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Regulation of Vascular Integrity

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Abstract

The integrity of blood vessels is critical to vascular homeostasis. Maintenance of vascular integrity has been conventionally regarded as a passive process that is largely dependent on continuous blood flow. Recent studies, however, have begun unveiling molecular processes essential for maintenance of vascular integrity and homeostasis under physiological conditions, leading to the notion that maintenance of the vasculature is an active biological process that requires continuous, basal cellular signaling. Failure of this system results in serious consequences such as hemorrhage, edema, inflammation and tissue ischemia. In this review, we will discuss the emerging concepts in regulation of vascular integrity with the emphasis on structural components of blood vessels that are essential for vascular maintenance.

Keywords

Vascular integrity; permeability; endothelial junction; cadherin; pericyte; integrin; extracellular matrix

Background

In the last 20 years we have accumulated an enormous amount of knowledge with regard to the new vessel formation [1,2]. As currently understood, the steps in new vessel formation include endothelial cell proliferation and migration followed by assembly into a new vascular structure, lumen formation and, finally, maturation of the newly formed endothelial tube. The latter part, tube stabilization and restoration of the barrier function, is crucial for newly formed vessels' maturation. This stabilization stage requires activation of distinct cellular signaling pathways which are different from the signaling pathways that initiate vascular cell proliferation and migration. Moreover, not only newly formed vessels, but also existing vessels need to be actively maintained in order to maintain their integrity and tissue homeostasis. Thus, how vessel stabilization and maintenance are achieved is a fundamental question in vascular biology that has not received sufficient attention.

This neglect is partly attributable to the view that vessel maintenance is a rather static process, and partly due to our failure to understand the importance of this process. However, the experience with various therapeutic angiogenic approaches has clearly demonstrated that it is not simply sufficient to induce new vessel growth to achieve a functionally meaningful improvement in perfusion, but that prevention of vessel regression and promotion of vessel maturation are equally important [2]. Moreover, an important emerging approach in cancer therapy is the normalization of the tumor vasculature. The conversion of a typically

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abnormal tumor vasculature characterized by excessive leakiness and inefficient perfusion to an efficient and well-sealed system results in improved tumor perfusion and facilitates delivery of cytotoxic compounds [3,4].

Studies in various animal models as well as mouse and human genetic studies have identified a number of factors that play critical roles in active maintenance of blood vessel integrity during the embryonic vessel development or in the adult vasculature (Table 1). It is now clear that these factors work coordinately over many steps of vascular stabilization and maintenance in an orchestrated manner. During the process of vascular formation, after nascent vessels are assembled, endothelial cells develop cell-cell junctions to establish an effective barrier, a process in which Ang1-Tie2 and FGF systems play pivotal roles [5,6]. Concomitantly, while mesenchymal progenitor cells differentiate into pericytes or smooth muscle cells through the action of TGF- β , PDGF-BB derived from endothelial tip cells promotes pericyte recruitment and proliferation [7-9]. Throughout this process, integrins mediate extracellular matrix (ECM)-cell signaling that further directs vessel stabilization [10].

Maintenance of vascular integrity

Vascular integrity is tightly regulated by a number of factors that ensure proper functions of various components of the blood vessel wall. One of the early hallmarks of deteriorating vascular integrity is increased permeability which is predominantly controlled by endothelial junction stability. Selective regulation of vascular permeability is achieved by regulation of the size and state of paracellular gaps and control of the transcellular transport. The normal vasculature demonstrates a certain level of basal permeability that varies from bed to bed. In fact, early studies have revealed constitutively open junctions in a subset of vascular beds [11]. Under normal conditions, about 30% of endothelial cell-cell junctions in postcapillary venules, where active permeability regulation occurs, are open and permeable to ~60 Å molecules. Upon stimulation with either histamine or 5-HT (5-hydroxytryptamine), cellular junctions in postcapillary venules selectively open up and allow passage of larger molecules; however, outflow through venular junctions is limited and restricted to the perivascular spaces [11]. This suggests the existence of an external barrier in the perivascular tissue.

