Controlling Vascular Permeability: How Does It Work and What Is the Impact on Normal and Pathological Angiogenesis

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Abstract

The permeability of the vasculature is a property of the capillary wall to obstruct movement of fluid or solutes driven by physiological force. The vasculature is essential for the health of normal tissues, hemostasis, lipid transport, and immune surveillance and is also an influential characteristic of many diseases in which it is greatly increased. The control mechanism of vascular permeability is a complex process that needs to be tightly regulated in order to preserve not only the vascular homeostasis but also its integrity. Here, transcellular and paracellular pathways play an important role as well as direct and indirect influence of the vascular permeability by molecules or blood

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pressure. In pathologies the vasculature is often affected by the disease process. This may result in neoangiogenesis, where an excessive formation of new, unstable, and hyperpermeable vessels with poor blood flow takes place. In this scenario the vascular endothelial growth factor (VEGF) plays a key role.

Keywords
Vascular permeability · Transcellular permeability · Paracellular permeability · Junctions · VE-cadherin · N-cadherin · VEGF · Inflammatory cytokines · Angiogenesis

Introduction

All cells require a continuing supply of nutrients and a means of clearing waste. Vertebrates have solved these problems by developing a vascular system that extends into all organs and tissues. The main function of the vasculature is to serve as a blood conduit; this way sufficient oxygenation of the peripheral tissues, followed by return of the deoxygenated blood to the lungs, is ensured. Secondly, the vasculature is essential for the health of normal tissues, hemostasis, lipid transport, and immune surveillance and is also an influential characteristic of many diseases in which it is greatly increased. While the vascular system of higher organisms is often described as "closed," it needs to be sufficiently "open" (i.e., "permeable") to allow an exchange of small molecules (gases, nutrients, and waste products) with the surrounding tissues.

In the healthy individual, the vasculature is a more or less stable system: in this arrangement endothelial cell survival is continuously maintained. During physiological conditions – such as the development of the embryo, ovulation, regrowth of the endometrium, or in conjunction with injury or disease – there is a demand of new vessel formation. This growth of all new tissues, whether healthy or not, is accompanied by blood vessel formation. In this setting, the new vessels form de novo in a process called vasculogenesis, while angiogenesis implies vessel formation from the preexisting vasculature.

In most organs, the endothelial cells form a dynamic barrier between the blood and the tissue via which plasma and its solutes cross the vascular barrier. This was investigated over the last century by physiologists including Pappenheimer, Landis, Starling, Renkin, Michel, Curry, Rippe, and Bates (Pappenheimer 1953; Rippe and Haraldsson 1994; Michel and Curry 1999; Bates and Harper 2002; Curry 2005). They found out that capillaries are the vascular segment involved in molecular exchange in normal tissues and that gases, water, and other small molecules cross the capillary endothelial cell barrier freely. In contrast, the passage of larger molecules such as plasma proteins is tightly restricted.

In many diseases including cancer or chronic inflammatory conditions – on the other side – it can be observed that the vascular barrier disintegrates and leakage occurs and increases. This leakage of especially larger molecules and cells results not only in edema and inflammation but also in disease progression.

The following chapter will discuss the current knowledge about the mechanism of vascular permeability as well as its impact on angiogenesis.

Molecular Mechanism of Vascular Permeability

The transport of nutrients and small solutes is constant in physiological conditions, and it is essential for both the homeostasis of the vascular system and the organs. In most normal adult tissues, endothelial cells preserve basal vascular permeability at a low level, while they increase permeability in response to physiological situations like inflammation. Therefore, vascular permeability must be tightly controlled by a number of extracellular stimuli and mediators to maintain tissue homeostasis. Accordingly – with the disintegration of these conditions – debilitated regulation of endothelial permeability causes various diseases, including chronic inflammation, asthma, edema, sepsis, acute respiratory distress syndrome, anaphylaxis, tumor angiogenesis, and diabetic retinopathy.

