Endothelial Cell-Cell Junctions in Tumor Angiogenesis

Quentin Roux and Julie Gavard

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Abstract

Angiogenesis is a complex and tightly regulated multistep process whose deregulations induce an aberrant growth of blood vessels, strongly associated with cardiovascular pathologies and also with tumor progression in most of the solid cancers. Tumor vessels are essentially smaller, disorganized, and leaky. In this scenario, the endothelial cells that mat the inner side of the vascular wall are excessively activated and exhibit higher proliferation rate and enhanced migratory phenotype. The loss of endothelial barrier integrity is one of the most striking phenotype of the tumor vasculature and contributes to exacerbate angiogenesis, tissular damage, stromal abnormalities,

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perivascular inflammation, and poor drug delivery.

Physiologically, the endothelial barrier controls the bidirectional passage and the flux of fluids, molecules, and cells from the blood stream to the irrigated tissues. In the tumor microenvironment, this barrier is strongly permeable, allowing thereby unrestricted, anarchic movements across the endothelium. Molecularly, the dismantlement of the endothelial cell-cell junctions, notably those formed by the cell-cell adhesion molecule VE-cadherin, supports vascular leakage in the tumor microenvironment.

There is now growing evidence that restoring the function of endothelial cell-cell junctions could help normalizing the tumor vasculature and further support the use of anti-permeability agents as potent means to interfere with tumor-driven angiogenesis.

Keywords

Adherens junctions · Endothelial barrier · Tight junctions · Tumor angiogenesis · Vascular leakage · Vascular permeability · VE-cadherin

Introduction

During embryonic development, the vasculature is established through de novo formation of blood vessels (termed as vasculogenesis) followed by the stereotyped organization of the vascular network from pre-existing vessels (termed as angiogenesis). High concentration of angiogenic factors, such as vascular endothelial growth factor (VEGF), drives neo-angiogenesis in embryos and adults. This morphogenetic program is also aberrantly reactivated in the tumor microenvironment to supply tumor cells with nutrients, oxygen, and growth factors. However, the tumor vasculature is comparatively disharmonized and rather inefficient.

Endothelial cells that line the luminal face of blood vessels sustain both vascular homeostasis and bidirectional exchanges with irrigated organs. The main functions of the endothelium are not only to act as transport tubes that fuel organs but also to form a physical yet flexible barrier. In functional vessels, adherens and tight junctions seal the endothelial cells together and orchestrate the endothelial barrier. Both adherens and tight junctions take the lead role in controlling the exchanges between blood and tissues. Notably, VE-cadherin, a cell-to-cell adhesion protein exclusively expressed in the endothelium, sets at the cornerstone in the assembly and disassembly of endothelial junctions, in health and diseases.

One striking feature of tumor blood vessels is the loss of barrier integrity and the abnormal elevation of vascular permeability. This is associated with enhanced vascular sprouting and many other abnormalities concerning origin, organization, and fate of tumor endothelial cells. In this chapter, we will summarize current knowledge in (1) tumor-induced angiogenesis mechanisms, (2) the organization of endothelial cell-cell junctions, and finally (3) how endothelial cell-cell junctions contribute to the different processes of tumor-induced angiogenesis, with an emphasis on the mechanisms involved in the disruption of the endothelial barrier and the increase of vascular permeability.

Molecular Basis of Tumor Angiogenesis

Formation of the Vascular Network During Development

The vascular system is established during embryogenesis and gives rise to a dense, structured blood vessel network of arteries, arterioles, veins, venules, and capillaries that ultimately perfuses all tissues throughout the body and fuels cells with oxygen and nutrients. The vascular tree originates from the primary vascular plexus in which mesoderm-derived progenitor cells differentiated into angioblasts (Carmeliet 2005). This embryonic vascular structure is formed upon vasculogenesis, which corresponds to de novo formation of a primitive vascular network. It is progressively remodeled by angiogenesis, allowing the formation and maturation of new

blood vessels from the pre-existing network (Carmeliet 2005). Although the vascular system is considered as mostly quiescent after birth, developmental angiogenesis program can be reactivated to form new blood vessels and adapt the network to cells' needs, such as postnatal retinal vascularization, pregnancy, body growth, etc. (Carmeliet 2005).

Blood vessels consist of an endothelial cell monolayer covered by a basement membrane that anchored perivascular muscle cells and smooth muscle cells for arteries and veins, and pericytes for capillaries, which together maintain the integrity of the vascular wall and allow contraction. Endothelial cells orchestrate the vascular barrier and form a stable, dynamic monolayer acting as a selective filter between the blood compartment and the irrigated tissues (Dejana 2004; Gavard 2013). Barrier properties are largely modulated by both adherens junctions, enriched in the VE-cadherin cell-cell adhesion molecule, and tight junctions with claudins, occludin, and junction adhesion molecules (JAMs), that bridge neighboring endothelial cells together and maintain the cohesiveness of this tissue. The adhesive properties of these molecules and their intracellular signaling capabilities are essential for endothelium homeostasis, with key roles in adhesion, migration, proliferation, division orientation, and adapted, coordinated responses to external cues (please see section "Endothelial Cell-Cell Junctions").

Angiogenesis mostly refers to sprouting angiogenesis during which pre-existing capillaries bud and form a neo-vessel that migrate and invade the avascular space, and further maturate and integrate within the vascular network (Fig. 1) (Potente et al. 2011). In hypoxic conditions, cells secrete growth factors to stimulate surrounding vessel sprouting, among which is the vascular endothelial growth factor family (VEGF), and more specifically VEGF-A_{165} (Olsson et al. 2006). Briefly, VEGF signaling pathway is implicated in several developmental processes, in the vascular system but also in the nervous and lymphatic systems (Olsson et al. 2006; Ferrara et al. 2003). Secreted by tissues in hypoxic conditions, VEGF-A can notably interact with its cognate receptors VEGF-R1 and VEGF-R2 expressed at the plasma membrane of endothelial cell. VEGF-A can also be presented to VEGF-R via the transmembrane binding receptor neuropilin-1 (NRP1) (Koch et al. 2014). VEGF-A binding to VEGF-R2 induces the dimerization and autophosphorylation of the receptor, allowing recruitment of SH2 (Src Homology 2) domain kinases that further activate intracellular signaling pathways implicated in cytoskeleton rearrangement, migration, and cell survival (Olsson et al. 2006). Through these signaling cascades, VEGF regulated the different steps of neo-vessel formation including endothelial differentiation, proliferation, and migration and controls as well vessel permeability by modulating the composition and localization of endothelial cell-cell junctions. Contrarily to VEGF-R2, VEGF-R1 bears a weak tyrosine kinase activity and instead may operate as a competitive inhibitor for VEGF-R2, limiting thereby endothelial cell responses to the growth factor. From the study of knockout mice, VEGF-A and VEGF-R appear essential for vascular development, VEGF knockout leading to the in utero death of embryos between days 8.5 and 10.5 because of severe defects in the establishment of the vascular network (Carmeliet et al. 1996; Dumont et al. 1994; Ferrara and Kerbel 2005).

In response to VEGF, pericytes located on the external face of capillaries detached and the basement membrane are degraded (Armulik et al. 2005; Betsholtz et al. 2005). Subsequently, VEGF signaling induces endothelial activation and differentiation in two separate phenotypes with nonredundant cellular functions and distinct genetic expression profiles: tip and stalk cells (Blanco and Gerhardt 2013). Tip cells notably develop numerous cytoplasmic protrusions termed as filopodia and lead and guide the vascular sprouts. Thus, tip cells allow endothelial cells to migrate, sense, and explore the environment and determine the orientation of the sprout. Guided by the tip cell, stalk cells proliferate at the rear and subsequently form the lumen of neo-vessels (Blanco and Gerhardt 2013).

From a molecular standpoint, two major pathways are implicated in this differentiation step: (i) the VEGF pathway that induces filopodia

formation and promotes the tip cell phenotype and (ii) the Notch/Dll4 pathway operating as a controller pathway that orientates endothelial cell toward the stalk phenotype (Fig. 1). The Notch signaling pathway is highly conserved through evolution and is implicated in numerous developmental processes and cell fate determination. Modulators of this pathway are Notch receptors (Notch 1 to Notch 4 in Vertebrates) expressed at cell membrane and their transmembrane ligands, namely, the Delta like ligands (DLL1, DLL3, DLL4) and Jagged ligands (Jag1 and Jag2) (Blanco and Gerhardt 2013). The membrane localization of ligands and receptors defines the Notch pathway as an intercellular contact-dependent pathway. Briefly, Notch activation in angiogenesis is initiated with the ligand DLL4 harbored at the membrane of neighboring endothelial cells. This DLL4/Notch interaction results in the proteolytic cleavage of the Notch intracellular domain (NICD) that translocates into the nucleus and activates Notch target genes including genes encoding for VEGF receptors (Blanco and Gerhardt 2013; Guruharsha et al. 2012). Consequently, Notch balances VEGF signaling in endothelial cells. Of note, mice knockout for Notch1, Notch4, or the ligand DLL4 die in utero because of vascular anomalies and an inability to remodel the primary vascular plexus into a hierarchized network (Xue et al. 1999; Krebs et al. 2000).

To ensure the formation of a stereotyped vessel network, endothelial cell phenotype is thus strictly controlled, and number of tip cells and sprouts remains limited during developmental and postnatal physiological angiogenesis.

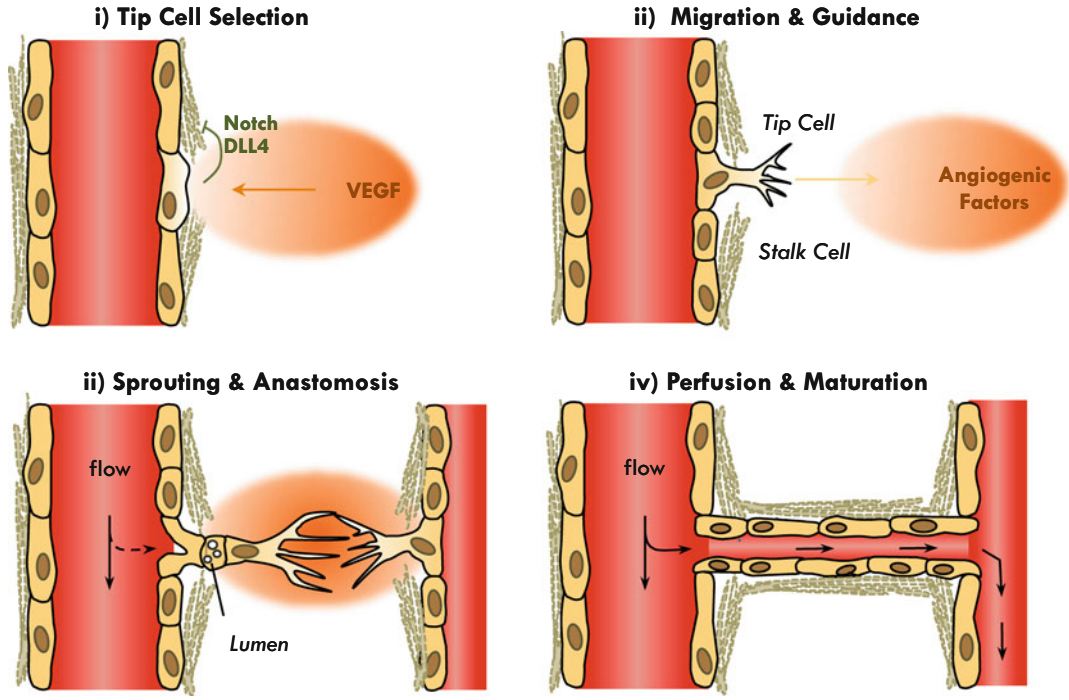

Fig. 1 Multistep processes of sprouting angiogenesis. (i) VEGF (vascular endothelial growth factor) gradient concentration allows DLL4 (delta-like ligand 4) expression in the receiving endothelial cell. Neighbor endothelial stalk cells express DLL4 receptor Notch. Basal matrix is locally degraded. (ii) Tip cell is selected to migrate and lead the sprout, as the endothelial cell with high VEGF-R2 activation, low VEGF-R1, and low Notch activation, opposed to proliferating stalk cells at the rear. (iii) Stalk cells multiply and allow nascent vessel expansion. At the front, tip cells between two adjacent vascular buds join. Vacuolization is initiated to drive lumen formation. (iv) Upon lumen formation and perfusion, a neo-vessel is functionally formed. Maturation includes strengthening of endothelial junctions, matrix deposit, and pericyte recruitment