Increased endothelial permeability, elicited by physiological and pathological stimuli, is usually reversible and does not permanently deteriorate vascular integrity. Interference with endothelial junctional components can, however, leads to severe impairment of vascular integrity. In this scenario, junctional disruption is usually accompanied by eventual endothelial detachment from the vessel wall followed by thrombus formation. Although the sequence of events in this process is not well understood, it is possible that duration of permeability-inducing stimuli may influence the outcome. Unlike transient increase of vascular permeability, which endothelial cells can quickly restore the barrier function by reestablishing VE-cadherin-based junctions, prolonged stimuli may leads to a more profound effect such as accumulation of reactive oxygen species (ROS). Excessive amounts of ROS, known to exert a number of adverse effects on endothelial function, may mediate such a scenario. In fact ROS can irreversibly inactivate protein tyrosine phosphatases (PTPs) by oxidizing a Cys residue in the active site, thereby affecting tyrosine phosphorylation-dependent signaling events [12]. Given the importance of VE-PTP and other tyrosine phosphatases in the VE-cadherin function and junction stability (discussed later), it is reasonable to hypothesize that accumulation of ROS impairs the built-in safety system and thus deteriorate endothelial barrier function.

Endothelial junctions –the primary gatekeeper

In endothelial cells, among the three types of intercellular junctions, namely adherens-, tight- and gap junction, adherens and tight junction contribute to the structural integrity of the endothelium [13]. Although it is difficult to precisely delineate the functional difference of these two types of junctions, it has been shown that assembly of tight junctions is dependent on prior formation of adherens junctions, and it is generally considered that adherens junctions are primarily important for the control of endothelial permeability, whereas tight junctions are implicated in blocking the movement of lipids and integral membrane proteins between the apical and basolateral surfaces of the cell (molecular fence) [13,14].

Each type of junction possesses a distinct set of proteins. Cadherins are a family of transmembrane proteins that constitute adherens junctions and mediate cell-cell contacts in a calcium-dependent manner through trans-homophilic interactions. In endothelial cells, VE-cadherin localizes at sites of cellular contacts, regulating the formation of adherens junctions and connecting the site of the junction to the actin cytoskeleton. While it has been assumed that cadherins directly link to α -catenin and thus to the actin cytoskeleton, a recent report demonstrated that α -catenin does not bind simultaneously to both actin and E-cadherin- β -catenin complex, suggesting that the linkage is more dynamic and potentially regulated by other mechanisms [15].

Stability of VE-cadherin at adherens junctions, which is controlled by binding to catenins, especially to p120-catenin, is critical to the maintenance of endothelial permeability and integrity. Src family kinases have been known to play an important role in VEGF-induced increase in endothelial permeability, and VE-cadherin phosphorylation via Src triggers disruption of cell-cell contacts, leading to VE-cadherin internalization [16]. This process is thought to be important for the establishment of endothelial motility and the angiogenic phenotype of “activated” endothelial cells. Thus, endothelial junctions are dynamic structures that actively assemble and disassemble even in the quiescent monolayer, suggesting that the balance of action controlling net VE-cadherin dynamics determines the endothelial behavior.

In the cytoplasmic tail of VE-cadherin, at least three tyrosine and one serine residues are reported to be phosphorylated and involved in regulation of permeability [17-20]. Among them, Y⁶⁵⁸ and Y⁷³¹ are implicated in p120-catenin and β -catenin binding, respectively. Phosphorylation of these sites, likely by Src, disrupts catenin binding and affects VE-cadherin stability at adherens junctions. Another proposed mode of regulation of VE-cadherin phosphorylation may involve Csk-dependent regulation of Src activity. Csk, a Src antagonist, has been reported to bind to phosphorylated Y⁶⁸⁵ of VE-cadherin [19], once bound, possibly blocking Src phosphorylation of other tyrosine residues of VE-cadherin or other Src substrates at adherens junctions. Although experimental evidence for this hypothesis is lacking, it is generally agreed that Src activation induces junction disruption and increases endothelial permeability [16]. Furthermore, it has been recently reported that serine phosphorylation plays a role in VE-cadherin internalization. In this scenario, Vav2 is a VEGF-induced Src substrate which, in turn, activates Rac1 and Pak, leading to S⁶⁶⁵ phosphorylation and internalization of VE-cadherin in a β -arrestin2-dependent manner [20].

Targeted disruption of VE-cadherin gene or truncation of β -catenin binding domain of VE-cadherin in mice causes lethality at 9.5 days of gestation due to severe defects in vascular remodeling [21]. Furthermore, endothelial-specific deletion of β -catenin is embryonic lethal starting at E11.5, presenting with vascular insufficiency including reduced numbers of endothelial junctions, hemorrhage and fluid extravasation [22].

