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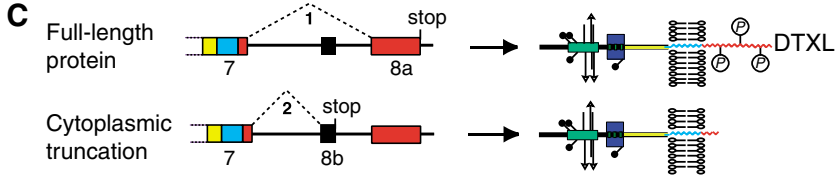
We apologise for an error that occurred in Fig. 1C of this article. The transmembrane region of the cytoplasmic truncation mutant is shown in red instead of blue. This error appeared in both the print and the online versions of this article.

The correct Fig. 1C is shown below.



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Novel functions of the CD34 family

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Summary

For almost 30 years, the cell-surface protein CD34 has been routinely used as a marker to assist in the identification and isolation of hematopoietic stem cells (HSCs) and progenitors in preparation for bone-marrow transplantation. In addition, it has increasingly been used as a marker to help identify other tissue-specific stem cells, including muscle satellite cells and epidermal precursors. Despite its utility as a stem-cell marker, however, the function of CD34 has remained remarkably elusive. This is probably because: (1) it is subject to a range of tissue-specific post-transcriptional and post-translational modifications that are expected to alter its function dramatically; (2) the simple interpretation of CD34 gain- and loss-of-function experiments has been confounded by the overlapping expression of the two recently discovered CD34-related proteins podocalyxin and endoglycan; and (3) there has been a glaring lack of robust in vitro and in vivo functional assays that permit the structural and functional analysis of CD34 and its relatives. Here, we provide a brief review of the domain structure, genomic organization, and tissue distribution of the CD34 family. We also describe recent insights from gain- and loss-of-function experiments and improved assays, which are elucidating a fascinating role for these molecules in cell morphogenesis and migration.

Key words: Podocalyxin, CD34, Endoglycan

Introduction

The CD34 family of cell-surface transmembrane proteins comprises the hematopoietic progenitor cell antigen CD34, podocalyxin (also known as podocalyxin-like protein 1, PODXL or PCLP1, thrombomucin, gp135, GCTM2, TRA-1-60 and TRA-1-81) and endoglycan (also known as podocalyxin-like protein 2, PODXL2 or PCLP2) (Doyonnas et al., 2001; Hilkens et al., 1992; Kerjaschki et al., 1984; Kershaw et al., 1997; Krause et al., 1994; McNagny et al., 1997; Miettinen et al., 1999; Orlando et al., 2001; Sassetti et al., 1998; Sassetti et al., 2000; Takeda et al., 2000; Meder et al., 2005; Schopperle et al., 2003; Schopperle and DeWolf, 2007). CD34 has been widely used as a marker to assist in the identification and isolation of hematopoietic stem cells (HSCs) and progenitors in preparation for bone-marrow transplantation; more recently it has been employed as a marker to help identify other tissue-specific stem cells, including muscle satellite cells and epidermal precursors. Notably, however, the function of CD34 and its family members has not yet been definitively determined, although several roles have been ascribed to the proteins. For instance, CD34 has been proposed to promote the proliferation, and block the differentiation, of progenitor cells, and podocalyxin and CD34 enhance the trafficking and migration of hematopoietic cells. Podocalyxin appears to have a role in cell morphogenesis, and members of the CD34 family have also been proposed both to promote and to block cell adhesion. Moreover, recent data suggest further functions for these proteins – in particular podocalyxin might have roles in chemotaxis and in asymmetric cell division.

The CD34-family proteins have overlapping expression patterns, but each protein is also expressed uniquely in certain tissues. As described above, CD34 is widely used as a marker of vascular endothelial cells and hematopoietic stem and progenitor cells (Andrews et al., 1989; Baumhueter et al., 1994; Berenson et al., 1988; Ema et al., 1990; Fina et al., 1990; Sato et al., 1999; Young et al., 1995), and podocalyxin is also widely expressed on vascular endothelial cells and hematopoietic stem and progenitor cells (Doyonnas et al., 2005; Horvat et al., 1986; Kershaw et al., 1997; McNagny et al., 1997; Sassetti et al., 1998). However, podocalyxin was initially characterized as a marker of kidney glomerular epithelial cells (podocytes) and is essential for kidney development; *Podxl*$^{-/-}$ mice exhibit perinatal lethality (Doyonnas et al., 2001; Kerjaschki et al., 1984). Aberrant expression of podocalyxin has also been implicated in a wide range of malignancies, including cancers of the breast and prostate (Casey et al., 2006; Heukamp et al., 2006; Kelley et al., 2005; Ney et al., 2007; Schopperle and DeWolf, 2007; Schopperle et al., 2003; Somasiri et al., 2004). The final family member, endoglycan, was identified through its sequence similarity to CD34 and podocalyxin; it is also expressed by a subset of hematopoietic cells (Sassetti et al., 2000).

In this Commentary, we describe the structures of the CD34-family proteins, their binding partners, and their normal expression patterns. We then consider the proposed functions of these proteins, insights from recent gain- and loss-of-function experiments, and possible new directions for research into the functional aspects of this protein family.

Domain structures of CD34-family proteins

All three CD34-family proteins have a serine-, threonine- and proline-rich extracellular domain that is extensively O-glycosylated and sialylated; this results in actual molecular masses of 90-170 kDa (substantially greater than the predicted masses of ~35-60 kDa) and characterizes the CD34 family as a subfamily of the sialomucins (Fig. 1A) (Doyonnas et al., 2001; Hilkens et al., 1992; Kerjaschki et al., 1984; Kershaw et al., 1997; Krause et al., 1994; McNagny et al., 1997; Miettinen et al., 1999; Orlando et al., 2001; Sassetti et al., 1998; Sassetti et al., 2000; Takeda et al., 2000). The extracellular part of each family member also includes a cysteine-bonded globular domain and a juxtamembrane stalk, as well as putative N-linked glycosylation sites. In addition, each protein contains a single transmembrane helix, as well as a highly conserved