Structurally the endothelium lining the vasculature constitutes a barrier which itself maintains the integrity between blood and interstitium and regulates extravasation of fluids and plasma proteins. Traditionally the term "vascular permeability" implies the basal vascular sieving of solute and small molecules. In this setting, molecules smaller than approximately 40 kDa can cross spontaneously the endothelial barrier, while larger molecules need active disruption of the vessel wall in order to extravasate to the surrounding tissues. Although such induced leakage preferentially takes place in postcapillary venules, capillaries and larger venules may also leak (Majno et al. 1969; Kohn et al. 1992; Roberts and Palade 1995).

Permeability is mediated by the strictly regulated opening and closing of cell-cell junctions. Here, the active transport occurs at least through two mechanisms called the *transcellular* or *paracellular* pathways, both for macromolecules and inflammatory cells. The transcellular permeability is probably governed by signaling pathways that are responsible for endocytosis and vesicular trafficking in cells (Mehta and Malik 2006). These pathways include vesicular transport systems, fenestrae, and biochemical transporters. On the other hand paracellular permeability is controlled by the dynamic opening and closing of endothelial junctions implying that a complex rearrangement of adhesion proteins with their related cytoskeleton must occur.

It is likely that the two described pathways are interconnected in some way, since many permeability-increasing agents expanse vesicular transport and also disrupt the integrity of endothelial cell-cell junctions (Feng et al. 1999; Dejana 2004; Weis and Cheresh 2005); however, whether this occurs in the same vessels and at the same time is still a matter of debate. It is possible that, in some areas of the vasculature – such as the microvasculature of the glands – the transcellular pathway is better developed, whereas in others, such as the postcapillary venules, the paracellular pathway is favored. This way it is reasonable to assume that the involvement of these different mechanisms may depend on factors like the type of vessel, the organ, the kinetics of the transport, and the nature of what is transported across the wall. It will be of interest once more knowledge is available on the vesicular transport systems in endothelial cells, to try to integrate both systems into a more comprehensive picture.

Transcellular Permeability

For transcellular permeability, the formation of vesiculo-vacuolar organelles (VVOs) or of fenestrae is required. Transcytosis is an important mechanism for delivery of macromolecules to tissues: during this process caveolae pinch off from the plasma membrane, form vesicular carriers, shuttle to the opposite side of the endothelial cell where vesicles fuse with the plasma membrane, and discharge their cargo into the perivascular space. The VVOs have been described and investigated primarily using electron microscopy analyses, which have shown that VVOs are prominent structures in both normal vessel endothelial cells and tumor-supplying cells (Caruso et al. 2001; Dvorak and Feng 2001). At the same time VVOs have been implicated as the primary pathway for macromolecular extravasation. However, up to date the origin of VVOs is not known precisely. Originally vesicles and vacuoles that form the VVOs were thought to derive from caveolae. A main protein in caveolae is caveolin-1. However, studies with caveolin-1 knockout mice showed a lack of caveolae with reduced permeability for macromolecules, while the vasculature still contained VVOs.

Paracellular Junctions/Permeability

The scientific findings about the molecular organization of the different types of endothelial cell-cell junctions have established a basis for understanding how these structures might crosstalk and interact reciprocally (Bazzoni 2004; Vestweber 2007; Wallez et al. 2007): endothelial cell junctions present a particularly complex network of adhesion proteins that are linked to intracellular cytoskeletal and signaling partners. These proteins are organized into

distinct structures called *tight junctions (TJs)* and *adherens junctions (AJs)*. In addition, several adhesion proteins (such as platelet endothelial cell adhesion molecule (PECAM1), MUC18, intercellular adhesion molecule 2 (ICAM2), CD34, endoglin, and others) cluster at cell-cell contacts that are distinct from TJs and AJs. Regarding the regulation of paracellular permeability, there are at least the above mentioned two different types of intercellular junctions involved: the AJs and TJs. These junctions are localized in the lateral cell membrane between neighboring endothelial cells sealing the space between these cells. The AJs and TJs consist of different families of transmembrane proteins that promote homophilic cell-cell interactions and transfer intracellular signals. Many reports support the concept that intercellular junctions are dynamically remodeled not only in embryogenic cells but also in resting cells (Dejana et al. 2009). Adhesive membrane proteins of AJs and TJs form adhesive complexes which act as zipper-like structures between the interacting cells (Nelson and Veshnock 1987; Yap et al. 1997; Chitaev and Troyanovsky 1998; Cavey et al. 2008). These proteins are localized hierarchically from the apical to the basal pole of the lateral membrane: in the most apical position, the TJ protein family is localized followed by occludin and the nectin family. Unlike the TJs, the AJs are localized more basal than apical, mainly consisting of the cadherin family.

