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REVIEW

Phosphatases and kinases as regulators of the endothelial barrier function

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Abstract The endothelial layer of blood vessels controls the passage of cells and solutes from the blood into the surrounding tissue. Crucial for this regulation is the integrity of endothelial cell–cell junctions. Various molecular mechanisms control junctional integrity of the endothelial layer including GTPases, modulation of the actomyosin cytoskeleton and phosphorylation and dephosphorylation of junctional proteins. Several kinases and phosphatases have been identified that are good candidates for the regulation of the endothelial barrier function. For some of them, in vivo evidence has recently been presented that highlights their importance in either the regulation of vascular permeability or leukocyte extravasation. This review will summarize current knowledge about the regulation of endothelial junctions by kinases and phosphatases. In particular, the role of the endothelial specific phosphatase VE-PTP in the context of endothelial cell contact stability will be highlighted.

Keywords Endothelium · Phosphatases · Kinases · Phosphorylation · VE-PTP

The endothelial barrier

The integrity of blood vessels is crucial for tissue homeostasis and appropriate functioning of the immune system. Consequently, vascular permeability and the extravasation of leukocytes are tightly regulated. A central player in controlling vascular integrity is the endothelium of the blood vessel wall, which serves as a semipermeable barrier between the blood and the surrounding tissue. Solutes and leukocytes can pass the endothelial barrier either by a trans-cellular route directly through the body of an endothelial cell or through a para-cellular pathway by mechanisms that reversibly open and close endothelial junctions. The constitutive passage of solutes through resting endothelium is mainly mediated by a transcellular pathway, whereas inflammation-induced enhancement of vascular permeability mainly relies on the opening of endothelial junctions (Majno and Palade 1961; Mehta and Malik 2006; Schulte et al. 2011). Leukocytes can also directly pass through endothelial cells or through junctions, as has been demonstrated in vitro as well as in vivo (Carman and Springer 2004; Feng et al. 1998; Ley et al. 2007; Schoefl 1972; Vestweber 2007). However, recent in vivo studies analyzing large numbers of extravasating leukocytes revealed that the paracellular route is the major pathway in several tissues (Küppers et al. 2013; Schulte et al. 2011; Woodfin et al. 2011). Thus, the control of endothelial junctions is of central importance for inflammation-induced vascular permeability and leukocyte recruitment.

Several transmembrane adhesion receptors at endothelial junctions are involved in leukocyte diapedesis (transmigration) but only some of them play a role for the regulation of junctional integrity (Muller 2011; Vestweber 2007). PECAM-1 was the first identified diapedesis-mediating adhesion receptor that binds in a homophilic way to PECAM-1 on leukocytes (Muller et al. 1993). Other receptors that are also found at endothelial contacts and on leukocytes and support the diapedesis process are CD99 and CD99L2 (Bixel et al. 2004, 2007; Schenkel et al. 2002). At least the latter does not seem to support leukocyte diapedesis via homophilic molecular interactions, although a heterophilic ligand has not yet been identified (Seelige et al. 2013). None

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of these three proteins are involved in the regulation of endothelial junctions, as antibodies against them or gene deficiency do not seem to modulate vascular permeability.

The prototype of an endothelial transmembrane protein that is located at junctions and plays a role for leukocyte diapedesis and vascular permeability is VE-cadherin (Dejana and Vestweber 2013; Vestweber et al. 2009). VE-cadherin is of dominant importance for the stability of endothelial junctions since antibodies against it can enhance vascular permeability and leukocyte extravasation (Corada et al. 1999; Gotsch et al. 1997) and gene deficiency disrupts vascular integrity (Carmeliet et al. 1999; Gory-Faure et al. 1999). VE-cadherin is a major component of endothelial composite junctions that consist in intermingled adherens and tight junctions in many vascular beds (Dejana et al. 2008). VE-cadherin is linked to the catenins inside the cells that connect it with the actin cytoskeleton, an essential requirement for its ability to form intact endothelial junctions (Kemler 1993).