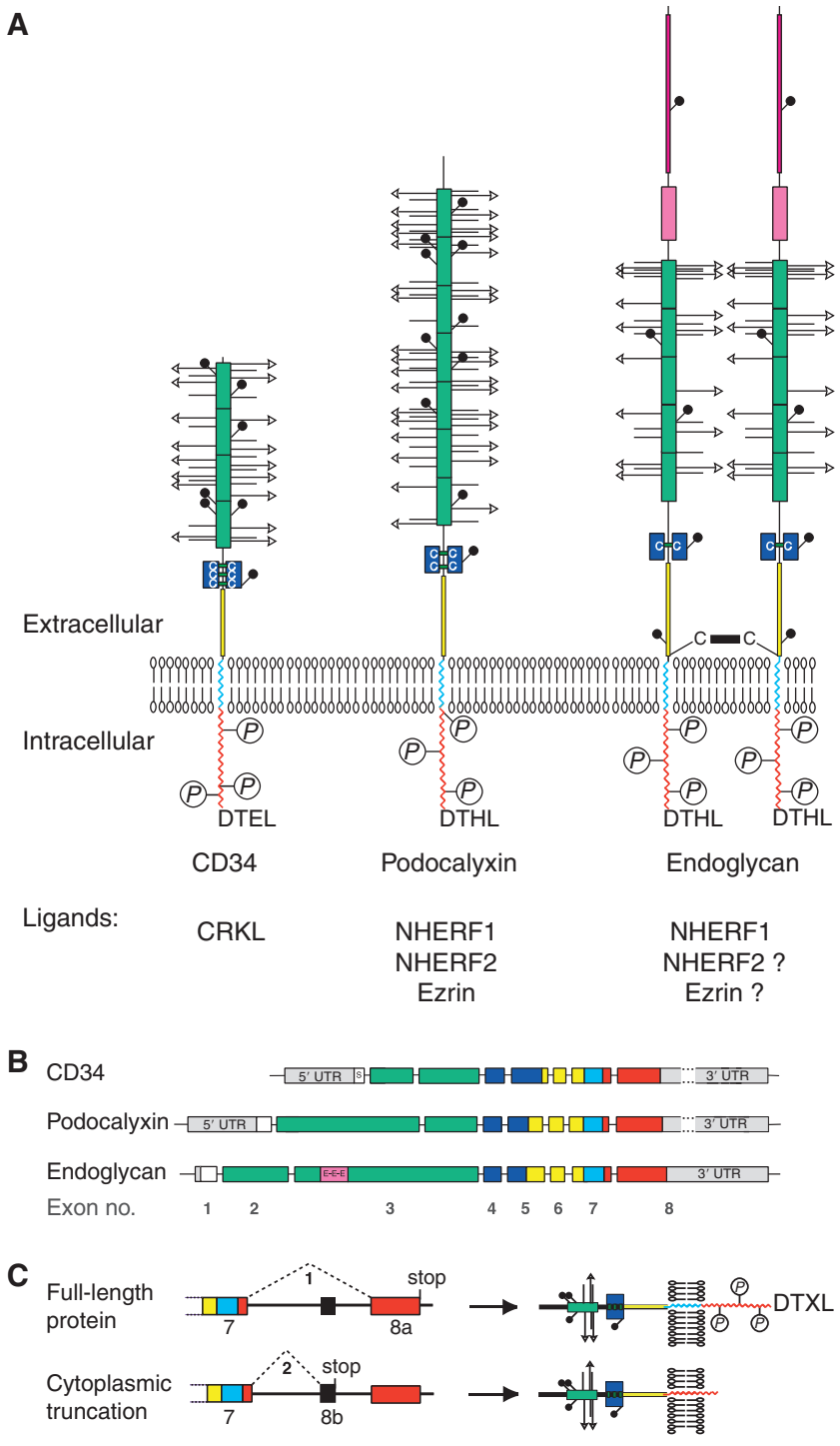

Fig. 1. Protein structures, genomic organization and splicing of the CD34 family. (A) Schematic of protein structures. CD34, podocalyxin and endoglycan each have an extensively O-glycosylated (horizontal lines) and sialylated (horizontal lines with arrowheads) serine-, threonine- and proline-rich extracellular mucin domain (green), putative sites of N-glycosylation (lines with circles), a cysteine-containing globular domain (dark blue) and a juxtamembrane stalk region (yellow). Their single-pass transmembrane domains (light blue) are followed by short cytoplasmic tails (red) containing putative phosphorylation sites and C-terminal PDZ-domain docking sites (DTEL or DTHL). The extracellular unpaired cysteine residue of endoglycan can facilitate homodimerization; endoglycan also contains an unusual polyglutamic-acid-rich extracellular domain (pink box). (B) Genomic organizations. Each protein is encoded by eight exons, with individual exons encoding the corresponding domain in each protein. (C) Alternative splicing. CD34 and podocalyxin display identical patterns of alternative splicing, and can exist as truncated versions that lack most of the cytoplasmic tail.

cytoplasmic tail that contains consensus phosphorylation sites and a C-terminal PSD-95–Dlg–ZO-1 (PDZ)-domain-binding motif.

Although CD34, podocalyxin and endoglycan are predicted to have a very similar domain structure, they differ in several notable respects (Fig. 1A). First, the mucin domains vary in length, with CD34 having the shortest mucin domain (and being the shortest protein overall) (Krause et al., 1994; Sassetti et al., 1998; Sassetti et al., 2000). Second, the globular regions of podocalyxin and CD34 contain even numbers of cysteine residues, whereas endoglycan has an unpaired juxtamembrane cysteine that is probably involved in its homodimerization (Brown et al., 1991; Fieger et al., 2003; Kershaw et al., 1997; Sassetti et al., 2000). Third, podocalyxin and endoglycan share an identical C-terminal PDZ-binding motif (DTHL), but the motif in CD34 is slightly altered (DTEL); this has functional consequences for intracellular ligand binding (described in greater detail below) (He et al., 1992; Kershaw et al., 1997; McNagny et al., 1997; Sassetti et al., 2000; Simmons et al., 1992). Last, endoglycan has a non-glycosylated N-terminal region that is not present in CD34 or endoglycan, and that contains glutamic-acid-rich repeats (Sassetti et al., 2000).