An important emerging concept is that intercellular junctions are dynamic structures undergoing continuous remodeling not only during morphogenesis in the embryo or upon exposure of cells to agents that increase permeability but also in confluent and resting cells. Continuous recycling of adhesive proteins and signaling partners may occur at AJs and also at TJs. Cadherins, and in particular VE-cadherin, show a flow-like movement in a basal to apical direction which is accompanied by actin reorganization.

Adherens junctions (AJs) initiate cell-to-cell contacts and promote their maturation and maintenance. AJs compromise the cadherin family of adhesion proteins. Endothelial cells express relatively high levels of two important cadherins: a cell-type-specific cadherin called *VE-cadherin* and the *neuronal cadherin (N-cadherin)*. N-cadherin is also present in other cell types such as neural cells and smooth muscle cells (Bazzoni 2004). VE-cadherin can be found in essentially all types of vessels, whereas other non-cell-type-specific cadherins, such as T-cadherin and P-cadherin, are variably expressed in different types of endothelial cells (Ivanov et al. 2001).

VE-cadherin is an endothelial-specific transmembrane protein consisting of five immunoglobulin-like domains in the extracellular region, a single transmembrane domain, and a short intracellular region. Each domain repeats a single transmembrane domain and a short cytoplasmic region from which each molecular region accounts for a different function. While the extracellular region is responsible for homophilic interactions in trans, the transmembrane domain is involved in cis interactions and lateral clustering. In contrast, the cytoplasmic tail of the protein forms complexes with catenins, such as b-catenin, p120, and plakoglobin, and many other signaling and cytoskeletal partners. Many reports in the literature support the idea that adherens junctions might influence the tight junction organization by modulating their expression and assembly. For instance, in confluent endothelial monolayers, vascular endothelial cadherin clustering inhibits the transcriptional activity of forkhead box protein O1 (FoxO1), which is a repressor of claudin-5 expression – a key component of tight junctions.

The second most important cadherin expressed in endothelial cells is *N-cadherin*. Although N-cadherin is expressed at levels that are comparable to VE-cadherin, it presents a diffuse distribution on the endothelial cell membrane and is poorly clustered at intercellular junctions. In 1998 Navorro et al. found out that when VE-cadherin is present at junctions, it excludes N-cadherin from those sites (Navarro et al. 1998). It is therefore possible to assume that in stabilized endothelial monolayers N-cadherin does not play a role at endothelial cell-cell junctions. Instead, N-cadherin seems to act at heterotypic cell-cell contacts between endothelial cells and pericytes (mesenchymal cells that associate with the walls of small blood vessels).

Adherens junctions dissolve in response to a number of stimuli: these catalysts include the vascular endothelial growth factor (VEGF) and inflammatory cytokines such as histamine and bradykinin. This dissolution allows extravasation of macromolecules. It is well known that concerning the molecular mechanism of vascular permeability phosphorylation of VE-cadherin and leukocyte extravasation are essential events. However, the available data is conflicting, and up to date it is not yet exactly clear how the molecular events trigger vascular permeability.

