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THE ROLE OF PLATELET ENDOTHELIAL ADHESION MOLECULE-1 (PECAM-1) IN MECHANOSENSING OF SHEAR STRESS AND OTHER ROLES OF THIS ADHESION MOLECULE IN VASCULAR BIOLOGY

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ABSTRACT
Platelet endothelial adhesion molecule-1 (PECAM-1 or CD41) is a molecule expressed on endothelial junctions between adjacent endothelial cells (EC). PECAM-1 is often used as a marker for vascular endothelium. However, recent studies found several other functions of PECAM-1 in vascular biology. Especially the role of PECAM-1 in mechanosensing of shear stress is interesting. Different signaling complexes for mechanosensing were proposed, such as the PECAM-1/SHP-2 complex and the mechanosensory complex. Both complexes initiate transduction pathways with downstream consequences. Besides the role of PECAM-1 in mechanosensing of shear stress, is PECAM-1 also known to play a role in: leukocyte transmigration, vascular permeability, atherosclerosis, angiogenesis, thrombosis and stability of the cytoskeleton. This makes PECAM-1 a unique and important molecule in vascular biology. Therefore, this review discusses what is known about the mechanosensing of shear stress by PECAM-1 and other roles of this adhesion molecule in vascular biology.

INTRODUCTION
ECs form the inner linings of blood vessels and participate in a variety of tasks: regulation of permeability, migration, remodeling, proliferation, apoptosis, the production, secretion and metabolism of biochemical structures, as well in regulating contractility of vascular smooth muscle cells (SMC) (1). The endothelial monolayer functions as gatekeeper, controlling infiltration of proteins and cells into the vessel wall. Specialized transport vesicles are important for transendothelial passage of soluble macromolecules (2). Furthermore, coordinated opening and closure of cell-cell junctions participate in transendothelial passage of molecules. Tight regulation of these cell-cell junctions is necessary for protecting blood vessels from inflammation, thrombotic reactions or leukocyte transmigration (2,3).

Adhesion of ECs occurs through junctional complexes formed by transmembrane adhesive proteins, with cytoplasmic tails, resulting in homophilic interactions with adhesion molecules on neighboring cells. Cytoplasmic tails of adhesive proteins bind to the cytoskeleton, anchoring those adhesive proteins to actin filaments and transferring intracellular signals inside the cell (3). The nectin-afadin complex was found to help anchoring junctional complexes. Nectin binds inside cells to afadin, and afadin binds to ponsin and actin (4).

Two major types of junctional complexes have been described; tight junctions (TJs) and adherens junctions (AJs) (2,3). At the apex of endothelial cells were TJs found adhering ECs to one another. Adhesion in TJs is mediated by claudins, occludins, junctional adhesion molecules (JAM) and endothelial cell selective adhesion molecules (ESAM). These molecules interact with each other and with other molecules. TJs regulate the ion influx between adjacent ECs and provide a barrier within the membrane by regulating paracellular permeability and maintaining cell polarity (3). AJs contain cadherins and catenins, attached to cytoplasmic actin filaments for stability of the cell-cell junctions. Vascular endothelial cadherin (VE-cadherin) is a specific calcium dependent transmembrane adhesion protein and is associated with vascular endothelial protein tyrosine

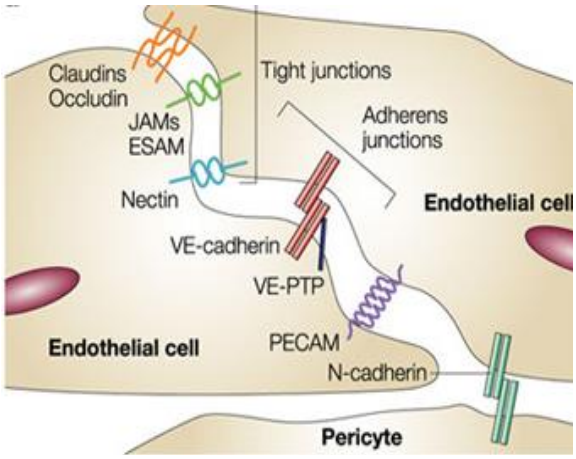

Figure 1: Dejana et al., 2004; The organization of endothelial cell-cell junctions.
Claudins, occludins, JAMs, and ESAMs mediate adhesion in tight junctions. VE-cadherin and VE-PTP mediate adhesion in adherens junctions. Nectin helps anchoring junctional complexes to endothelial cells. PECAM-1 contributes outside junctional structures to endothelial cell-cell adhesion. Finally N-cadherin induces adhesion of endothelial cells to pericytes.

phosphatase (VE-PTP). VE-cadherin ensures to a large extent the adhesion in AJs and cannot be found in other cell types than ECs (2,3). AJs are important for contact inhibition of endothelial cell growth, paracellular permeability to circulating leukocytes and angiogenesis (3).