Genomic organization of the CD34 family

The genomic organizations of the *Cd34*, *Podxl* and *Podxl2* loci strongly suggest that there is an evolutionary relationship between the three genes (Fig. 1B) (Li et al., 2001; Nielsen et al., 2002). Each protein is encoded by eight exons, and, across the family, individual exons encode equivalent protein motifs and are consistent in length (Li et al., 2001; Nielsen et al., 2002; Satterthwaite et al., 1992). Throughout the family, intronic distances are also strikingly similar and, for *Podxl* and *Cd34* at least, alternative splicing to the seventh intron generates a longer transcript with a premature stop codon that encodes a protein lacking much of the cytoplasmic tail (Fig. 1C) (Kershaw et al., 1997; Li et al., 2001; McNagny et al., 1997; Nakamura et al., 1993; Nielsen et al., 2002; Sassetti et al., 2000; Suda et al., 1992); such a splice variant has yet to be described for endoglycan. Thus, these three sialomucins have been grouped into a single family on the basis of domain structure, genomic organization and patterns of alternative splicing.

Intracellular binding partners of the CD34 family

Clues to the function of novel proteins can often be obtained by identifying binding partners with known functions. The high degree of sequence conservation in the cytoplasmic regions of CD34 and its relatives has suggested that intracellular binding partners exist; these potential interacting proteins have therefore been the focus of much research (Fig. 1A). The first true intracellular ligands to be identified for a CD34 family member were the closely related PDZ-family proteins ezrin-radixin-moesin-binding phosphoprotein 50 (NHERF) and Na^+/H^+ exchange regulatory cofactor NHE-RF2 (NHERF2), which both interact with podocalyxin (Li et al., 2002; Takeda et al., 2001; Tan et al., 2006), and the adapter protein Crk-like (CRKL), which binds CD34 in hematopoietic progenitor cells (Felschow et al., 2001).

NHERF1, NHERF2 and ezrin

NHERF1 and NHERF2 are two members of the NHERF family of scaffolding proteins (reviewed in Donowitz et al., 2005; Shenolikar et al., 2004; Thelin et al., 2005; Voltz et al., 2001; Weinman, 2001). NHERF2 was identified as an intracellular ligand for podocalyxin by two independent groups who screened for podocyte-specific podocalyxin-interacting proteins in lambda phage and yeast two-hybrid expression screens, respectively (Li et al., 2002; Takeda et al., 2001). Later, NHERF1 was identified as a ligand in a similar lambda-phage screen of hematopoietic cell-specific podocalyxin-binding proteins (Tan et al., 2006). Both NHERF1 and NHERF2 have a C-terminal ezrin-radixin-moesin (ERM)-binding domain and two tandem PDZ domains. ERM-binding domains facilitate interaction with ERM family members and provide other binding partners with an indirect linkage to the actin cytoskeleton; for instance, podocalyxin interacts with the actin-binding ERM protein ezrin through NHERFs (Takeda et al., 2001). PDZ domains recognize specific sequences that are usually found at the C-terminus of proteins. The PDZ2 domains of both NHERF1 and NHERF2 recognize the consensus sequence x[S/T]x[I/V/L/M], where x represents any amino acid. This sequence is found in all three CD34 family members, but only the DTHL sequence of podocalyxin and endoglycan facilitates interaction with NHERF proteins; CD34 (which instead contains the sequence DTEL) does not interact with NHERF proteins (Li et al., 2002; Orlando et al., 2001; Takeda et al., 2001; Tan et al., 2006). Because the multiple protein-interaction domains of NHERF proteins have the potential to facilitate dimerization and the formation of large protein complexes that are connected to the actin cytoskeleton, they are thought to be involved in a wide variety of biological processes, including trafficking, transport and signaling. Thus, podocalyxin and endoglycan may participate in several cellular functions through their interactions with NHERF proteins. In addition to the ability of podocalyxin to associate with the actin cytoskeleton through NHERF proteins, it has also been shown to interact directly with ezrin through a juxtamembrane ezrin-binding sequence; this indicates a second, more direct mechanism by which it might interact with the actin cytoskeleton (Schmieder et al., 2004).

CRKL

Although CD34 does not interact with NHERF proteins, in hematopoietic progenitor cells it does interact with the CRKL, a member of the Crk family of adaptor proteins (Felschow et al., 2001). Crk proteins contain one Src-homology 2 (SH2) domain (phosphotyrosine-binding) and two Src-homology 3 (SH3) domains (proline-rich-sequence-binding), and link proteins that do not possess intrinsic kinase activity to intracellular signaling cascades, thereby enabling them to transmit signals indirectly. Although the exact binding site for CRKL on CD34 has not yet been determined, it is dependent on a highly conserved intracellular juxtamembrane sequence that is present in both isoforms of CD34 (Felschow et al., 2001). The intracellular sequences of podocalyxin and endoglycan are more similar to each other (43% identity) than to CD34 (23% identity to podocalyxin) so the existence of diverse binding partners in this family is not surprising (He et al., 1992; Kershaw et al., 1997; McNagny et al., 1997; Sassetti et al., 2000; Simmons et al., 1992). Importantly, the difference in binding partners suggests that, although the three proteins probably have overlapping functions, there are likely to be some notable differences.

Normal expression pattern of CD34 family members

Table 1 shows a general overview of the known tissue and cell-type distribution of CD34-family proteins. Because of the potential for functional redundancy among CD34-family members,

Table 1. Tissue and cell-type distribution of CD34-family proteins

Distribution	CD34	Podocalyxin	Endoglycan
Multipotent precursors	+	+	+
T cells	–	–	+ (thymocytes)
B cells	–	–	+ (TLR activated)
Mast cells	+	–	–
Eosinophils	+	–	–
Macrophages	–	–	–
Erythrocytes	–	+ (anemic)	–
Platelets	–	+	–
Muscle satellite cells	+	–	–
Hair follicle stem cells	+	–	–
Vascular endothelia	+	+	+
Smooth muscle	–	–	+
Podocytes	–	+	±
Neurons	±	+	+ (30%)
Fibrocytes	+	–	–
Mesothelia	–	+	–

+, expression; ±, weak expression; –, no expression.

(Andrews et al., 1989; Baumhueter et al., 1994; Beauchamp et al., 2000; Berenson et al., 1988; Blanchet et al., 2007; Doyonnas et al., 2005; Fina et al., 1990; Horvat et al., 1986; Kershaw et al., 1997; McNagny et al., 1997; Sassetti et al., 1998; Sassetti et al., 2000; Trempus et al., 2003; Young et al., 1995.)