The junctional adhesion molecules (JAMs) form a subgroup of the Ig-supergene family, which are closely located to endothelial and epithelial tight junctions and can mediate homophilic and heterophilic interactions (Bradfield et al. 2007; Monteiro and Parkos 2012; Weber et al. 2007). JAM-A and JAM-C participate in leukocyte extravasation (Martin-Padura et al. 1998; Woodfin et al. 2011), whereas JAM-A is known to affect junctional stability in epithelial cells (Laukoetter et al. 2007) and JAM-C was suggested to affect VE-cadherin function (Orlova et al. 2006). In contrast to the JAMs, ESAM is a more distantly related tight junction-associated member of this family that is selectively expressed only in endothelial cells and that also supports leukocyte extravasation and the induction of vascular permeability (Wegmann et al. 2006). The typical tight junction components such as occludin and some of the claudins are of course also expressed in endothelial cells but participation in the regulation of leukocyte extravasation has not yet been described and permeability regulating activities are focused on small molecular weight components (Furuse et al. 1993, 1998; Morita et al. 1999).

Tyrosine phosphorylation and cell contact stability

Several studies have suggested that the increase of tyrosine phosphorylation of junction associated proteins correlates with a decrease in junctional integrity. Inhibitors of tyrosine phosphatases, with very broad specificity for almost all phosphatases, deregulate junction integrity in epithelial (Staddon et al. 1995) and endothelial cells (Young et al. 2003).

In agreement with this, it was demonstrated that vascular endothelial growth factor (VEGF) leads to strong tyrosine phosphorylation of the adherens junction molecules VE-cadherin, β-catenin and plakoglobin, which correlated with an increase in endothelial permeability (Esser et al. 1998). Moreover, the same study also described that PECAM-1 was tyrosine phosphorylated upon VEGF stimulation. Importantly, the cadherin association of the catenins was not altered and the cadherin–catenin complex retained its junctional localization, indicating that catenin and cytoskeletal association might not be affected by tyrosine phosphorylation. In line with this study, the inhibition of the kinase Src prevented the VEGF-induced increase in permeability, showing that a kinase is involved in the VEGF-induced opening of endothelial junctions (Weis et al. 2004).

Thrombin, another permeability-increasing agent, promotes tyrosine phosphorylation of VE-cadherin-associated β-catenin, plakoglobin and p120-catenin (Ukropec et al. 2000). Additional studies revealed that other permeability-increasing agents such as histamine (Andriopoulou et al. 1999), tumor necrosis factor-α (TNF-α) (Angelini et al. 2006) and platelet-activating factor (PAF) (Hudry-Clergeon et al. 2005) also induce tyrosine phosphorylation of VE-cadherin, β-catenin, γ-catenin and p120-catenin. Taken together, these studies suggest a correlation between the induction of permeability triggered by a variety of different stimuli and the induction of tyrosine phosphorylation of components of the VE-cadherin–catenin complex.

These studies prompted a more detailed analysis of certain tyrosine residues within the cytoplasmic tail of VE-cadherin. It has been shown that certain tyrosine/phenylalanine (Y/F) point mutants (Y658F and Y731F) of VE-cadherin, when overexpressed in cultured endothelial cells, inhibited transmigration of myeloid cells (Allingham et al. 2007). In another report, it was found that overexpression of each of three VE-cadherin mutants (Y731F, Y645F and Y733F) but not the Y658F VE-cadherin mutant, reduced transmigration of lymphocytes through transfected endothelial cell monolayers (Turowski et al. 2008). Analyzing the relevance of Y658 and Y731 of VE-cadherin for VEGF-induced permeability, it was shown that an Y658/731F VE-cadherin double mutant inhibited the VEGF effect (Monaghan-Benson and Burridge 2009). Parts of the VEGF-induced signaling mechanism leading to the phosphorylation of the VE-cadherin–catenin complex were revealed as activation of Rac-1 and subsequent production of reactive oxygen species (ROS). As a possible consequence of this, β-catenin dissociated from VE-cadherin (Monaghan-Benson and Burridge 2009). The importance of the cadherin–catenin complex for the adhesive function of cadherins makes the dissociation of this complex indeed an attractive target for mechanisms that counteract cadherin-mediated cell adhesion. Besides the study above, several other studies have reported that catenin phosphorylation correlated with the dissociation of β-catenin (Lilien and Balsamo 2005; Piedra et al. 2001, 2003) and plakoglobin (Miravet et al. 2003) from various cadherins. VEGF-induced tyrosine phosphorylation of β-catenin was also found to dissociate this catenin