Endothelial cell specification is initiated by the gradient of VEGF-A and VEGF/VEGF-R2 signaling that induces the formation of filopodia, tip cell differentiation, and an increased expression of DLL4 at the endothelial tip cell membrane (Hellstrom et al. 2007; Jakobsson et al. 2009, 2010; Bentley et al. 2009). Tip cells are migratory cells, characterized by high levels of DLL4 and VEGF-R2, while stalk cells have a proliferative phenotype and express less DLL4 and VEGF-R2. Instead, stalk cells express high levels of Notch Jagged ligand (Jag1) and VEGF-R1 (Hellstrom et al. 2007; Phng et al. 2013). Concurrently, VEGF induces the transcriptional activation of the DLL4 promoter in tip cells. The interaction between DLL4 (expressed by tip cell**s**) and Jag1 (expressed by stalk cells) leads to Notch activation in the neighboring stalk cells. Consequently, Notch signaling is activated through DLL4 in the endothelial cells surrounding the tip cell. Notch activated-endothelial cells adopt a stalk cell phenotype and proliferate to elongate the sprout. Downstream, Notch activation in stalk cells increases the expression of the low kinase activity VEGF receptor VEGF-R1, while decreasing VEGF-R2 levels, ultimately desensitizing stalk cells from VEGF signaling and therefore modulating proliferation versus migration phenotype (Hellstrom et al. 2007; Phng et al. 2013). Thus, the activity of the Notch pathway is inversely regulated in tip and stalk cells. Experimental loss of DLL4 expression results in an excessive sprouting and branching phenotype due to excessive number of formed tip cells and endothelial proliferation (Hellstrom et al. 2007). Notch-depleted endothelial cells indeed adopt features of tip cells and present migratory phenotype by sprouting and branching, whereas Notch activation in stalk cells promotes proliferation (Blanco and Gerhardt 2013). Recently, Notch activity, but not DLL4, was reported to contribute to specify arterial endothelial cell phenotype (Pitulescu et al. 2017). Notch signaling is thus essential for the establishment of a hierarchical and functional vascular network, by restricting endothelial cell tip specification but also by coupling angiogenesis to arteriogenesis during blood vessel formation.

Sprout progression is guided by the tip cell in response to attractive and repulsive guidance molecules allowing the formation of a vessel adapted to the need and the topology of the perfused tissue (Michaelis 2014). Attracted toward the same gradient of pro-angiogenic factors, migrating sprouts fuse via anastomosis and connect their respective lumen. In turn, blood perfusion reduces VEGF tissue expression and orientates endothelial cell toward a quiescent state notably through activation of the shear stress responsive transcription factor KLF2 (Kruppel-Like Factor 2), further promoting endothelial cell survival and strengthening of cell-cell junctions (Dekker et al. 2006; Wu et al. 2008). A fully mature branch is established after deposition of the basement membrane, recruitment of mural cells through PDGF-B (platelet-derived growth factor B), and stabilization of endothelial cell-cell junctions that maintain endothelial cell quiescence, polarity, and survival (von Tell et al. 2006; Armulik et al. 2005). In case of abnormal or not suitable vessel formation, angiogenesis can be reversed and vessel pruned (Ferrara and Kerbel 2005) while ensuring the integrity and the functionality of the vascular network.

Overview on Tumor-Induced Angiogenesis

Following Judah Folkman's postulate in 1971, the development of a dense and anarchic vascular network within tumors was shown to be essential for exponential tumor growth and metastasis. This pioneer work demonstrated the need for solid tumors to develop a specific vasculature and be perfused to grow over a limited size of 2 mm^3. This paves the way for the anti-angiogenic concept in clinics, i.e., anticancer strategy by which therapeutic drugs are designed to block neo-vessel formation and inhibit tumor progression (Folkman 2006). For instance, the crucial role for VEGF signaling in tumor growth justifies the development of anti-angiogenic therapies based on blocking this mechanism (Folkman 2006; Ferrara and Kerbel 2005). Notably, bevacizumab, a humanized mouse anti-VEGF antibody, is the first clinically approved

anti-angiogenic drug and has proven efficiency in combination with standard chemotherapies in non-small cell lung cancers and advanced colorectal cancers (Folkman 2006; Ferrara and Kerbel 2005). However, blocking angiogenesis does not appear adequately effective to starve tumor cells and may even be deleterious for patients, suggesting that instead of destructing the vasculature, normalizing its function might prove better clinical benefits (Jain 2005). Indeed, tumor vasculature consists in a dense, badly structured and poorly functional network of leaky capillaries that fail to maturate. The formation of this network relies on the unreasonable reactivation of developmental processes, sprouting angiogenesis being the more studied and most likely the more prevalent. Alternate strategies of neo-vessel formation rely on reciprocal interaction between cancer cells and endothelial cells, in processes defined as co-option, mimicry, and transdifferentiation (Fig. 2).

Growing tumors rapidly develop a hypoxic core where oxygen concentration is not sufficient to sustain inordinate cell expansion. Similarly to normal conditions, hypoxia-regulated pathways induce pro-angiogenic factor secretion to stimulate surrounding vessel growth. Tumors release considerable amounts of VEGF that aberrantly activate neighboring endothelial cells, multiplying tip cell number, filopodia development, and network ramifications. Interestingly, tumor-emanating VEGF is disseminated and made available to the microenvironment, under multiple forms including within extracellular vesicles (Skog et al. 2008; Andre-Gregoire and Gavard 2017; Feng et al. 2017). VEGF signaling in endothelial cells is tightly regulated during physiological angiogenesis. VEGF controls endothelial differentiation and guides sprout migration in the activated endothelium, while in quiescent endothelial cells, VEGF autocrine and paracrine signaling are required to maintain vascular homeostasis, endothelial cell survival, and junctional stability (Lee et al. 2007). In the tumor microenvironment, high concentration of VEGF results in the growth of numerous unstable tumor capillaries, where endothelial cells fail to establish stable cell-cell junctions, recruit perivascular cells, and form permeable blood vessels sustaining limited tumor oxygen and nutrient needs (Carmeliet and Jain 2011). According to its pivotal function in tumor-induced angiogenesis, VEGF is the primary target for anti-angiogenic therapy (Folkman 2006; Ferrara and Kerbel 2005; Carmeliet and Jain 2011), as exemplified by the anti-VEGF monoclonal blocking antibody bevacizumab, whose efficacy has been proven as adjuvant therapy in certain cancer types, although individual responses vary between patients and are opposed to resistance.

In addition to sprouting angiogenesis, other mechanisms such as the recruitment of endothelial progenitors from the bone marrow can be initiated by cancer cells to develop their vascular network, mimicking thus vasculogenesis developmental program (Folkins et al. 2009). New branches can also be formed by induction of intussusception to split existing blood vessels (Fig. 2).

Moreover, the plasticity of cancer cells allows them to become integral components of the tumor vasculature. Although marginal, cancer cell-based vascular-like structures actively participate in tumor blood supply, dissemination, and resistance to anti-angiogenic therapies. In a process defined as vascular or vasculogenic mimicry, cancer cells acquire the ability to form de novo a tubular network perfused with plasma and red blood cells within the tumors (Kirschmann et al. 2012). This phenomenon is observed in a large variety of high-grade cancers, including melanoma, sarcomas, carcinomas, and glioma. Tumor cells adopt an endothelial phenotype and display modified gene expression of the vascular (VE-cadherin, EphrinA2, VEGF-R1) and embryonic/stem cell (Nodal, Notch4) repertoires (Folberg et al. 2000). The activation of endothelial-specific pathways, including the ectopic expression of VE-cadherin normally restricted to the endothelial lineage, induces the establishment of cell-cell junctions between cancer cells and endothelial cells, resulting in endothelial-like cancer cells integration within the endothelium (Fig. 2). In response notably to the activation of tumor-derived VEGF/VEGF-R1 pathway, endothelial-like cancer cells form

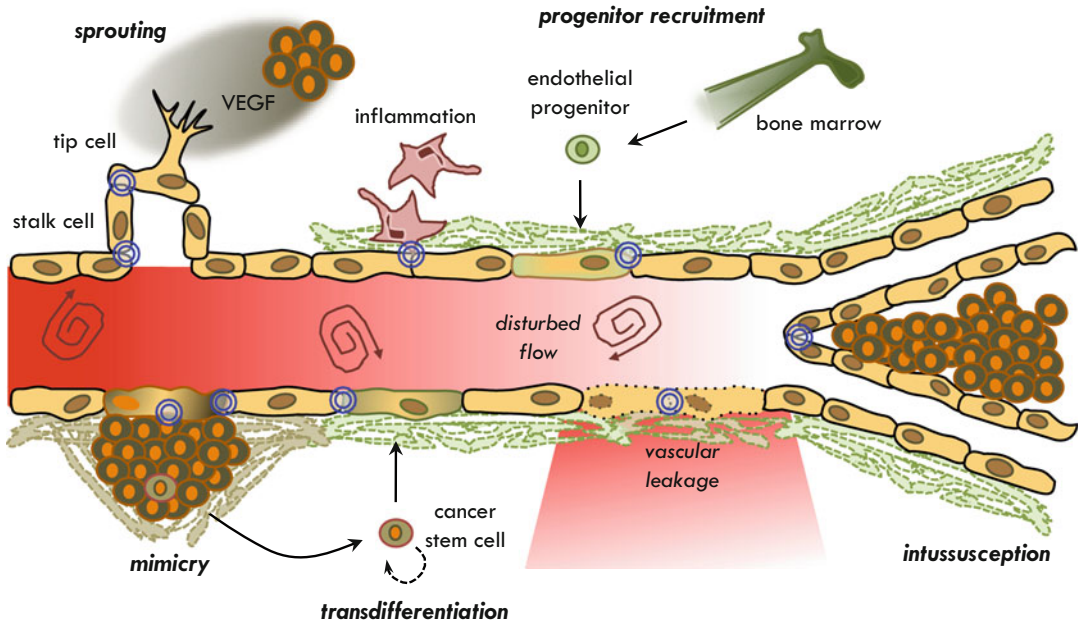

Fig. 2 Cell-cell interactions in tumor-induced angiogenesis. Neovascularization of tumor mass can occur via multiple mechanisms: (i) reactivation of developmental sprouting angiogenesis, (ii) homing of circulating endothelial progenitor cells in a process reminiscent of vasculogenesis, (iii) vascular mimicry where tumor cells adopt a endothelial-like phenotype and associate with tumor vessel, (iv) transdifferentiation is the ability of cancer stem-like cells to recapitulate endothelial differentiation program and integrate tumor vessels, and (v) intussusception and co-option correspond to the ability of tumor cells to employ the existing vessel network to their own benefit by splitting and sequestrating it, respectively. Tumor vasculature features abnormal number of tip cells and sprouts, perivascular inflammation, vascular leakage, disturbed blood flow

pseudo-vascular structures (Seftor et al. 2012; Kirschmann et al. 2012).

