout and proliferate in response to VEGF increasing the length of the incipient capillary. It is the coordination of Notch and VEGF signaling that regulates the extent to which cells become leaders (tip cells) and which become followers (stalk cells). Activation of Notch represses the tip cell in favor of the stalk cell phenotype, in part, by regulating the levels of VEGFR2. The resolution of the endothelial activation phase requires synthesis and organization of the basement membrane and the recruitment of pericytes and smooth muscle cells. This chapter focuses on the molecular regulation of these signaling pathways, and it contrasts our current understanding of endothelial cell activation in development and in disease.

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Introduction

The term endothelial cell activation makes reference to the series of events by which a fully differentiated, non-motile and non-proliferative cell acquires an angiogenic phenotype. The process entails the development of invasive, migratory, and proliferative capacities by the endothelial cell. This same term has also been used to describe the phenotypic alterations of the endothelium in response to inflammatory mediators and that result in the retention and recruitment of inflammatory cells from the blood stream into the stroma. In this chapter, we will focus on angiogenic endothelial cell activation.

Endothelial cells are the basic and constant component of the vascular system. These are also the cells that initiate the angiogenic response and are responsible for establishing the pattern of the future capillary plexus. Once specified as endothelial, these cells enclose the genetic information that pre-determines their contribution to either veins or arteries, as well as their association with presumptive smooth muscle cells. Thus, early decisions in vascular morphogenesis carry important consequences for the overall formation of the vascular tree. During the last decade, the implementation of targeted gene inactivation in whole animals has provided an explosion of information regarding the genetic circuitry that mediates endothelial cell activation. The vascular system is one of the first fully functional organs to be established in vertebrate embryos and it is essential for viability and survival. This dependency has been extremely advantageous to vascular biologists through the recent rush of genetic knockout models. The unsuspected contribution of several regulatory molecules has been revealed by phenotypes that include hemorrhage and embryonic lethality. Indeed, genetic inactivation in whole animals has provided major breakthroughs in our understanding of vascular development. Based on information from loss- and gain-of-function studies, today we know that the key signaling pathways in endothelial cell activation include vascular endothelial growth factor (VEGF) and Notch. Subsequently, Slit, Ephrins, Cadherins, Wnts and Angiopoetins, Transforming

Growth factor β and integrins participate in stages post-activation, to guide, remodel, stabilize and differentiate the newly formed vessels [1,2]. This chapter will focus on the cellular events that regulate endothelial cell activation during development and in pathological conditions.

Activation of Endothelial Cells During Developmental Angiogenesis

The elucidation of the molecular underpins that regulate endothelial cell activation are critical to a concrete understanding of how blood vessels are formed. After the formation of the primary vascular plexus, which arises from the direct differentiation of mesenchymal cells into endothelial cells (vasculogenesis); the subsequent expansion of the vascular system occurs through angiogenic growth. That is, endothelial cells depart from their vascular beds and sprout into the avascular stroma.

Initiation of the Angiogenic Response

What initiates the vascular sprout? Current experimental evidence indicates that VEGF, through activation of its tyrosine kinase receptors, VEGFR1 and VEGFR2, is likely to be the initiating factor [3–8]. Activation of VEGFR2 results in significant cytoskeletal changes with extension of filopodia and acquisition of a migratory phenotype [9–16] (Fig. 3.1). As the vascular sprout continues to grow it is followed by a solid cord of cells which differ from the initial sprouting cells, as they display less filopodia and are not as migratory. Instead, these "followers" appear to proliferate more frequently than the sprouting cell [17,18]. Thus, the just beginning vessel is formed by two morphologically and functionally distinct cell types: the *tip cell* that provides directional migration and the *stalk cells* that compose the body of the rapidly expanding capillary (Fig. 3.1). Although both cell types have been shown to respond to VEGF, the tip cell appears to migrate and not proliferate, while the stalk cells mostly proliferate in response to this growth factor [17]. What mediates such alternative responses? While the answer to this question is unclear, it is likely that the selection of a particular outcome lies on either the phosphorylation of distinct tyrosine residues in the VEGFR2 with consequent recruitment of alternative second messengers; or the contribution of additional signaling pathways or a combination thereof.