from VE-cadherin (Chen et al. 2012). Interestingly, however, tyrosine phosphorylation of α-catenin was reported to correlate with enhanced binding to β-catenin (Burks and Agazie 2006). In addition, other groups have found that stimuli that trigger enhanced vascular permeability and tyrosine phosphorylation of the VE-cadherin–catenin complex do not necessarily lead to the dissociation of β-catenin from VE-cadherin (Adam et al. 2010; Andriopoulou et al. 1999; Konstantoulaki et al. 2003; Nottebaum et al. 2008; Timmerman et al. 2012). Despite these differences, most of these studies have in common that they argue for a role of tyrosine phosphorylation of the VE-cadherin–catenin complex in the regulation of endothelial junctions.

Kinases that influence endothelial barrier integrity

Several kinases have been identified that affect endothelial barrier integrity and phosphorylate proteins at endothelial junctions. The first of them to be described were members of the Src kinase family. VEGF-stimulated induction of vascular leaks was blocked in mice deficient for either Src or the Src family member Yes, whereas Fyn-deficiency had no effect (Eliceiri et al. 1999). Furthermore, blocking of endothelial Src also resulted in the inhibition of neutrophil transmigration through endothelial monolayers (Allingham et al. 2007). Binding of the catenin p120 to VE-cadherin was able to reduce Src-mediated VE-cadherin phosphorylation and this again resulted in decreased transendothelial migration of leukocytes (Alcaide et al. 2008). Various tyrosine residues of VE-cadherin have been described as being phosphorylated, directly or indirectly, by the activity of Src. Whereas Y658 and Y731 were described as targets downstream of Src in studies using corresponding commercially available site-specific anti phospho-tyrosine antibodies (Monaghan-Benson and Burridge 2009), a study based on peptide mapping suggested that Y685 was the exclusive tyrosine residue being phosphorylated upon VEGF mediated stimulation of Src (Wallez et al. 2007). VEGF was also described to stimulate a Src-dependent signaling cascade that leads to the activation of p21-activated kinase (PAK) followed by the phosphorylation of serine 665 on VE-cadherin, which creates a binding site for the association of β-arrestin. This in turn initiates clathrin-dependent endocytosis of VE-cadherin leading to the weakening of endothelial junctions (Gavard and Gutkind 2006). More recently, it was demonstrated that shear-induced junctional Src-activation leads to the phosphorylation of Y658 and Y685 of VE-cadherin in veins but not in arteries. Src-inhibition in this study blocked VE-cadherin phosphorylation and bradykinin-induced permeability (Orsenigo et al. 2012). Taken together, these studies suggest various mechanisms whereby Src stimulates phosphorylation of VE-cadherin, which in turn leads to weakening of endothelial junctions. However, it has also been reported that Src-mediated tyrosine phosphorylation of VE-cadherin alone is not sufficient to induce contact opening, as shown by overexpression of dominant-negative c-terminal Src kinase (Csk) (Adam et al. 2010). Csk inhibits Src by phosphorylation at Y527 (Okada and Nakagawa 1989). Interestingly, Src-mediated phosphorylation of Y685 of VE-cadherin creates a specific binding site for Csk (Baumeister et al. 2005). This interaction was implicated in cell density-dependent inhibition of cell growth. Whether binding of this negative regulator of Src might also initiate a negative feed-back loop that might serve to restrict junction opening is not yet known.

Apart from Src-family kinases, the redox-sensitive proline-rich tyrosine kinase 2 (Pyk2) is also involved in modulating endothelial integrity. Inhibition of Pyk2 prevents β-catenin phosphorylation and the Rac1-mediated loss of endothelial cell contact stability, a signaling pathway that is initiated upon loss of VE-cadherin function (van Buul et al. 2005). In addition, Pyk2 mediates the ICAM-1-triggered phosphorylation of VE-cadherin and downregulation of Pyk2 activity results in decreased leukocyte transmigration (Allingham et al. 2007). Also, the Pyk2-related focal adhesion kinase (FAK) has been shown to phosphorylate β-catenin and this β-catenin phosphorylation upon FAK-recruitment to VE-cadherin is necessary for VEGF-induced permeability (Chen et al. 2012). On the other hand, FAK was also reported to support the strengthening of endothelial junctions upon stimulation with sphingosine-1-phosphate (Belvitch and Dudek 2012).