Cancer stem-like cells are also a rare, self-sustained population of cancer cells with multipotency properties. They reside in tumor vascular niche and are in tight interaction with endothelial cells (Lathia et al. 2015). For instance, experimental animal models for brain tumors suggest that human cancer cells bearing stem properties can differentiate and integrate the host vasculature (Ricci-Vitiani et al. 2010; Wang et al. 2010; Cheng et al. 2013). Such cells could be tracked and identified as pseudo-endothelial and pericyte-like cells. How much they contribute to tumor vascularization and promote vascular functionality is not completely clear.

Finally, cancer cells can also be fueled through the pre-existing vasculature notably in highly perfused organs (lungs, brain, and liver) where tumors can be initiated and grow without inducing new vessel formation in a process termed as vessel co-option (Leenders et al. 2002). For cancer cells, co-option consists in maintaining tissue vessels quiescent and fully functional, forming thus a stable and efficient blood supply beneficial for tumor growth.

All these different tactics elaborated by cancer cells to enhance vascularization have to be considered when designing anti-angiogenic therapies, as they may impact directly on response to treatment and development of resistance.

Vascular Leakage in Tumor Angiogenesis

The vascular endothelium forms a semipermeable barrier separating blood stream from surrounding tissues while permitting constant, directional passages between the two compartments. The

vascular system represents indeed a 5000 m^2 exchange surface between the blood and the irrigated organs. This barrier controls the passage of plasma molecules and solutes, as well as circulating cells into the adjacent tissue. Reciprocal movements across the endothelium can occur via two different routes, namely, a transcellular and paracellular transport, i.e., through and between endothelial cells, respectively (Fig. 3).

The transcellular transport takes place through endothelial cells and operates as follows via (i) passive diffusion of ions or lipophilic molecules; (ii) gradient diffusion from high to low concentrations; (iii) active diffusion which requires energy to transport large molecules, such as fatty acids or vitamins; and (iv) transcytosis where macromolecules are transported within a membrane-bound carrier from one side of the cell to the other (Vestweber 2012; Azzi et al. 2013). This latter mode occurs either through a system of clustered vesicles, called vesicular vacuolar organelles (VVOs), which direct link vascular lumen and albumen, or through more typical intracellular vesicles (caveolae) (Kiss 2012).

The paracellular transport corresponds to the molecular and cellular fluxes between adjacent endothelial cells. This requires the weakening of endothelial cell-cell junctions that are normally sealed by cell-to-cell adhesion molecules. For instance, low vascular permeability blocks delivery and diffusion of xenobiotics and drugs, as observed at the blood-brain barrier (Zlokovic 2008). Conversely, high endothelial permeability occurs in demand to specialized functions, such as blood filtration in the kidney where glomerular endothelial cells are doted of fenestrations, i.e., transcytoplasmic holes that allow crossing the glomerular capillary wall (Satchell and Braet 2009). The selectivity of the endothelial barrier is thus adapted to the tissue needs. The expression profile of adhesion molecule and their organization can be modified, as observed in the context of the highly selective, protective blood-brain barrier and conversely to facilitate exchanges in lung capillaries. The integrity of the endothelial junctions has to be tightly regulated, since barrier dysfunctions can directly alter the homeostasis of perfused tissues and blood flow. Physiologically, endothelial cell-cell junctions mechanically mediate adhesion between neighboring endothelial cells. This sealed contact is not permeable to albumin (69 kDa) and other large molecules and presents as well selectivity toward the passage of much smaller molecules (<1 kDa) (Vestweber 2012; Azzi et al. 2013). Additionally to endothelial cell-cell adhesive contacts, blood-tissue permeability is controlled by endothelial cell/extracellular matrix and endothelial cell/pericyte interactions that constitute an additional filter (Vestweber 2012; Betsholtz et al. 2005).

Permeability elevation is a hallmark of neo-angiogenesis. While transient and regulated in normal angiogenesis, excessive angiogenesis frequently correlated with uncontrolled vascular

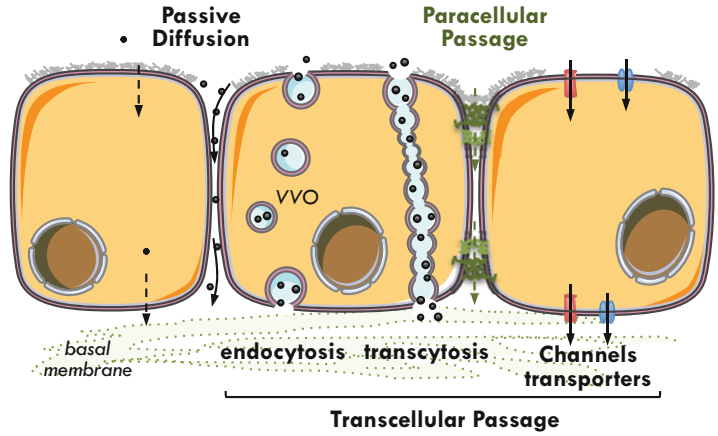

Fig. 3 Routes through the endothelial barrier. There are two major ways to cross the endothelial barrier: (i) the paracellular passage in between the endothelial cells and (ii) the transcellular pathway via passive and active diffusion, via transcytosis, or via a specific network of intracellular organelles, named as vesiculo-vacuolar organelles (VVO)

leakage (Weis and Cheresh 2005). In this context, the constantly elevated VEGF concentration found in the tumor microenvironment is the principal cause of formation, instability, and high permeability of capillaries. VEGF affects pericytes and smooth muscle cells in which VEGF signaling induces detachment from the basement membrane and gives rise to barely covered tumor capillaries (von Tell et al. 2006). Pericytes are indeed loosely attached to tumor vessels, and this therefore contributes to disproportionate permeability increase (Goel et al. 2011). Loss of pericytes induces rapid degradation of the basement membrane further destabilizing endothelial cells and impairs mural-endothelial Ang1/Tie2 signaling that usually maintain endothelial quiescence and junction stability in covered vessels (Saharinen et al. 2008; Gavard et al. 2008). In tumor capillaries, endothelial junctions are also directly impaired by elevated activation of VEGF/VEGF-R2 signaling that induces VE-cadherin destabilization and internalization (Gavard and Gutkind 2006; Gavard et al. 2008).

The resulting increased tumor vessel permeability contributes to deviant neo-angiogenesis directly profitable to tumor cells that receive an unrestrained, yet not optimal access to nutrients, oxygen, and growth factors (Fig. 4) (Le Guelte et al. 2011). Elevated permeability manifests early in the angiogenic process and serves for endothelial cell sprouting out of the vascular bud. Administration of monoclonal antibodies engineered against immature vessels with high permeability and relaxed junctions (Corada et al. 2002; May et al. 2005) decreases tumor vascularization and decelerates tumor growth in animal models (Corada et al. 2002). Moreover, this loss of tumor vessel integrity hijacks regulated leukocyte transmigration pathways (diapedesis), increasing immune cell recruitment within the tumor and exaggerating the inflammatory responses, a hallmark of tumor microenvironment that again benefits to tumor growth, progression, and metastasis (Grivennikov et al. 2010; Vestweber 2012). Blood flow is also particularly altered in the tumor vasculature, vessel permeability being associated with an increased interstitial pressure leading to interstitial fluid accumulation in the tumor microenvironment (Azzi et al. 2013). This vascular leakage is thus a limiting factor for chemotherapeutic and other blood-delivered treatments (Jain 2005).

Endothelial Cell-Cell Junctions

The endothelial cell-cell junctions rely on transmembrane cell-cell adhesion molecules that

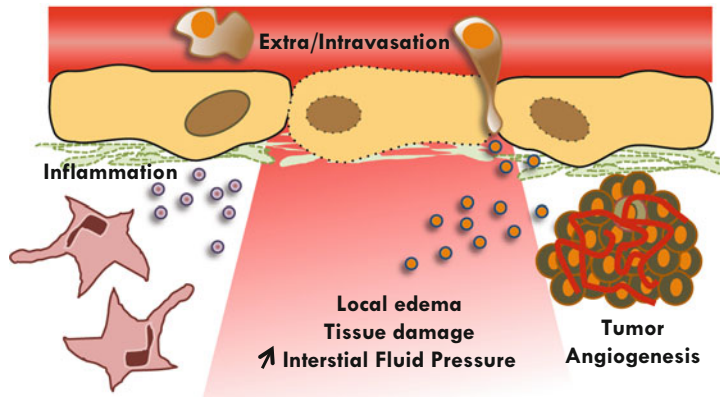

Fig. 4 Impact of vascular leakage on the tumor microenvironment. Enhanced, uncontrolled vascular permeability is a hallmark of tumor vessels. The loss of barrier integrity can affect tumor growth by encouraging: (i) metastasis and dissemination of cancer cells in and out of the blood stream (intra- and extravasation); (ii) inflammation process where macrophages and neutrophils are recruited into the perivascular bed; (iii) extravasation of plasma solutes and fluids that contribute to edema, tissue damage, and increased interstitial fluid pressure; and (iv) tumor angiogenesis

orchestrate the vascular barrier in a selective and dynamic manner. Two interrelated structures are found at the endothelial-endothelial junctions: the tight junctions and the adherens junctions (Fig. 5). Tight junctions are highly impermeable cell-cell contact structures that play a role in barriers and cell polarity. They are organized around a heterogeneous family of cell-cell adhesion molecules: occludin, claudins, and junctional adhesion molecules (JAM). Adherens junctions are believed to form more dynamic contacts than tight junctions and rely on four main cell-cell adhesion molecules, namely, VE-cadherin, PECAM, Nectin, and JAMs.

