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Endothelial functions of PECAM-1 (CD31)

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Abstract

Purpose of review—The purpose of this article is to describe the function of the vascular cell adhesion and signaling molecule, PECAM-1, in endothelial cells, with special emphasis on its role in maintaining and restoring the vascular permeability barrier following disruption of the endothelial cell junction.

Recent findings—In addition to its role as an inhibitory receptor in circulating platelets and leukocytes, PECAM-1 is highly expressed at endothelial cell-cell junctions, where it functions as an adhesive stress-response protein to both maintain endothelial cell junctional integrity and speed restoration of the vascular permeability barrier following inflammatory or thrombotic challenge.

Summary—Due to the unique ability of antibodies that bind the membrane-proximal region of the extracellular domain to trigger conformational changes leading to affinity modulation and homophilic adhesion strengthening, PECAM-1 might be an attractive target for treating vascular permeability disorders.

Keywords

PECAM-1; CD31; endothelial cell junctions; vascular permeability

INTRODUCTION

Platelet/endothelial cell adhesion molecule-1 (PECAM-1) was originally described in the mid-1980's as the CD31 differentiation antigen expressed on the surface of human granulocytes, monocytes and platelets [1–4]. At the same, several other groups

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independently reported the presence of an endothelial cell surface antigen - variously known as glycoprotein (GP) IIa [5], GPIIa' [6], hec7 antigen [7], and EndoCAM [8] - that became highly enriched at cell-cell junctions. Screening of an endothelial cell cDNA expression library with an antibody specific for platelet integral membrane proteins led to the cloning of a 130 kDa protein having homology with recently cloned cell adhesion molecule members of the immunoglobulin gene (Ig) superfamily, and the protein was named platelet/endothelial cell adhesion molecule-1 (PECAM-1) to denote its cloning origins, its family membership, and its likely function [9]. Immunochemical and biochemical characterization, together with its subsequent cloning from two different leukocyte libraries [10,11] established that the endothelial cell junctional protein, the CD31 hematopoietic differentiation antigen, and platelet PECAM-1 were identical entities, facilitating investigation of the role that this cell adhesion and signaling molecule plays in the biology of blood and vascular cells. A number of excellent reviews exist on the function of PECAM-1 in platelet biology [12], in signal transduction [13,14], and on its role in leukocyte transendothelial migration and inflammation [15,16]. In contrast, this chapter will focus primarily on the role that PECAM-1 plays in endothelial cell biology, with a special emphasis on the homophilic adhesive properties of the PECAM-1 extracellular domain and how it functions to regulate the endothelial cell vascular permeability barrier.

STRUCTURAL FEATURES OF THE EXTRACELLULAR AND CYTOPLASMIC DOMAINS, AND TISSUE DISTRIBUTION

PECAM-1 (Figure 1) has a molecular mass of 130 kDa, approximately 40% of which is carbohydrate [9,19]. The 574 amino acid extracellular domain is comprised of six Ig homology domains of approximately 100 amino acids each, the amino terminal two of which belong to the I2 set of Ig-superfamily folds [17]. Extensive mutagenesis studies, coupled with both functional and structural information, have shown that IgD1 and IgD2 function in (1) mediating homophilic PECAM-1/PECAM-1 interactions between leukocytes and endothelial cells, and (2) concentrating PECAM-1 at endothelial cell-cell borders, where it functions as both a major endothelial mechanosensor [20–23], and as regulator of vascular permeability. The former will be covered extensively in an accompanying article by **Tzima** in this volume, while the role of PECAM-1 in maintaining endothelial cell barrier function will be described in detail below. Further down the molecule, membrane-proximal Ig domains 5 and 6 each contain calcium coordination sites [24], and antibodies that bind IgD6 have the interesting property of increasing the homophilic binding affinity of the receptor [25,26], a property with potential translational applications that will be examined in more detail below.