Not only cytosolic kinases but also receptor tyrosine kinases (RTKs) are able to influence the stability of endothelial junctions. One is the VEGF receptor-2 (VEGFR-2) and another the angiopoietin receptor Tie-2. As already stated, stimulation of VEGFR-2 by VEGF results in VE-cadherin phosphorylation and loosening of endothelial contacts (Esser et al. 1998) and furthermore increases angiogenesis (Detmar et al. 1998). Ang1/Tie-2 signaling in turn increases the barrier function of endothelial junctions (Gamble et al. 2000; Mammoto et al. 2007). In addition to physiological Ang1/Tie-2 signaling, a short synthetic peptide that activates Tie-2 also increases the barrier function of cell contacts (David et al. 2011; Kumpers et al. 2011). Ang1-mediated Tie-2 signaling is capable of counteracting the VEGF-induced permeability increase in blood vessels (Thurston et al. 1999). Overexpression of Ang1 in vivo strongly stabilizes the endothelial barrier function, rendering the endothelium insensitive to increases in VEGF-induced permeability. Thus, the interplay between VEGFR-2-signaling and Tie-2-signaling is crucial for controlling endothelial barrier function.

Collectively, these reports illustrate that diverse kinases and therefore numerous regulatory pathways are involved in the control of endothelial barrier integrity. To ensure precise regulation of endothelial junction opening, the kinase-mediated phosphorylation has to be balanced by phosphatase-mediated dephosphorylation.

PTPs counteract kinases that influence endothelial barrier function

Protein-tyrosine-phosphatases (PTPs) can be grouped into classical PTPs, dual-specific PTPs and low-molecular-weight PTPs (Alonso et al.; 2004, Kappert et al. 2005). The classical PTPs, hereafter referred to as PTPs, can further be divided into membrane-spanning receptor PTPs (RPTPs) and cytosolic non-receptor PTPs (NRPTPs) or cytosolic PTPs. The latter consist in the catalytic domain and additional sequences that regulate their activity or localization. RPTPs display high variability in their extracellular region and possess one or two intracellular phosphatase domains. Extracellular ligands of RPTPs are to date largely unknown. However, RPTPκ and also RPTPμ can interact in trans in a homophilic way (Brady-Kalnay et al. 1993; Gebbink et al. 1993; Sap et al. 1994) and DEP-1 (CD148) interacts with components of matrigel (Sorby et al. 2001). Furthermore, heparan sulfate proteoglycans were shown to bind to RPTP-σ (Aricescu et al., 2002; Johnson et al. 2006).

Higher cell density and decreased phosphorylation of junctional proteins is accompanied by increased phosphatase activity in the membrane fraction (Gaits et al. 1995) and by increased phosphatase expression and higher cell contact localization of several phosphatases, including DEP-1, RPTPμ, and VE-PTP (Campan et al. 1996; Gaits et al. 1995; Nottebaum et al. 2008; Östman et al. 1994). Furthermore, a large scale of all PTPs by sodium orthovanadate or phenylarsine oxide results in higher phosphorylation of the cadherin–catenin complex and simultaneously increases transendothelial permeability and leukocyte transmigration (Young et al. 2003). Thus, cytosolic PTPs and RPTPs are important for the regulation of junctional integrity in endothelial cells. Several phosphatases are known to interact with junctional proteins and thus influence endothelial barrier function, among which are PTP1B, SHP-1, SHP-2, RPTPμ, DEP-1, and VE-PTP.

The first PTP to be discovered was the cytosolic PTP1B in the late 1980s (Charbonneau et al. 1989; Tonks et al. 1988a, b). Later, it was found that PTP1B binds to N-cadherin and dephosphorylates β-catenin, thus maintaining N-cadherin-mediated adhesion (Balsamo et al. 1996, 1998). PTP1B is in addition known to interfere with VEGF-mediated VEGFR-2-signaling, since it counter-regulates VEGF-induced phosphorylation of VEGFR-2. In line with this, PTP1B is involved in stabilizing VE-cadherin-mediated cell–cell adhesions by reducing VE-cadherin tyrosine phosphorylation (Nakamura et al. 2008).