Studies conducted in the retina indicate that the presentation of VEGF is likely aided by astrocytes [18–21]. These cells provide spatial guidance into pre-determined tracks. In other organs, this function might be provided by functionally analogous cell types or by the nature and composition of the matrix. Nonetheless, it has been considered that the presentation of VEGF, either bound to the matrix (or to the surface of adjacent cells) or in a soluble form, alters the responses of endothelial cells to the growth factor [22–24]. Thus, the

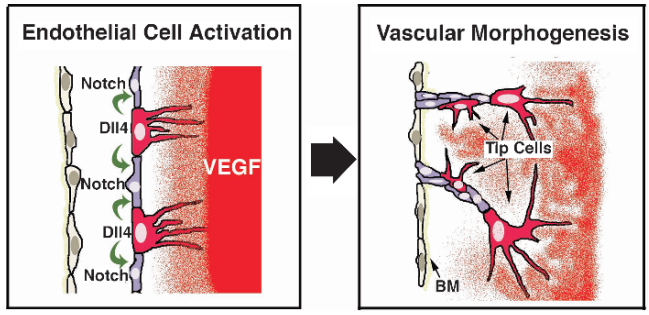

FIG. 3.1. *Endothelial Cell Activation* (*left*) results in the departure of a subset of endothelial cells from parental vessels. The process requires the acquisition of a tip cell phenotype whereby some endothelial cells are specified to become "leaders" in the sprout and their immediate neighbors are the "followers" or simply remain in the original vessel. Several loss- and gain-of-function studies in mouse and zebrafish have determined that the Notch signaling pathway is critical for this process. Thus, cells expressing the ligand Delta 4 (*Dll4*) activate the *Notch* 1 receptor in adjacent cells. Once activated, Notch mediates down-regulation of VEGFR2 and increased levels of VEGFR1. As consequence, the Dll4-expressing cells are more susceptive than their neighbors in sensing gradients of VEGF. The outcome is the formation of a vascular sprout (*Vascular Morphogenesis*; *right*) with highly migratory *tip cells* and proliferating stalk cells.

ability of VEGF to be immobilized allows for the formation of a gradient that is sensed by filopodia on the tip cell and provides directional migratory cues. In contrast, when VEGF is soluble there is no gradient formed and endothelial cells tend to lose directionality and be less migratory [23,24].

The ability of VEGF to interact with the extracellular matrix is regulated by two mechanisms: (1) splicing, and (2) extracellular processing. Encoded by a single gene, VEGF-A can originate multiple transcripts as the result of alternative splicing. Seven isoforms have been identified: the most frequently detected forms are VEGF 121, 165, and 189 (the names represent number of amino acids) [22]. Interestingly, the differences amongst the isoforms reside in a region coded by exons 6a, 6b and 7 and that is targeted by the splicing machinery. These exons code for domains that interact with heparin and other matrix proteins. There is a direct correlation between the ability of VEGF to bind to the matrix and the extent of the carboxy-terminal tail coded by the exons mentioned above. Thus, VEGF 189 binds more avidly to matrix proteins than VEGF 165. In contrast to these, VEGF 121 is considered to be the soluble VEGF form.

The biological significance of each VEGF isoform has only been revealed recently and has relevance to our understanding of endothelial cell activation. Using a knockin strategy, a group of investigators decided to integrate the cDNAs of VEGF 121, 165 and 189 into the VEGF locus, i.e., under the regulatory control of its promoter [23]. The resulting mice were only able to generate 121, 165 or 189. The approach not only restricted the production of one particular transcript,

it also resulted in an overexpressor for such isoforms, as the full activity of the promoter was confined to only one isoform. The findings were remarkable as they clarified the relevance of matrix-bound VEGF: the longest VEGF isoform (able to bind tightly to matrix) was essential for directional filopodial growth. Mice exhibited increased vascular density and thinner vessels than wild-type mice. In contrast, expression of 121 resulted in lower capillary density, enlarged vessels, and poor directionality of the vascular sprouts [23]. VEGF 121 mice exhibited patterning anomalies in larger vessels, including Tetralogy of Fallot [25].