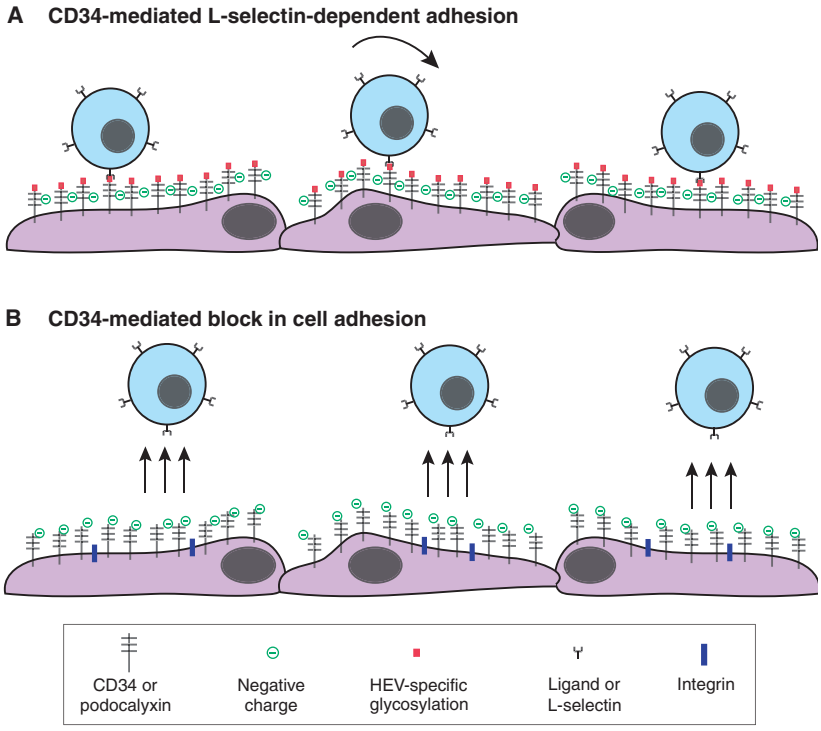

Fig. 2. Proposed functions of CD34-family proteins: promoting and blocking adhesion. (A) L-selectin expressed on naive lymphocytes recognizes HEV-specific glycosylation motifs on CD34-family members and mediates cell adhesion. (B) The bulky, negatively charged extracellular domains of CD34 family members block cell adhesion by charge repulsion and steric hindrance; this prevents interaction of integrins with their ligands.

knowledge of their distribution is essential to interpreting any phenotypes (or lack thereof) observed in in-vivo gene-inactivation studies. In this regard, it is important to note that the expression patterns of these family members are both unique and overlapping. As examples of overlapping distribution, it has been shown that CD34, podocalyxin and endoglycan are co-expressed by early hematopoietic precursors and by vascular endothelial cells (Delia et al., 1993; Doyonnas et al., 2005; Hara et al., 1999; Horvat et al., 1986; Kershaw et al., 1997; McNagny et al., 1997; Miettinen et al., 1990; Sassetti et al., 1998; Sassetti et al., 2000). Thus, there is potential for functional compensation, which suggests that it is unlikely that profound defects in these tissues will be apparent in mice that lack the gene encoding any one of these proteins. Instead, more information is likely to be gleaned from analysis of tissues in which only one of these genes is expressed (see below). For example, the proteins are uniquely expressed in mast cells (CD34), kidney podocytes (podocalyxin) and lymphocyte subsets (endoglycan) (Drew et al., 2002; Kerjaschki et al., 1984; Sassetti et al., 2000) (K.M.McN., unpublished).

Proposed functions of CD34 family members

Remarkably, the functions of CD34 and its family members remain to be definitively ascertained. A variety of potential functions have been considered, including enhancing proliferation and blocking differentiation of stem or progenitor cells, and promoting the adhesion of lymphocytes to specialized vascular endothelium in lymphoid tissues. In addition, recent gain- and loss-of-function experiments have suggested that the proteins have several interesting new functions. The proposed functions of CD34-family proteins are discussed below.

Enhancing proliferation and blocking differentiation

There are two reasons why CD34 has been hypothesized to have a role in enhancing cell proliferation and/or blocking differentiation. First, its expression on the cell surface of multipotent hematopoietic progenitors and its progressive downregulation on more mature cells suggest that it has a role in the maintenance of the undifferentiated progenitor/stem-cell phenotype (reviewed by Krause et al., 1996). Second, in one strain of *Cd34*-knockout mice there are fewer hematopoietic progenitor cells in embryonic and adult tissues, and adult-derived progenitors appear to have a proliferation defect (Cheng et al., 1996). In comparison with wild-type animals, these mice have a significantly decreased total number of progenitor cells in the adult, as well as reduced numbers of fetal-liver-derived erythroid and myeloid progenitors; this is despite the fact that there is no clear decrease in absolute numbers of mature cells in the hematopoietic system of adult mice (Cheng et al., 1996). Furthermore, there is one report that shows that CD34 can block the differentiation of one particular cell line (Fackler et al., 1995). However, given the more recent reports, which suggest that CD34 can alter the adhesive and migratory properties of cells (Blanchet et al., 2007; Drew et al., 2005; Nielsen and McNagy, 2007) (see below for more details), and the fact that the proliferative defect was not consistent in a second *Cd34*-knockout strain (Suzuki et al., 1996), it is possible that at least some of the apparent effects of CD34 expression on differentiation and proliferation actually reflect defects in adhesion or migration.

Promoting lymphocyte adhesion

The best-documented function for the CD34 family of proteins is the promotion of lymphocyte adhesion to specialized vascular endothelium in lymphoid tissues (Fig. 2A). It is well known that naive lymphocytes are recruited into secondary lymphoid organs in a multi-step process that involves, as a first step, their L-selectin-dependent low-affinity binding (‘rolling’) to specialized lymphoid vascular endothelia termed ‘high endothelial venules’ (HEVs). This is followed by integrin-mediated firm arrest and transendothelial