SHP-2 is a cytosolic PTP that was reported to associate via β-catenin with the VE-cadherin–catenin complex. Thrombin stimulation increases SHP-2 phosphorylation and dissociates SHP-2 from the VE-cadherin–β-catenin complex, resulting in increased phosphorylation of the cadherin–catenin complex and decreased endothelial barrier integrity (Ukropec et al. 2000). SHP-2 function is necessary for maintaining endothelial cell contact integrity (Grinnell et al. 2009) and is moreover involved in the recovery of endothelial junctions after thrombin stimulation (Timmerman et al. 2012). SHP-2 furthermore interacts with the phosphorylated PECAM-1 cytoplasmic domain (Jackson et al. 1997; Masuda et al. 1997) and regulates its phosphorylation status (Cao et al. 1998), which in turn influences the association of SHP-2 to PECAM-1 (Cao et al. 1998; Newman and Newman 2003).

RPTPμ was probably the first PTP shown to interact with various cadherins, among them E-, N- and R-cadherin, as was analyzed in different cell types (Brady-Kalnay et al. 1995, 1998). RPTPμ also binds directly to VE-cadherin at endothelial cell contacts, which leads to its dephosphorylation and is accompanied by increased endothelial barrier function (Sui et al. 2005).

DEP-1, a member of the R3 subfamily of RPTPs, has also been reported to influence endothelial junction integrity. DEP-1 expression and activity are strongly enhanced upon increasing cell density (Östman et al. 1994), where DEP-1 plays a role in contact inhibition of growth by interfering with VEGFR-2 triggered cell proliferation (Lampugnani et al. 2003). On the other hand, it has been shown that DEP-1 dephosphorylates the inhibitory Y529 of Src and thereby supports VEGF-induced and Src-mediated stimulation of endothelial permeability (Spring et al. 2012). In addition, DEP-1 interacts with occludin and DEP-1 overexpression enhances epithelial barrier function, during tight junction assembly (Sallee and Burridge 2009). These studies indicate the complex roles of DEP-1 in regulating endothelial junction integrity. To further elucidate DEP-1 functions, gene-deficient mice were generated by different approaches. However, depending on the approach, results differed. Takahashi et al. (2003) observed that an in-frame replacement of DEP-1 cytoplasmic sequences with enhanced green fluorescent protein leads to embryonic lethality at embryonic day E11.5 due to disorganized vascular structures and growth retardation. Mutant yolk sacs and embryos exhibited strong defects in vascular remodeling. In contrast, DEP-1-deficient mice, generated by targeted disruption of the DEP-1 gene directly after the signal peptide sequence, were viable, healthy and fertile and showed no signs of embryonic defects. No obvious alterations in anatomy, life span, or spontaneous tumor appearance were detected (Trapasso et al. 2006). In line with this, DEP-1-loss-of-function mice generated by constitutive deletion of the DEP-1 transmembrane exon also did not display embryonic lethality (Zhu et al. 2008). While, in the first study, a mutant form of DEP-1 lacking the phosphatase domain is still present in the plasma membrane, no cell surface-located DEP-1 remains in the last two studies. Dominant negative effects in the former study are possible but have not yet been analyzed in detail.

The impact of VE-PTP on endothelial junction stability

Like DEP-1, VE-PTP belongs to the R3 subtype of RPTPs. VE-PTP is so far the only known phosphatase that is exclusively expressed in endothelial cells (Baumer et al. 2006; Fachinger et al. 1999). It consists in 17 FNIII-like extracellular domains, a transmembrane region and a single cytoplasmic phosphatase domain. VE-PTP is the murine homologue of human RPTPβ (Fachinger et al. 1999) and is crucial for embryonic development, as VE-PTP-deficient mice die around embryonic day E9.5/E10.0 due to severe vascular malformations (Baumer et al. 2006; Dominguez et al. 2007). Since the first vascular plexus develops normally in the absence of VE-PTP, this phosphatase is dispensable for the initiation of vasculogenesis but is essential for the following maturation and remodeling of vessel structures during angiogenesis (Baumer et al. 2006; Carra et al. 2012; Dominguez et al. 2007).