The Endothelial Tight Junctions

Occludin is a four membrane-spanning domain protein with two extracellular loops and intracellular N- and C-terminal parts (Furuse et al. 1993). The C-terminal tail connects plasma membrane proteins to the actin cytoskeleton through intracellular adaptors, such as the zona occludens family proteins (ZO). The C-terminal tail harbors multiple tyrosine, serine, and threonine phosphorylation motifs that modulated the interaction between occludin and ZO. For instance, Src-mediated tyrosine phosphorylation of occludin unleashes ZO-1 from the tight junction protein and therefore destabilizes cell-cell contact (Elias et al. 2009). Conversely, chronic shear stress promotes the recruitment of tight junction proteins to the plasma membrane and the increase of their transcription (occludin and claudin-5) (Walsh et al. 2011). The stabilization of cell-cell contacts via chronic shear stress involves the inhibition of occludin tyrosine phosphorylation, favoring in turn the interaction between occludin and ZO. This ultimately connects tight

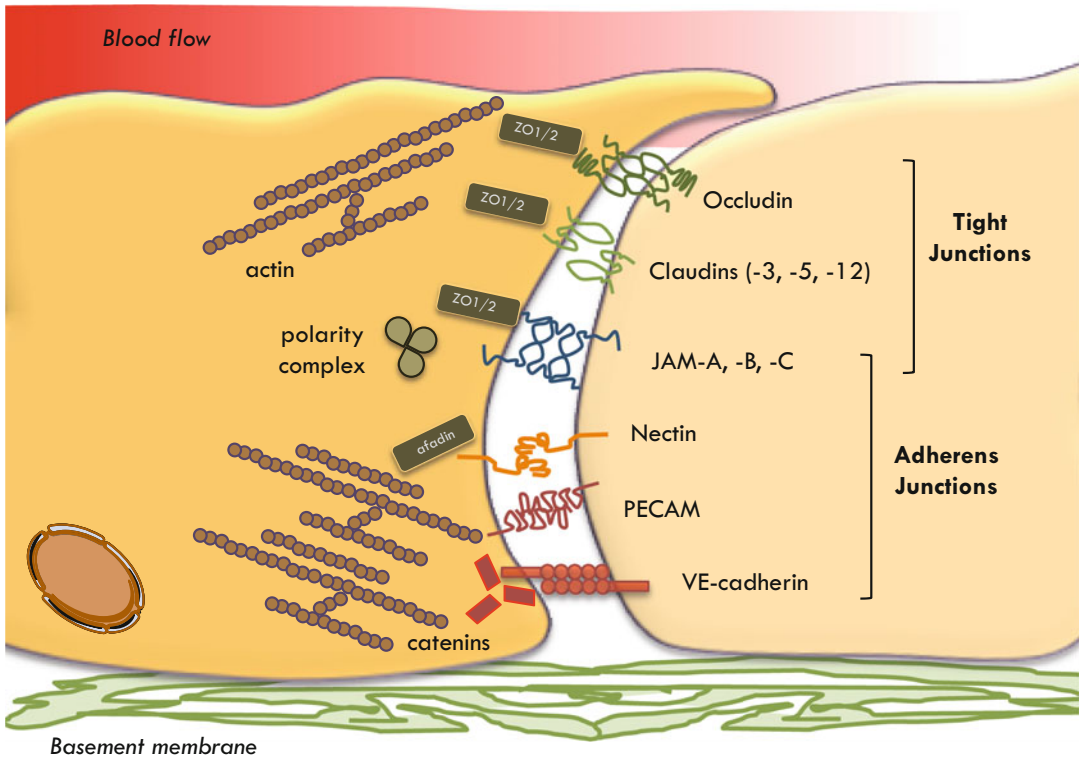

Fig. 5 Endothelial cell-cell contacts. Representation of tight and adherens junctions between two neighbor endothelial cells. Cell-cell adhesion molecules tethered adjacent cell, cell membrane, to the intracellular compartment and the actin cytoskeleton. (*JAM* junctional adhesion molecules, *ZO* zona occludens)

junction to the actin cytoskeleton and reinforces cell-cell contacts (Walsh et al. 2011). Conversely, acute shear stress triggers occludin tyrosine phosphorylation, prevents ZO recruitment, and consequently promotes endothelial permeability (Walsh et al. 2011). Moreover, the N-terminal part of occludin interacts with the E3 ubiquitin ligase Itch that can in turn drive occludin degradation upon VEGF challenge (Murakami et al. 2009).

Likewise, claudins are integral components of tight junctions (Matter and Balda 2003). They can be engaged in homophilic and heterophilic interactions with identical and different adhesion molecules, respectively. Interestingly, claudin-5 deficient mice do not show any embryonic vascular defects, at neither the morphological nor the functional level. However, such mice display higher permeability of the blood-brain barrier for small molecules, provoking to postnatal death (Nitta et al. 2003). Others claudins expressed by endothelial cells are claudins-3 and 12 (Schrader et al. 2012), but to date there are few information available on their functions. Of note, endothelial tight junction formation depends on the establishment of VE-cadherin-based adherens junctions (Taddei et al. 2008). In details, VE-cadherin mediates AKT-dependent phosphorylation of the transcriptional forkhead box factor (FoxO1), which results in the nuclear export of its phosphorylated form. When at the plasma membrane, VE-cadherin traps β-catenin away from a FoxO1/β-catenin repressor unleashed in turn the claudin-5 promoter (Taddei et al. 2008) and thereby allows expression of claudin-5. Similar mechanisms might occur at the occludin promoter (Leclair et al. 2016).

Junctional adhesion molecules (JAMs) belong to the immunoglobulin (Ig) transmembrane superfamily of cell-cell adhesion proteins expressed in epithelial and endothelial cells, as well as lymphatic cells, smooth muscle cells, and some blood cells (Bauer et al. 2014). JAM-B and JAM-C are predominantly expressed in endothelial cells, whereas JAM-A is present in endothelial and epithelial cells. In brain endothelial cells, only JAM-A and JAM-C are expressed (Aurrand-Lions et al. 2001). The intracellular domain of JAM-B and JAM-C can interact with partitioning defective protein 3 (PAR-3), which is instrumental in the establishment of endothelial cell polarity (Ebnet et al. 2003). Moreover, both JAM-B and JAM-C associate via their C-terminal part with ZO and PAR proteins through the PDZ domain (PDZ stands for post synaptic density protein (PSD95), *Drosophila* disc large tumor suppressor (Dlg1), and ZO-1). The biological functions of JAMs have been initially explored in the epithelium, where the role of JAM-A was established in the epithelial barrier function and to allow inflammatory responses (Laukoetter et al. 2007). Interestingly, JAM-C exhibits different cellular localization in micro- and macro-vascular endothelial cells. Indeed, in macrovascular cells, JAM-C constitutively accumulated at cell-cell junctions while recruited upon stimulation (such as VEGF or histamine) in microvascular endothelial cells (Orlova et al. 2006). Beside well-described JAM function in leukocyte diapedesis, the overall role of JAMs in the intact and damaged endothelium is not fully elucidated.

The Endothelial Adherens Junctions

Unlike epithelial cells where tight junctions and adherens junctions are distinctly structured along the apicobasal plan, both junctions are intertwined in endothelial cells. JAMs illustrated this interface between tight and adherens junctions (Fig. 5). In addition, weakening and strengthening of adherens junctions echo on the organization, composition, and localization of tight junctions (Gavard and Gutkind 2008).

Platelet endothelial adhesion molecule (PECAM or CD31) is a single-span transmembrane glycoprotein from the immunoglobulin superfamily, exclusively expressed in endothelial cells and blood circulating cells (monocytes, neutrophils, T and B lymphocytes). While its extracellular N-terminal part composed of six immunoglobulin domains is mainly involved in homophilic interactions (i.e., PECAM-PECAM), PECAM was reported to bind to glycosaminoglycans (GAG) from the extracellular matrix or to alternate membrane receptors such as $\alpha_V\beta_3$ integrin, CD38, and CD177 (Gandhi et al. 2008; Privratsky and Newman 2014). PECAM intracellular domain harbors several tyrosine and

serine/threonine phosphorylable sites, which can serve as docking sites for signaling molecules (Privratsky and Newman 2014). Interestingly, upon phosphorylation, PECAM recruits scaffolding molecules such as γ-catenin and thus associates with the actin cytoskeleton (Ilan et al. 2000). In this scenario, γ-catenin preferentially binds to phosphorylated PECAM in migratory cells (Ilan et al. 2000), while it associates with VE-cadherin in confluent endothelial cells and contributes to strengthen mature adherens junctions (Lampugnani et al. 1995).

PECAM operates in leukocyte transendothelial migration (diapedesis) during inflammatory processes. Unlike VE-cadherin knockout (see next section), PECAM1 gene invalidation does not impair developmental angiogenesis program (Carmeliet et al. 1999; Cao et al. 2009). Instead, the phenotype of mice depleted for PECAM1 unveils a diminution of neutrophil recruitment to inflammatory sites, a reduction of endothelial filopodia, and a lower incidence of subcutaneous tumor development (Cao et al. 2009). From a molecular standpoint, PECAM promotes heterotypic and homophilic interactions between monocytes and endothelial cells. Interestingly, the expression of PECAM (prodiapedesis) and VE-cadherin (anti-diapedesis) inversely correlates during transcellular passage of monocytes (Hashimoto et al. 2011). Indeed, in the course of monocyte transmigration, the levels of surface-exposed PECAM are increased, as opposed to VE-cadherin junctional localization (Hashimoto et al. 2011). The association of neutrophils and leukocytes to the endothelium induces Src-dependent destabilization of VE-cadherin-based cell-cell contacts and inversely correlates with PECAM bioavailability at the plasma membrane (Alcaide et al. 2012, 2008; Garnacho et al. 2008). PECAM may have broad impact on vascular barrier function, as its depletion quells neutrophil infiltration and perturbs angiogenesis (Solowiej et al. 2003). PECAM was also proposed to function as a sensor for mechanic shear stress generated by the blood flow (Tzima et al. 2005). Loss of PECAM expression in endothelial cells is associated with a defect in the activation of atherosclerosis-mediated pro-inflammatory pathways (Tzima et al. 2005; Conway and Schwartz 2012, 2013; Conway et al. 2013).

Nectins exist as four isoforms, among which Nectin-2 and Nectin-3 localized at endothelial cell junctions. Similarly to cadherins, they function as dimers that bridge neighboring cells together. Intracellularly, Nectins are bound to the afadin molecule, which associates in turn to the actin cytoskeleton and shuttles between junctions (Dejana 2004). Nectins are most likely cooperating with adherens junctions by impacting on the actin cytoskeleton organization at cell-cell junctions, but their exact contribution to vascular homeostasis and plasticity remains to be fully examined (Dejana 2004; Rehm et al. 2013).

VE-cadherin is an instrumental transmembrane adhesion molecule of the adherens junctions. This adhesion molecule actively and dynamically participates in cell-cell contact formation and remodeling and regulates the homeostasis of the endothelial barrier (Fig. 6). VE-cadherin known roles in the endothelial barrier is developed in the next section.