Binding of adhesive proteins with actin filaments is crucial for not only the stabilization of junctions, but specific for the dynamic regulation of opening and closing of the relevant junction (2). TJs and AJs were intermingled found throughout the cleft between two adjacent ECs. Therefore, a possible interaction or influence between TJs and AJs could be observed. Furthermore, variation was found in junctional complexes in different vascular beds and organs (2). For example, TJs are poorly organized in post-capillary venules, but complex and well organized in the blood brain barrier, were permeability is important and strictly controlled (5).

Besides the specialized junctional structures, other cell-specific homophilic adhesion proteins were expressed at EC intercellular junctions. Neuronal-cadherin (N-cadherin) is diffusely distributed on the cell membrane, and promotes adhesion and communication of ECs with pericytes or astrocytes (6). Furthermore, platelet endothelial cell adhesion molecule-1 (PECAM-1), also known as CD31, could be important for endothelial functioning. Figure 1 shows an overview of the organization of endothelial cell-cell junctions.

PECAM-1 is encoded by a 75-kb gene at the end of chromosome 17 and is a member of the immunoglobulin (Ig) gene superfamily. PECAM-1 is expressed at 1 or 2 x 10^6 molecules per cell. Mature PECAM-1 consists of six Ig-like homology domains, a 574-amino acid extracellular domain, 19-residue transmembrane domain and a 118-amino acid cytoplasmic tail (7-10). The first two Ig-like homology domains of the extracellular domain of PECAM-1 are involved in homophilic binding between adjacent (neighboring) ECs for stabilization of endothelial cell-cell junctions (10). The cytoplasmic domain of PECAM-1 binds to β-catenin and γ-catenin and contains two tyrosine residues that could be phosphorylated. If these two tyrosine residues of PECAM-1 are phosphorylated, they start several transduction pathways with downstream consequences (9,11,12). PECAM-1 is highly expressed in all human ECs of existing and newly formed blood vessels, making it a useful and often used endothelial marker nowadays (13). Low density expression of PECAM-1 was found on the surface of hematopoietic and immune cells, including monocytes, macrophages, neutrophils, mast cells, natural killer cells, lymphocytes and platelets (14).

Furthermore, is PECAM-1 known to have different important roles in vascular biology, such as: mechanosensing of shear stress, regulation of leukocyte transmigration, vascular permeability, atherosclerosis, angiogenesis, thrombosis and stabilisation of the cytoskeleton. Therefore, the aim of this review is to discuss the different roles of PECAM-1 (especially the role of PECAM-1 in mechanosensing of shear stress) in vascular biology.

The role of PECAM-1 in mechanosensing of shear stress .

Shear stress is the force that flow exerts per unit area of the vessel wall and can be expressed in units of dyn/cm^2 (15). Exercise or diseases associated with chronically increased cardiac output causes increased shear stress. Decreased shear stress was found during heart failure or hypotension. During cardiac arrest or hemorrhagic shock, were sudden and abrupt decreases of shear stress found (16). Normal laminar blood flow increases cytoskeletal tension and promotes cell-cell contact. Meanwhile disturbed blood flow decreases cellular forces leading to possible dislocation of cell-cell contacts and endothelial dysfunction (17,18).

Alterations in shear stress are sensed by ECs. After exposure to decreased shear stress are ion channels activated within seconds. Potassium channels and certain stretch-sensitive ion channels are activated resulting in hyperpolarization, generation of reactive oxygen species (ROS) and increased calcium influx. Hereafter, closing of potassium channels causes depolarization, activation of kinases and release of nitric oxide (NO) (11,19). Several hours later, various cellular micro domains and membrane adhesive proteins react to changes in shear stress and act as mechanosensors.