As discussed above, phosphorylation of VE-cadherin and, presumably, of its binding partners is an important step leading to junctional instability. There are several protein tyrosine phosphatases (PTPs) that may reverse negative effects of VE-cadherin phosphorylation, maintaining it in a dephosphorylated state. Among them, VE-PTP, an endothelial-specific protein tyrosine phosphatase, has been shown to closely relate to VE-cadherin functions [23]. In fact, targeted disruption of VE-PTP largely recapitulates the phenotype of VE-cadherin null mice, strongly suggesting the importance of VE-PTP-mediated dephosphorylation of VE-cadherin for junctional stability [24]. Intriguingly, a recent report demonstrated that inhibition of VE-PTP compromises endothelial barrier function not only via the VE-cadherin phosphorylation, but also by modulating γ -catenin (plakoglobin) phosphorylation and functions [25].

The premise of the importance of adherens junctions in the maintenance of blood vessel integrity also holds for the fully developed, adult vascular system. It has been shown that treatment of adult mice with VE-cadherin function-disrupting antibodies leads to an irreversible breakdown of the endothelial barrier function and detachment of endothelial cells from the basement membrane. As a result, mice subjected to the antibody treatment show marked lung and heart edema and thrombotic complication [26]. Similarly, a series of studies demonstrated that VE-cadherin plays an important role in the regulation of tumor vessel stability, in which the tumor vasculature is successfully disrupted with VE-cadherin monoclonal antibodies [27,28]. Moreover, downregulation of VE-cadherin in endothelial cell-derived tumor increases incidence of hemorrhage and exaggerated tumor growth, suggesting inhibitory role of this protein in tumor progression [29].

In contrast to VEGF which has been shown to activate Src at adherens junctions and induce VE-cadherin internalization, FGF appears to affect VE-cadherin differently even though both are considered as potent angiogenic factors. Interestingly, capillaries forming in response to VEGF or FGF stimulation are morphologically distinct: while VEGF-induced capillaries have many fenestrae, capillaries induced by FGF are tightly sealed [30]. A recent study has unveiled the molecular basis of this difference. Disruption of FGF signaling in endothelial cells in vivo and in vitro results in disassembly of adherens and tight junctions progressing to severe impairment of the endothelial barrier function and finally disintegration of the newly formed and existing vasculature [31]. Thus, physiological FGF signaling on the endothelium plays a key role in the maintenance of vascular integrity and possibly counteracts the VEGF effect which is, in the long run, potentially harmful for vessels to keep homeostasis (Figure 1). The primary target of FGF signaling appears to be VE-cadherin as a loss of FGF signaling in endothelial cell affects VE-cadherin localization at endothelial junctions prior to affecting cell viability [31]. Besides regulation of endothelial permeability, VE-cadherin also controls endothelial quiescence through contact inhibition [32]. Loss of VE-cadherin readily leads to endothelial proliferation by activating VEGFR2 and, in some cases, to apoptotic cell death.

Another factor which is crucial for the maintenance of endothelial quiescence is angiopoietin-1 (Ang-1) and its receptor Tie2. While Tie2 expression is largely specific to endothelial cells, low level Ang-1 production by mural and perivascular cells appears to facilitate basal Tie-2 signaling input in endothelial cells that, in turn, is required for endothelial homeostasis [5]. Ang-1 or Tie2 deficient mice have similar phenotypes; both die in mid-gestation due to cardiovascular failure, showing severe vascular remodeling defects leading to perturbed vascular integrity [33-35]. These animals also show reduced pericyte coverage and detachment of pericytes from the endothelium. The molecular mechanisms of Ang-1-mediated vascular quiescence have begun to be unveiled in recent studies. Ang-1 inhibits VEGF-induced Src activation through mDia, a RhoA downstream target. RhoA activation by Ang-1 leads to mDia association with Src, thereby uncoupling Src to VEGFR2

and inhibiting Src activation [36]. Moreover, Ang1 induces Tie2 translocation to cell-cell contacts and bridges Tie2 proteins, resulting in trans-association of Tie2. Although functional contribution of the Tie2 homophilic interaction to junctional stability is unclear, Ang-1 preferably transmits Akt signaling in the presence of Tie2 trans-dimers in comparison to Erk signaling being activated by Ang-1 in isolated endothelial cells [37,38].

