Modulators of endothelial cell filopodia
PECAM-1 joins the club

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Filopodia are an important feature of actively motile cells, probing the pericellular environment for chemotactic factors and other molecular cues that enable and direct the movement of the cell. They also act as points of attachment to the extracellular matrix for the cell, generating tension that may act to pull the cell forward and/or stabilize the cell as it moves. Endothelial cell motility is a critical aspect of angiogenesis, but only a limited number of molecules have been identified as specific regulators of endothelial cell filopodia. Recent reports, however, provide evidence for the involvement of PECAM-1, an endothelial cell adhesion and signaling molecule, in the formation of endothelial cell filopodia. This Commentary & View will focus on these studies and their suggestion that at least two PECAM-1-regulated pathways are involved in the processes that enable filopodial protrusions by endothelial cells. Developing a more complete understanding of the role of PECAM-1 in mediating various endothelial cell activities, such as the extension of filopodia, will be essential for exploiting the therapeutic potential of targeting PECAM-1.

The formation of new vessels typically involves the initial outward proliferation and migration of endothelial cells (ECs) from a pre-existing vasculature. The tips of these angiogenic sprouts are made up of highly polarized ECs, characterized by the presence of numerous long filopodia that probe the environment, directing the cell toward angiogenic factors. Surprisingly, our understanding of the processes that regulate the formation of these cellular protrusions in ECs is still incomplete. Of note, only a limited number of molecules have been identified as specifically involved in the formation of endothelial filopodia. These include VEGF receptors and the neuroplins. Recent reports from our group, however, provide evidence of a role for PECAM-1 as a regulator of filopodia formation in ECs.

PECAM-1 is a vascular-associated molecule of the Ig superfamily expressed on leukocytes and platelets as well as ECs, where it is enriched at intercellular junctions. PECAM-1 consists of a 574 amino acid extracellular region organized up of tight bundles of actin filaments. With respect to directed cell migration, filopodia are particularly important, probing and sensing the pericellular environment for chemotactic and other molecular cues in the extracellular matrix (ECM) that guide the direction and movement of the cell. They also act as points of attachment to the ECM for the cell, generating tension that may act to pull the cell forward and/or stabilize the cell as it moves. Given these roles, it is not surprising that filopodia contain diverse receptors for ECM proteins and an array of signaling molecules.

The regulated polymerization of actin filaments result in two morphologically distinct protrusive structures, lamellipodia and filopodia, at the leading edge of motile cells. Filopodia are slender, finger-like extensions (diameter 0.1–0.3 μm), often emanating from lamellipodia, made up of tight bundles of actin filaments. With respect to directed cell migration, filopodia are particularly important, probing and sensing the pericellular environment for chemotactic and other molecular cues in the extracellular matrix (ECM) that guide the direction and movement of the cell. They also act as points of attachment to the ECM for the cell, generating tension that may act to pull the cell forward and/or stabilize the cell as it moves. Given these roles, it is not surprising that filopodia contain diverse receptors for ECM proteins and an array of signaling molecules.

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into six cystine linked Ig-like domains, a 19 amino acid transmembrane domain and a 118 amino acid cytoplasmic tail. It was initially identified as an adhesion molecule capable of binding interactions with itself or with a number of non-PECAM-1 molecules, including heparin containing proteoglycans. However, subsequent studies led to the recognition that PECAM-1 also participates in intracellular signaling. Although it does not have any intrinsic catalytic activity, the cytoplasmic domain of PECAM-1 contains two tyrosine residues (Y663 and Y686) that each fall within a conserved signaling sequence known as the immunoreceptor tyrosine-based inhibitory motif (ITIM). Phosphorylation of these two tyrosine residues in PECAM-1 creates docking sites for the binding and activation of several cytosolic signaling molecules containing src homology-2 (SH2) domains. Included among the SH2-containing molecules that associate with PECAM-1 are the protein tyrosine phosphatases, SHP-1 and SHP-2, and phospholipase C-γ. PECAM-1 may also associate with phosphoinositide 3-kinase and β- or γ-catenin. The ability of PECAM-1 to bind to these various cytosolic molecules enables it to potentially modulate the activity of a number of intracellular signaling pathways.