Our group and others have analyzed the role of VE-PTP in regulating the endothelial barrier function in more detail during the last years. An important interaction partner of VE-PTP is the adhesion molecule VE-cadherin (Nawroth et al. 2002; Nottebaum et al. 2008). VE-cadherin specifically coprecipitates with VE-PTP and, for this interaction, the membrane proximal extracellular domains of both proteins are sufficient (Nawroth et al. 2002). When elucidating the influence of VE-PTP on VE-cadherin function in more detail, it was shown that VE-PTP expression reverses VEGFR-2-induced tyrosine phosphorylation of VE-cadherin and increases the VE-cadherin-mediated barrier function (Nawroth et al. 2002). Furthermore, we found that VE-PTP is redistributed to endothelial contacts with increasing cell density, which is accompanied by an increased interaction of VE-PTP with VE-cadherin (Nottebaum et al. 2008). Downregulation of VE-PTP expression reduces VE-cadherin adhesiveness, leading to increased transendothelial permeability and leukocyte transendothelial migration. In addition, VE-PTP downregulation increases tyrosine phosphorylation of plakoglobin, which was identified as a direct substrate of VE-PTP. Plakoglobin is crucial for the contact-stabilizing function of VE-PTP (Nottebaum et al. 2008).

Importantly, VEGF-induced endothelial permeability correlates with the dissociation of VE-PTP from VE-cadherin. This dissociation was also detected upon leukocyte binding to TNF-α-inflamed endothelium and was accompanied by increased tyrosine phosphorylation of the VE-cadherin–catenin complex (Nottebaum et al. 2008). Recently, we were able to show that this VE-PTP-VE-cadherin dissociation is in fact necessary for efficient opening of endothelial contacts in vivo (Broermann et al. 2011). We demonstrated that specific stabilization of the VE-PTP-VE-cadherin interaction results in a lack of permeability induction after VEGF- or LPS-stimulation and reduces leukocyte extravasation in IL-1β- or LPS-stimulated tissues (Broermann et al. 2011). Stabilization of the VE-PTP-VE-cadherin association was achieved by fusing two additional protein domains (FKBP and FRB*) to the C-terminus of either VE-cadherin or VE-PTP, respectively. These domains contained different binding sites for a small molecular weight chemical compound (rapalog) that was able to strongly stabilize the interaction between VE-PTP and VE-cadherin. To analyze the effect of these modifications in vivo, we inserted the cDNAs for VE-cadherin-FKBP and VE-PTP-FRB* into the VE-cadherin gene locus of mice, thereby replacing endogenous VE-cadherin and ensuring endothelial specific expression. We found that administering the rapalog compound to these knock-in mice strongly inhibited the induction of vascular permeability by VEGF and LPS as well as the cytotkine-stimulated recruitment of neutrophils in vivo, whereas no such affect was seen with this compound in wild-type mice (Broermann et al. 2011). This established that the dissociation of VE-PTP from VE-cadherin is required for the opening of endothelial junctions in vivo.

Recently, the endothelial leukocyte-binding receptor and the downstream signaling pathway that trigger the dissociation of VE-PTP from VE-cadherin were identified. We found that binding of lymphocytes to VCAM-1 is necessary to induce this dissociation (Vockel and Vestweber 2013). In addition, we found that the signaling steps involved and required for this process comprised the activation of Rac1, the production of reactive oxygen species (ROS) via NADPH-oxidase and the activation of the kinase Pyk. Importantly, the same signaling cascade was also required for the VEGF-induced dissociation of VE-PTP from VE-cadherin (Vockel and Vestweber 2013). This is in good agreement with previous studies that showed that VEGF-induced phosphorylation of the VE-cadherin–catenin complex also required Rac activation and ROS production (Monaghan-Benson and Burridge 2009). The actual dissociation of VE-PTP from VE-cadherin is probably mediated by the binding of a phosphorylated substrate to VE-PTP, which leads to an allosteric change that dissociates the extracellular domains of these two proteins. The idea for this was based on the finding that a phosphatase-dead trapping mutant of VE-PTP could not bind to VE-cadherin, although robust binding of VE-PTP and VE-cadherin is mediated via their extracellular domains. This initiated the hypothesis that binding of other substrates to the phosphatase domain of VE-PTP would inhibit the association with VE-cadherin via allosteric effects. Testing this hypothesis, it was indeed possible to show that a model substrate of VE-PTP comprising a phosphorylated peptide of Tie-2 when introduced into endothelial cells via a fused Tat peptide could trigger the dissociation of VE-PTP from VE-cadherin, whereas the non-phosphorylated substrate had no such effect. Collectively, these results establish a Rac1, NADPH-oxidase, Pyk-dependent signaling pathway that contributes to the VEGF- and lymphocyte-induced destabilization of