The second mechanism for alteration of matrix binding is proteolytic processing. Several enzymes including plasmin and a cohort of matrix metalloproteinases (MMPs) are able to cleave VEGF in the extracellular space [24]. Processing of the growth factor occurs at aa113 and severs the molecule to separate the receptor binding domain from the extracellular binding region. This intramolecular processing event is extremely effective at dissociating VEGF from its matrix anchorage, and capable of releasing a soluble form fully able to activate VEGF tyrosine kinase receptors. Thus, depending upon the availability of enzymes, the extracellular environment can interfere with the well-orchestrated control provided by alternative splicing [24]. The contribution of extracellular enzymes to VEGF processing is likely to be a process associated with inflammation and other pathological events, such as cancer and less likely to be an active participant of developmental angiogenesis.

In addition to matrix-bound VEGF, the direction of migration is aided and subsequently regulated by plexins, slits, and semaphorins [26]. The contribution of these molecules to the process of endothelial cell activation will be discussed later.

Acquisition of Tip *Versus* Stalk Endothelial Cell Identities

As mentioned previously, VEGF is essential for endothelial cell activation, as it regulates both migratory and proliferative activities. However, signaling via VEGF alone is not sufficient to organize a well-orchestrated vasculature. A critical step in endothelial cell activation is to establish leadership: Who will be the leader cell that initiates the vascular sprout and who will follow? Recent studies have demonstrated that the Notch signaling pathway is critical for specifying stalk versus tip and for generating the required functional hierarchy that allows a vascular cord to emerge from a field of equivalent endothelial cells (Fig. 3.1) [27–29].

Prior to angiogenic growth, the local (in situ) differentiation of mesenchymal cells into endothelial cells results in the formation of a homogenous capillary plexus (vasculogenesis). This *"vascular rete"* expands quickly and remodels into a hierarchic vascular tree consisting of arteries, veins and interconnecting capillaries. Thus, angiogenesis, and therefore endothelial cell activation, are the first steps towards achieving vascular remodeling. However, to be functional, only a subgroup of endothelial cells must lead (i.e., be activated). The obvious question is how can hierarchic leadership be established in the context of a primary plexus where all endothelial cells are equal? Furthermore, how can this be accomplished if all these cells are exposed to the same VEGF gradient? A recent "boon" in the literature has shown that the Notch pathway enables endothelial cells to differentially "read" the same VEGF gradient by altering the levels of VEGF receptors [30–36]. Activation of the Notch receptor represses VEGFR2 and increases VEGFR1. The outcome are cells with a lower ability to "sense" VEGF [33]. In this manner, Notch provides suppressive signals that enable only a few cells to respond more avidly to the VEGF gradient (those in which Notch was not activated) and initiate the vascular sprout.

The first piece of evidence implicating the Notch pathway in the suppression of sprouts came from expression studies. Delta-like 4 (Dll4), one of the five mammalian Notch ligands, is specifically and conspicuously expressed by tip cells [37,38]. While the majority of endothelial cells within the vascular plexus display some degree of Notch receptor at their cell surface, expression of the ligands is not detected prior to tip cell specification [37,38]. Presence of Dll4 in the incipient tip cell rapidly results in the activation of Notch in the immediately adjacent neighbors (Fig. 3.1). The process leads to a reduction in their ability to detect VEGF signals and the suppression of the tip cell phenotype. This interpretation is consistent with findings from genetic deletion of Dll4 in both mouse and zebra fish [30,31,35]. Lack of Dll4 results in excessive sprouting and capillary hyperfusion during active angiogenesis, indicating that activation of a Notch receptor via Dll4 is necessary for inhibition of excessive sprouting events. The large number of sprouts in these mutants is not compatible with the organization of interconnected patented vessels. The outcome is the formation of a non-functional vascular bed that precipitates in embryonic lethality, despite the excessive number of activated endothelial cells.