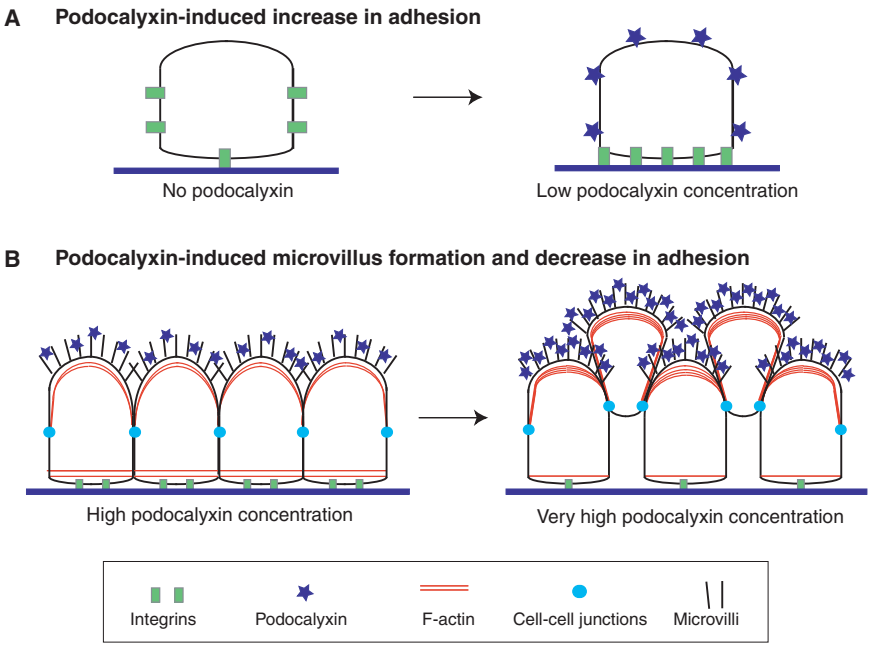

Fig. 3. Levels of podocalyxin expression might explain its apparently contradictory roles in cell adhesion. (A) Membrane-protein-segregation model. Low levels of podocalyxin establish apical domains and force integrins to the basal surface of cells, thereby enhancing cell adhesion. (B) High levels of podocalyxin strongly induce microvillus formation. Dramatic relocalization of actin to the apical membrane to support formation of microvilli might deplete basolateral actin, thereby disrupting integrin-mediated adhesion.

migration (reviewed by Butcher and Picker, 1996; Lasky, 1992). In the first step, L-selectin on lymphocytes recognizes the sulfated form of Sialyl Lewis X (SLeX), a tetrasaccharide carbohydrate that is present as a post-translational modification on certain proteins that are expressed by HEV cells. When CD34 and podocalyxin, and possibly endoglycan, are expressed by HEVs, they are appropriately glycosylated (with the SLeX modification) for interaction with L-selectin and thereby provide ligands for this adhesive interaction (Baumhueter et al., 1993; Fieger et al., 2003; Sarangapani et al., 2004; Sassetti et al., 1998). Notably, however, despite the widespread vascular expression of CD34-family proteins, only the rare HEV cells (which represent <1% of the total vascular endothelium) glycosylate these proteins appropriately for L-selectin binding. Thus, although the discovery in the early 1990s that CD34 binds to L-selectin (Baumhueter et al., 1993) quickly led to speculation that the CD34 family has a global role in enhancing adhesion (Healy et al., 1995; Hu and Chien, 1998; Larrucea et al., 2008; Larrucea et al., 2007; Majdic et al., 1994), this interaction is likely to represent an important exception rather than providing a general insight into their function.

Blocking cell adhesion

In contrast to the proposed role of CD34-family proteins in promoting adhesion, other studies have suggested that CD34-family proteins act in most situations as regulated blockers of adhesion – that is in the opposite capacity (Fig. 2B). This role was first postulated for podocalyxin in the early 1980s when it was found that podocalyxin and, in particular, its negatively charged, heavily glycosylated extracellular domain was required for appropriate spacing of podocyte foot processes in the kidney (Kerjaschki et al., 1984; Schnabel et al., 1989). This was later confirmed in *Podxl*-knockout studies, in which kidney podocytes failed to dissolve tight junctions and form foot processes appropriately during development (Doyonnas et al., 2001). Similarly, in-vitro overexpression of podocalyxin in Madin-Darby canine kidney (MDCK) and Chinese hamster ovary (CHO) cell lines has been shown to decrease cell-cell aggregation and to reduce the ability of cells to form appropriate junctional complexes in monolayers (Takeda et al., 2000). These studies strongly support a 'molecular Teflon' role for CD34-related proteins in blocking cell adhesion.

It is noteworthy that other studies that have examined the expression of podocalyxin, even in the same cell models as described above, have reached the diametrically opposing conclusion: that podocalyxin overexpression enhances adhesion (Larrucea et al., 2008; Larrucea et al., 2007). An explanation for these discrepancies probably lies in the level of podocalyxin expression and the effects that varying podocalyxin levels have on the localization of other molecules (Fig. 3). Several groups have shown that podocalyxin has an 'apicalizing' effect on cells – it is potently targeted to the apical domain, and its overexpression can even lead to the expansion of apical domains in cells at the expense of basolateral domains (Fig. 3B) (Meder et al., 2005; Nielsen et al., 2007; Yu et al., 2007). We have noticed that low-level expression of podocalyxin in human breast epithelial cell lines (MCF-7) tends to aid in driving the exclusion of integrins from the apical domains of cells (Jane Cipollone, Marcia Graves, Calvin D. Roskelley, Poh Tan and K.M.McN., unpublished observations). We therefore propose that – when expressed at low levels – podocalyxin can help to drive integrins to the basolateral surface of cells (essentially aiding in polarization), although it itself blocks adhesion; thereby, it can enhance integrin avidity at the basolateral face (Fig. 3A). Similarly, we have noticed that inhibition of podocalyxin expression in hematopoietic precursor cells that are bound to fibronectin can lead to inappropriate migration of integrins toward the 'apical' surface, effectively diluting their expression on the adhesive face of cells (Jane Cipollone, Marcia Graves, Calvin D. Roskelley, Poh Tan and K.M.McN., unpublished observations). By contrast, when podocalyxin is expressed at very high levels it might decrease cell adhesion by two mechanisms. First, by further expansion of the apical domain at the expense of the basolateral domain, cells might eventually have insufficient basolateral surface area to remain attached (Fig. 3B). Second, we have shown that the formation of podocalyxin-induced, actin-rich microvilli at the apical domain eventually leads to the titration of actin away from integrins and, thereby, lessens their ability to function as high-affinity anchors. The different effects of podocalyxin

at low and high levels are probably responsible for many of the apparent discrepancies in structure-function studies of these molecules and highlight the importance of very careful mechanistic analyses in all gain- and loss-of-function experiments.