There is scientific evidence that C-Src and Src family kinase are required for VEGF-, histamine-, and bradykinin-induced VE-cadherin phosphorylation and for vascular permeability (Esser et al. 1998; Weis et al. 2004; Wallez et al. 2007; Orsenigo et al. 2012; Hox et al. 2015). In addition, focal adhesion kinase (FAK) phosphorylates VE-cadherin downstream of VEGF in a Src-independent manner, inducing vascular permeability. Moreover several protein tyrosine phosphatases (PTP) act as regulators of junctional stability. For example, PTP1b overexpression reduces VEGF-induced VE-cadherin phosphorylation, leading to destabilization of the junction (Nakamura et al. 2008); density-enhanced phosphatase (DEP)-1 inhibition reduces Src activity and VEGF-induced permeability. In addition, VE-PTP (vascular endothelial-PTP) dissociation from VE-cadherin is required for vascular permeability in vivo and VE-PTP inhibition induces Y685 VE-cadherin phosphorylation (Broermann et al. 2011; Wessel et al. 2014). Phosphorylation of distinct serine and tyrosine residues on VE-cadherin induces molecular and leukocyte extravasation. In vitro, VEGF induces phosphorylation of serine (S)665, thereby modulating VE-cadherin endocytosis by recruitment of β-arrestin to the phosphorylated serine. In addition, phosphorylation of tyrosines (Y)658 and Y731 causes the dissociation of β-catenin and p120. Phosphorylation of Y658 and Y731 in vitro is triggered by binding of leukocytes to intercellular adhesion molecula 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) in endothelial cells. In vivo, basal VE-cadherin phosphorylation of Y658 and Y685 is present in capillaries and venules of certain tissues (Orsenigo et al. 2012). However, Wessel et al. (2014) showed basal phosphorylation of Y731 and phosphorylation of Y685 in capillaries and venules only after induction with peroxyvanadate (Wessel et al. 2014). These seen differences may be dependent on the studied tissue and the specificity of the phosphoantibodies.

It is well investigated that VE-cadherin phosphorylation in capillaries and venules (not in arterioles and arteries) correlates with the sites of vascular permeability. Indeed, leakage takes place in postcapillary venules, occasionally in capillaries and larger venules, but not in arteries. Bradykinin and histamine induce the loss of Y658 and Y685 phosphorylation in the sites of vascular permeability, inducing VE-cadherin endocytosis (Orsenigo et al. 2012). Another study showed that histamine and VEGF induce vascular permeability through the phosphorylation of Y685. In the same study, IL-1β and TNF-α led to leukocyte extravasation accompanied by a decrease in the phosphorylation of Y731 (Wessel et al. 2014).

In physiological conditions, the dissolution of adherens junctions is transient, and the junctions will soon close again in part due to VE-cadherin recycling and reappearance on the cell surface. Some reports show that junctions can remain in their open state (Baluk et al. 1997). However, in pathological situations where vascular permeability is increased (also denoted vascular leak), the regulation of junction dynamics is lost and the junctions remain open, thus indicating chronic permeability.

Tight junctions (TJs) are located at the most-apical side of the lateral interendothelial membrane. They consist of three prevailing families of transmembrane proteins: claudins, occludin, and junctional adhesion molecules (JAMs). TJs are known to regulate the passage of ions and solutes through the paracellular route (Bazzoni 2004; González-Mariscal et al. 2008)**.** TJs may also act as a membrane "fence" to limit the free movement of lipids and proteins between the apical and the basolateral cell surfaces. The core components of TJs that promote cell-to-cell adhesion are members of the claudin family (Furuse and Tsukita 2006; Van Itallie and Anderson 2006).

The claudin family consists of more than 20 members, only a few of which are expressed by endothelial cells. Claudin 5 is more or less ubiquitous along the vascular system whereas non-cell-specific claudins are also found in endothelial cells; in responding to the different needs of the perfused organ, the combination of claudins may vary.

Multiple intracellular partners of TJ adhesive proteins have been described. Among the best investigated factors, the members of the zonula occludens-familiy (ZO) family (ZO1 and ZO2 in the endothelium) are found – a closely related subgroup of the membrane-associated guanylate kinase (MAGUK) family that localizes at TJs in most tissues including the endothelium. Other intracellular TJ proteins include signaling and actin-binding proteins.

In many vessels, endothelial cells make the vasculature uninterrupting. This is due to the fact that in certain organs the endothelial cells display specialized structures to facilitate rapid transport across the endothelium. Examples for fenestrated endothelium in vessels are endocrine glands, digestive tract mucosa, or the kidney peritubular capillaries. Here, the endothelial cells are equipped with so-called endothelial fenestrae, circular pores, covered by diaphragm. A key protein is the plasmalemmal vesicle-associated protein-1 (PV1). In addition there are naturally occurring fenestrae without diaphragm, i.e., in the kidney.