Several suggested mechanosensors were described and discussed in recent literature. One of the proposed mechanosensors are cytoskeleton proteins. These proteins are linked with components of the EC matrix by integrins, these integrins function as mechanosensor. Integrins are

transmembrane receptors important for cell-cell adhesion. Mechanical forces induced by blood flow, may be transferred from cytoskeleton towards integrins, hereby dividing mechanical forces by rearranging interlinked microtubule and microfilaments (15). In addition, PECAM-1 could function as mechanosensor for shear stress (12,20,21). Blood flow stretches actin, a cytoskeleton protein which is bound to PECAM-1 via an unknown cytoskeletal linker complex, and the force is transduced and sensed by PECAM-1 (22). See also figure 2.

A complex response, due to the sensing of mechanosensors, occurs in ECs. This response, called mechanotransduction, describes the response to cellular physical or mechanical stimuli, such as shear stress, and translate these into biochemical signals that result in a physiological response (15). Mechanotransduction induced by shear stress requires several steps: first, deformation of the apical surface of endothelium; second, intracellular transmission of stress; third, mechanical forces have to be converted into biological activity; and fourth, further downstream intracellular signaling (23). No individual mechanosensor has been identified definitively and mechanisms of transduction remain poorly understood. However, there are mechanosensing complexes initiated by PECAM-1 as reaction to shear stress described in literature, such as: the PECAM-1/SHP-2 complex and the mechanosensory complex. Both complexes result in transduction and a variety of downstream consequences and will be discussed in the next session.

PECAM-1/SHP-2 complex

Forming of the PECAM-1/SHP-2 complex for mechanosensing starts with phosphorylation of tyrosine residues in the cytoplasmic tail of PECAM-1, as a response to cellular stimulation caused by shear stress. PECAM-1 cytoplasmic membrane phosphorylation regulates signaling complexes and interactions of PECAM-1 with the cytoskeleton. Recent studies found a role for sarcoma (Src) family kinases in PECAM-1 tyrosine phosphorylation as response to shear stress. See also figure 3. No shear stress makes it impossible for Src-family kinases to reach the phosphorylation sites of PECAM-1 (7,9,10,20,21). However, Src family kinase inhibitors does not block the PECAM-1 phosphorylation entirely (24). Therefore, more research is necessary to determine if PECAM-1 is phosphorylated by Src family kinases or non-Src family kinases and whether the tyrosine kinases able to phosphorylate PECAM-1 are dependent of each other.

In human PECAM-1 are tyrosine residues at position 663 and 686 the tyrosine phosphorylation sides, because phosphorylation of PECAM-1 failed after replacement of those tyrosine residues by phenylalanine (25). Recent studies found that PECAM tyrosine phosphorylation is not induced through simply increased calcium or potassium levels (26,27). ECs which were treated with thrombin, adenosine triphosphate (ATP), histamine, bradykinin or acetylcholine to increase calcium levels, were not able to induce tyrosine phosphorylation of PECAM-1. Also barium and tetraethyl ammonium ion, which are potassium channel inhibitors, failed to block PECAM-1 tyrosine phosphorylation. Furthermore, stretch activated channel blockers, such as gadolinium failed to inhibit PECAM-1 tyrosine phosphorylation. As well there was no tyrosine phosphorylation of PECAM-1 through activated protein kinase C (PKC) (26,27). Together these results suggest that the tyrosine phosphorylation of PECAM-1 is self-contained and independent of ion channels.

Around tyrosine 663 and 686 were two immunoreceptor tyrosine-based inhibitory motifs (ITIM) found. Paired ITIM within the PECAM-1 cytoplasmic domain ensures that PECAM-1 is part of the immunoglobulin-ITIM family of inhibitory receptors. Tyrosine phosphorylated PECAM-1 is able to recruit Src homology 2 (SH2) domain containing signaling proteins, which leads to downstream signaling. SHP-2, a SH2 domain containing protein tyrosine phosphatase, is the most reported and investigated protein with a PECAM-1 interaction. SHP-2 transmits mechanical force between the extracellular matrix and interacting cell. PECAM-1 phosphorylation causes both recruitment and activation of SHP-2 (20).

Several other SH2 domain containing proteins and adaptor molecules have abilities to associate with PECAM-1. For example Gab1 is an important mediator of cellular growth and apoptosis, which co-localizes with PECAM-1 and SHP-2 after exposure to shear stress (8). Furthermore, PECAM-1 is the only known ITIM-containing receptor on human platelets and SHP-2,

which binds to PECAM-1 and transmits both stimulatory and inhibitory signals to cell. This makes it interesting to look whether PECAM-1 has positive or negative regulatory effects on endothelium (8).