endothelial junctions via dissociation of VE-PTP from VE-cadherin (Fig. 1).

VE-PTP has also been reported to associate with the VEGFR-2. Although this interaction does not support direct co-immunoprecipitation, it could be documented based on proximity ligation assays (Mellberg et al. 2009). In line with this, silencing of VE-PTP was accompanied by enhanced VEGFR-2 phosphorylation and downstream signaling. VE-PTP was also implicated in Tie-2-mediated balancing of VEGFR-2 effects and was suggested to be relevant for endothelial cell polarity and vessel lumen formation (Hayashi et al. 2013).

The tyrosine kinase receptor Tie-2 was actually the first VE-PTP-binding partner that was identified (Fachinger et al. 1999). According to this study, VE-PTP substrate trapping mutants co-precipitated with Tie-2 but not with VEGFR-2, and VE-PTP specifically dephosphorylated Tie-2. When elucidating the role of the VE-PTP–Tie-2 interaction in more detail, we found that antibodies against VE-PTP dissociate it from Tie-2 and trigger VE-PTP endocytosis whereas VE-cadherin-bound VE-PTP was not affected (Winderlich et al. 2009). This release of VE-PTP from Tie-2 led to the activation of this kinase receptor. This in turn activated Erk and Akt signaling and promoted cell proliferation, leading to circumferential growth and widening of vessel structures in newborn mice (Winderlich et al. 2009). These results recapitulated embryonic defects that had been described in VE-PTP gene-deficient mice (Baumer et al. 2006; Dominguez et al. 2007). In conclusion, this study showed that VE-PTP balances the activity of Tie-2, which helps in determining vessel diameter and remodeling of the vasculature (Winderlich et al. 2009).

It is well documented that Angiopoietin-1 (Ang-1) strongly supports stabilization of endothelial junctions and counteracts VEGF-induced vascular permeability in vivo (Thurston et al. 1999). This raises the question whether VE-PTP counteracts endothelial cell contact integrity by deactivating Tie-2. A first hint for this was suggested in a recent report (Goel et al. 2013). To understand the physiological relevance of VE-PTP for the regulation of vascular permeability it will be important to analyze this in more detail in the future. Interestingly, Ang-1