The cytoplasmic domain of PECAM-1 is comprised of eight separate exons that are subject to alternative splicing [27], yielding isoforms that are expressed in a tissue- and differentiation-specific manner [28,29], and that have the potential to differ in their functional properties [30–32]. Though largely unstructured, the cytoplasmic domain contains a single lipid-associated segment that is susceptible, upon cellular activation, to inducible, sequential phosphorylation [18,33]; first of serine residues that release a membrane-associated control region from the inner face of the plasma membrane (see

Figure 1), and then of tyrosines 663 and 686, each of which exist within immunoreceptor tyrosine-based inhibitory motifs (ITIMs). PECAM-1 ITIMs, when phosphorylated, recruit the protein-tyrosine phosphatase, SHP-2 [34], resulting in formation of a PECAM-1/SHP-2 complex that functions in circulating blood cells to inhibit a plethora of tyrosine kinase-initiated cellular activation events [35]. Endothelial PECAM-1 is able to recruit cytosolic SHP-2 to the inner face of the plasma membrane in a phospho-ITIM-specific manner [36,37] to form a complex that functions to increase endothelial cell motility and migration in a process that will be discussed in **Section 5** below.

THE CONTRIBUTION OF IgD1 AND IgD2 TO HOMOPHILIC INTERACTIONS

The adhesive properties of PECAM-1 largely depend on its ability to form PECAM-1/PECAM-1 homophilic interactions. Such interactions are essential for concentrating PECAM-1 at endothelial cell-cell borders [38] where it functions both to regulate the vascular permeability barrier (see below) and leukocyte trafficking [39]. Sun et al. were the first to demonstrate that PECAM-1 homophilic interactions require PECAM-1 IgD1 and IgD2 [25], however this interaction is species-specific, as substituting human PECAM-1 with murine IgD1, abrogates PECAM-1 homophilic interactions [25,40]. Studies by Newton et al. demonstrated that five residues (D11, D33, K50, D51, and K89) are required for homophilic binding [41]. Disruption of at least one of these, K89, results in loss of both endothelial cell border localization [38] and the ability of PECAM-1 to contribute to endothelial cell junctional integrity [42,43]. The structure of the homophilic binding domain of PECAM-1 has been recently been solved [17], and reveals that both IgD1 and IgD2 participate importantly in the formation of the trans homophilic binding interface, with a total buried interface area of more than 2300 $Å^2$. Such extensive contacts likely enable PECAM-1 to maintain vascular integrity and to resist mechanical force under conditions of fluid shear stress. A space-filling model of IgD1/D2 based on the crystal structure is shown in Figure 1, while select residues participating in formation of the homophilic binding interface are listed in Table 1.

LECTIN-LIKE PROPERTIES OF PECAM-1

PECAM-1 is heavily glycosylated [19], with nine *N*-glycosylation sites within the extracellular domain, three of which are in IgD1 and IgD2 [9,17]. Kitazume et al. were the first to demonstrate a role for carbohydrate residues in mediating PECAM-1 homophilic interactions [44], and proposed that PECAM-1 possess lectin-like properties similar to the Siglec family of cell adhesion receptors [45]. Interestingly, homophilic binding ability was linked to the presence of α2,6-sialic acid modified glycan residues, which appeared to be necessary for the ability of PECAM-1 to traffic normally to the cell surface and confer a cell survival advantage to endothelial cells in culture [46]. α2,6-linked sialic acids were also shown to be necessary for endothelial cells to form tube-like structures in vitro [47]. An important caveat of each of these studies is that they were performed using primarily murine cells and murine PECAM-1, More recent studies [48] have identified important species-specific requirements for PECAM-1-mediated homophilic binding, as α2,3- but not α2,6-, sialylated glycans, appear to participate in PECAM-1/PECAM-1 interactions in humans. Structural and functional studies aimed at identifying and characterizing the specific role of

glycans in human PECAM-1/PECAM-1 interactions are the subject of an ongoing investigation in our laboratory.

PECAM-1 AND ENDOTHELIAL MIGRATION AND CELL SURVIVAL

Cell migration

The first hint that PECAM-1 might be involved in cell migration and angiogenesis came from findings that anti-PECAM-1 antibodies inhibit the ability of endothelial cells grown on Matrigel to form tube-like structures [49–52]. This concept was supported shortly thereafter by the observation that antibodies specific for PECAM-1 inhibit tumor-induced angiogenesis *in vivo* in mice [53,54], and later by the observation that tumor angiogenesis is impaired in PECAM-1-null mice [55]. The mechanism by which PECAM-1 promotes cell migration appears to be due to the ability of the PECAM-1/SHP-2 complex to alter the cytoskeleton, both by dephosphorylating focal adhesion kinase [56,57], as well as by altering the activity of the small G-protein, RhoA [58,59]. Taken together, these findings provide strong rationale for targeting PECAM-1 in endothelialopathies such as tumor angiogenesis and the growth and development of hemangiomas.