Pericyte –the endothelial guard

Pericytes, classified with vascular smooth muscle cells as mural cells, are mesenchymal-derived cells that support microvessels. They are embedded in the basement membrane and make special interfaces with endothelial cells at peg-socket contacts where basement membrane is absent, and where gap- and adherens junction constituents are deposited [39-41]. The peg-socket contact is thought to facilitate not only a physical attachment but also communication between these two cell types.

There are a number of factors that potentially influence pericyte and, consequently, vascular functions. Of these, TGF- β family of growth factor and related signaling proteins are crucially important for this aspect of vascular development. The mode of action of this growth factor is highly context-dependent, with in vitro observations, using the endothelial cell/pericyte coculture system, suggesting that TGF- β is inhibitory to endothelial functions including proliferation [42]. In fact, the presence of pericytes in the endothelial monolayer leads to TGF- β activation, which, in turn, inhibits endothelial proliferation and migration and reduces VEGFR2 expression [43,44]. Therefore one of the important roles of mural cells is to subdue proliferating endothelial cells, thereby limiting vessel overgrowth and resolve an active angiogenic process. This appears to be a built-in, self-limiting system in endothelial cells since endothelium-derived TGF- β promotes pericyte differentiation that is required for this negative feedback system[45]. Genetic studies in mice have further endorsed this essential role of TGF- β in pericyte functions and vascular development. Inactivation of TGF- β 1 gene in mice leads to extraembryonic vascular defects exemplified by failure of endothelial differentiation and increased vascular fragility in the yolk sac vasculature [46]. Moreover, mouse phenotypes resulting from targeted disruption of components of TGF- β signaling pathway, such as TGF- β RII, endoglin or activin receptor-like kinase 1(ALK1) are highly reminiscent of TGF- β 1 null mice, all leading to severe vascular abnormality and embryonic lethality at mid gestation [47-50]. Finally, in humans, mutations in endoglin or ALK1 gene are known to cause hereditary hemorrhagic telangiectasia (HHT), an autosomal dominant disorder characterized by systemic vascular dysplasia and recurrent hemorrhage [51].

The other important factor for pericyte-endothelial interaction is PDGF. Both PDGF-B and PDGF-R β null mutant mice die perinatally, displaying lethal hemorrhage and edema [52,53]. Underlying cause of hemorrhage is a pericyte loss from microvessels and microaneurysm formation [54]. Along the line of the results obtained from TGF- β depletion, absence of pericyte causes endothelial hyperplasia and increased capillary diameter, confirming that pericytes negatively control endothelial cell proliferation. The further analysis of PDGF-R β mice revealed that, in the endothelium, normal distribution of junction protein is slightly altered, raising the possibility that the loss of PDGF signaling directly affects endothelial junction formation. This is countered by the observation that the onset of endothelial hyperplasia precedes the endothelial junction abnormality which seems to occur in conjunction with systemic VEGF-A upregulation in these animals. Therefore, it is thought that the abnormality in endothelial junctions is secondary effects associated with VEGF-A upregulation [55]. The more precise understanding of the role of the PDGF system in pericyte development and recruitment arose from the observation that pericyte populations are differently sensitive to the loss of PDGF signaling in a tissue specific manner. This

suggests that initial induction of pericyte differentiation from undifferentiated mesenchymal cells is independent of PDGF signaling and is mediated by other factors such as TGF- β . In the angiogenic vessel growth and the subsequent maturation process, PDGF-B released by endothelial cell, especially tip cells, drives pericyte and vascular smooth muscle cell proliferation and migration [56].

Besides the developing vasculature, adult vessels can also lose their integrity due to a loss of pericyte support. Retinal vessels, which are the most extensively pericyte-covered vessels in the body, are susceptible to diabetic microvascular complications. Diabetic patients are prone to developing diabetic retinopathy, of which one of the earliest morphological indicators is the loss of pericyte coverage. Pericyte dropout from the retinal microvessel wall has been associated with the formation of capillary microaneurysms and subsequent hemorrhage in patients, and a number of studies strongly suggest that loss of pericytes may be a causal pathogenic event in this disease [57,58].