VE-Cadherin in the Endothelial Barrier

VE-cadherin is a type II classical cadherin, exclusively expressed in vascular and lymphatic endothelial cells. Classical cadherins are all defined by five repeated immunoglobulin-like cadherin motifs (EC) in their extracellular domain, while the presence of a large hydrophobic region in the first domain (EC1) differentiates between type I and type II cadherins (Yagi and Takeichi 2000). The intracellular domain is a highly conserved region, which bridges cadherins to the actin cytoskeleton through different catenins (α, β, γ, and p120) and other intracellular and plasma membrane components, most of them being specific of the endothelial compartment. VE-cadherin extracellular domain assembles as a hexamer from three VE-cadherins (Bibert et al. 2002; Hewat et al. 2007). This association allows the torsional flexibility of VE-cadherin bridges and enables the interaction between VE-cadherins harbored by neighboring cells (Bibert et al. 2002; Hewat et al. 2007). The transmembrane

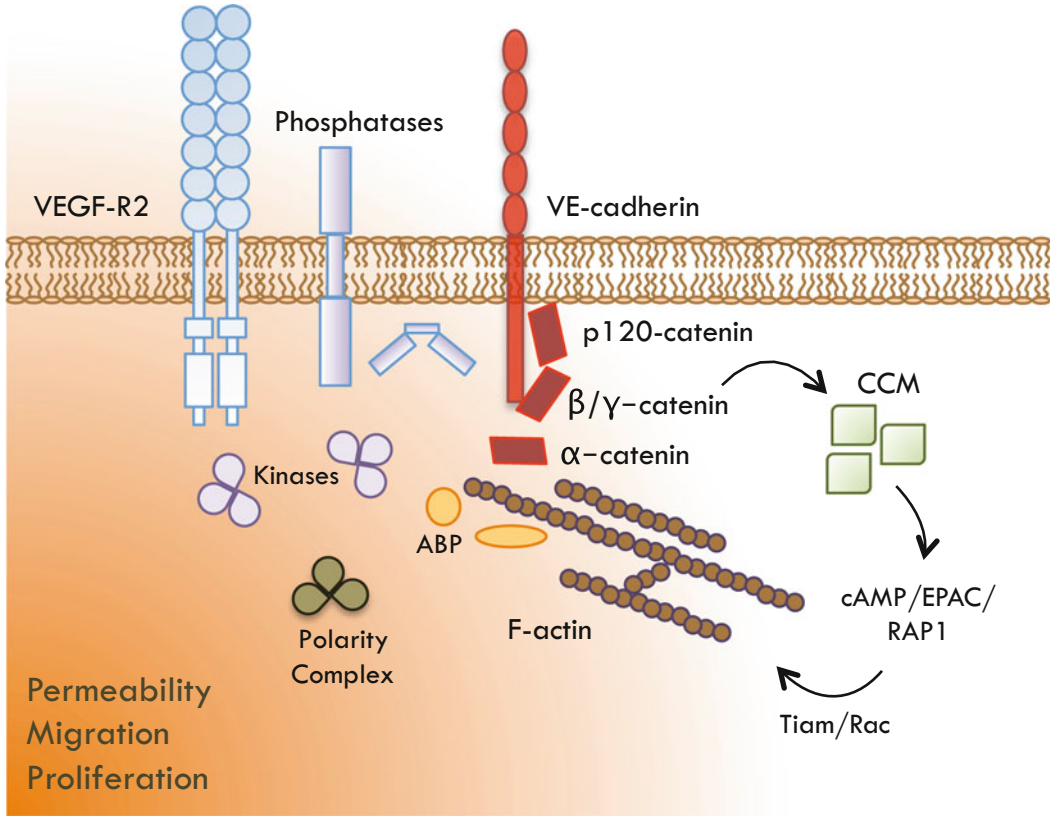

Fig. 6 VE-cadherin and partners at the endothelial junctions. Endothelial cells express the cell-cell adhesion molecule VE-cadherin. Cytosolic catenins (p120, α, β, and γ) bridge VE-cadherin to the actin cytoskeleton and actin-binding proteins (*ABP*). This adhesive complex interacts with the VEGF-R2 (vascular endothelial growth factor receptor 2, receptor and non-receptor phosphatases, cytosolic kinases, polarity complex, and CCM (cerebral cavernous malformation proteins)

domain entails VE-cadherin clustering at the plasma membrane. Catenins, namely, p120, α, β, and γ, are cytoplasmic proteins bound to cadherins that ensure a physical connection between the plasma membrane and the actin cytoskeleton.

The core domain of β-catenin consists of 12 repeated sequences (each of 42 amino acids) called armadillo repeats (arm) and is engaged into protein-protein interaction with the negatively charged C-terminal tail of cadherins. As part of the Wnt signaling pathway, β-catenin can shuttle to the nucleus and interacts with DNA binding sites, resulting in the activation of genes instrumental in embryogenesis and carcinogenesis (Clevers 2006). To reconcile its dual involvement in cell-cell adhesion and Wnt signaling, β-catenin coexists under different conformations, which most likely confers its distinct functions. Indeed, a β-catenin/α-catenin heterodimer is implicated in cadherin-dependent adhesive function, whereas β-catenin transcriptional activity resides exclusively in its monomeric closed conformation form. Additionally, the monomeric open conformation bears both adhesive and transcriptional functions (Gottardi and Gumbiner 2004). Interestingly, mice null for β-catenin were engineered by site-specific recombinase technology (CRE) under the control of the endothelial cell-specific promoter Tie2 (the tyrosine kinase receptor for Angiopoietin-1). These embryos exhibit severe perturbations in the vascular patterning of the head, vitelline membrane, umbilical cord, and placenta (Cattelino et al. 2003). Moreover,

β-catenin depletion significantly weakens endothelial cell integrity, hinders α-catenin expression, and promotes the accumulation of plakoglobin and desmoplakin at cell-cell contacts (Cattelino et al. 2003). Conversely, exon3 deletion mutant of β-catenin behaves as a gain of function (GOF) mutant, whose expression can be forced in the endothelial compartment of transgenic mice (Corada et al. 2010). This mutant cannot be phosphorylated and further degraded and therefore remains constantly active. Interestingly, this augments Wnt/β-catenin and Notch signaling activation in endothelial cells. Alteration of vascular remodeling and endothelial differentiation manifested by vessel branching defects and lack of arteriovenous specification further characterized the phenotype of mutant mice (Corada et al. 2010).

The p120-catenin belongs as well to the armadillo family and binds to the juxtamembrane domain of cadherins (Kowalczyk and Reynolds 2004). Mice in which the p120-catenin gene was silenced in the endothelial lineage die early in utero because of embryonic and extraembryonic vascular defects (Oas et al. 2010). Molecularly, both N-cadherin and VE-cadherin expression are dramatically reduced in vessels, explaining the phenotype of reduced microvessel density, low pericyte recruitment, and hemorrhages in knockout embryos (Oas et al. 2010). A highly conserved sequence of 10 amino acid (644–654 on the human sequence) bears the binding region to classical cadherins. In particular, mutation within the DEE motif results in VE-cadherin/p120-catenin uncoupling (Nanes et al. 2012). p120 is thought to mask putative internalization motifs on VE-cadherin and therefore contributes to maintain VE-cadherin at the plasma membrane (Nanes et al. 2012; Kowalczyk and Reynolds 2004). Additionally, p120-catenin has been described to orchestrate cell morphology, motility, and adhesion by modulating the activity of Rho, Rac, and Cdc42 small GTPases (Anastasiadis 2007). Indeed, p120-catenin can directly interact with Rho GTPase-activating protein (p190RhoGAP) via its C-terminal tail and subsequently tunes the Rho/Rac balance involved in cell-cell contact formation (Zebda et al. 2013). Furthermore, p120 binding to VE-cadherin modulates in a Rac-dependent manner the ability of endothelial cells to spread and thereby controls the adhesive contact area, while the adhesive strength rather relies on β-catenin association (Oas et al. 2013, 2010).

γ-catenin (or plakoglobin) can be recruited to VE-cadherin in place of β-catenin. In fact, VE-cadherin/plakoglobin association exists in mature confluent cells, while β-catenin preferentially interacts with VE-cadherin in nascent contacts (Lampugnani et al. 1995). Thus, VE-cadherin/β-catenin and VE-cadherin/plakoglobin interactions are inversely correlated. Different phases of endothelial cell-cell contact formation can be discriminated. The "initiation stage" corresponds to nascent junctions that are organized around VE-cadherin, β-catenin, and α-catenin. Next, the "extension stage" involves the reinforcement of cell-cell contact through plakoglobin recruitment. Finally, upon maturation, VE-cadherin is connected to the actin cytoskeleton through plakoglobin, and this complex can be biochemically isolated in triton-insoluble fractions (Lampugnani et al. 1995).

α-catenin actively participates in cadherin-mediated cell-cell contacts and connects the VE-cadherin/catenin complex to the actin cytoskeleton. Unlike other catenins, it is not an armadillo protein and operates as an actin-binding protein. Interestingly, α-catenin cannot simultaneously link actin and be part of the cadherin/β-catenin complex (Yamada et al. 2005). In fact, α-catenin coexists as a monomer or a dimer, while its conformation balances its binding affinity for the cadherin/β-catenin complex and the actin filaments. The monomeric α-catenin preferentially binds to cadherin/β-catenin, whereas the α-catenin dimer can only associate with actin filaments (Drees et al. 2005). This VE-cadherin/catenin complex connects cell-cell adhesion molecules to the actin cytoskeleton. In line with this, actomyosin contractility is enhanced upon junctional remodeling, where cytoskeletal pulling forces rely on the direct interaction of α-catenin with the actin-binding protein, vinculin. Here, vinculin prevents from the dismantlement of adherens junctions upon thrombin

challenge (Huveneers et al. 2012). Moreover, endothelial cell-cell contacts can be strengthened upon high intracellular concentration of cyclic (−3–5)monophosphate (cAMP) (Sakurai et al. 2006). Increase in cAMP concentration elicits a signaling pathway involving Epac (exchange protein activated by cAMP) and the small GTPase Rap1, to form circumferential actin bundles. α- and β-catenins from the VE-cadherin adhesion complex seem to play essential roles in the accumulation of VE-cadherin on the actin bundles (Noda et al. 2010). Thus, the cAMP/Epac/Rap1 signaling pathway regulates actin dynamics and reinforces cell-cell contact in endothelial cells. Besides, VE-cadherin plays a pivotal role in the regulation of small GTPases Rho and Rac activity (Lampugnani et al. 2002). Interestingly, the expression of VE-cadherin causes the reorganization of actin stress fibers through Rac activation and Rho inhibition. Furthermore, VE-cadherin requires the Rac1-specific guanine nucleotide exchange factor (GEF) Tiam to associate with the cytoskeleton (Lampugnani et al. 2002).