Another unique endothelial barrier system is the *blood-brain barrier (BBB)*. The BBB has the purpose to prevent the brain from exposure to the blood and the adverse consequence of edema. In addition to the presence of adherens junctions, the brain vasculature consists of high-resistance tight junctions and an abundant basement membrane. Perivascular components such as astrocytes, pericytes, and neurons participate functionally in creating the BBB. A potentially unique feature of the BBB is the transendothelial vesicular transport of a range of nutrients and metabolic waste products. Up to date, there is still limited information on to what extent the BBB can be transiently opened in response to growth factors and inflammatory cytokines (Hudson et al. 2014). Also comparative information on molecular mechanisms in the central nervous system and peripheral permeability is until now lacking.

Vascular Permeability in Health and Disease

In physiological conditions regulated vascular permeability occurs in a well-controlled manner. Here, vascular permeability to solutes and small molecules takes place constitutively and appears not to require an active process. It is likely that the constant sieving of solutes is important in maintaining the interstitial pressure in the tissue. It is also evident for maintaining the immune surveillance function of the lymphatics. Interstitial fluid collected by the lymphatics is carried via lymphatic capillaries to lymph nodes where foreign antigens will be exposed to the immune system. The extravasation of macromolecules serves diverse purposes, for example, to maintain the balanced blood and interstitial pressures, to act in immune surveillance, and to carry other molecules, such as hormones and lipid, across the vessel wall. Extravasated fibrinogen, processed to fibrin, may form a provisional matrix on which new blood vessels extend. Extravasation of inflammatory and immune cells serves specific purposes in different pathologies. The cells are necessary for the healing of an acute disease process but may also propagate a chronic disease. In pathologies excess leakage leads to tissue deterioration and to the progression in severity of diseases. In this setting, exaggerated and uncontrolled vascular permeability is associated with many diseases, among them cancer, myocardial infarction, ischemic stroke, and retinopathies.

However, a clear role for *physiological* vessel permeability is not yet established. Overall, studies on the regulation of vascular permeability often suffer from the lack of physiological interpretation.

Regulation of Vascular Permeability

The vascular permeability can be regulated directly or indirectly.

The direct influence is given by molecules that make the barrier to disintegrate; this can occur via a transvessel pore or a junction that needs to be opened. Indirectly the vascular permeability may be regulated by the blood pressure.

Molecular regulators of vascular permeability include angiogenetic growth factors like VEGF (which will be discussed later on in this chapter) and inflammatory cytokines like histamine and bradykinin.

Inflammatory Cytokines

The two best-studied *inflammatory cytokines* in vascular permeability are histamine and bradykinin. Histamine is produced by mast cells and binds to G-protein-coupled H1- and H2-histamine receptors (GPCRs) on endothelial cells (Marshall 1984). Bradykinin is cleaved from kininogen; it acts via GPCRs B1 and B2. It is quite well investigated that both inflammatory cytokines (histamine and bradykinin) mediate activation of the serine/threonine kinase Akt, which itself phosphorylates and thereby activates endothelial nitric oxide synthase (eNOS). Thereby, p-eNOS catalyzes the generation of NO. NO is a key regulator of the vascular tone; it mediates vasodilation by stimulating soluble guanylyl cyclase and increasing cyclic guanosine monophosphate (GMP) in smooth muscle cells (Forstermann and Sessa 2012). Akt is not the only kinase that can phosphorylate and activate eNOS, but it is the best-studied pathway. Another target effect of nitric oxide is S-nitrosylation of beta-catenin that will cause its dissociation from VE-cadherin and consequently the disassembly of adherens junctions. The eNOS-NO pathway is implicated also in VEGF-regulated vascular permeability since ablation of eNOS expression blocks the VEGF response (Fukumura et al. 2001).

The Impact of Vascular Permeability on Physiological and Pathological Angiogenesis

Angiogenesis (the growth of new blood vessels) is a complex multistep process that involves multiple cell types, numerous growth factors, and complex regulatory checks and balances. The families of proteins that appear to be most critical to blood vessel growth are the various members of the vascular endothelial growth factor (VEGF), angiopoietin (Ang), and ephrin (Eph) families.