In support of a role for CD34-family proteins in blocking cell adhesion, we have shown conclusively that loss of CD34 from mast cells increases cell aggregation (Drew et al., 2005). This phenotype is fully reversible upon ectopic expression of CD34 in *Cd34*-deficient mast cells (Drew et al., 2005). The homotypic aggregation of *Cd34*-deficient mast cells was also dramatically enhanced when the gene encoding sialophorin (*Spn*, also known as CD43), a distantly related transmembrane mucin, was deleted; this again suggests that functional redundancy can mask the full phenotype of CD34-related proteins (Drew et al., 2005). Interestingly, the naturally occurring splice variant of CD34 (described above) lacks the bulk of the cytoplasmic tail and acts as a more potent inhibitor of adhesion than full-length CD34 (Drew et al., 2005). As described for podocalyxin above, this probably reflects subtle differences in CD34-isoform localization: the full-length form of CD34 retains the ability to be cleared from the adhesive face of cells through its interaction with intracellular ligands, whereas the isoform that lacks the cytoplasmic domain is cleared less efficiently and can therefore act as a more powerful inhibitor of adhesion.

Trafficking of hematopoietic cells

Mice that lack members of the CD34 family have provided a wealth of information about the functional roles of these proteins in normal development and disease. As CD34 is particularly well known for its expression in the hematopoietic system, this is a major area of study in $Cd34^{-/-}$ animals. Despite the relatively minor hematopoietic phenotypes noted in $Cd34^{-/-}$ mice, one dramatic functional defect is observed in multiple cell types: $Cd34^{-/-}$ cells have a profound migratory impairment. Typically, intravenous transplantation of HSCs into recipient mice results in their trafficking via the circulation to the bone marrow where they undergo extravasation between endothelial cells, enter the bone marrow parenchyma and chemotax towards a specific subendosteal niche for their maintenance. In short-term homing assays that compared wild-type and $Cd34^{-/-}$ fetal-liver hematopoietic cells (an enriched source of HSCs) we have demonstrated a 20% reduction in cell migration of $Cd34^{-/-}$ fetal liver to bone marrow (Doyonnas et al., 2005). Similarly, $Podxl^{-/-}$ cells demonstrate a 30% decrease in short-term homing, and cells that lack both proteins are further impaired (Doyonnas et al., 2005). Moreover, $Cd34^{-/-}$ HSCs exhibit markedly reduced bone-marrow repopulation in competitive transplantation assays, in which HSCs are allowed to fully engraft recipient bone marrow over a 12- to 16-week interval (Drew et al., 2005; Nielsen and McNagny, 2007). Interestingly, $Cd34^{-/-}$ and wild-type HSCs display an equivalent ability to engraft the bone-marrow compartment of lethally irradiated mice, but $Cd34^{-/-}$ cells exhibit <20% of the engrafting capacity of wild-type cells when transplanted into sublethally irradiated or non-irradiated animals (Drew et al., 2005; Nielsen and McNagny, 2007). Again, these dramatic effects in non-irradiated or weakly irradiated recipient mice probably reflect increased adhesion of $Cd34^{-/-}$ HSCs to the vasculature and an impairment in their ability to traffic between endothelial cells and enter the underlying bone-marrow microenvironment for their maintenance. It is possible that, under physiological conditions, CD34 is required for migration of HSCs between endothelial cells and into hematopoietic stem-cell niches within the bone marrow. Irradiation causes considerable inflammation and leads to increased vascular permeability (Mazo et al., 2002), which probably lessens the requirement for an anti-adhesive sialomucin coating on HSCs to enable their efficient extravasation (Nielsen and McNagny, 2007).

Using three separate experimental approaches, we have shown that, similar to its role in HSCs, CD34 facilitates the trafficking of mast cells and eosinophils to peripheral tissues. In the first approach, ablation of intraperitoneal mast cells by injection of water demonstrated that mast cells in $Cd34^{-/-}$ mice display delayed repopulation kinetics (Drew et al., 2005). Second, $Cd34^{-/-}$ mice are profoundly resistant to allergic inflammation of the lung; this reflects impaired migration of $Cd34^{-/-}$ mast cells and eosinophils into lung tissue and airways (Blanchet et al., 2007). Finally, mice that carry $Cd34^{-/-}$ mast cells are remarkably resistant to intestinal polyposis and cancer, owing to the impaired trafficking of mast cells to the intestine (where they have a role in angiogenesis in preneoplastic lesions) (Gounaris et al., 2007). Taken together with the data on HSCs, these results indicate that podocalyxin and CD34 enhance trafficking and migration of hematopoietic cells. Although the precise mechanism of action of CD34 and podocalyxin in migration has yet to be proven, most evidence indicates that these proteins decrease non-specific adhesion and enhance mobility.

Cell morphogenesis

Podocalyxin is essential for maintaining the elaborate structure of kidney podocytes (Doyonnas et al., 2001; Kerjaschki et al., 1984; Schnabel et al., 1989). These epithelial cells are comprised of a cell body with multiple extensions that are termed major processes, and smaller, interdigitating 'foot processes' that extend from the major processes. The apical surfaces of podocyte cell bodies and processes are normally coated with podocalyxin, and $Podxl^{-/-}$ mice lack foot processes entirely (Doyonnas et al., 2001; Kerjaschki et al., 1984; Schnabel et al., 1989). The clear effect of podocalyxin on podocyte morphology and the discovery of its association with the actin cytoskeleton (Orlando et al., 2001; Takeda, 2003) led us to investigate the role of podocalyxin in establishing shape in other cell types (Nielsen et al., 2007). Podocalyxin is frequently expressed on the surface of cells with complex membrane extensions. For example, podocalyxin is expressed by a subset of neurons (Vitureira et al., 2005); similar to podocytes, neurons have highly branched networks of cytoskeletal constituents. Furthermore, many cytoskeletal proteins are found both in podocytes and in neurons, which indicates that similar mechanisms underlie the formation of cellular extensions in both cell types (reviewed by Kobayashi et al., 2004). In addition, podocalyxin is expressed by megakaryocytes, which extend long processes when generating platelets (Miettinen et al., 1999).