Cell survival

Exposure of endothelial cells to a variety of apoptotic and/or inflammatory stimuli results in endothelial injury and dysfunction (reviewed in [60]), and their ability to resist programmed cell death is crucial for endothelial cells to maintain vascular homeostasis. PECAM-1 homophilic binding [61,62] and subsequent signaling through the PECAM-1 cytoplasmic domain [63,64] play important roles in endothelial cell cytoprotection. Interestingly, although PECAM-1 ITIMs are required to inhibit the *intrinsic* pathway of Bax-induced apoptosis [64], they appear to do so independent of their ability to recruit and activate SHP-2 [65] – at least in endothelial cells exposed to genotoxic chemotherapeutic drugs. PECAM-1 has also recently been reported to endow the vascular endothelium with the ability to maintain vascular integrity during inflammation-induced activation of the *extrinsic* pathway of apoptosis [66]. As in chemotherapy-induced endothelial cell death, PECAM-1 ITIM tyrosines appear to be required for cytoprotection. The distinct signaling pathways employed downstream from PECAM-1 ITIM tyrosine phosphorylation leading to protection of endothelial from pro-apoptotic stimuli remain to be fully elucidated.

ORGANIZATION OF THE ENDOTHELIAL CELL JUNCTION

The vascular endothelium regulates the flow of fluids and cells via a number of mechanisms. Cell surface negatively-charged glycans located on the luminal surface of the endothelium form a charged repulsive surface that prevents platelets, red cells, and leukocytes from adhering to the endothelium under normal conditions [67], while membrane compartments like caveolae regulate transendothelial transport of soluble macromolecules [68]. Most trafficking, however, takes place at the endothelial cell-cell junction, the integrity of which is tightly regulated by the coordinated action of a series of cell surface receptors and cytoskeletal elements that work together to regulate fluid exchange with the underlying tissue while retaining blood cells within the vessel [69]. There are two types of junctional

adhesive structures (Figure 2); Tight Junctions (TJ) and Adherens Junctions (AJ). Tight junctional components, comprised of claudins, occludins, and JAMs, are present to various degrees in different endothelial cell beds – especially those that require tight regulation of vascular permeability such as in the blood-brain barrier [70]. Adherens Junctions, on the other hand, are made up of the vascular-specific cadherin, VE cadherin, linked to the actin cytoskeleton via members of the catenin family, and play probably the most important role in regulating vascular permeability [71,72]. Finally, the most abundant component of the endothelial cell junction, PECAM-1, is present in neither tight nor adherens junctions [73], rather becoming concentrated deep within the junction as a consequence of "diffusion-trapping [38] – a process in which N-terminal IgD1 and IgD2 mediate *trans* homophilic interactions between PECAM-1 molecules on adjacent cells.

PECAM-1 AND THE MAINTANENCE OF THE ENDOTHELIAL CELL PERMEABILITY BARRIER

A plethora of studies support the concept that PECAM-1 contributes importantly to the maintenance of the endothelial cell permeability barrier. Ferrero demonstrated twenty years ago that addition of anti-PECAM-1 antibodies to endothelial cell monolayers in culture increases the rate of albumin transit in transwells, that transfection of PECAM-1 into cultured fibroblasts reduces albumin transit, and that injection of the PECAM-1 mAbs into mice results in fluid leak into the hepatic and renal vasculature [74]. Though PECAM-1-deficient mice exhibit no vascular abnormalities while sitting quietly in a cage in an animal facility, they have a profound, easily observable phenotype when subjected to inflammatory [75–77] or hemostatic [78] challenge.