Physiological angiogenesis is coordinated by these molecules to produce viable, patent, and mature vessels in which vascular permeability is low. These vessels are often of arterial or venular (rather than capillary) phenotype. Overexpression of special vascular growth factors, such as VEGF, becomes evident in *pathological* angiogenesis where a new, immature vasculature develops; in this condition the blood flow is often unregulated, and there is an inappropriate relationship between metabolic demand and flow.

Originally the mechanism of angiogenesis was described in 1946 when Abell showed that dyes, such as India ink blue, leak out of capillaries growing into a recent wound (Abell 1946). An ultrastructural study in 1963 showed that intravenous injection of large molecular weight tracers, such as colloidal carbon, resulted in deposits in the interstitium outside growing capillaries in wound healing models. Increased vascular permeability during angiogenesis is now recognized as a cardinal feature of pathological angiogenesis. Many diseases are associated with an uncontrolled sprouting angiogenesis process; among the best known diseases are particularly cancer, psoriasis, arthritis, and retinopathies. The novel vasculature is often characterized by weak, friable vessels that are inherently leaky and that often bleed. At the same time the tissue itself may become edematous as a result of this increase in permeability, and this effect is compounded by unregulated flow through these vessels seen in the clinical appearance such as cerebral edema in glioblastoma multiforme and in ascites and pleural effusions in liver metastasis and ovarian cancer (Xu et al. 2000; Yano et al. 2000; Bekes et al. 2016).

Physiological angiogenesis, on the other hand, can occur throughout adult life as well as during both pre- and postnatal development. However, in physiological angiogenesis increased vascular permeability appears to have a minor effect. Physiological angiogenesis has been shown to occur during muscle remodeling after exercise-induced training; during hair growth, fat deposition, and wound repair; as well as during the female reproductive cycle in the endometrium and the developing follicle. In pathological conditions the dysregulation of vessel growth contributes to disease progression.

Molecular Mechanism of Physiological Angiogenesis

Angiogenesis entails a sequence of steps including vessel branching (defined by the activation of quiescent endothelial cells), sprouting, anastomosis, regression, and finally maturation to a new stable quiescent status. In the quiescent state, endothelial cells form monolayers where cells are interconnected by junctional molecules. Alone, endothelial cells are unable to establish a mature and functional vasculature. Peri-endothelial mural cells, such as smooth muscle cells (SMCs) for large vessels and pericytes for small vessels, surround and support endothelial cells, suppressing their proliferation and transducing cell survival signals. Quiescent vessels become activated and start the branching process as a result of stimulation by angiogenic factors, such as VEGF-A, VEGF-C, Ang-2, FGFs, or chemokines. These factors are released from inflammatory cells and tumor cells, e.g., through hypoxia-inducible factor-1α (HIF-1α)-dependent regulation. Lumen formation depends on the establishment of apical basal polarity and cell-cell contacts. Adhesion receptors such as E-cadherin in adherens cell-cell junctions and integrins at cell-extracellular matrix attachment sites are responsible for apical-basal polarity of endothelial cells and for lumen formation. In addition, tight junctions are, as a rule, localized to the apical-lateral membrane. The anastomosis with another vessel branch, reestablishing the junctions at the point of cell-cell contact, allows for blood flow. Mural cells and endothelial cells deposit new basement membrane to ensure an optimal flow distribution and a functional vessel network. Now the new vessel is mature and endothelial cells reattain a quiescent status.

Microenvironmental feedback is essential for matching angiogenesis to the requirements of the tissue. Thus, tissues that are stressed due to an inadequate supply of oxygen and nutrients signal for induction of angiogenesis by inducing the expression of vascular endothelial growth factor (VEGF); this signaling is suppressed when perfusion meets the needs of the tissue. However, the process of invasion of capillaries into a tissue and establishment of circulation cannot meet rapid fluctuations in vascular needs, and excessive cycles of vascular invasion and regression can be destructive. Thus, the vascular bed must include a sufficient reserve for matching fluctuations in tissue requirements, and it must have the ability to respond to acute changes by recruiting those reserves and to utilize proper angiogenesis only when its needs exceed the available reserves. Acute physiological needs for increased perfusion are met by local regulation of blood flow, mediated by neuronal control of vasodilation through perivascular contractile cells. Induction of local vascular dilation and hyperpermeability are affected not only by VEGF but also by NO.