With respect to its endothelial cell functions, PECAM-1 regulates leukocyte transendothelial migration, protects against endotoxic and apoptotic stresses and contributes to the molecular sensing of fluid shear stress. PECAM-1 is also involved in angiogenesis. The initial evidence for this came from studies demonstrating that anti-PECAM-1 antibodies inhibit corneal neovascularization induced by angiogenic implants, vascularization of subcutaneous Matrigel plugs and tumor angiogenesis. Subsequent studies in PECAM-1-null mice have helped to confirm and further refine our understanding of the involvement of PECAM-1 in blood vessel formation. PECAM-1-deficient mice are viable, suggesting that vascular development is sufficiently preserved in the absence of PECAM-1 to permit adequate embryogenesis. However, the angiogenic response in a model of foreign body-induced chronic inflammation and the vascularization of subcutaneous Matrigel implants and subcutaneous tumors are inhibited in PECAM-1-null mice.

In addition, post-natal lung development (a process dependent on angiogenesis) and the initial post-natal vascularization of the murine retina are impaired in PECAM-1-deficient mice. Significantly, the vascular pattern and density in the eyes and lungs are similar in wild type and PECAM-1-null adult mice (unpublished observations), suggesting that the loss of PECAM-1 delays, but does not prevent the eventual development of the retinal or pulmonary vasculature. Together, these data implicate PECAM-1 in pathological angiogenesis, as well as in post-natal vascular developmental processes, such as those occurring in the eyes and lungs.

One of the mechanisms of PECAM-1’s involvement in blood vessel formation appears to be an ability to stimulate endothelial cell motility. Support for this conclusion comes from studies which have shown that anti-PECAM-1 antibodies inhibit the migration of murine and human ECs and endothelial cells from PECAM-1-deficient mice are less motile compared to their wild-type counterparts and the expression of PECAM-1 in non-PECAM-1 expressing cells enhances cell motility. This activity in stimulating cell motility appears to involve the dephosphorylation of paxillin by the SHP-2 phosphatase.

Its involvement in angiogenesis and endothelial cell motility led to an exploration of whether PECAM-1 might also play a role in the formation of endothelial cell filopodia. Under various conditions it was observed that filopodia were more numerous and/or longer in length in (1) wild type versus PECAM-1-null murine ECs, (2) control HUVEC compared to HUVEC treated with PECAM-1 siRNA and (3) cell transfectants expressing human PECAM-1 versus non-transfected controls. The significance of these in vitro data was confirmed by the finding that the length and number of filopodial extensions from ECs at the leading edge of the developing retinal vascular plexus were suppressed in PECAM-1-null mice. The concentration of PECAM-1 in the tips of endothelial filopodia (Fig. 1, unpublished observation) is similar to what has been reported for VEGFR-2 (reviewed in ref. 9) and is consistent with a role for PECAM-1 in the formation of these cellular protrusions. PECAM-1 thus represents a new addition to a relative short list of molecules that have been identified as specific regulators of endothelial filopodia formation.

These data may help in part to explain the vascular phenotype of the PECAM-1-null mice. If PECAM-1 is understood as increasing both the length and number of endothelial filopodia, then its presence enables the endothelial cell to more quickly and/or more efficiently assess the pericellular matrix environment for chemotactic or other pro-migratory factors. In this way, rather than being essential for cell migration, PECAM-1 facilitates the process by promoting the efficiency of endothelial cell motility. This might account for why the loss of PECAM-1 does not compromise the formation of the vasculature in the embryo, and delays, but does not arrest, post-natal vascular development in the eyes and lungs.

Although the specific mechanisms by which PECAM-1 promotes the formation of filopodia remain to be determined and are the subject of ongoing studies, both published and unpublished data are suggestive of some possible processes. First, PECAM-1 antibodies that block heterophilic (non-PECAM-1) ligand binding interactions inhibit filopodia formation by subconfluent HUVEC. This suggests that in the context of an angiogenic, motile endothelial cell, PECAM-1 heterophilic binding interactions with matrix proteins (e.g., proteoglycans) may transduce signals that activate the formation of the bundled, parallel arrays of actin that provide structural support for the filopodia.

Second, the expression of PECAM-1 in cellular transfectants stimulates the formation of filopodial extensions and wound-induced migration, processes that are both associated with increased ERK activation. These phenomena are inhibited by mutations of Y663 and Y686 in the cytoplasmic domain that lead to a loss of the ability of PECAM-1 to bind SHP-2. They are also suppressed by molecular or pharmacological inhibition of the...
catalytic activity of the SHP-2 phosphatase. Both cell motility and filopodia are downstream consequences of ERK activation. Further, SHP-2 dephosphorylation of paxillin, with the subsequent activation of src kinase, is one mechanism for the activation of ERK. Our data therefore suggest that the stimulation of filopodia formation mediated by PECAM-1 involves SHP-2-mediated activation of ERK.