Formation of the PECAM-1/SHP-2 complex for mechanosensing, as mentioned above, is required for the activation of signaling transduction pathways. The mitogen-activated protein kinase (MAPK)/extracellular signal regulated kinase (ERK) pathway could be initiated by the PECAM-1/SHP-2 complex (8,20,21,27). Based on these data Fujiwara (11) hypothesis that ERK activation is an event downstream of the PECAM-1/SHP-2 complex. This proposed signaling pathway starts with PECAM-1 tyrosine phosphorylation, followed by the recruitment and activation of SHP-2 as mentioned above, resulting in phosphorylation and thus activation of RAS, B-RAF binds RAS and phosphorylation of ERK occurs. See also figure 2. Removal of a phosphate group inactivates this MAPK/ERK pathway. After activation of ERK are AP-1 transcription factors activated and reach the cell nucleus. Several genes, such as growth factors and cytokines are translated and expressed (11). Thus, the MAPK/ERK pathway, regulated by PECAM-1, functions as an on-off switch by adding phosphate groups to a neighboring protein resulting in gene activation.

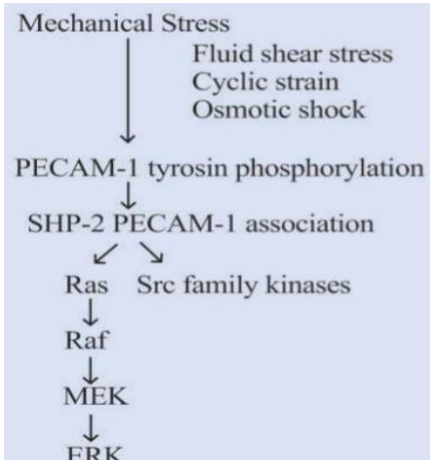

Figure 2: Fujiwara, 2006; PECAM-1 dependent mechanosignaling cascade.
Shear stress causes PECAM-1 tyrosine phosphorylation, resulting in the PECAM-1/SHP-2 complex. This complex activates the MAPK/ERK signaling pathway, finally resulting in gene activation.

Mechanosensory complex

Another interesting observation is the mechanosensory complex (mechanosome) proposed by Tzima et al. (28). This study found a connection between PECAM-1, VE-cadherin and vascular endothelial growth factor receptor 2 (VEGFR-2) in sensing shear stress. In this identified complex functions VE-cadherin as an adaptor protein and ensures contact between VEGFR2 and PECAM-1. See also figure 3. This new formed complex of VEGFR2 and PECAM-1 allow Src-family kinases to phosphorylate VEGFR-2 and in this way is the phosphatidylinositol-3-OH-kinase (PI3K) signaling pathway activated (22). PI3K ensures integrin activation within fifteen seconds after disturbances in blood flow and activates protein kinase B, also known as Akt. This phosphorylated and thus activated PI3K/Akt pathway has several functions downstream, such as inhibition of apoptosis, stimulation of the translation of mRNA into protein and stimulating proliferation (28). Thus, the mechanosensory complex causes responsiveness of ECs to blood flow and activates pro-inflammatory signaling pathways in response to disturbed flow.

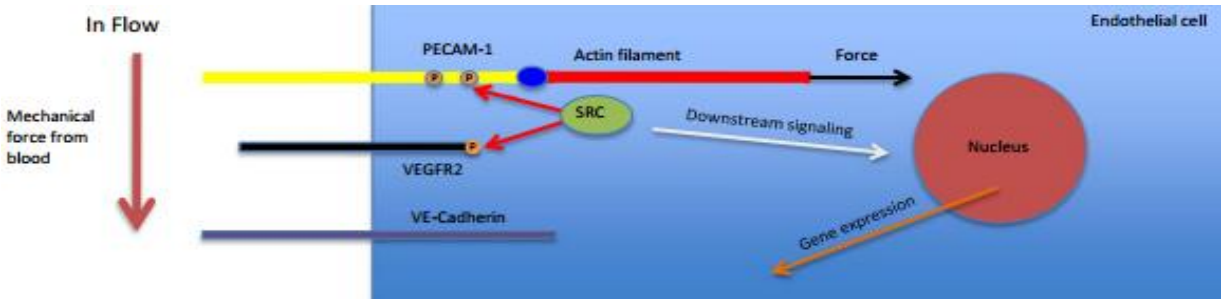

Figure 3: Wragg et al., 2014; Mechanosensing of shear stress by PECAM-1
Mechanical force from blood stretches the actin filaments and the force is transduced to PECAM-1. PECAM-1 is pulled into a linear state and the phosphorylation sites of PECAM-1 are attainable for Src-family kinases, for example SHP-2. This results in activation of the MAPK/ERK pathway, as described in the PECAM-1/SHP-2 complex. In addition, VE-cadherin ensures contact between VEGFR2 and PECAM-1. Now is the phosphorylation site of VEGFR2 attainable for Src-family kinases. This results in activation of the PI3K/Akt pathway, as described in the mechanosensory complex.