As has been discussed, the endothelial-pericyte interaction plays a fundamental role in vascular homeostasis. Although structural and functional basis of endothelial-pericyte cell contacts has not been well characterized, N-cadherin is reported to localize, albeit transiently, at the interface of these two cell types in the embryonic brain vasculature. This N-cadherin-based junction is considered to mediate pericyte adhesion to endothelial cells, thereby contributing to vessel maturation and stabilization [59,60]. Underlying mechanisms in promoting the endothelial-pericyte interaction presumably involve sphingosine 1-phosphate (S1P), a sphingolipid metabolite found in high concentrations in platelets and blood. S1P stimulation of endothelial cells results in activation of Rac1, leading to N-cadherin trafficking to plasma membrane and establishment of the N-cadherin-catenin complex formation [61]. Moreover, inhibition of N-cadherin expression with small interfering RNA profoundly attenuates the process of vascular stabilization in vitro and in vivo, suggesting specific contribution of S1P signaling to N-cadherin-induced pericyte recruitment.

Extracellular matrix –the scaffold and more

Being a major structural component of tissues, it is reasonable to assume that the extracellular matrix (ECM) plays an important role in the maintenance of vascular integrity. In the vascular system, the ECM exists mainly in two forms: the interstitial matrix which occupies intercellular spaces, and the basement membrane, a sheet-like structure that supports the endothelium [62]. Type I collagen is a member of the fibrillar collagen family which accounts for a large percentage of the ECM in the body and provides structural support to tissues. A mouse knockout model of type I collagen dies between day 12 and 14 of gestation due to rupture of large blood vessels [63]. Furthermore, lack of type III collagen, another fibrillar collagen, also leads to rupture of major vessels in mice, confirming the idea that these collagens serve to ensure structural integrity of vessels as the high blood pressure system develops in the embryo and are indeed essential for the maintenance of mechanical stability of the vasculature [64]. Notably, patients with osteogenesis imperfecta and Ehlers-Danlos syndrome caused by disorders of type I collagen and type III collagen, respectively, often manifest aortic aneurysms and their rupture in early adult life [65].

The basement membrane is a highly organized sheet-like structure, with major components being type IV collagen, laminins, nidogens, and a heparin sulfate proteoglycan perlecan [66]. In the vessel wall, the basement membrane provides a physical barrier to both soluble molecules and migrating cells and functions as the scaffold that allows pericyte interaction with endothelial cells, thereby contributing to vessel stabilization. Hence, reflecting the

difference of functional properties of the interstitial ECM and the basement membrane, the abnormality of basement membrane appears to affect microvessel integrity. Genetic disruption of major type IV collagen isoforms by targeting the *Col4a1/2* locus in mice results in normal deposition and assembly of the basement membrane up to E9.5. However, lethality occurs between E10.5-E11.5, due to structural deficiencies in the basement membranes, manifesting as bleeding into the pericardium of the heart and dilation of blood vessels [67].

Another important constituent of the basement membrane is the family of laminins. Two laminin α chains ($\alpha 4$ and $\alpha 5$), one β chain ($\beta 1$) and one γ chain ($\gamma 1$) which are found in two laminin molecules: laminin-8 ($\alpha 4\beta 1\gamma 1$) and laminin-10 ($\alpha 5\beta 1\gamma 1$), have been shown to be expressed in blood vessels [66]. The laminin $\alpha 4$ chain is expressed by all endothelial cells regardless of their stage of development, and null mutation of this laminin in mice leads to microvascular hemorrhages during the embryonic and neonatal periods [68]. In contrast, the laminin $\alpha 5$ chain, being expressed in vessels 3-4 weeks after birth, is thought to contribute to vessel maturation by influencing pericyte function; however, a tissue-specific knockout strategy is required for further evaluation [69].