While signal transduction events initiated by phosphorylation of PECAM-1 cytoplasmic domain ITIM tyrosines dominate the function of PECAM-1 in circulating platelets and leukocytes, the homophilic binding properties of PECAM-1 appear to be critical for its "firewall" role supporting endothelial cell junctional integrity. Recent mechanistic studies employing Electric Cell-substrate Impedance Sensing technology found that, compared with PECAM-1-deficient endothelial cells, PECAM-1-expressing endothelial cell monolayers exhibit increased steady-state barrier function, as well as more rapid restoration of barrier integrity following thrombin-induced perturbation of the endothelial cell monolayer integrity [42]. This effect was found to be dependent upon the ability of PECAM-1 to interact homophilically and become localized to cell–cell junctions, because a homophilic binding-crippled mutant form of PECAM-1 that could not localize to cell-cell borders was unable to support efficient barrier function. In contrast, cells expressing ITIM-less forms of PECAM-1 exhibited normal to near-normal barrier integrity. Whether non-ITIM sequences with the cytoplasmic domain play a role in stabilizing endothelial cell-cell junctions is not known, nor is the role that PECAM-1-linked carbohydrate residues play in this process understood. Both are the subject of ongoing investigations.

Perhaps most intriguing from a translational point of view is the observation that the adhesive properties of PECAM-1 are subject to affinity modulation [25,26] – a property well known for members of the integrin family of adhesion receptors, but relatively rare for

members of the Ig superfamily. In a process that is not yet understood mechanistically, addition of antibodies that bind membrane-proximal IgD6 are able not only to increase the homophilic binding affinity of PECAM-1, but are able to actually enhance the rate of endothelial cell migration and barrier restoration in endothelial cell monolayers subjected to physical or inflammatory challenge. The finding that the adhesive properties of PECAM-1 are regulatable may allow for the development of novel approaches and reagents that can enhance endothelial cell migration and restore barrier function in a wide variety of vascular permeability disorders.

CONCLUSION

There is growing appreciation that the fields of thrombosis and inflammation are inextricably and mechanistically linked. PECAM-1, via its ability to inhibit the activation of circulating platelets and leukocytes, while at the same time supporting the integrity of endothelial cell-cell junctions and providing protection of the vascular bed to apoptotic stimuli, appears to play a significant role in each of these interrelated processes. PECAM-1 has predictably been implicated in a number of clinically-relevant disorders, ranging from thrombosis and cardiovascular disease to inflammation and cancer. It is hoped that this brief review will spur additional efforts to improve our understanding of the structure and function of this novel cell adhesion and signaling molecule in the vascular cells in which it is expressed, and allow for translational opportunities to be exploited.

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KEY POINTS

- PECAM-1 is enriched at endothelial cell intercellular junctions, where it regulates leukocyte trafficking, mechanotransduction, and vascular permeability.
- Extensive homophilic contacts between amino acids located in amino terminal Ig homology domains 1 and 2 of the molecule enable PECAM-1 to maintain vascular integrity and to resist mechanical force under conditions of fluid shear stress.
- The adhesive properties of PECAM-1 are subject to affinity modulation – a rather unique property for a member of the Ig-superfamily – and may be physiologically important in thrombosis, inflammation and the immune response.