The Role of the Vascular Endothelial Growth Factor (VEGF)

In the recent decades, the molecular basis of angiogenesis has been extensively studied, and a variety of signaling systems, such as the vascular endothelial growth factor (VEGF), angiopoietin-Tie, ephrin-Eph, and Delta-Notch, were found to play important roles in angiogenesis. Among them, VEGF, the strongest angiogenetic growth factor, is crucial not only for vascular maintenance and permeability regulation but also for physiological angiogenesis from early embryonic to adult stages and especially pathological angiogenesis, such as in cancer (Senger et al. 1983; Murakami 2012). Although it is more marked during disease states, there has also been evidence demonstrating that there is a regulated increase in vascular permeability to both solutes and water as capillaries grow and form new vessels in physiological systems (Spanel-Borowski and Mayerhofer 1987; Dejana et al. 2001).

The immediate response to stimulation by VEGF leads to vasodilation and increased vessel permeability. These changes occur within minutes after stimulation and thus may be useful as biomarkers for activation of endothelial VEGF receptors. The mammalian genome encodes five VEGF family members, VEGF-A (also known as VEGF), placenta growth factor

(PlGF), VEGF-B, VEGF-C, and VEGF-D, which regulate vasculogenesis, angiogenesis, and lymphangiogenesis. Particularly VEGF-A is crucial for blood vessel formation during early embryogenesis. Not only the VEGF-A homozygous knockout mice but also its heterozygous mice (VEGF-A fl/_) exhibit an embryonic lethal phenotype due to immature blood vessel formation, indicating that the local concentration of VEGF-A in the embryos has to be tightly regulated for proper angiogenesis. Several VEGF-A subtypes are generated by alternative splicing. Among these, VEGF-A165 has the highest biological activity with binding affinity for a coreceptor, neuropilin-1 (Nrp1). VEGF-A was first described as early as 1983 by Senger et al. who partially purified a factor secreted by hepatocarcinoma cell lines that increased dye extravasation into the skin of guinea pig (Senger et al. 1983). Further characterization resulted in a publication in 1986 describing the dose dependency and some details of the protein (Senger et al. 1986). However, the protein structure and amino acid sequence of this factor were not described until 1990 (Senger et al. 1990). VEGF was originally denoted vascular permeability factor (VPF) implying its essential role in regulation of the vascular barrier (Senger et al. 1983). VEGF exerts its action by binding to VEGFRs, a family of three tyrosine kinase receptors: VEGFR1, VEGFR2, and VEGFR3. VEGFRs share a similar structure characterized by immunoglobulin-like loops in the extracellular domain, a transmembrane domain, a juxtamembrane domain, a tyrosine kinase domain, and a C-terminal tail (Koch et al. 2011). Preferentially VEGF2 has been implicated in the regulation of permeability and angiogenesis. VEGFs can be presented in cis to the VEGFRs, when the coreceptor is expressed together with the VEGFR in the endothelial cell, or in trans, when the coreceptor is expressed in a different endothelial cell or another cell type (Jakobsson et al. 2006; Koch et al. 2011). The binding of VEGFs to VEGFRs induces dimerization of the receptors, creating homo- or heterodimers (Lemmon and Schlessinger 2010; Nilsson et al. 2010). Dimerization is followed by conformational changes that allow the subsequent activation and autophosphorylation of the tyrosine kinase. A number of phosphorylation sites in VEGFR2 have been identified (Matsumoto et al. 2005). Several of these phosphorylation sites have been studied in loss-of-function analyses by phenylalanine knock-in, in vivo and/or in vitro. The most interesting site at this point appears to be the Y949 site in the VEGFR2 kinase insert. It serves as a binding site for an adaptor molecule, T cell-specific adaptor (TSAd), which binds to the cytoplasmic tyrosine kinase c-Src. Silencing or gene inactivation of TSAd makes endothelial junctions unresponsive to VEGF, resulting in loss of VEGF-induced vascular permeability (Sun et al. 2012). Several studies from the David Cheresh lab implicate c-Src in phosphorylation of the critical adherens junction protein VE-cadherin (Eliceiri et al. 1999; Weis et al. 2004). According to the model, c-Src-induced phosphorylation of VE-cadherin promotes dissolution of VE-cadherin contacts between cells, followed by internalization and degradation or recycling of VE-cadherin (Fukuhra et al. 2006). The other VEGFR2 phosphorylation sites induce signaling pathways that also contribute to vascular permeability regulation. These sites include Y1173 (Y1175 in the human VEGFR2), which binds phospholipase Cg, as well as the adaptor molecule Shb, and Y1212 (Y1214 in the human VEGFR2), which binds the adaptor Nck. Whether other growth factors for which there are receptors on endothelial cells, such as placenta growth factor (binding exclusively to VEGFR1) or fibroblast growth factors (FGFs, binding to FGFR1 and FGFR2), mediate acute or chronic vascular permeability has not yet been addressed in detail.