Degradation of the ECM in the vascular wall predisposes to compromised vascular integrity in the adult vasculature. One such example is aortic aneurysm, a vascular disease in which normal architecture of large vessels is severely destructed, potentially resulting in catastrophic rupture if left untreated [70]. Besides the disturbed vessel wall structure, it is also characterized by chronic inflammatory infiltrate and loss of ECM components such as medial elastin. ECM degradation that occurs in this disease is largely attributed to increased matrix metalloproteinase (MMP) production/activity in the vascular wall. Specifically MMP-2 and MMP-9 have been implicated in the pathogenesis of aortic aneurysm due to their intrinsic elastin-lytic activity and increased expression levels in the aneurysm tissue and the plasma of affected individuals [71]. In the process of aneurysm progression, the imbalance of MMPs and tissue inhibitors of MMPs (TIMPs) is believed to play a key role. In fact, mice deficient in MMP-2 or MMP-9 are resistant to CaCl_2 -induced aneurysm formation [72], whereas genetic disruption of TIMP-1, a MMP-9 inhibitor, leads to exaggerated aneurysm growth in a mouse model of thoracic aortic aneurysm [73]. These results confirmed that ECM degradation by MMPs promotes aneurysm progression by deteriorating structural integrity of large vessels. Another line of evidence indicating the contribution of ECM to vessel integrity maintenance is brought by an observation of MMP expression in the embryonic development. Histone deacetylase 7 (HDAC7) is exclusively expressed in the vascular endothelium in the early embryonic stage, where it maintains vascular integrity by repressing the expression of MMP-10. HDAC7 null mice presents embryonic lethality after E11.0 due to a failure in endothelial cell adhesion and consequent dilatation and rupture of blood vessels [74].

Integrins mediate ECM-cell interactions and have been known to influence a number of vascular functions including angiogenesis [10]. In the vessel formation, integrins are required for endothelial cell migration for their engagement to the ECM, and newly formed vessels are stabilized by the synthesis of the basement membrane and the recruitment of mural cells. Among many integrins identified in blood vessels and implicated in vascular development, the αv and $\beta 1$ subunit appear to play a role in vessel integrity in a different manner [75].

Ablation of the αv gene does not affect vasculogenesis and early angiogenesis since all αv -null embryos develop normally up to E9.5. However, lethality becomes evident in two waves: between 10 and 12 days of gestation due to placenta defects and the perinatal period due to intracerebral and intestinal hemorrhages [76]. While deletion of both $\beta 3$ and $\beta 5$

integrin subunits does not impair embryonic vascular development, inactivation of $\beta 8$ gene phenocopies the cerebral hemorrhage of αv knockout mice, suggesting $\alpha v \beta 8$ is responsible for the cerebral blood vessel defects observed in αv null mice [77,78].

Detailed investigations revealed somewhat unexpected, but intriguing underlying mechanisms. In the cerebral vasculature of αv null mice, the cause of hemorrhage is not due to defects in pericyte recruitment. Moreover, most αv -null vessels display ultrastructurally normal endothelium-pericyte associations and normal inter-endothelial cell junctions, thus suggesting that endothelial cells and pericytes establish normal relationships in cerebral microvessels. However, defective interactions were detected between cerebral microvessels and the surrounding brain parenchyma, composed of neuroepithelial cells, glia, and neuronal precursors [79]. The follow up study using cell type-specific gene inactivation confirmed that the absence of αv integrin subunit in astrocyte endfeet interfered with normal apposition of the glia with the growing vessels and led to vessel dilatation and eventual rupture [80]. This series of studies has important implications from three standpoints. First, they revealed the active contribution of parenchymal cells that have not been regarded as important players to the vessel stabilization process. Second, even without deterioration of the endothelium, vascular integrity can be compromised. Third, the role of the glia may at least partly explain the unique structural and functional property of the blood-brain barrier, which has been characterized with fully developed tight junctions between endothelial cells and high pericyte coverage in the central nervous system vasculature.

Although a global knockout of the $\beta 1$ integrin gene leads to early embryonic lethality before blood vessels form, the essential role of this subunit in embryonic vascular development has been established by cell-specific gene inactivation approaches [81,82]. While endothelium-derived $\beta 1$ subunit is indispensable for embryonic vascular remodeling, deletion of the $\beta 1$ gene from the smooth muscle cell lineage does not seem to affect the embryonic vascular development; however, these mice showed perinatal and postnatal lethality due most likely to widespread hemorrhage and systemic edema [83,84]. Morphological analyses revealed abnormal smooth muscle cell and pericyte coverage to endothelium, suggesting that $\beta 1$ integrin is required for the mural cell interaction to endothelial cells.