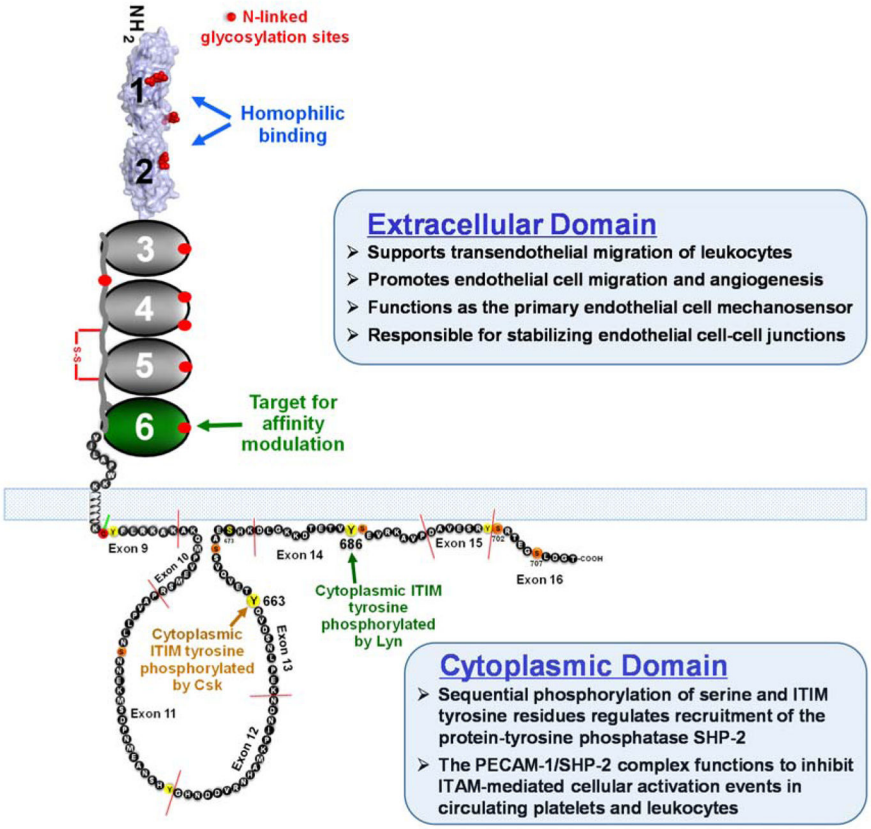

Figure 1.

Schematic diagram of PECAM-1. The extracellular domain is comprised of six Ig-like domains, the first two of which are shown as a space-filling model of the recently determined homophilic binding domain (reference 17) that is involved in cell adhesion. The structure of the cytoplasmic domain was determined by 2D NMR (reference 18), and is characterized by two lipid-associated regions separated by a large unstructured region. PECAM-1-mediated signaling is initiated by phosphorylation of serine 702, which releases ITIM tyrosine 686 from its association with the plasma membrane, facilitating its phosphorylation by the Src-family kinase, Lyn. Sequential phosphorylation of ITIM tyrosine 663 completes the process, and allows PECAM-1 to now recruit SH2 domain-containing proteins, the most notable of which is the protein-tyrosine phosphatase, SHP-2.

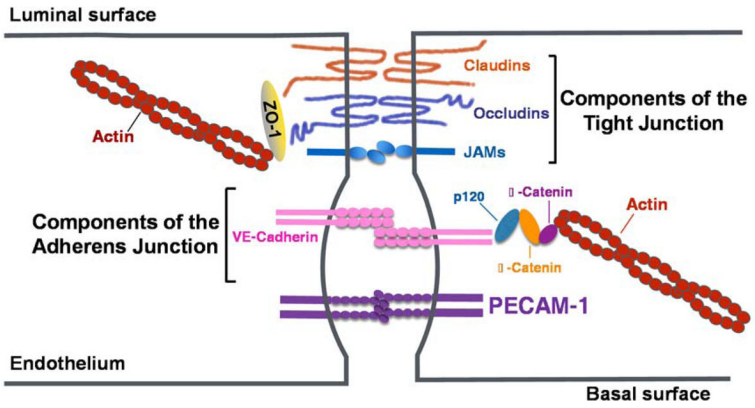

Figure 2.

Adhesive molecules of the endothelial cell-cell junction. The vascular permeability barrier is maintained by tight junctions comprised of claudins, occludins, and JAMs, followed by adherens junctions comprised primarily of vascular endothelial cadherin (VE-cadherin) associated with the actin cytoskeleton via members of the catenin family. Underneath these specialized compartments lies the most abundant endothelial cell surface receptor, PECAM-1, which is expressed at 1–2 x 10^6 molecules per cell. Figure adapted from E. Dejana, Nature Reviews Molecular Biology 5:261, 2004 (69).

Table 1

Amino acid interacting * pairs on the IgD1 and IgD2 inter-chain interfaces present in the crystal structure of the homophilic binding domain of human PECAM-1

Interacting interfaces	Amino acid pairs
IgD1-IgD1	V34-K13, T37-K24/P16, T36-P16, S38-P16/L15, K62-T64/A32/S63/F31, T64-F31, V40-L15, P42-L15, H39-L15/F68/T27/N25, F3-D11, K41-S66/Q29, F68-S35, T27-S35/N8, L15-N8, Q29-N8/S9/D33, S66-D33
IgD1-IgD2	Y49-R122/E165, D51-K154, D51-K154, D52-R122, K81-E165
IgD2-IgD2	R157-K131, D158-K131, A132-R157

* Multiple amino acids interacting with a single amino acid are separated by a "/"