In "normal" physiological angiogenesis, there is an association between the VEGF expression and hypoxia, for instance, in exercise-induced angiogenesis in the skeletal muscle, in the corpus luteum, and in the endometrium. However, nonhypoxic angiogenesis can also be stimulated, for instance, by increasing shear stress in skeletal muscle or by hormonal control in the female reproductive system (Mukhopadhyay et al. 1998). In pathological states, the same growth factors are overexpressed (Damert et al. 1997),

but despite significant understanding of the mechanisms underlying regulation of transcription and translation of VEGF (Sandner et al. 1997; Wenger et al. 1997), the underlying mechanisms of permeability regulation by growth factors in pathology are still not well understood.

The actions of VEGF on permeability and angiogenesis have been extensively studied. However, the vasodilator actions of VEGF may underlie its actions in physiological and pathological angiogenesis to an extent not currently appreciated. Increasing blood flow to a tissue is a more controllable and direct mechanism for increasing tissue growth than stimulating angiogenesis or permeability, particularly in the short term. The fact that VEGF is uniquely able to stimulate angiogenesis directly, act as a potent vasodilator, and is able to increase vascular permeability means that its upregulation in all known endogenous physiological and pathological forms of angiogenesis is a fundamental switch in tissue perfusion.

Anti-angiogenic Therapy in Cancer via Suppression of the VEGF-VEGFR System

In general, the possibility of controlling vascular permeability has several therapeutic implications. An uncontrolled and lasting increase in permeability that is not balanced by the reabsorption of lymphatic fluid causes edema, which, in turn, increases ischemic tissue injury in conditions such as stroke or myocardial infarction. Furthermore, vascular leakage in tumors not only facilitates tumor cell penetration into the vessels and metastatic dissemination but also contributes to the accumulation of fluid in the stroma and the elevated interstitial pressure that are common to several solid tumors (Weis and Cheresh 2005). Elevated interstitial pressure is probably the cause of altered tumor perfusion, the development of necrotic areas, and impaired drug delivery. Conversely, increasing vascular permeability in a reversible manner might be beneficial because it might increase drug accessibility to different tissues in which fluid exchange between blood and tissues is limited, such as in the brain.

Summary

Vascular permeability and angiogenesis occur in physiological and pathological conditions. Whereas vascular permeability is the process where blood vessels exchange nutrients, solutes, and inflammatory cells with the surrounding tissues, angiogenesis is characterized by endothelial cell sprouting, migration, and anastomosis. Both processes – vascular permeability and angiogenesis – are tightly regulated physiological processes. However, uncontrolled, increased permeability and angiogenesis lead to pathological conditions such as the progression of several diseases. VEGF is one of the most important players in this scenario, being regulated by a multifactor system, and can be therapeutically influenced in controlling vascular permeability and angiogenesis in diseases.

Cross-References

- ▶ Endothelial Cell-Cell Junctions in Tumor Angiogenesis
- ▶ Mechanisms of Tumor Angiogenesis
- ▶ The Role of the VEGF Signaling Pathway in Tumor Angiogenesis

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