VE-cadherin can associate directly and indirectly with other molecules through its intracellular or extracellular domains to control endothelial cell proliferation, survival, polarity, migration, and the overall barrier function. In quiescent confluent endothelial cells, the expression of VE-cadherin adhesive complex at the cell surface allows VE-cadherin/VEGF-R2 association and thereby regulates VEGF-induced proliferation (Grazia Lampugnani et al. 2003). Indeed, this interaction promotes the activation of the phosphatidylinositol 4,5-bisphosphate 3-kinase (PI3K)/AKT survival pathway. In genetically modified VE-cadherin null endothelial cells or when VE-cadherin is excluded from junctions, VEGF-R2 phosphorylation and VEGF-R2-mediated proliferation are enhanced. Indeed, VEGF stimulation in VE-cadherin-depleted cells leads to rapid clathrin-dependent internalization of VEGF-R2 (Lampugnani et al. 2006). However, its endocytosis does not abort its signaling but rather elicits MAPK activation and enhances cell growth (Lampugnani et al. 2006). Thus, VE-cadherin junctional distribution hinders VEGF-R2 internalization and MAPK pathway activation. Early studies showed that β-catenin is required for VEGF-R2 and VE-cadherin functional interaction (Carmeliet et al. 1999). Likewise, intravenous delivery of VEGF in mice transiently disrupts the VE-cadherin/VEGF-R2 complex in heart endothelial cells (Weis et al. 2004b). Importantly, the inhibition of the proto-oncogene tyrosine-protein kinase (Src) can prevent VEGF-R2/VE-cadherin dissociation caused by VEGF. Next, the membrane-associated phosphatase (DEP-1) plays an important role in VEGF-mediated cell proliferation. Indeed, DEP-1 is localized at the endothelial cell-cell junctions in the proximity of VE-cadherin and VEGF-R2. Moreover, it has been reported that DEP-1 specifically interacts with VE-cadherin through β, γ, or p120-catenins (Holsinger et al. 2002). In DEP-1 knocked-down endothelial cells, VEGF-R2 phosphorylation and cell proliferation are boosted (Grazia Lampugnani et al. 2003). Interestingly, DEP-1 can also dephosphorylate the Src kinase; for example, DEP-1-dependent Y418 Src dephosphorylation limits its downstream signaling and participates in the inhibition of endothelial proliferation (Spring et al. 2012). Altogether, β-catenin- and DEP-1-dependent VEGF-R2/VE-cadherin coupling has a substantial impact on cell-cell contact growth inhibition in endothelial cells (Grazia Lampugnani et al. 2003; Lampugnani et al. 2006). In keeping with this idea, VE-cadherin can interact with cytosolic C-terminal Src kinase (Csk) and reduce Src kinase activity. In growing endothelial cells, VE-cadherin is strongly phosphorylated at tyrosine residues and Csk exclusively binds to the phosphorylated form of VE-cadherin on Y685 (Baumeister et al. 2005). The Csk/pY685-VE-cadherin interaction requires the Csk SH2 domain, while Csk co-immunoprecipitates with wild-type VE-cadherin, but not with a non-phosphorylable (Y685F) VE-cadherin mutant, even upon Src activation. Consequently, the association of Csk with pY685-VE-cadherin increases with cell density and modulates endothelial cell proliferation and permeability.

VE-cadherin has been described to associate with different phosphatases that stabilize endothelial cell-cell contacts. One of the most well-

documented interactions between VE-cadherin and phosphatases is illustrated by vascular endothelial protein tyrosine phosphatase (VE-PTP). VE-PTP is an endothelial-specific membrane protein, which impairs VE-cadherin tyrosine phosphorylation (Nawroth et al. 2002). Moreover, unlike most of the other VE-cadherin partners, VE-PTP association with VE-cadherin does involve neither its intracellular domain nor β-catenin, suggesting that this interaction is most likely mediated through VE-cadherin transmembrane and/or extracellular domains. The use of full-length and several deletion mutants of VE-PTP and VE-cadherin allows the mapping of this association and the identification of the 17th FNII repeat domain of VE-PTP and VE-cadherin fifth extracellular immunoglobulin domain (EC5). At the functional level, VE-cadherin is linked to VE-PTP in quiescent endothelial cells. However, this interaction can be abolished during lymphocyte and neutrophil infiltration and in response to angiogenic and inflammatory agents, such as VEGF or tumor necrosis factor (TNFα), respectively (Nottebaum et al. 2008). In this scenario, VE-cadherin/VE-PTP dissociation is accompanied with tyrosine phosphorylation of VE-cadherin, β-catenin, and γ-catenin (plakoglobin) resulting in the opening of VE-cadherin-based junctions. Interestingly, VE-PTP-dependent dephosphorylation of VE-cadherin orchestrates adhesion and permeability. This might rely rather on plakoglobin than β-catenin. Indeed, the absence of plakoglobin exacerbates the effects of the in vitro knockdown of VE-PTP on adhesive and barrier properties of endothelial cells. Thus, VE-cadherin/plakoglobin/VE-PTP association seems to be essential to stabilize endothelial cell-cell contacts (Nottebaum et al. 2008). Additionally, VE-PTP was indirectly implicated in VEGF-R2 dephosphorylation in the process of lumen polarization during zebra fish development (Hayashi et al. 2013). Simultaneous stimulation of endothelial cells with VEGF and Angiopoietin-1 (Ang1) leads to the accumulation of VE-PTP, Angiopoietin-1 receptor (Tie2), and VEGF-R2 at the endothelial junctions, causing further VEGF-R2 dephosphorylation. Overall, VE-PTP-dependent dephosphorylation of VEGF-R2 mediates Tie2-mediated action on endothelial cell lining and vessel maturation, while VEGF-R2 regulates VE-cadherin tyrosine phosphorylation, endothelial cell polarity, and lumen formation (Hayashi et al. 2013). The phosphatase SHP2 (Src homology two-domain-containing tyrosine phosphatase) associates with the VE-cadherin adhesive complex through β-catenin (Ukropec et al. 2000). Upon thrombin stimulation, β-catenin is phosphorylated and subsequently SHP2 dissociated from the VE-cadherin complex, leading to endothelial permeability increase (Ukropec et al. 2000). However, SHP2 can dephosphorylate β-catenin, when engaged in endothelial junctions (Timmerman et al. 2012). Conversely, β-catenin tyrosine phosphorylation levels are increased in SHP2-depleted cells. Moreover, SHP2 contributes to VE-cadherin-associated β-catenin dephosphorylation after thrombin stimulation, suggesting that SHP2 could play an important role in the recovery of disrupted endothelial adherens junctions. Protein phosphatase 2A (PP2A) was also found coupled to VE-cadherin in brain endothelial cells, while its activity governs barrier integrity. PP2A is a serine/threonine phosphatase, which is potentially able to dephosphorylate VE-cadherin at S665, and thereby contributes to maintain low brain endothelial cell permeability (Le Guelte et al. 2012; Gavard and Gutkind 2006). For instance, tumor-derived factors elicit a signaling pathway leading to the dissociation of the VE-cadherin/PP2A complex in endothelial cells. This causes VE-cadherin S665 phosphorylation and its further internalization (Le Guelte et al. 2012). Again, vascular permeability is augmented.

VE-cadherin orchestrates and maintains the endothelial barrier integrity and homeostasis. Of note, most of the tumor-derived factors released in the tumor microenvironment converge on modulating the VE-cadherin biology. Recovering VE-cadherin physiological behavior could emerge as a promising strategy to promote vascular normalization in anticancer therapies.

Endothelial Junctions in Tumor-Induced Angiogenesis

Dynamics of Endothelial Junctions in Migration and Sprouting

As mentioned in the previous section, the vascular sprout is a dynamic structure where endothelial cells compete for the tip position (Jakobsson et al. 2009, 2010). During elongation and migration of the growing vessel, endothelial cells exhibit a mixed pattern of tip and stalk phenotypes; a phenomenon coined as "salt-and-pepper" distribution. This lively process depends on individual cell behavior, with the coexistence of opposing molecular signaling pathways: in one hand the activation of the VEGF pathway and, on the other hand, the lateral inhibition through the Notch pathway under the control of DLL4 (Blanco and Gerhardt 2013) (please see above section).

VEGF signaling is tightly linked to VE-cadherin dynamics that orchestrate endothelial cell adhesion, behavior, and endothelium properties (Gavard 2009, 2013). Interestingly, there is substantial evidence that proliferative and migratory traits of endothelial cells rely on VE-cadherin (Grazia Lampugnani et al. 2003). The importance of VE-cadherin during development has been formally demonstrated with mice missing the VE-cadherin intracellular domain, while the early embryonic lethality of these embryos (E9.5) is associated with hemorrhagic vessels (Carmeliet et al. 1999). VEGF-R2 activation induced endothelial permeability by a mechanism involving VE-cadherin phosphorylation and subsequent endocytosis (Gavard and Gutkind 2006). In keeping with this idea, the differential VE-cadherin-dependent adhesion between endothelial cells in response to VEGF stimulation was recently shown to mechanically orchestrate tip and stalk cell rearrangement within the sprout (Bentley et al. 2014). VE-cadherin, actin rearrangement, and VEGF-R2/Notch balance are indeed involved. High VEGF-R2 activity in tip cells leads to VE-cadherin phosphorylation and its further internalization (Bentley et al. 2014). VE-cadherin internalization is known to elicit cell-cell junction weakening and transient elevation of endothelial permeability (Gavard and Gutkind 2006). This might ultimately governs the migratory phenotype of tip cells, especially protrusion formation. In this scenario, endothelial cells with weaker adhesion potential are oriented toward a tip cell phenotype and progress to the tip of the sprout (Bentley et al. 2014). In contrast, Notch induces downregulation of VEGF-R2 in stalk cells, low VE-cadherin phosphorylation, and Rac/Tiam activation. This collectively results in the inhibition of filopodial protrusions and favors their cell-cell adhesive phenotype. Likewise, low level of VEGF-R2 phosphorylation in stalk cells can be attributed to VE-PTP activity and depends on the presence of the Angiopoietin-1 receptor Tie2 at the endothelial cell-cell junctions (Hayashi et al. 2013). In this context, VE-PTP limits both VEGF-R2 signaling and VE-cadherin-mediated vascular permeability and thereby promotes neo-vessel stabilization.

Endothelial-to-mesenchymal transition (EndMT) is characterized by a series of morphological alteration including disruption of intercellular junctions, loss of cell polarity, accompanied with enhanced both proliferation and migration of endothelial cells that escape from the vasculature (Zeisberg et al. 2007b; Dejana et al. 2017). In pathological conditions, the acquisition of mesenchymal and stem-cell-like traits by endothelial cells contributes to fibrosis and accumulation of ectopic stromal cells and myofibroblasts (Zeisberg et al. 2007b; Dejana et al. 2017). Factors, including inflammation, shear stress, and TGF-β signaling (transforming growth factor), promote endothelial-to-mesenchymal transition and participate in tumor microenvironment heterogeneity in cancers (Zeisberg et al. 2007a; Xiao et al. 2015). This also suggests that anti-angiogenic treatments may also impact on the stromal cellular composition. Molecularly, the endothelial-specific deletion of cerebral cavernous malformation protein CCM1 leads to endothelial-to-mesenchymal transition and provokes vascular abnormalities in mice (Maddaluno et al. 2013). Interestingly, the role of CCM1 in

VE-cadherin-based adherens junction formation has been documented. CCM1 mutations were originally identified in patients affected by cerebral cavernous malformations (CCM), a disease characterized by cerebral blood leakage and abnormal vessel structure (Dejana et al. 2009). VE-cadherin indeed indirectly connects to CCM1 thanks to β-catenin and recruits Rap1, a GTPase that stabilizes endothelial adherens junction (Lampugnani et al. 2010). It has been further suggested that CCM1 via its direct interaction with the VE-cadherin/catenins complex triggers Rap1 and Tiam-dependent Rac activation, whereas CCM2 bound to the VE-cadherin adhesion complex through CCM1 inhibits RhoA (Lampugnani et al. 2010).