Further research at the mechanosensory complex and disturbance or stop in blood flow, related to loss of shear stress was conducted. Several more effects were found after the activation of PI3K. Sensing of flow by the mechanosome results in closure of potassium ATP channels. Subsequently, EC membrane depolarization takes place, resulting in the phosphorylation of several kinases including PI3K. This phosphorylation leads to activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase 2 (NOX2). This protein is a superoxide generating enzyme which forms eventually ROS. Increased ROS production causes damage and is often found as response to impeded blood flow. These increased levels of ROS transmit signals towards transcription factors as nuclear factor kappa-light-chain-enhancer of activated B cells (NFκβ), resulting in increased cell proliferation and neovascularization attempting to restore the impeded blood flow and loss of shear stress (15).

Moreover, after EC membrane depolarization as a consequence of the mechanosensory complex, T-type voltage gated calcium channels are opened. As a result of increased cytoplasmic calcium concentrations endothelial is nitric oxide synthase (eNOS) activated and converted into NO. Increased levels of NO are important for the regulation of the vascular tone. Production of NO results in vasodilatation and is also an attempt to restore the impeded blood flow. All these processes are initiated by PECAM-1, which measures in the endothelium the changes in blood flow and corresponding shear stress (15). See also figure 4.

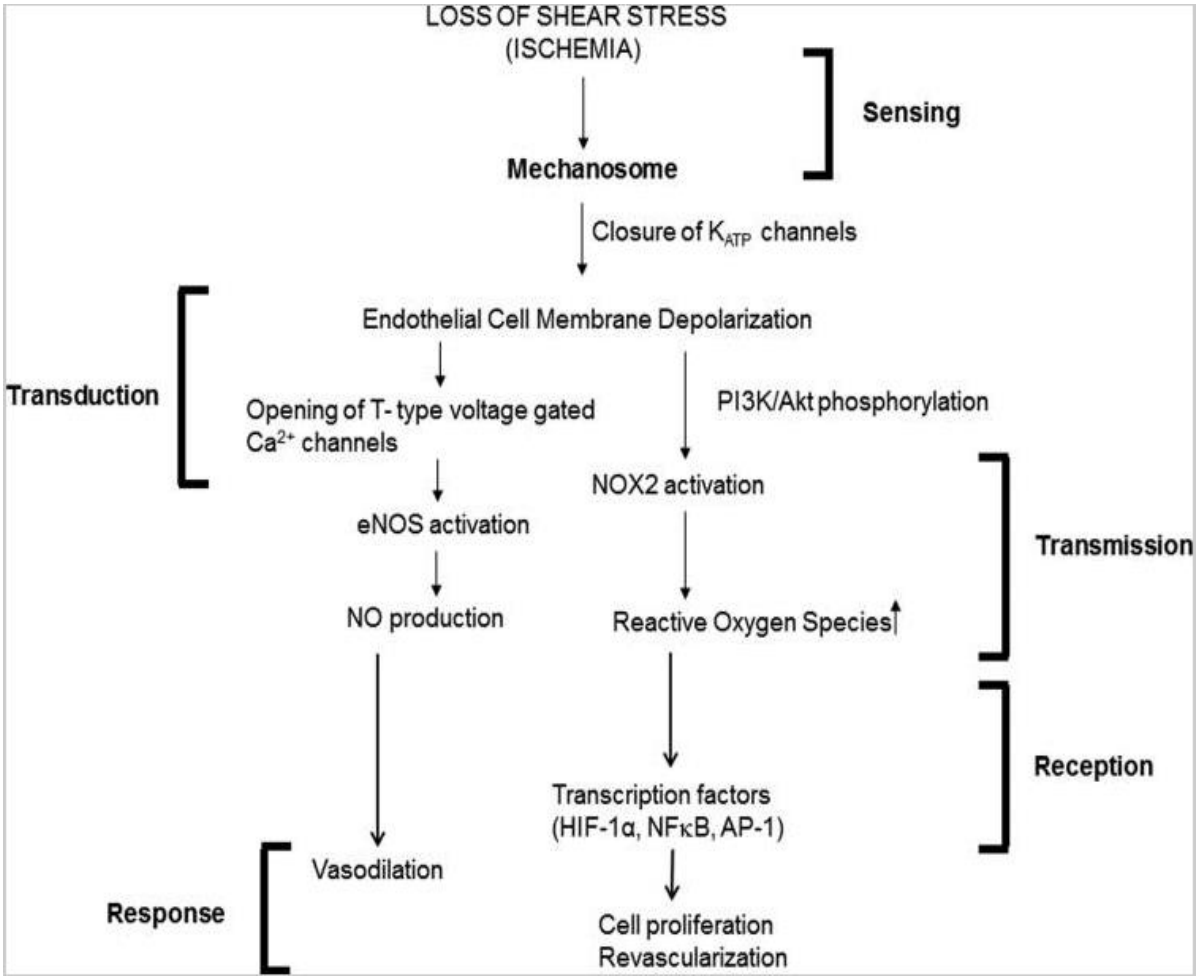

Figure 4: Chatterjee & Fischer, 2014; Schematic representation of the mechanotransduction cascade after loss of shear stress. The mechanosome senses blood flow and consist of PECAM-1, VEGFR-2 and VE-cadherin. This sensing results in closing of potassium channels after loss of shear stress or stop in blood flow. Closure of potassium channels causes membrane depolarization, resulting in the opening of calcium channels and PI3K/Akt phosphorylation. Opening of calcium channels causes NO production and finally vasodilatation, as attempt to restore impeded blood flow. PI3K/Akt phosphorylation causes production of ROS. ROS production ensures the activation of transcription factors, finally resulting in neovascularization, also as attempt to restore impeded blood flow.

Other roles of PECAM-1 in vascular biology

Leukocyte transmigration