Additional genetic loss- and gain-of-function has identified Notch1 as the primary receptor of Dll4 during these events [32]. Although, in contrast to Dll4, no haploinsufficiency was observed in Notch1, inactivation of this gene or pharmacological inhibition of the pathway also leads to excessive sprouting events. In the case of targeted inactivation, mice die at E9.5 with absence of vascular remodeling [39]. Interestingly, excess of Notch also results in embryonic lethality at a similar point in time. In this case, however, mice showed enlarged vessels [40], a phenotype that is consistent with the absence of tip cells and with the increase in stalk cells that proliferate but are unable to coordinate the organization of vascular sprouts.

Considering the critical requirement for Notch signaling for stalk / tip cell specification, it is not surprising that genetic ablation of genes involved in the regulation of this pathway, as well as major downstream targets, all lead to embryonic lethality. Interestingly, inactivation of all these molecules (a total of 14 KOs) die between E9.5 and E11.5 with no or extremely poor vascular remodeling [29,41].

While it is likely that Notch also regulates later aspects in vascular morphogenesis and homeostasis, its ability to suppress the tip cell phenotype is essential for productive vascular growth and it is critical for endothelial cell activation.

Guiding Cues for Activated Endothelial Cells

The highly ordered pattern of a fully developed vascular tree has implied the existence of a well-orchestrated molecular machinery able to provide guidance cues at the onset of vascular remodeling. In fact, the activated endothelial tip cell follows a VEGF gradient, but it is aided by attractive and repellent factors that fine-tuned its directionality. Originally identified as axon guidance molecules, semaphorins, plexins and slits are currently known to be more widely expressed and to play significant roles in vascular patterning [26,42–44].

Semaphorins comprise a family of membrane bound or secreted proteins that provide signals to facility navigational control during neuronal growth and, more recently, also acknowledged to provide vascular directionality [42–45]. They signal through plexins and neuropilins. In general, membrane-bound semaphorins bind to plexins, whereas secreted semaphorins bind to neuropilins [45,46]. A large cohort of genetic studies in Drosophila indicate that semaphorin signaling acts as a repulsive cue in axon guidance, in addition to suppressing neuronal migration. Nonetheless, other studies showed that these same molecules might also provide stimulatory signals depending upon the levels of intracellular cGMP [47–50]. These two functions, attraction and repulsion, are a theme of the so-called “guidance molecules” and offer a Yin and Yang balance essential for the fine-tune trajectory of endothelial navigations.

Semaphorin4A (Sema4A) suppresses VEGF-mediated endothelial cell migration and angiogenesis in vivo. Genetic targeting of Sema4A in mice results in enhanced angiogenesis in response to VEGF or inflammatory stimuli [51]. The effects of Sema4A on endothelial cells are mediated by Plexin D1 that blocks VEGF-mediated Rac activation and integrin-dependent cell adhesion. Combined, the findings indicated that Sema4A-Plexin-D1 signaling negatively regulates angiogenesis [51]. In addition to Sema4A, Sema3A has been shown not to compete with VEGF165 for binding to neuropilin1, functioning as an antagonist for the VEGF-VEGFRs proangiogenic signals [52–57].

The second group of ligands and receptor molecules involved in vascular patterning are the Netrins and UNC5 / DCC receptor families [58]. In neurons, Netrins have been shown to attract and repel neurons depending upon the nature of the receptor that is receiving the signal. Thus, attraction is generally mediated by DCC, while repulsion is conveyed by UNC5 [59]. Consistent with this notion, genetic deletion of UNC5B leads to excessive vascular branching and increased filopodia, particularly in the tip cells, suggesting a role for this receptor in vascular retraction [60]. Furthermore, exposure of growing sprouts to Netrin 1 results in retraction of filopodia in wild type mice, but not in UNC5B knockout mice [60]. These findings are also supported by studies in zebrafish. Morpholino knockdown of UNC5B results in excessive capillary branching and aberrant vessel patterning. Intersomitic vessels migrate laterally, invading somites, instead of migrating dorsally [60–62]. This is perhaps the most clear demonstration that the activated endothelial cell requires Netrin-UNC5B for directionality.