In parallel to the VEGF/VE-cadherin axis dominating endothelial junction remodeling and dynamics, tumor-secreted basic fibroblast growth factor (bFGF) is another known inducer of endothelial cell migration whose signaling have been associated to JAM-A. In resting endothelial cells, JAM-A is present at endothelial cell-cell contacts where it can complex with $\alpha_V\beta_3$ integrin, a mediator of endothelial cell adhesion on vitronectin (Naik and Naik 2006). Upon bFGF stimulation, JAM-A is partially delocalized from endothelial junctions and dissociates from $\alpha_V\beta_3$. In turn, this provokes the activation of the mitogen-activated protein kinase (MAPK) signaling and modulates endothelial cell adhesion, spreading, and migration (Naik et al. 2003). Accordingly, the stimulation of endothelial cells with bFGF fails to induce angiogenesis in JAM-A deficient mice (Cooke et al. 2006). Interestingly, bFGF opposes to endothelial permeability and VE-cadherin internalization and instead stabilizes adherens junction (Murakami et al. 2008).

JAMs may exert multiple functions in the course of tumor progression. For example, JAM-C inhibition using blocking antibodies was shown to abolish ex vivo angiogenesis in the aortic ring model of endothelial sprouting, while this treatment impairs tumor growth in vivo in a syngenic mouse model of lung carcinoma (Lamagna et al. 2005a, b). More recently, endothelial-specific JAM-C gene deletion has been associated with a reduced tumor growth in a mouse model of ovarian cancer, as compared to wild-type animals (Leinster et al. 2013). In this context, the overall tumor vessel density is not affected, but rather tumor vessel permeability is increased together with a reduction in pericyte coverage, suggesting that JAM-C function in tumor-induced angiogenesis is more likely related to vessel functionality through mediating cell-cell junction stability and/or pericyte anchorage.

JAM-B, also known as vascular endothelial, VE-JAM was recently reported to be a negative regulator of pro-angiogenic pathways interfering with VEGF/VEGF-R2 signaling (Meguenani et al. 2015). Interfering with JAM-B at endothelial junctions using a blocking antibody inhibited endothelial tube formation in vitro and reduced VEGF-induced aortic ring vessel outgrowth without altering pericyte coverage ex vivo. Moreover, blocking JAM-B in JAM-B-expressing murine endothelial cells and JAM-B-transfected HUVECs reduced ERK1/2 (extracellular regulated kinase) phosphorylation upon VEGF stimulation, further indicating that JAM-B-based adhesion in resting endothelial cells interferes with VEGF/ERK pathway activation, favoring in turn endothelial junction stability. Mice knockout for JAM-B are viable and do not display vascular abnormalities, while aortic rings from those mice stimulated with VEGF show an increased vessel branching, thus suggesting the existence of compensatory mechanisms regarding JAM-B functions in angiogenesis. Additionally, JAM-B anti-angiogenic function does not extend in vivo in inhibition of tumor vascularization. Indeed, treatment of mice with a JAM-B blocking antibody did not affected endothelium-derived tumor growth (hemangioma), and no effects on tumor vasculature and progression were observed in a model of pancreatic tumor. Similarly, in an ectopic model of Lewis lung carcinoma, tumor development was similar between wild-type animals and JAM-B deficient mice (Meguenani et al. 2015).

Endothelial Cell-Cell Junctions in Polarity

The overall organization of the vascular tubes with luminal and basolateral sides implies

polarity. The description of the endothelial polarity suffers from the comparison to the morphological and functional detailed knowledge of epithelial cell polarity. The molecular mechanisms governing the establishment of polarity involve cell-cell adherens and tight junctions in epithelial cells and are thus thought to be transposable to endothelial cells. However, the flattened endothelial morphology in blood vessels and the fact that tight and adherens junctions are intermingled might explain disparities between the two systems. In epithelial cells, cell polarity relies on JAM-A recruitment and stabilization at early cell-cell contacts through its association with Afadin and ZO-1 (Ebnet et al. 2000). The cell polarity complex composed of PAR-3, PAR-6, and aPKC is then recruited by the binding of PAR-3 to JAM-A intracellular PDZ binding domain. In an ectopic expression model, JAM-A expression leads to the recruitment of PAR-3 at cell-cell contacts (Suzuki and Ohno 2006; Ebnet et al. 2001). aPKC is activated by small Rho GTPase family members, Rac1 and Ccd42, and phosphorylates JAM-A on Ser285 to promote maturation of cell-cell junctions. Moreover, it was recently demonstrated that transient activation of Cdc42 by JAM-A during mitosis regulates cortical dynein localization to control planar spindle orientation (Iden et al. 2012; Tuncay et al. 2015; Ebnet 2013). These molecular mechanisms involving JAM-A polarity regulation are thought to be transposable to endothelial cells, as endothelial cells deficient for JAM-A exhibit spontaneous and random motility (Bazzoni et al. 2005). However, JAM-A deficient mice have a normal vascular system development (Bazzoni et al. 2005). In the context of endothelial polarity, JAM-C and JAM-B are also expressed at endothelial junctions and are able to recruit PAR-3 via their PDZ binding domain (Ebnet et al. 2003). Interestingly, JAM-C-dependent assembly of the cell polarity complex was shown to be critically required for round spermatid polarization and subsequent differentiation, further suggesting that endothelial JAMs might cooperate in endothelial polarity establishment and maintenance (Gliki et al. 2004).

It has been reported that VE-cadherin associates as well as with polarity proteins Par3 and Par6 that further participate in the formation of the apicobasal polarity complex in endothelial cells. However, unlike epithelial cells, this occurs independently of aPKC (Ebnet et al. 2003; Iden et al. 2006). VE-cadherin silencing causes irregular expression of apical and basal markers in endothelial cells (Lampugnani et al. 2010). Reminiscent phenotype is observed upon endothelial depletion of β1 integrin and can be partially rescued by Par3 (Zovein et al. 2010). In keeping with this idea, β1 integrin mediates endothelial sprouting while governing VE-cadherin localization and vessel maturation (Yamamoto et al. 2015). Additionally, VE-cadherin and cell-cell junctions were recently implicated in the control of lumen formation during embryonic development. The formation of the VE-cadherin/CCM complex allows lumen polarization, while the absence of any of these proteins causes severe alterations of the lumen (Lampugnani et al. 2010). Interestingly, mice bearing in the endothelial compartment a loss-of-function mutation in the CCM2 gene present aberrant vascular lumens (Whitehead et al. 2009). Moreover, CCM2 might regulate RhoA activity and vascular permeability (Whitehead et al. 2009). Similarly, it has been established in VE-cadherin null or partially depleted zebra fish embryos that VE-cadherin is crucial to vascular lumen formation (Montero-Balaguer et al. 2009). Indeed, vessel fusion is dramatically altered because of the weakening of cell-cell contacts between two newly formed vessels (Montero-Balaguer et al. 2009). Indeed, VE-cadherin plays an important role in vascular connection and lumen formation and stability, in association with membrane dynamics and actin cytoskeleton-based forces (Phng et al. 2015; Gebala et al. 2016). How and whether these pathways are pirated in tumor-induced angiogenesis will require in-depth investigation.

Remodeling of Endothelial Junctions in Tumor Vascular Permeability

The action of tumor cells and their secreted factors with the endothelium has also been extensively demonstrated to promote the loss of barrier

integrity, in many solid cancers (Le Guelte et al. 2011). For example, upon interaction between breast cancer cells and endothelial cells, VE-cadherin is phosphorylated on tyrosine residues and re-localized away from cell-cell contacts, contributing to increased permeability (Cai et al. 1999). VE-cadherin can also modulate endothelial permeability by recruiting the scaffolding molecule β-arrestin (Gavard and Gutkind 2006). Indeed, the phosphorylation of VE-cadherin upon VEGF stimulation can serve as a docking site for β-arrestins (Gavard and Gutkind 2006; Hebda et al. 2013). This connection causes VE-cadherin endocytosis and the elevation of endothelial permeability and can be turned down by anti-permeability agents, such as the maturation angiogenic factor Angiopoietin-1 (Gavard and Gutkind 2006; Gavard et al. 2008; Saharinen et al. 2008). Likewise, co-culture of ovarian cancer cells with endothelial cells demonstrated that tumor cell-secreted VEGF increases endothelial permeability in association with decreased VE-cadherin localization at the plasma membrane (Hu et al. 2006). Indeed, VEGF expression is broadly augmented in cancer cells, mainly upon hypoxia stress, although this heightened production is maintained upon ex vivo normoxic culture. Tumor-derived VEGF has been shown to be delivered in the milieu through extracellular vesicles and functionally to contribute to endothelial cell-cell destabilization, permeability, and angiogenesis (Skog et al. 2008; Treps et al. 2016; Feng et al. 2017). Thus, these data provide evidence for a critical role for VE-cadherin in cancer-associated vascular permeability.

Importantly, the non-receptor proto-oncogene kinase Src is instrumental in vascular leakage, upon challenge of VEGF and other tumor-emanating permeability mediators. Indeed, the integrity of both skin macrovascular and cerebral microvascular endothelial barriers was maintained in Src knockout animals upon acute VEGF challenge (Eliceiri et al. 1999; Paul et al. 2001). Src was indeed shown to take part in vascular permeability in tumors and influence tumor cell extravasation and metastasis (Weis et al. 2004a). Mechanistically, Src has been well established to modify adherens junction either directly via phosphorylation of VE-cadherin and its associated catenins (Lambeng et al. 2005; Weis et al. 2004a, b), or indirectly via the activation of intracellular pathways leading to VE-cadherin phosphorylation (Gavard and Gutkind 2006). Alternatively, Src kinase tunes the activity of the focal adhesion kinase (FAK), which is in turn recruited at cell-cell junctions, and thereby operates on vascular permeability and tumor dissemination (Chen et al. 2012; Jean et al. 2014). Some studies also document the impact of Src on tight junction stability at the plasma membrane via occludin phosphorylation (Elias et al. 2009; Takenaga et al. 2009).

The Wnt pathway, which is frequently aberrantly activated in cancers, is also a key regulator of the endothelial cell-cell junctions (Gavard and Mege 2005). β-catenin is the essential downstream effector of the canonical Wnt pathway and operates at the cell-cell junctions where it connects cadherins to the actin cytoskeleton and serves as an intermediate between cadherins and other intracellular signaling pathways (Carmeliet et al. 1999; Cattelino et al. 2003; Gavard and Mege 2005). Additionally, Fzd7, one of seven transmembrane domain receptor of the Wnt family, associates with VE-cadherin complex through its cysteine-rich extracellular domain (Ferreira Tojais et al. 2014). In vivo experiments show that the depletion of Fzd7 in endothelial cells promotes vascular leakage, as measured by Evans blue dye extravasation upon VEGF stimulation. Likewise, the silencing of Fzd7 gene in vitro causes the disorganization of tight junctions and the dissociation of the VE-cadherin/β-catenin complex. Fzd7 can further regulate the expression of VE-cadherin and β-catenin both in vitro and in vivo. In this scenario, the activation of the canonical Wnt/β-catenin axis rescues Fzd7 deficiency in terms of vascular permeability and VE-cadherin/β-catenin disruption (Ferreira Tojais et al. 2014). An additional interplay between VE-cadherin expression and tight junction organization resides in the β-catenin-dependent modulation of the FoxO pathway that negatively controls the transcription of occludin and claudin-5 (Taddei et al. 2008; Leclair et al. 2016).