Besides the role of PECAM-1 in mechanosensing of shear stress, several other roles of PECAM-1 in vascular biology are known. To start with leukocyte transmigration. After an immunological response leukocytes bind to activated ECs. Adhesion of leukocytes is followed by diapedesis. Leukocytes squeeze through the borders between ECs and penetrated out of the circulatory system towards underlying damaged inflamed tissue. Chemoattractants on EC surface capture leukocytes and leukocytes are carried along with the blood flow with a certain degree of velocity. This rolling of leukocytes occurs until leukocytes slow down and bind to the endothelium. After firm adhesion, are leukocytes transmigrated through the endothelium towards tissue. These processes are controlled and regulated by selectins and integrins (29). Furthermore, proteins at cell-cell junctions, such as PECAM-1, have an important role in transmigration of leukocytes. Muller et al. (30) were the first who determined that PECAM-1 is required for transendothelial migration of leukocytes. A monoclonal antibody, or recombinant soluble to PECAM-1, blocks the migration of monocytes by 70-90% (30).

Besides mediating leukocyte transmigration through ECs, PECAM-1 also mediates leukocyte transmigration through the perivascular basement membrane after trespassing the EC layer (31). The mechanisms by which PECAM-1 mediate leukocyte transmigration through blood vessel walls remain unclear, because PECAM-1 has not been reported to interact directly with molecules and mechanism of leukocyte transmigration depend on the signaling properties of PECAM-1.

However, several explanations for the effects of PECAM-1 on leukocyte transmigration were found in literature. A possible explanation could be enhanced migration of neutrophils through the vessel wall, as result of signal transduction caused by PECAM-1/PECAM-1 interactions at EC junctions. The mechanisms of these homophilic interactions of PECAM-1 with its own ligand are not completely understood, but may be regulated by the large cytoplasmic domain of PECAM-1. Processes of neutrophil transmigration are accelerated, suggesting the ability of PECAM-1 to regulate neutrophil mobility. Furthermore, the ligation of PECAM-1 at EC junctions facilitates movement of neutrophils through the basement membrane by triggering integrins, which assists in the interaction of leukocytes with components of the basement membrane. The ligation of PECAM-1 regulates expression of leukocyte proteolytic enzymes at the cell surface, facilitating in this manner also movement of neutrophils through the basement membrane (32).