Lastly, the presence of PECAM-1 increases the expression of Cdc42 in murine ECs and in PECAM-1-expressing transfectants. This is not surprising given the abundance of data linking this Rho-GTPase to the formation of filopodia. It is important to note, however, that PECAM-1 is expressed at persistently high levels on ECs and thus acute increases in PECAM-1 levels are unlikely to directly mediate an increase in Cdc42 expression during angiogenesis. This therefore suggests that if PECAM-1 regulates Cdc42 expression during angiogenesis, this activity involves changes in the activation state of PECAM-1 and/or its interaction with other molecules involved in the regulation of Cdc42. In addition, although disturbance of the PECAM-1-SHP-2 interaction suppresses PECAM-1-dependent filopodia formation, this does not alter the expression of Cdc42 (unpublished data). Together, these data suggest that (i) the influence of PECAM-1 on Rho GTPases in filopodia formation is likely to involve or impact more than just changes in the expression levels of Cdc42 and (ii) filopodia formation mediated by PECAM-1 includes pathways that are dependent as well as independent of SHP-2-regulated ERK activation.

The data generated to date are admittedly descriptive in a number of respects, and the mechanisms of PECAM-1’s activity as a regulator of endothelial filopodia are still being defined. However, studies of PECAM-1-transfectants suggest a dynamic interaction between PECAM-1 and SHP-2 in which the level of PECAM-1 tyrosine phosphorylation, and thus SHP-2 binding, are regulated by bound, catalytically-active SHP-2. Based on these data, along with what was noted above, we suggest the following working model for the involvement of PECAM-1 in the formation of filopodia (Fig. 2). In the setting of post-natal developmental angiogenesis, or during certain forms of pathological angiogenesis, PECAM-1 on angiogenic and/or motile endothelial cells is tyrosine phosphorylated. These phosphorylation events occur either in response to angiogenic growth factor stimulation or are a result of the binding of PECAM-1 to matrix proteins elaborated in the angiogenic context. PECAM-1 phosphorylation subsequently induces the binding of SHP-2 to PECAM-1 and thus its recruitment to the cell membrane. We propose that this binding interaction with PECAM-1 activates the phosphatase activity of SHP-2, with the activated SHP-2 dephosphorylating the PECAM-1 molecule to which it is bound. This leads to the release of SHP-2 from PECAM-1. The liberated but now membrane localized SHP-2 in turn targets paxillin, dephosphorylating it, to activate Src and eventually ERK-dependent signaling. One of the many consequences of ERK activation that might be relevant to PECAM-1-dependent filopodia formation could be the activation of myosin light chain kinase and the subsequent phosphorylation of the light chains of actin-associated myosins. Additionally, in processes that do not appear to directly involve SHP-2-mediated ERK signaling, and which are poorly understood, PECAM-1 is involved in the regulation of Cdc42 expression. Studies are underway to evaluate the validity of this model.

The involvement of PECAM-1 in pathological angiogenesis has raised the possibility of PECAM-1 as a future target for anti-cancer therapy. The appeal of PECAM-1 is further enhanced by the fact that loss of PECAM-1 does not cause a debilitating vascular phenotype and significant vascular-related side effects have not been observed in mice treated with anti-PECAM-1 antibodies. This suggests that anti-PECAM-1 therapy is likely to be well tolerated. However, the involvement of PECAM-1 in the trafficking of white cells and in providing resistance to endotoxic and apoptotic stresses means that consideration must still be given to the potential for immunosuppression and/or increased sensitivity to vascular insults during anti-PECAM-1 therapy. Consequently, developing a full understanding of the role of PECAM-1 in mediating endothelial cell functions will be essential for exploiting the therapeutic potential of targeting PECAM-1.

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Figure 2. A proposed model for the involvement of PECAM-1 in the formation of endothelial cell filopodia during angiogenesis. During pathological or certain forms of developmental angiogenesis, angiogenic factors and/or cell-matrix interactions stimulate PECAM-1 tyrosine phosphorylation. These phosphorylation events lead to the binding of SHP-2 to PECAM-1 and the activation of SHP-2’s phosphatase activity. The activated SHP-2 dephosphorylates the PECAM-1 molecule to which it is bound, leading to its release from PECAM-1. The liberated SHP-2 subsequently targets paxillin, dephosphorylating it, to trigger ERK-mediated activation of filopodia formation. Also, in processes that are still undefined, PECAM-1 may be involved in the regulation of Cdc42 during angiogenesis.
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