Endothelial JAMs are important modulators of barrier integrity that are differentially expressed

throughout the vasculature, JAM-A being highly expressed in the blood-brain barrier where vessel permeability is reduced at the minimal (Aurrand-Lions et al. 2001). JAM-A and JAM-C were reported to maintain barrier integrity in several models. Indeed, impairing with JAM-A adhesive function with blocking antibodies results in corneal swelling due to impaired barrier function in rabbits (Mandell et al. 2006). Likewise, homozygous JAM-C mutation in humans was associated with the development of brain hemorrhages, suggesting an important role for JAM-C in the maintenance of the blood-brain barrier (Mochida et al. 2010). Contrarily to macrovascular endothelial cells where JAM-C is predominantly present at cell-cell contacts, its expression in quiescent microvascular cells is mostly cytoplasmic and is redirected to the junctions only upon challenge. In this context, JAM-C was shown to increase endothelial permeability through the modulation of VE-cadherin cell-cell contacts in a mechanism dependent of the small GTPase Rap1 (Orlova et al. 2006). Moreover, JAM-C overexpression in endothelial cells was reported to increase vascular permeability in thrombin-stimulated endothelial cells (Li et al. 2009).

While VEGF that was first identified as the vascular permeability factor (VPF) is the most studied factor, other cytokines found to be released in the tumor microenvironment can favor vascular permeability in a synergic manner, including the CXCL8 (IL-8) chemokine, TNFα, and more (Le Guelte et al. 2011). They frequently converge on the modulation of endothelial cell-cell junctions, notably regulating VE-cadherin-based adhesion.

Involvement of Endothelial Junctions in Tumor Vascular Aberrations

Inflammation is a hallmark of the tumor microenvironment playing critical roles in tumor initiation, progression, metastasis, and responses to therapy. While in developing tumors anti-tumorigenic and pro-tumorigenic inflammatory mechanisms coexist, inflammation and immune cells appear to be ultimately beneficial for tumors, notably by supplying chemokines and cytokines favoring cancer cell survival and proliferation, as well as angiogenesis (Grivennikov et al. 2010).

Immune cell recruitment to the site of inflammation is tightly regulated by endothelial junctional molecules, such as PECAM-1, MIC2 (also known as CD99, the Ewing's sarcoma marker), ICAM-2 (intercellular adhesion molecule 1), ESAM (endothelial cell adhesion molecule), and members of the JAM family that are partially re-localized in the activated endothelium to mediate leukocytes rolling, adhesion, as well as paracellular and transcellular transendothelial migration (Muller 2011). Endothelial JAM-A, JAM-B, and JAM-C are known regulators of this process (Arcangeli et al. 2013). JAM-A and JAM-C can notably be detoured from endothelial lateral borders under inflammatory stimulation with TNF-α, IFN-γ (Interferon) and oxidized LDL (low density lipoprotein) (Ozaki et al. 1999; Keiper et al. 2005). Briefly, partial JAM-A apical localization contributes to leukocyte adhesion on the endothelium through heterotypic interaction between JAM-A and lymphocyte function-associated antigen 1 (LFA-1) leukocyte integrin (Ostermann et al. 2002). Interactions between JAM-A and LFA-1 have been shown to destabilize endothelial JAM-A homodimers that therefore favor leukocyte transmigration (Wojcikiewicz et al. 2009). Impairing JAM-A function with a blocking antibody in vivo decreased inflammation and transendothelial migration (Woodfin et al. 2009; Martin-Padura et al. 1998), while the diapedesis of polymorphonuclear leukocytes is significantly reduced in JAM-A knockout mice (Cera et al. 2004). Endothelial JAM-B and JAM-C that preferentially form heterotypic JAM-B/JAM-C interactions at endothelial contacts are also actively involved in transendothelial migration, where JAM-B and JAM-C engage in both homophilic interaction with JAM-C found at the leukocyte membrane and heterophilic interactions with α4β1 and αMβ2 (Mac1) leukocyte integrins, respectively (Johnson-Leger et al. 2002; Cunningham et al. 2002; Lamagna et al. 2005b; Ludwig et al. 2009). JAM-C is essential to ensure unidirectional leukocyte transmigration from the blood to inflammatory sites. Indeed, JAM-C

pharmacological inhibition using blocking antibodies impairs leukocyte recruitment in several models of inflammation through an increased reverse transendothelial migration, rather than an inhibition of leukocyte transmigration, this process being recently described in vivo using real-time beam laser confocal microscopy and three-dimensional imaging in real time (Vonlaufen et al. 2006; Bradfield et al. 2007; Scheiermann et al. 2009; Woodfin et al. 2011). Importantly, in the inflammatory endothelium, endothelial cells express disintegrin metalloproteinase ADAM10 and ADAM17 that were shown to cleave JAM-A and JAM-C but also VE-cadherin, increasing endothelial permeability and facilitating leukocytes diapedesis (Schulz et al. 2008; Koenen et al. 2009; Rabquer et al. 2010). Additionally, processed soluble JAM-C mediates human microvascular endothelial cell migration and induces tube formation in vitro and angiogenesis in vivo in the tridimensional matrix-based plug and sponge granuloma models, this mechanism being potentially involved in tumor growth inhibition reported with JAM-C blocking antibodies and JAM-C endothelial depletion (Rabquer et al. 2010; Lamagna et al. 2005a, b; Leinster et al. 2013).

The elevated permeability found in tumor blood vessels interferes with the endothelial regulation of the inflammatory responses, further promoting endothelial instability and angiogenesis but also cancer cell metastasis. This latter dynamic process actively relies on JAM-mediated interactions between cancer cells and endothelial cells. First, JAM-C expressed on lung carcinoma cell lines mediates in vitro cancer cell adhesion to endothelial cells through JAM-C/JAM-C interaction and may thereby be involved in tumor metastatic processes (Santoso et al. 2005). Moreover, it was demonstrated that B16 melanoma cells metastasis to the lung was significantly decreased in JAM-C-deficient mice, as well as in mice with an endothelial-specific JAM-C depletion. Corroborating this, treatment of mice with soluble JAM-C prevented melanoma lung metastasis (Langer et al. 2011). Thus, JAM-C homophilic interaction contributes to melanoma cell transendothelial migration and lung metastasis. This involves endothelial JAM-B in a JAM-C/JAM-B heterophilic mode, as B16 melanoma cell metastasis was significantly reduced in JAM-B knockout mice (Arcangeli et al. 2012).

Several studies have unveiled the involvement of eNOS (endothelial nitric oxide synthase) in endothelial proliferation, in the context of inflammatory pathologies and central nervous system neoplasms (Argaw et al. 2012; Bulnes et al. 2010). Corroborating this, VEGF can no longer enhance vascular permeability in response to VEGF challenge in eNOS-deficient animals (Fukumura et al. 2001). Conversely, eNOS is over-expressed in vessels from brain tumors and might contribute to tumor edema (Bulnes et al. 2010). The loss of barrier integrity exacerbates this phenotype in a positive feedback loop, as the vascular leakage provokes erythrocyte invasion in the tissue and hemoglobin secretion. Hemoglobin drives in turn high eNOS expression together with reduced claudin-5, ZO-1, and JAM-A expression (Yang et al. 2013).

Several cancers, such as highly aggressive brain tumors (glioblastoma), hepatocarcinoma, and melanoma, share the ability to form de novo vascular networks composed of non-endothelial vascular channels that take part to blood perfusion of the tumor mass and facilitate the expansion of tumor cells (Kirschmann et al. 2012; Seftor et al. 2012). This event, also known as vascular/vasculogenic mimicry, is unique to tumors with a highly angiogenic, aggressive, and plastic phenotype. There is notably a strong correlation between VE-cadherin expression and aggressiveness of melanoma cells (Seftor et al. 2012). Cancer-derived endothelial-like cells express VE-cadherin and produce metalloproteases (MMP) that help vascular remodeling and promote vascular mimicry. In hepatocellular carcinoma cells, the overexpression of the transcription factor Twist1 is linked to vascular mimicry (Sun et al. 2010). Conversely, knockdown of Twist1 inversely correlated with invasiveness, migration, and vascular mimicry. Molecularly, high level of Twist1 in the nucleus coincides with the upregulation of VE-cadherin and MMP and the downregulation of E-cadherin in cells isolated from patients with hepatocellular carcinoma and found histologically positive for

vascular mimicry. To conclude, high level of Twist1 activity governs the plasticity of hepatocellular carcinoma cells. Vascular mimicry and epithelial-mesenchymal transition (EMT) operate using similar mechanisms and signaling pathways, but it still remains unclear whether the epithelial-mesenchymal transition is upstream vascular mimicry and how they are orchestrated. In addition to this vascular mimicry that concern a population of aggressive tumor cells, tumor stem-like cells that are mostly quiescent can also gain endothelial-like properties. These fake endothelial cells have been identified in aggressive brain tumors, where a fraction of cancer stem-like cells expresses VE-cadherin, and, together with normal endothelial cells, contribute to the tumor vasculature (Wang et al. 2010). Anywhere from 20 to 90% of tumor-associated endothelial cells have been scored to be of tumor origin (Ricci-Vitiani et al. 2010). They could also incorporate the tumor vasculature by mimicking pericyte, in a transdifferentiation process (Cheng et al. 2013).

Future Directions

In the course of tumor progression, new vessels are produced to sustain tumor cell growth. The tumor vasculature perverts the rules of harmonized, developmental program and displays a network of tortuous, leaky, and misconnected vessels. The increased vascular permeability was observed early in medicine, and considerable progresses have been achieved to delineate the signaling mechanisms underlying endothelial permeability. For instance, VEGF is one the best representative of the pro-permeability, pro--angiogenic factor involved in tumor-induced angiogenesis and modulating the endothelial barrier. We also learned that endothelial cell-cell junctions, and especially VE-cadherin-based contacts, are actively contributing to neovascularization and tumor growth, as well as tumor heterogeneity and plasticity. It can be envisioned that vessels with a normalized, functional endothelium barrier could restore vascular homeostasis and may favor chemotherapy delivery. One challenging question will be therefore to evaluate whether rescuing an intact endothelial barrier will be clinically valuable.

Cross-References

- ▶ Anti-angiogenic Targets: Angiopoietin and Angiopoietin Receptors
- ▶ Benefits and Pitfalls of Tumor Vessel Normalization
- ▶ Controlling Vascular Permeability: How Does It Work and What Is the Impact on Normal and Pathological Angiogenesis
- ▶ Mechanisms of Tumor Angiogenesis
- ▶ The Impact of Endothelial Transcription Factors in Sprouting Angiogenesis
- ▶ The Role of the VEGF Signaling Pathway in Tumor Angiogenesis
- ▶ The Role of VEGF in Controlling Vascular Permeability

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