When podocalyxin is ectopically expressed in MDCK and MCF-7 cells, we observe a striking increase in surface microvillus formation upon electron-microscopic examination (Fig. 3B) (Nielsen et al., 2007). This effect is dependent on the extracellular domain of podocalyxin, as cytoplasmic-domain deletion mutants can induce microvillus formation, whereas deletions of the extracellular domain abolish the effect (Nielsen et al., 2007). This suggests that podocalyxin has a global role in modifying cell shape. In support of this, podocalyxin has also been shown to have a role in kidney epithelial cell tubulogenesis in vitro (Cheng et al., 2005). MDCK cells that express podocalyxin can be induced to form tubules in semisolid matrices; strikingly, the depletion of podocalyxin by RNA interference blocks this tubulogenesis (Cheng

et al., 2005). These results might be relevant to other tissues in which podocalyxin is typically expressed (Dekan et al., 1990; Takeda et al., 2001). For example, podocalyxin in mammary epithelial cells (Somasiri et al., 2004) and vascular endothelia (Horvat et al., 1986) might facilitate remodeling and angiogenesis, respectively.

Cancer and the CD34 family

There are many recent papers that implicate podocalyxin in malignancy, including breast cancer, prostate cancer, embryonic carcinomas, leukemia and pancreatic cancer (Casey et al., 2006; Heukamp et al., 2006; Kelley et al., 2005; Ney et al., 2007; Schopperle and DeWolf, 2007; Schopperle et al., 2003; Somasiri et al., 2004). An early report described podocalyxin as a marker of testicular cancer and postulated that it might be a useful serum biomarker (Schopperle et al., 2003). Shortly thereafter, we found that upregulation of podocalyxin correlates with poor outcome in invasive breast carcinoma (Somasiri et al., 2004). Interestingly, tumors with high podocalyxin levels showed no correlation with lymph-node involvement (the majority were 'node-negative') but later went on to become life-threatening tumors (Somasiri et al., 2004). This suggests that high expression levels of podocalyxin are a better marker to predict poor outcome than other indicators currently in use. Moreover, podocalyxin mutations have been associated with increased cancer aggressiveness in prostate-cancer patients (Casey et al., 2006). In addition, podocalyxin expression is dysregulated in acute myeloid leukemia and acute lymphoblastic leukemia (Kelley et al., 2005), hepatocellular carcinoma (Chen et al., 2004) and Wilms tumors (Stanhope-Baker et al., 2004). Podocalyxin has thus been associated with a wide variety of cancers and, strikingly, it is often associated with more aggressive cases. The most likely functional implication of podocalyxin upregulation is increased metastasis, although this has yet to be proven experimentally.

New directions for the CD34 family?

Sialomucins and chemokine-dependent trafficking

Interestingly, in two recent studies, transmembrane sialomucins have been shown to increase the fidelity of chemokine signaling, and therefore to enhance chemotaxis. For example, it has recently been shown that cell-surface expression of P-selectin glycoprotein ligand 1 (PSGL-1, also known as SELPL), a well-characterized sialomucin that is a hematopoietic ligand for P-selectin (which is expressed on activated endothelium), can greatly enhance the ability of naive lymphocytes to home to peripheral lymph nodes (Veerman et al., 2007). This enhanced chemotaxis was shown to be independent of P-selectin binding and was instead attributed to the ability of PSGL-1 to enhance binding of the C-C motif chemokines 19 and 21 (CCL19 and CCL21, respectively) on T cells, and their subsequent signaling through chemokine receptors (Veerman et al., 2007). Intriguingly, a similar role for the sialomucin CD164 in enhancing signaling via the chemokine CXCL12 has been demonstrated in human hematopoietic precursors (Forde et al., 2007). In this elegant study, silencing of *CD164* gene expression was shown to impair the trafficking of cells towards CXCL12 (Forde et al., 2007). Moreover, in wild-type cells, CXCL12 was shown to stimulate association of CD164 with the G-protein-coupled receptor CXCR4 (the transmembrane receptor for CXCL12); this association with CD164 enhanced downstream CXCR4 signaling and the activation of integrins for adhesion and chemotaxis (Forde et al., 2007). Thus, in these two scenarios, sialomucins facilitate chemotactic cell migration. Intriguingly, we have recently noticed a similar role for podocalyxin in chemotactic signalling: podocalyxin associates with CXCR4 and enhances its ability to transmit downstream chemotactic signals, a pathway that is defective in *Podxl*-deficient cells (Poh Tan and K.M.McN.). The mechanism by which these sialomucins facilitate chemotactic signaling remains to be clarified (i.e. do they act directly, via their association with chemokine receptors, or indirectly, by aiding in the establishment of

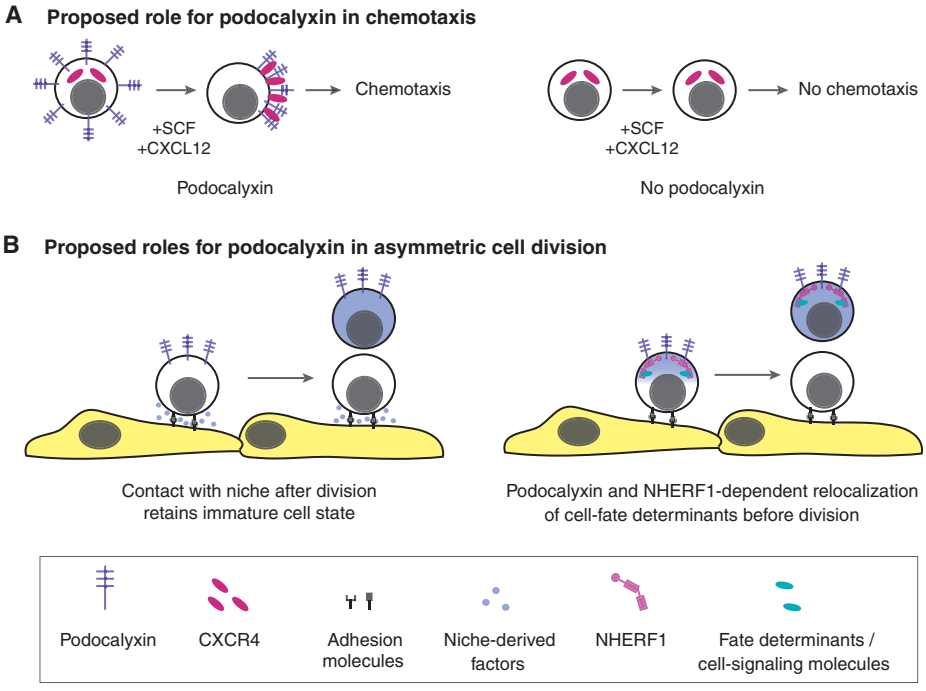

Fig. 4. Possible functions of CD34-family proteins: chemokine-mediated trafficking and regulation of asymmetric cell division. (A) Model of podocalyxin-dependent chemotaxis. (B) Two proposed mechanisms by which podocalyxin might facilitate asymmetric cell division. Segregation of podocalyxin to the apical surface might enable the interaction of adhesion molecules with the stem-cell niche; after division, only the cell that remains in contact with the niche would receive signals to maintain pluripotency. Alternatively, podocalyxin- and NHERF1-dependent segregation of cell-fate determinants might instruct the cell to divide asymmetrically.

apical-basal polarity and integrin segregation in activated cells?), and this will probably be an area of intense study in the future (Fig. 4A).