Slits and roundabouts (Robo) are the last family of ligand/receptor that contributes to neuronal and vascular patterning [63,64]. Signaling through slit has been shown to act as a repulsive factor, preventing axons that have crossed the midline from re-crossing [65]. Four Robo receptors (named 1–4) have been identified in mammals, and from these, Robo-4 appears to be endothelial-specific. The contribution of Slit-Robo to the guidance of the tip cell is controversial in vitro with reports demonstrating promigratory and others inhibitory activity [66–68]. Morpholino knockdown of Robo4 in zebrafish, however, results in spatio-temporal disruption of intersomitic vessels. The outcome includes vessels sprouting from the aorta in the wrong direction and premature interruption of their trajectory [68]. Together, the data indicate that Robo4 functions to direct vessel growth to the correct path.

Formation of the Vascular Lumen

The resolution of endothelial cell activation requires the differentiation of endothelial cells and acquisition of a lumen. This is perhaps the step in the angiogenic cascade that is least understood. As of now, genetic analysis using targeting inactivation has been unable to identify molecules responsible for lumen formation. However, as previously discussed, Notch contributes to lumen diameter, by regulating the ratio of stalk to tip cells. Thus, more tip cells (less Notch) reduces vascular lumens [39], while excess of stalk cells (more Notch) leads to vascular hyperplasia and distended lumens [40]. In addition to Notch, it has been shown that soluble VEGF favors enlarged vessels, in contrast to bound VEGF (both during development and in the adult) [23,24].

More recently, elegant morphological descriptions of lumen formation have been reported in zebrafish. These combined with in vitro analysis indicate that formation of vacuoles precedes lumen development within a vessel and that flow is not required for the event, but it facilitates the process [69].

Activation of Endothelial Cells in Pathological Conditions

Angiogenesis induced during pathological events results in vessels that are structurally and functionally altered, when compared to capillary beds from normal organs and tissues [70–73]. In contrast to developmental angiogenesis, the growth of capillaries during pathology is disorganized, exces-

sive, and dysfunctional. Endothelial cells under pathology become activated by an irregular set of stimuli, too much VEGF, altered levels of Notch and Notch ligands, and variations in the levels of guiding molecules (plexins, slits and semaphorins) [73].

Excessive tissue growth, such as tumors, results in decreased oxygen tension that increases production of a number of genes, including VEGF [74,75]. A strong mitogen in vivo, VEGFA induces proliferation and permeability. Extravasation of plasma provides both matrix components, such as vitronectin, fibrinogen and fibronectin, and an additional cohort of growth factors. These include TGF-β, FGF and PDGF, all of which contribute to vascular growth and to further up-regulate VEGF expression [76]. The final outcome is an irregular and dysfunctional vascular plexus. Specifically, tumor blood vessels differ from their normal counterparts by altered morphology and blood flow, enhanced leakiness, abnormal pericytes and basement membrane [71]. Many of these phenotypes have been associated with excess of VEGF. In particular, vascular tortuosity, dilation and permeability mimic situations when VEGF has been locally delivered to an otherwise "normal" tissue [24,77,78].

In contrast to developmental vascular growth, activation of the endothelial cell in pathological conditions requires the digestion of a well-organized and cross-linked basement membrane. Thus, MMPs and their inhibitors are essential [79–81]. However, as could be expected, the system is extremely redundant. Multiple MMPs are able to perform the job, i.e., digest the basement membrane. In fact, genetic inactivation of most MMPs has been innocuous to post-natal angiogenesis [79–81]. MMPs also modulate exposure of cryptic extracellular matrix domains and regulate growth factor function. We have shown, for example, that MMP-mediated proteolytic processing of VEGF alters its association with the matrix and it induces distinct modes of vascular expansion [24]. Specifically, excess of MMPs result in VEGF cleavage, increasing the levels of its soluble form. This leads to the formation of highly tortuous and hyperplastic vessels that are unable to perfuse tissues with the same effectiveness as thin vessels [24].

The information gathered from development studies has significantly helped in generating therapeutic strategies for suppression of vascular growth that target, in particular, the activated endothelial cell. For example, suppression of VEGF through the monoclonal specific antibody bevacizumab has resulted in increased survival and reduction of tumor growth [82]. Excess of Semaphorin3F, as a means to modulate the function of the activated endothelial cell, has also been employed for vascular suppression [83,84].