Moreover, PECAM-1 is not only observed in junctions, but also in the lateral border recycling compartment (LBRC) (33). The transmigration of leukocytes through EC junctions is regulated by LBRCs. LBRCs are membrane networks, located just below the cell borders of adjacent ECs, and contain several elements including PECAM-1. During transmigration of leukocytes is PECAM-1 recycled in LBRC and targeted towards segments of the junction where migration of monocytes is occurring. In addition, if antibodies for PECAM-1 were used, is the recruitment and translocation of LBRC (and PECAM-1) towards zones of leukocyte migration blocked. Monocytes were unable to transmigrated through the junctions (33). Unfortunately, no literature was found about the regulation of opening and closing of cell-cell junctions controlled by PECAM-1 and other elements of the LBRC.

Vascular permeability

Correct and precise regulation of vascular permeability is essential for leukocyte transmigration and response to inflammation. Recent studies found a role for PECAM-1 in the regulation of vascular permeability between ECs. The contribution of PECAM-1 expression towards experimental autoimmune encephalomyelitis (EAE), a mouse model for inflammation, was investigated. EAE is an inflammatory disease of the central nervous system in mice and was used as model to study human multiple sclerosis (34). PECAM-1 mediated signaling or adhesion could play a role in these processes. PECAM-1 knock out mice showed an earlier onset of clinical symptoms during EAE compared with wild-type control mice, associated with increased migration of leukocytes across the blood brain

barrier. Furthermore, an impaired ability to restore vascular integrity was found in PECAM-1 knock out mice (34).

PECAM-1 deficient mice exposed to lipopolysaccharide (LPS), triggering an acute inflammatory shock comparable with human septic shock, show reduced survival and increased mortality compared with wild-type control mice (35,36). Deficit PECAM-1 expression at ECs junctions could be the cause for this increased mortality. Moreover, PECAM-1 deficient mice showed increased vascular permeability, increased apoptosis and higher expression of tumor necrosis factor-αlfa (TNF-α), interferons and cytokines compared with wild-type control mice (35,36).

Atherosclerosis

A connection between endothelial dysfunction and low shear stress was found in the development of atherosclerosis. Low shear stress and thus slow blood flow can be observed in certain areas of the vascular system. Branch points with low shear stress demonstrate appearance of leukocyte adhesion molecules, transmigration of leukocytes, increased uptake of lipoproteins, secretion of growth and chemotactic factors, proliferation of macrophages and SMC resulting in atherosclerotic plaques (37). Furthermore, is NF-κβ activated by disturbed shear stress. Consequences of NF-κβ activation are the expression of NF-κβ dependent genes such as: vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), endothelial (E)-selectin and platelet derived growth factor (PDGF) at atherosclerosis susceptible sites (37). On the other side are in blood vessels with steady shear stress these events, as mentioned above, downregulated. This downregulation protects to a certain degree against atherosclerotic plaques (28).

Furthermore, apolipoprotein E (ApoE) deficient mice, a mouse model for atherosclerosis, were used to investigated the effects of PECAM-1 in atherosclerosis (38,39). Double knock out mice for both PECAM-1 and ApoE result in fewer lesions in their aorta compared to single knock out mice on high-fat Western diet. By these single knock out mice, only ApoE was knocked out and PECAM-1 expression remain normal as in wild-type controls. Reduced macrophage infiltration, as well reduced expression of ICAM-1, VCAM-1 and NF-κβ were found in double knock out mice compared with single knock out mice. Thus, changes in shear stress are sensed by PECAM-1 and promotes atherosclerotic lesions and plaques. Deficiency of PECAM-1 results in atheroprotective effects, but do not prevent atherosclerosis (38,39). However, interesting results were found about effects of PECAM-1 deficiency in developing atherosclerosis in low-density lipoprotein (LDL) receptor deficient mice. These results suggest that PECAM-1 has both atheroprotective and atherosclerotic effects, depending on which segment of the aorta is affected (40).