Podocalyxin and regulation of stem-cell fate

It is noteworthy that proteins of the CD34 family are expressed by a variety of pluripotent cells and tissue stem cells. All three family members are expressed by HSCs and/or more-differentiated multipotent hematopoietic progenitors (Andrews et al., 1989; Berenson et al., 1988; Doyonnas et al., 2005; McNagny et al., 1997; Sassetti et al., 2000; Young et al., 1995). Podocalyxin is also expressed by embryonic stem cells and hemangioblasts (Hara et al., 1999; Laslett et al., 2007), and CD34 is expressed by dermal stem cells and muscle satellite cells (Beauchamp et al., 2000; Trempus et al., 2003; Trempus et al., 2007). It is possible that this family has a specific role in stem-cell behavior, and one could envision several stem-cell-related functions for anti-adhesion proteins that have a potent ability for establishing apical domains. HSCs, for example, are dependent on adhesion to selective stromal niches within bone marrow for their maintenance (reviewed by Jones and Wagers, 2008; Raaijmakers and Scadden, 2008; Wilson and Trumpp, 2006). The stromal niche is akin to the well-studied germ-cell niche in *Drosophila melanogaster* (Fuller and Spradling, 2007). Low-level expression of CD34-type proteins by HSCs within these niches could lead to enhanced integrin segregation and adhesion to the niche in an analogous manner to the scenarios illustrated in Fig. 3A and Fig. 4B. Further upregulation of CD34-family proteins on HSCs could lead to decreased adhesion of these cells and migration away from the niche, followed by subsequent differentiation (as illustrated in Fig. 3B and Fig. 4B).

Another possibility is that apically targeted CD34-type proteins could have a role in fate determination of HSCs during cell division. One model for HSC behavior is that key molecules that govern differentiation are redistributed unequally before division, such that those that are required to maintain an immature cell are localized at one pole, whereas the molecular determinants of a more mature cell are segregated to the opposite pole. Subsequent cell division gives rise to daughter cells that have distinct cellular compositions that destine these cells for different fates. This 'asymmetric division' model has been proposed as one mechanism for regulating stem-cell self-renewal and differentiation (reviewed by Morrison and Spradling, 2008; Jones and Wagers, 2008; Raaijmakers and Scadden, 2008; Wilson and Trumpp, 2006). Because podocalyxin associates with the actin cytoskeleton at the apical, non-adhesive surface of cells and recruits NHERF1 to these apical domains (Meder et al., 2005; Nielsen et al., 2007; Schmieder et al., 2004), it would be well positioned for a role in asymmetric cell division (Fig. 4B). For example, we have noticed previously that hematopoietic cells that are activated with interleukin 3 (IL3; a proliferation and differentiation factor) rapidly relocalize podocalyxin and its cytoplasmic ligand NHERF1 to uropods (Tan et al., 2006). NHERF1 has been shown to bind to numerous fate-determining signaling molecules (PDGFRA, PDGFRB, EGFR, β-catenin, PTEN and many G-protein-coupled receptors) (reviewed by Weinman et al., 2005) in various cell types. Therefore, recruitment of NHERF-bound factors to the podocalyxin-rich apical domain during cell division would provide a mechanism for the asymmetric segregation of signaling molecules during cell division (Fig. 4B). Podocalyxin is also upregulated in differentiating embryonic stem cells (Laslett et al., 2007), so a similar mechanism might be important when pluripotent cells differentiate. This model, though speculative, warrants further investigation.

Concluding remarks

Several seemingly unrelated functions have been proposed for the members of the CD34 family. Although it might be difficult to imagine how these proteins perform such diverse roles, it is not entirely surprising when one considers several factors. First, these sialomucins have clearly defined domains that could contribute to different functions: the extensively glycosylated extracellular mucin domain might regulate cell adhesion, whereas the cytoplasmic domain could provide a link to the actin cytoskeleton and several intracellular proteins. Second, distinct proteins might interact with the CD34-family members, depending on the phosphorylation state of the intracellular domain. Third, NHERF1 and NHERF2 are scaffolding proteins that have many interaction partners, so the association of podocalyxin and endoglycan with these proteins might lead to a wide range of downstream functions. Finally, the ability of podocalyxin to act as either a pro- or anti-adhesin might be closely linked to its capacity to generate apical-domain structures such as microvilli. The expression of appropriately glycosylated forms of podocalyxin on microvilli, which are known to express adhesion molecules at their tips, might facilitate L-selectin-dependent leukocyte rolling and transendothelial migration in HEVs (Girard et al., 1999; Picker et al., 1991; von Andrian et al., 1995). Conversely, in most other cell types the podocalyxin-coated, microvillus-rich apical domain might protect cells from non-specific adhesion. Thus, upregulation of podocalyxin in breast cancer cells might promote metastasis by disrupting cell adhesion, particularly during apical membrane expansion (Nielsen et al., 2007; Somasiri et al., 2004). This would mean that tumor cells could be disseminated by a different, although not necessarily mutually exclusive, mechanism from the well-described epithelial-mesenchymal transition (Kang and Massague, 2004). It is also tempting to speculate that the recruitment of filamentous actin to the apical membrane of cells to generate microvilli might deplete actin from the basal surface of these cells, thereby preventing stable interactions of integrins with the extracellular matrix (Fig. 3B) (Nielsen et al., 2007; Schmieder et al., 2004). Further studies that investigate the formation of intracellular multiprotein complexes will enable us to delineate more clearly the precise roles and mechanisms of action of CD34, podocalyxin and endoglycan in a range of tissues.

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