The link between vascular integrity and the integrin function is also derived from a human disease which presents cerebral hemorrhage in affected individuals. Cerebral cavernous malformations (CCM) are sporadic or inherited vascular anomalies of the central nervous system characterized by dilated, thin-walled, leaky vessels. Linkage studies have identified autosomal dominant mutations to three loci: CCM1 (KRIT1), CCM2 (MGC4607, Malcavernin, OSM), and CCM3 (PDCD10) [85]. Although neither of these CCM proteins is structurally related each other nor implicated in angiogenesis, recent studies indicated a possible clue to the pathogenesis of the abnormal vasculature seen in patients. KRIT1, originally identified as a Rap1 interacting protein, has been shown to bind integrin cytoplasmic domain-associated protein-1 alpha (ICAP-1 α), a modulator of $\beta 1$ integrin signal transduction. ICAP-1 α binds to the NPXY motif of the $\beta 1$ integrin tail, thereby modulating integrin functions [51]. Intriguingly, KRIT-1 also has the NPXY motif required for the ICAP-1 α binding, suggesting the possibility that there is a competition for ICAP-1 α binding between $\beta 1$ integrin and KRIT-1. It is known that all CCM proteins exist as a large protein complex; therefore, a defect of one protein can influence integrin signaling in the same manner [86]. Given the similar phenotype obtained from αv and $\beta 1$ null mice, of particular interest is the pathogenesis of CCM in relation to integrin signaling regulated by CCM proteins.

In addition, there is a strong possibility that the loss of KRIT1 directly affects endothelial junctions via a Rap1-dependent manner. KRIT1, being identified as a Rap1 specific effector,

maintains the integrity of endothelial junctions; hence, deficiency of this protein may contribute to the development of CCM [87].

Conclusions and Perspectives

Regulation of vascular integrity is a relatively new yet rapidly growing field in vascular biology as our realization of vessel stabilization being a critical step in neovascularization became a realistic concern for therapeutic angiogenesis. It is also becoming apparent that maintenance of existing vessels requires active cellular signaling and that signaling pathways involved share common features with the vessel maturation process.

In the last decade, we have gained significant knowledge as to how vessels mature and maintain. This is largely attributed to the power of mouse genetic and developmental studies using tissue specific gene inactivation and gene modification strategies. In this review we have discussed players actively contributing to the maintenance of vascular integrity. It is now clear that vessel maintenance can be successfully achieved by cooperation of many constituents of blood vessels such as endothelial cells, pericytes and the ECM. However, there has been a strong focus on endothelial cells, with less attention paid to pericytes and very little to the parenchymal cells surrounding blood vessels. Although the indispensability of endothelial cells for the maintenance of vessel integrity has been clearly demonstrated by a number of studies, it is important to extend our view to other components as well as to the multicellular complex architecture that requires organized functions of many constituents. The major barrier to study this is a gap between *in vivo* and *in vitro* systems. Even though we have found critical players that mediate vessel stability in mouse genetic studies, *in vitro* systems that still largely rely on monoculture systems have limitations to investigating interplay of different cell types that occurs in blood vessels. It is, therefore, important to try to mind the *in vitro-in vivo* gap by utilizing coculture systems or developing more sophisticated experimental models and rigorously validating the data obtained in various systems.

As regulation of vascular integrity is a fundamental process for human physiology and pathology, elucidating the precise mechanisms will lead to the next generation of therapeutic angiogenesis and cancer treatments.

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Figure 1.

Proposed model of VEGF-FGF mediated regulation of EC permeability VEGF increases vascular permeability by Src-dependent phosphorylation of VE-cadherin (right half). FGF may counteract the VEGF effect by two mechanisms: (1) facilitating Csk association with VE-cadherin, thereby inhibiting local Src activation and/or (2) activating VE-cadherin phosphatases such as VE-PTP or PTP μ , by which VE-cadherin stays dephosphorylated at catenin binding sites and stably binds to catenins. p120-catenin binding is critically important for cadherin retention at adherens junctions.