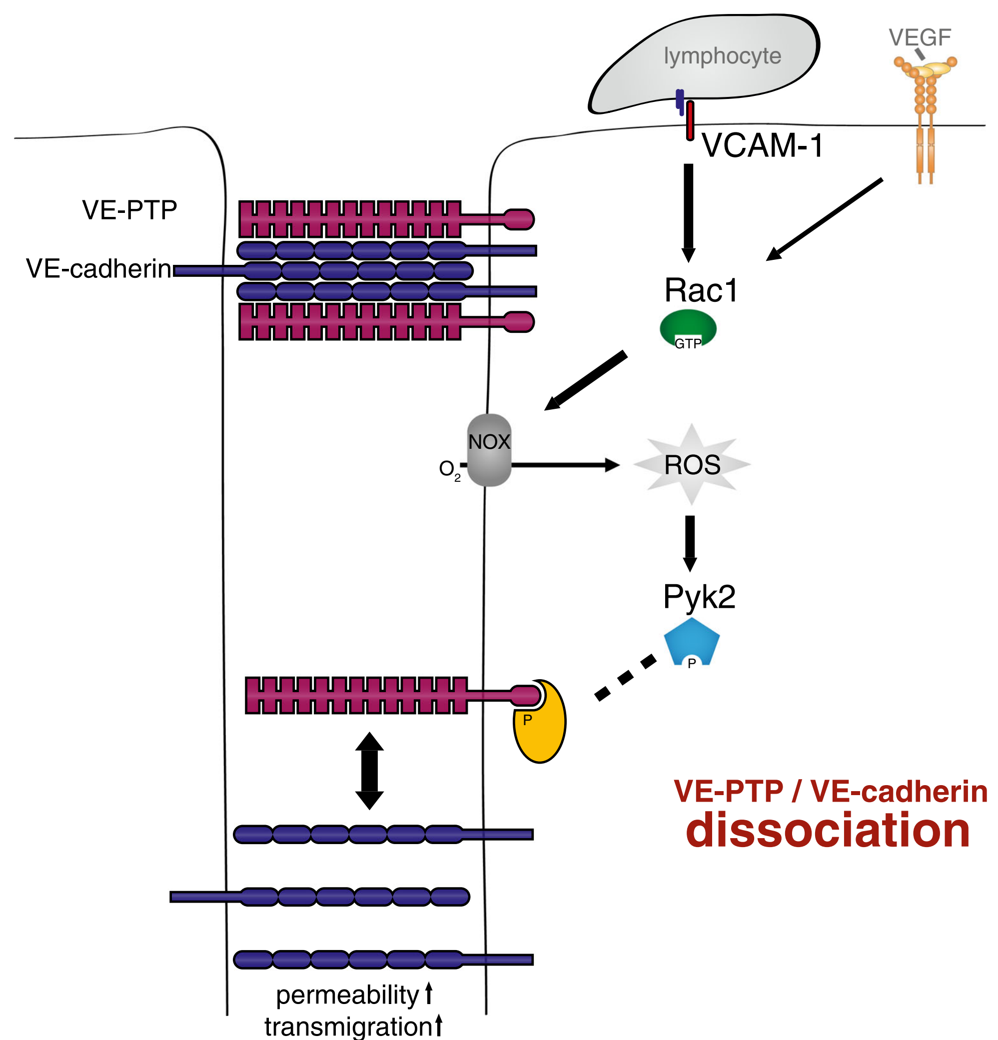

Fig. 1 Proposed signaling mechanism for the lymphocyte-induced dissociation of VE-PTP from VE-cadherin. Lymphocyte-binding to VCAM-1 or stimulation by VEGF triggers the production of reactive oxygen species (ROS) via Rac1-mediated activation of NADPH oxidase (NOX). This leads to activation of the redox-sensitive kinase Pyk2 that triggers directly or indirectly the phosphorylation of a VE-PTP substrate that in turn binds to VE-PTP. This binding may cause structural or conformational changes across the membrane that lead to the detachment of the extracellular domain of VE-PTP from VE-cadherin. This facilitates phosphorylation of components or associated factors of the VE-cadherin–catenin complex that participates in the destabilization of endothelial cell contacts. This figure was originally published in (Vockel and Vestweber 2013), ©The American Society of Hematology

stimulation induces the redistribution of Tie-2 to junctions where it probably connects Tie-2 of neighboring cells in trans (Saharinen et al. 2008). Together with Tie-2, the associated VE-PTP molecules are also redistributed to junctions. It will be interesting to analyze whether and how this re-distribution is relevant for the increase in junctional integrity.

Concluding remarks

As illustrated in this review, a complex set of membrane receptors and adhesion molecules as well as signal transducing kinases and phosphatases are involved in the regulation of endothelial junctions. These molecular mechanisms regulate the subcellular distribution, clustering cell surface expression of adhesion molecules such as VE-cadherin and their cytoskeletal linkage. In addition, these signaling mechanisms and others, such as GTPases and additional scaffolding proteins, regulate cytoskeletal activities that modulate the formation of cortical actin fibers and radial actin stress fibers. It is the interplay of these mechanisms that finally determines endothelial junction integrity. A deeper understanding of these mechanisms will allow the development of new strategies to interfere with a loss of junctional integrity of the vascular endothelium and thereby stop vascular leaks and harmful leukocyte invasion in inflammation.

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