Angiogenesis

PECAM-1 is also important in the process of angiogenesis. Recent studies found decreased angiogenesis of transplanted tumors in mice treated with anti PECAM-1. Anti PECAM-1 also causes inhibition of tube formation and transmigration by human umbilical vein endothelial cells (HUVEC) (41). A possible hypothesis could be that PECAM-1/PECAM-1 homophilic reactions between ECs in blood vessels with stable flow promote stable associations between ECs by modulating the phosphorylation of catenins. This suppresses the transmigration of cells through the endothelial layer. However, by angiogenetic stimuli is PECAM-1 removed from the endothelial junctions, resulting in the loss of inhibition of cell migration and more surface PECAM-1 available for heterophilic interactions. Later on as ECs are reorganized into new blood vessels, are the PECAM-1/PECAM-1 interactions reestablished and facilitate stabilization for the EC interactions. Hereby, suppressing EC motility and finally ceasing movement of ECs, resulting in formation of new vascular tubes (41).

Transfectants expressing PECAM-1 or mutant PECAM-1, in which tyrosine residues 663 and 686 were mutated, were used in another study investigating the role of PECAM-1 in angiogenesis (42). This study found that mutant PECAM-1 cannot be phosphorylated and binding of PECAM-1 and SHP-2, forming the PECAM-1/SHP-2 complex as earlier mentioned, does not occur. SHP-2 transmits mechanical force between the extracellular matrix and the interacting cell, which is essential for

efficient cell motility, an important condition for angiogenesis. So there was no phosphorylation of PECAM-1 and SHP-2, but presence of PECAM-1 at intercellular junctions and homophilic bindings remain. However, motility and new blood vessel formation were reduced in the PECAM-1 mutant (42). These results suggest, that binding of SHP-2 to PECAM-1 through tyrosine residues, promotes endothelial cell motility and the forming of new blood vessels.

The role of PECAM-1 in angiogenesis was also investigated in several other studies. An antibody to human PECAM-1, which cross-reacts with rat PECAM-1, blocks new blood vessel growth and corneal neovascularization in rats. (43). Moreover, PECAM-1 deficiency has impact on cell-cell interactions in endothelium, resulting in less migration, less capillary morphogenesis and lower rates of apoptosis. These changes were associated with a decreased expression of eNOS and NO. Upregulation of PECAM-1 was sufficient for the recovery of migration and morphogenesis in those mice (44).

Thrombosis

Falati et al. (47) showed that in PECAM-1 deficient mice larger, more rapidly and more stable thrombi were found compared with wild-type control mice. In control mice, who received transplants of PECAM-1 deficient bone marrow, were also larger thrombi found compared with mice that received control transplants. Furthermore, the time in which 75% of vessel occlusion was developed is significantly shorter in PECAM-1 deficient mice compared with wild-type control mice (47). Thus these data provide evidence for involvement of PECAM-1 in the regulation of thrombus formation.

Cytoskeleton

Suggestions were made in literature about PECAM-1 and the underlying cytoskeleton. In confluent ECs is 25% of PECAM-1 associated with the cytoskeleton, during migration is 65% of PECAM-1 associated with the cytoskeleton (45). PECAM-1 and the actin cytoskeleton are connected or linked by catenins. Specific β-catenin and γ-catenin, these catenins are scaffolding proteins anchoring cadherins to the actin cytoskeleton. PECAM-1/β-catenin complex forming requires ITIM tyrosine phosphorylation of β-catenin, because β-catenin binds to the same tyrosine residues (663 and 686) as SHP-2. Therefore, SHP-2 possibly regulates the phosphorylation of β-catenin (45). PECAM-1 is also proposed to act as an integrin modulator. Integrins have two main functions: attachment of cells to the extracellular matrix and signal transduction from the extracellular matrix to the cell. Anti PECAM-1 mice showed an upregulation of integrin functioning. PECAM-1 influences several integrin dependent processes, such as leukocyte transmigration as mentioned before (46).

CONCLUSION

In the last 15 years is enormous progress made in understanding the diverse roles of PECAM-1 in vascular biology. Now it is known that PECAM-1 has an important role in mechanosensing of shear stress by forming sensing complexes which activates several transduction pathways with downstream consequences. Furthermore, roles of PECAM-1 were found in regulating leukocyte transmigration, vascular permeability, atherosclerosis, angiogenesis, thrombosis and stability of the cytoskeleton. Although many functions of PECAM-1 are discovered, new observations are still made about the role of PECAM-1 in vascular biology. This suggests that not yet everything is known about PECAM-1. Future research could possibly define more roles of PECAM-1 in vascular biology. If the molecular pathways and mechanisms of PECAM-1 are defined in more detail, PECAM-1 could be an interesting molecule for therapeutic targeting. All these different roles of PECAM-1, also in vascular disease, makes PECAM-1 an interesting and hot topic in research.

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