

## The Multiple Functions of PECAM-1

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The past few years have seen increasing research interest in the mechanisms by which cells integrate and modulate their responses to the complex array of environmental stimuli to which they are exposed. This has seen advances in the characterisation of co-stimulatory and inhibitory receptors whose intracellular delivery of antagonistic or synergistic signals serve to modify other cellular signalling pathways. Advances have also been made in understanding the methodology by which cells physically organise signalling molecules into multimolecular complexes (through the action of adapter/scaffold proteins and lipid rafts) permitting efficient cross-talk between diverse signals.

With these advances, it has become apparent that Platelet Endothelial Cell Adhesion Molecule-1 (PECAM-1), once viewed largely as an adhesion protein, plays multiple roles in cellular signalling. PECAM-1 has recently been identified as a member of the Ig-ITIM (immunoreceptor tyrosine-based inhibitory motifs) superfamily which modulates activation responses through the delivery of inhibitory dephosphorylating signals of ITAM (immunoreceptor tyrosine-based activatory motifs)-dependent pathways in immune cells and platelets. PECAM-1 is also expressed on both haemopoietic and non-haemopoietic cells and forms multiple interactions with a range of extracellular ligands and intracellular signalling molecules which may be subject to regulation by tissue-specific splicing. The interaction of PECAM-1 with multiple intracellular molecules (including protein-tyrosine phosphatases, protein-tyrosine kinases and adaptors) has led certain workers to suggest that it may act as a scaffolding protein in the formation of extended intracellular signal complexes. Perhaps unsurprisingly, PECAM-1 has been linked to an increasing number of diverse physiological and pathological events. Thus, PECAM-1 is a multifaceted signalling molecule whose full biological significance is only starting to be appreciated.

### PECAM-1 Structure and Expression

PECAM-1 is a single pass transmembrane glycoprotein which has recently been defined as a member of the Ig-inhibitory receptor superfamily (1). This is based on the presence of six Ig domains in the 574-amino acid extracellular region and two tyrosines (Y663 and Y686) in sequences conforming to ITIM in the 118-amino acid residue intracellular region (I/VxxYxxL/V/I>20aa.I/VxYxxL/V/I) (Fig. 1).

PECAM-1 is expressed on various cell and tissue types - prominently on endothelial cells and at a lower level on leukocytes, with differential expression during the development of human immune cell populations and platelets. PECAM-1 mRNA has been detected in lung, brain, kidney and liver. The human PECAM-1 gene is

found on chromosome 17, with 16 exons of open-reading frame. Splicing of the transmembrane and intracellular regions (encoded by exons 9-15) has been detected in a tissue specific manner (reviewed in 1, 2). Although the expression of these isoforms at the *in vivo* protein level is not well defined, *in vitro* examination of splice variants has demonstrated functional differences in ligand binding, while variants lacking ITIM domains would presumably demonstrate altered signalling capacity. Posttranslational modifications of PECAM-1 include N- and O- linked glycosylation (1, 2) and palmitoylation at the cysteine at amino acid position 595 (unpublished data).

### Molecular Interactions and Signal Transduction of PECAM-1

PECAM-1 forms multiple molecular interactions with extracellular and intracellular molecules (Fig. 1). The extracellular Ig domains of PECAM-1 interact with ligands on adjacent cells including homophilic interactions with PECAM-1 and proposed heterophilic interactions with CD51/CD61 ( $\alpha v\beta 3$ ), CD38 and 120-kDa ligand on T-cells (1).

As PECAM-1 has no intrinsic enzymatic activity, transduction of extracellular signals is dependent on its non-covalent interaction with intracellular signalling molecules. Classical docking sites for Src homology 2 (SH2) domains are found at tyrosines Y663 and Y686, which become phosphorylated in response to a number of stimuli including PECAM-1 cross-linking, collagen-dependent aggregation or thrombin activation and aggregation of platelets, stimulation of ITAM-containing receptors on immune cells (e.g. B-cell and T-cell receptors, high affinity IgE receptor), shear stress or fibronectin/collagen adhesion of endothelial cells (1).