Table.1

Knock out mouse studies that are shown to affect vascular integrity

Type	knockout	Mode*	Vascular Phenotype	Ref.
Endothelial function	VE-cadherin	global	Die at E9.5 due to severe defects in vascular remodeling and maturation	[21]
	β -catenin	EC	Die at E11.5-13.5, defective vascular pattern, increased vascular fragility	[22]
	VE-PTP	truncation	Die by E10, defects in vascular patterning and remodeling	[24]
	claudin-5	global	Viable, Size-selective loosening of the blood-brain barrier, no hemorrhage or edema	[88]
	Fak	EC	Die at E10.5, hemorrhage, increased EC apoptosis, disorganized capillaries	[89,90]
	adrenomedulin	global	Die at E13.5, widespread hemorrhage, EC detachment from basement membrane	[91,92]
	Ramp2	global	Die at E14.5, edema, hemorrhage, reduced junction protein expression	[93]
	Ena/VASP	global	Die at E16.5-P0, edema, hemorrhage from venules, impaired stress fiber formation	[94,95]
	BMK1/ERK5	global	Die at E9.5-10.5, defects in angiogenesis, vessel maturation, increased EC death	[96,97]
	survivin	EC	Die at E13.5, wide spread hemorrhage, EC apoptosis, EC-PC gap	[98]
	SRF	EC	Die at E14.5, hemorrhage, defects in angiogenesis, reduced junction proteins	[99]
EC-PC crosstalk	Ang1	global	Die by E12.5, severer defects in angiogenesis, vascular remodeling, poor pericyte attachment	[33]
	Tie2	global	Die at E10.5, severer defects in angiogenesis, vascular remodeling	[34,35]
	Fli-1	global	Die at E11.5 due to hemorrhage, downregulation of Tie2	[100]
Pericyte differentiation	TGF- β 1	global	Die at E10.5, reduced vascular wall integrity, hematopoietic defect	[46]
	TGF- β RII	global	Die at E10.5, reduced vascular wall integrity, hematopoietic defect, similar to TGF- β 1 knockout	[47]
	endoglin	global	Die at E11.5, defects in vSMC development and endothelial remodeling	[48]
	ALK1	global	Die by E11.5, dilatation of major vessels, defects in vSMC development	[49,50]
	ALK3	EC, SMC	Die by E11.5, defects in vascular remodeling and maturation	[101]
	ALK5	global	Die by E11.5, dilated and fragile vessels, reduced vSMC	[102]
	smad4	EC	Die at E10.5, defects in vascular remodeling and vSMC development	[103]
	smad5	global	Die at E10.5-E11.5, enlarged vessels, decreased vSMC	[104,105]
Pericyte recruitment	CD148	global	Die by E11.5, dilated vessels, reduced pericyte investment, and endoglin expression	[106]
	PDGF-B	global	Die perinatally due to hemorrhage, pericyte loss and microaneurysm, EC hyperplasia	[52,54,55]
	PDGF-B	EC	Similar to PDGF-B null mice, but survive into adulthood	[57]
Pericyte function	PDGF-R β	global	Die perinatally, almost identical to PDGF-B null mice	[53,55]
	Ephrin-B2	SMC	Die perinatally, edema, extensive hemorrhage, defects in SMC FA formation and motility	[107]
ECM stability	Crk	global	Die at perinatally, edema, hemorrhage, reduced SMC	[108]
	α 1(I) collagen	global	Die by E14 due to rupture of major blood vessels	[63]
	α 1(III) collagen	global	90% die perinatally, 10% shorter life span with death due to rupture of major vessels	[64]
	α 1(IV) α 2(IV) collagen	global	Die at E10.5-11.5, defects in basement membrane, bleeding in the heart	[67]

Type	knockout	Mode*	Vascular Phenotype	Ref.
	Laminin $\alpha 4$	global	Elevated mortality at P0-2, multiple microvessel hemorrhages starting at E11.5	[68]
	biglycan	global	50% of male die suddenly within 3 months due to aortic dissection and rupture	[109]
	HSP47	global	Die by E11.5 due to blood vessel rupture	[110]
	pVHL	global, EC	Die at E10.5-12.5, hemorrhage, impaired endothelial ECM assembly	[111,112]
	HDAC7	global, EC	Die by E11, wide spread vascular rupture and pericardial effusion	[74]
Integrin	αv	global	Die at E10.5-11.5 or perinatally, cerebral hemorrhage	[76]
	αv	glia	Viable, cerebral hemorrhage, defects in glia attachment to vessels	[79,80]
	$\beta 1$	EC	Die at E9.5-10, defects in vascular patterning and remodeling, cerebral hemangioma	[83]
	$\beta 1$	SMC	Die by P10, dilated, fragile vessels, poor PC attachment to EC	[84]
	$\beta 8$	global	Die at E11.5 or perinatally, cerebral hemorrhage, defects in vascular patterning and EC hyperplasia	[78]

* Mode: global, global knockout; EC, endothelial-specific knockout; SMC, vascular smooth muscle-specific knockout; truncation, mutation causing truncation of the protein; glia, glial cell-specific knockout

EC, endothelial cell; SMC, vascular smooth muscle cell; PC, pericyte; FA, focal adhesion; ECM, extracellular matrix