The Notch pathway provides another example of harnessing signaling molecules towards therapeutic exploitation. Pharmacological suppression of Dll4 signaling in tumors results in a dramatic enhancement of tip cells unable to interconnect and organize functional vascular networks. The end result is poor blood perfusion and tumor mass reduction [33,34,85]. It is interesting to consider that an enhancement in the number of tip cells (activated endothelial cells) could lead to such an outcome; i.e., vessel suppression in the context of excessive endothelial cells [86]. These results bring to light the exquisite balance between tip and stalk cells and their relevance to the organization of a functional vasculature.

An Alternative Mode for Endothelial Cell Activation: Mechanical Forces and Angiogenesis / Arteriogenesis

There is vast experimental support for the concept that endothelial cells can sense changes in blood flow and pressure [87–90]. More importantly, these physical forces appear to dynamically transmit this information to the cytoskeleton and surrounding extracellular matrix [91]. The relevance of this statement stems from the fact that the level of flow and shear stress can result in either an angiogenic or an arteriogenic event that is triggered at the time of endothelial cell activation [89,92,93]. The distinction lies in whether the resulting sprout will recruit smooth muscle cells (arteriogenic event) or remain as a single capillary, with or without pericytes (angiogenic event). Several studies have now demonstrated that multiple physical forces participate to maintain homeostatic balance in the vascular endothelium. They also serve to maintain endothelial responsiveness while preserving the integrity of the endothelial monolayer and barrier properties. Shear stress triggers arteriogenesis events, including remodeling of arterio-arteriolar anastomoses and enlargement of vascular wall [94,96]. However, at which point do physical forces contribute to the angiogenic event? The answer to this question is not clear, and while not necessarily initiators, hemodynamic forces play a role in remodeling events during postnatal angiogenesis, although the molecular details remain unclear.

In addition to physical forces, it appears that the contribution of monocytes is required for arteriogenesis [97,98]. These cells release specific chemokines, growth factors and proteases that work to mediate vascular growth and contribute to the formation of new arterioles. The process occurs at sites of pre-existing arterio-arteriolar anastomoses [89]. The initial trigger appears to be altered shear stress within the collateral arteriole after an increase in blood flow. Subsequently, large pressure differences in pre-existing arterioles connecting up- and downstream leads to induction of cell proliferation, migration and vascular remodeling [99]. The increased diameter of collateral arterioles to arteries proceeds as an active growth rather than a passive dilatation [100,101].

Fluid forces also contribute to the primary triggering events associated with endothelial cell activation. Integrins, ion channels and tyrosine kinase receptors are the ini-

tial sensors for changes in physical forces [87,91,102]. The combination of initiating signaling events and transmission of information via the cytoskeleton to the nucleus culminates in the activation of a subset of shear stress responsive genes [103,104]. The cellular responses to shear stress include endothelial swelling and [102] and changes in the profile of cell surface/chemokine production that eventually result in recruitment of monocytes, as well as production of MMPs that initiate the digestion of the basement membrane [79,80].

Termination of the Angiogenic Endothelial Activation

Timely termination of the angiogenic response is as important as its initiation. A persistent or exaggerated angiogenic growth may lead to detrimental effects. Therefore, and in accordance with the complex and highly coordinated activation phase, negative regulatory processes have evolved and function at multiple levels to imposse termination of vascular sprouting.

Several mechanisms are operative in endothelial cells to shut down the activity of proangiogenic signaling pathways and transcription factors. Combined, they provide the stage for termination that is already set early in the activation phase of the angiogenic response. Unfortunately, little is known about these events, yet their further molecular elucidation might provide novel strategies for therapeutic intervention and suppression of vascular growth.