Phosphorylation appears to primarily involve Src family kinases although some residual phosphorylation in the presence of Src kinase inhibitors indicates that Csk-related kinases may also be involved; but not other kinases such as Syk, Itk and Pyk2 (3-5). Phosphorylation of these tyrosines creates docking sites for recruitment and activation of SH2 domain-containing SHP-2, SHP-1, SHIP and PLC- $\gamma$ . The interaction with phosphatases, particularly SHP-2, are likely to be responsible for the inhibitory functions of PECAM-1 in immune receptor signalling and platelet signalling (see below) (6).

Numerous interactions have been described for PECAM-1 which occur independently of the ITIM region. Recently it has been shown that PECAM-1 is constitutively associated with calmodulin. This interaction is dependent on the sequence <sup>599</sup>RKAKAK<sup>604</sup> in the PECAM-1 intracellular region,

which conforms to the calmodulin binding consensus and appears to regulate PECAM-1 cleavage (Wong, M.X., Harbour, S.N., Moseley, G.W., Lau, L.M., Andrews, R.K., and Jackson, D.E., unpublished data). Direct interactions with PECAM-1 have also been reported with  $\beta$ - and  $\alpha$ - catenins and STAT-3 and 5 (7, 8). Interactions with PI3 kinase and the adaptor protein Gab1 appear to be indirect. In this sense, it may be that PECAM-1 interacts with and localises a number of distinct signalling and cytoskeletal proteins (1). Further interactions of these proteins (e.g. SHP-2 and Grb-2/Gab-1, catenins and F-actin) would extend into the context of larger functional complexes. It has also been suggested recently that the Y686 ITIM of PECAM-1 resembles an ITSM (immunoreceptor tyrosine-based switch motif) which may associate with adaptor regulatory proteins (9). Thus PECAM-1 may act as a scaffolding protein for multiple signalling and structural proteins.

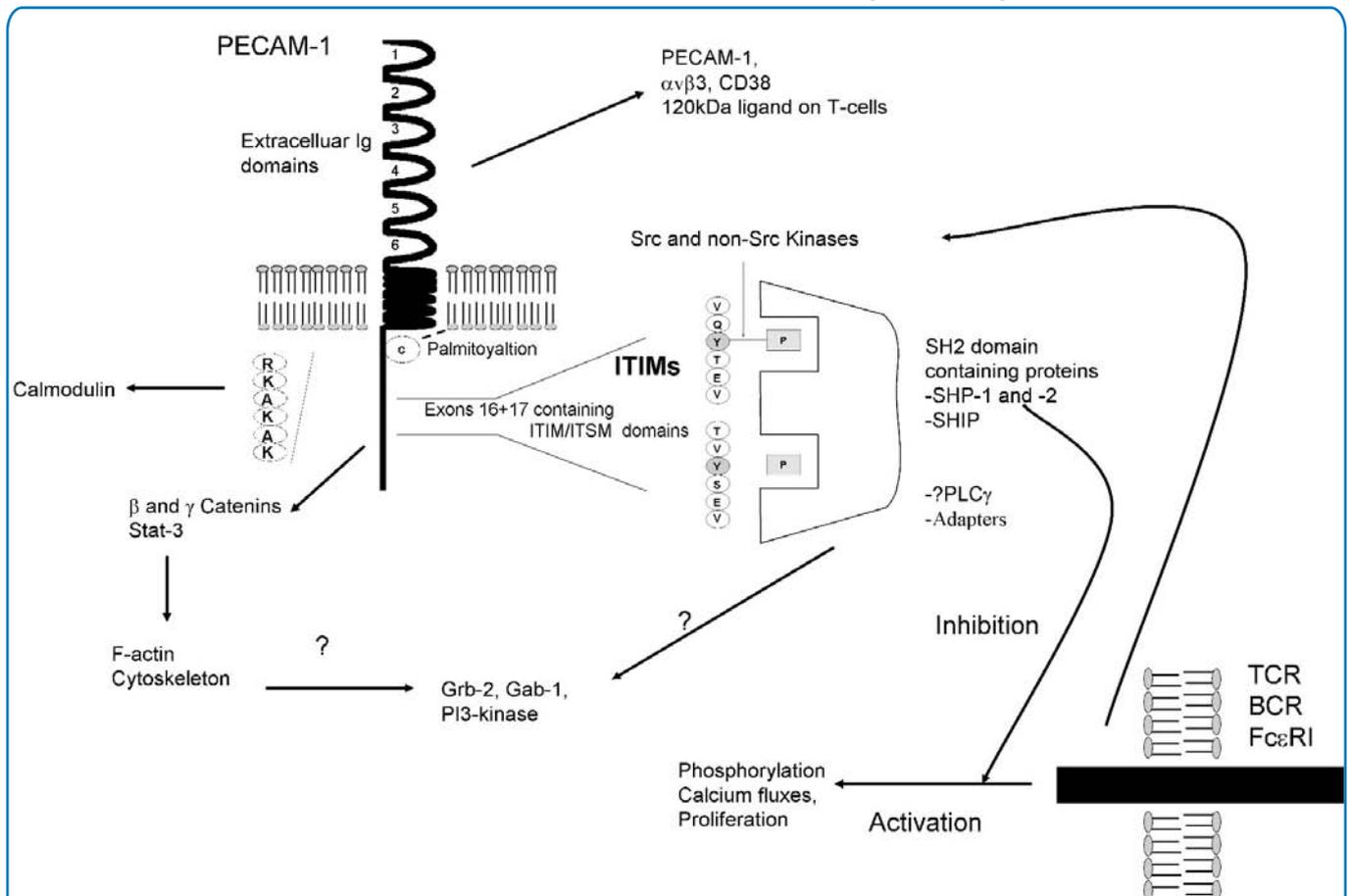
Other than the tyrosine phosphorylation described above, PECAM-1 also undergoes largely PKC-dependent serine phosphorylation in response to certain stimuli (7). The function of this modification is unclear, although there is evidence that it can regulate the interaction with  $\gamma$  catenin, thus regulating the link

of PECAM-1 with the cytoskeleton (8).

Having introduced the interactions described for PECAM-1 it is important to appreciate that a number of the associations are somewhat controversial. The details of this have been discussed elsewhere (1), but the controversy revolves largely around the poor reproducibility of some reported interactions (e.g. with PLC $\gamma$ -1), the considered *in vivo* significance of certain *in vitro* systems used to identify associations, and the poor affinity detected for certain interactions. As such, while the association of PECAM-1 with SHP-2 is widely acknowledged as genuine and biologically significant, confirmation of many of the other interactions awaits more definitive studies.

### Cellular Functions of PECAM-1

Perhaps the most clearly characterised cellular role of PECAM-1 is its inhibitory signalling in certain immune cells and platelets, which is likely to reflect its interaction with protein tyrosine phosphatases. PECAM-1 has been classified as an Ig-ITIM family member and, in common with other family members (e.g. Fc $\gamma$ RIIB, SIRP $\alpha$ ), it negatively modulates activating signals delivered through immune cell ITAM-bearing activating receptors such as the BCR,



**Fig. 1. PECAM-1 structure and molecular associations.** The six extracellular Ig domains variously contribute to associations with extracellular ligands. Phosphorylation of tyrosines Y663/Y686 in the ITIM/ITSM regions encoded in exons 16 and 17 occurs following ligation of PECAM-1 or activation of other receptors (e.g. BCR, TCR, Fc $\epsilon$ RI). This produces docking sites for SH2 domain-containing proteins including SHP-1, SHP-2 and SHIP which deliver inhibitory signals to the cell. The site for calmodulin association and the proximal palmitoylation site are also shown. Less well defined associations include ITIM phosphotyrosine interactions with PLC $\gamma$ -1 and non-ITIM interactions with catenins, STATs and PI3 kinase.