A key step during the termination phase is the formation of the basement membrane and the incorporation of pericytes and / or smooth muscle cells into the recently formed endothelial tubes (Fig. 3.2) [105,106]. Pericyte-induced stabilization appears to involve inhibitors of matrix metalloproteinases. In particular, endothelial cell-derived tissue inhibitor of metalloproteinase-2 (TIMP-2) and pericyte-derived TIMP-3 are shown to co-regulate human capillary tube stabilization following endothelial-pericyte interactions, through a combined ability to block tube morphogenesis and regression in three-dimensional collagen matrices [107]. TIMP-3 expression by pericytes is only induced upon association with endothelial cells. Blockade of TIMP3 leads to capillary tube regression, but it also requires MMP-1, MMP-10-, and ADAM-15 (a disintegrin and metalloproteinase-15). It has been demonstrated the proteinase inhibitory function of TIMP3 is essential for its capillary-stabilizing activity. These findings indicate that vascular networks are predisposed to undergo regression unless they acquire a vascular coat able to produce TIMP3 [107]. A large number of pharmacological studies concur with a key role of pericytes in vascular stability [105]. However, this vast cohort of data does not explain why certain capillaries remain highly stable in the absence of pericytes. Are there other cells responsible for this function or is the endothelium induced to secrete TIMP3 in the absence of pericytes? Clearly much remains to be understood within this particular step.

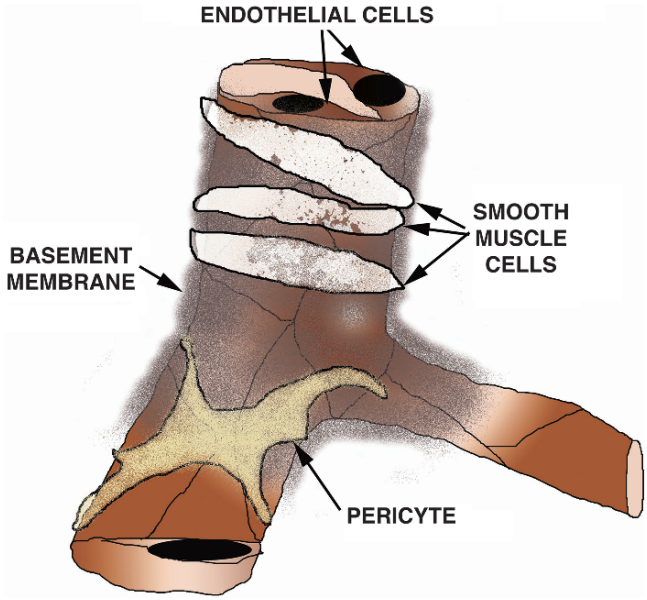

FIG. 3.2. Termination of the *endothelial cell* activation state requires the acquisition of a differentiated phenotype. In addition, the deposition of a highly organized *basement membrane* made by the contribution of both endothelial and mural cells (*smooth muscle* and *pericytes*) is an indication of vascular stability and prevents regression of newly formed vessels. The association of a coat of mural cells (common in most vessels) also prevents vascular regression and marks the end of the angiogenic cascade.

Concluding Thoughts

The consequences of vascular occlusion are devastating for organ function. Today, this pathology remains the most significant cause for morbidity and death in the industrialized world. Current therapies associated with myocardial infarction, stroke, and peripheral artery disease are limited to angioplasty, palliative interventions, and / or bypass. Therefore, a comprehensive understanding of how to modulate vascular growth in a manner that is appropriate for the resolution of a particular pathology can bring unequivocal value to a large number of diseases. Furthermore, the implementation of tissue engineering for wound healing and organ regeneration requires a sophisticated understanding of vessel growth and stabilization.

The last decade has marked the initiation of the molecular era in vascular biology. The advent of target genetic manipulation combined with the interdependency of the vascular system for embryonic survival have led to a remarkable expansion in our mechanistic understanding of how blood vessels are formed. Key genes and central signaling pathways have been identified, and the evolution of this field has allowed for

the implementation of therapeutic strategies that aim at suppressing or enhancing the vasculature. Yet, much remains to be learned. While we can therapeutically induce vessels, they tend to be unstable or lack the hierarchic structure essential for function. On the other side of the coin, while we have been able to suppress neovascularization, the strategy is not as effective as predicted and it frequently leads to the development of resistance.

As we improve our knowledge of how endothelial cells are activated and guided to navigate in different tissues; we must strive to think therapeutically and translate the information into meaningful tools that would enable the modulation of vascular growth during pathological conditions.

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