TCR and FcεRI (10-12). A similar inhibitory range of functions dependent on ITAM-bearing receptors including FcR-γ associated with GPIb/V/IX and GPVI, and FcγRIIIa is apparent in platelets (13-15). These functions are attributable to the well-established interplay between ITAMs and ITIMs. Roles have been described for PECAM-1 in the inhibition of signalling systems which do not involve ITAMs (e.g. G-protein coupled platelet activation by thrombin) but the mechanism of action here is poorly understood (16). In addition, it would appear that PECAM-1 has opposing roles in the regulation of Fcγ receptor signalling in macrophages, as PECAM-1 null macrophages display reduced TNF secretion by IgG immune complex stimulation of the PECAM-1 deficient macrophages in the presence of normal Fcγ receptor expression (17). This regulation is independent of ITAM-associated Fcγ receptor signalling.

PECAM-1 is also involved in adhesion and migration, whether by forming direct adhesive interactions itself or affecting the adhesive properties of other receptors. PECAM-1 is expressed at high levels at the lateral borders of endothelial cells where it is involved in the formation of cell-cell contacts and the vascular permeability barrier, and plays a vital role in leukocyte transmigration (18, 19). The influence of PECAM-1 in cell-cell interactions is also clear in its regulation of phagocytosis of apoptotic cells by macrophages (20), while a role in cell survival and the regulation of expression of apoptosis-protective genes has been described (21). Through modulation of the activity of integrins and assembly of the cellular cytoskeleton, PECAM-1 is also involved in cell motility and adhesion on the extracellular matrix (2).

### PECAM-1 as a Potential Therapeutic Target in Pathological Conditions

The role of PECAM-1 as a negative regulator of immune cell and platelet activation indicates that PECAM-1 may provide a viable target for therapy in pathological conditions such as thrombosis, allergies and autoimmune diseases. Notably PECAM-1 null mice show increased susceptibility to the development of collagen-induced arthritis (22) and IgE-mediated anaphylaxis (12), presumably reflecting hyperactivity of lymphocytes and mast cells lacking PECAM-1. PECAM-1 null mice also show early onset of experimental autoimmune encephalomyelitis, a condition homologous to human autoimmune multiple sclerosis (23).

In other studies PECAM-1 expression has been linked with the development of cancers, possibly reflecting roles in cell migration and adhesion (see above) or tumour angiogenesis (24), while a polymorphism of PECAM-1 is associated with arteriosclerosis (25). Finally, PECAM-1 has been identified as one of the major recognition receptors for *Plasmodium falciparum* erythrocyte membrane protein-1 indicating a role in malarial virulence (26). Therefore, PECAM-1 may be of clinical importance in pathological disorders, including cancer, thrombosis, inflammatory and infectious diseases.

### Concluding Remarks

Our knowledge concerning the functions of PECAM-1 has increased dramatically in the last few years. Certain areas of PECAM-1 biology are reasonably well defined, for example the importance of the ITIM region inhibitory functions in *in vitro* systems. However, the precise functions of the PECAM-1 ITIM *in vivo* remain poorly understood. While PECAM-1 null mice display phenotypes expected for an inhibitory receptor knockout in certain cellular systems (e.g. hyper-responsiveness of immune cells and GPVI-dependent platelet signalling), the importance of the Y663 and Y686 residues in these functions awaits the production and analysis of transgenic animals expressing PECAM-1 in which these tyrosines have been mutated.

Many other areas of PECAM-1 biology remain particularly mysterious; for example, the nature and significance of PECAM-1 interactions with signalling molecules other than SHP-1 and SHP-2. If PECAM-1 does indeed interact with a number of signalling proteins, does this impart the capacity for PECAM-1 to link to numerous signalling pathways, or do the molecules docking at PECAM-1 interact with and regulate one another? An interesting possibility is that these interactions may be modulated by tissue specific splicing of the intracellular region of PECAM-1. However, the expression of these splice variants at the protein level has rarely been confirmed. Further research in these areas will provide a much greater understanding of the potentially wide ranging functions of PECAM-1.

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