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The Biology of CD44 and HCELL in Hematopoiesis: The “Step 2-bypass Pathway” and other Emerging Perspectives

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Abstract

Purpose of Review—The homing and egress of hematopoietic stem/progenitor cells (HSPCs) to and from marrow, respectively, and the proliferation and differentiation of HSPCs within marrow, are complex processes critically regulated by the ordered expression and function of adhesion molecules that direct key cell-cell and cell-matrix interactions. The integral membrane molecule CD44, known primarily for its role in binding hyaluronic acid, is characteristically expressed on HSPCs. Conspicuously, human HSPCs uniquely display a specialized glycoform of CD44 known as Hematopoietic Cell E-/L-selectin Ligand (HCELL), which is the most potent ligand for both E-selectin and L-selectin expressed on human cells. This review focuses on recent advances in our understanding of the biology of CD44 and HCELL in hematopoiesis.

Recent Findings—New data indicate that CD44-mediated events in hematopoiesis are more complex than previously imagined. Ex vivo glycan engineering has established that HCELL serves as a “bone marrow homing receptor”. Moreover, biochemical studies now show that CD44 forms bimolecular complexes with a variety of membrane proteins, one of which is VLA-4. Engagement of CD44 or of HCELL directly induces VLA-4 activation via G-protein-dependent signaling, triggering a “Step 2-bypass Pathway” of cell migration, and extravascular lodgment, in absence of chemokine receptor engagement.

Summary—Recent studies have further clarified the roles of CD44 and its glycoform HCELL in hematopoietic processes, providing key insights on how targeting these molecules may be beneficial in promoting hematopoiesis and in treating hematologic malignancies.

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Keywords

HCELL; Step 2-bypass pathway of cell migration; multistep paradigm; CD44; bimolecular complex

Introduction

There is a rather bewildering array of structures and activities related to CD44. By intent, this review focuses on recent findings related to the biology of CD44 in hematopoiesis. From all current data, a unifying perspective for CD44 biology in hematopoiesis is proposed here, with profound implications for both normal and malignant hematopoiesis. To provide requisite foundation for understanding these contemporary data and notions, however, it is necessary to first summarize key features of CD44 multistructural and multifunctional properties. It is recognized that the foregoing description is limited in scope, and, for more comprehensive background information, the reader is referred to several excellent reviews on CD44 that are cited herein.

STRUCTURE AND FUNCTION OF CD44

CD44 is a ubiquitously expressed integral membrane glycoprotein which displays tremendous structural heterogeneity resulting from a wide variety of protein polymorphisms and post-translational modifications. The CD44 gene of all mammals is arranged into two groups comprising “standard” exons and “variant” exons, yielding single-chain proteins that, in fully processed form, range in size from ~80 kDa to more than 200 kDa [1]. The standard exon group comprises 10 exons, numbered usually as exons 1–5 and 16–20, but here designated as exons s1–s5 and s6–s10, respectively (see Figure 1). CD44 protein containing only standard exons is known as “CD44s” (also, “CD44H”, see below). This isoform consists of 341 amino acids in humans (predicted core molecular weight (m.w.) of ~37 kDa). Fully processed CD44s ranges in m.w. from ~80–110 kDa (depending on cell type), and includes an extracellular domain of 248 residues, a transmembrane region of 21 residues, and a 72 amino acid cytoplasmic tail. Exons s1–s5 encode an N-terminal globular domain which is stabilized by intermolecular disulfide bonds (via three pairs of cysteines), and exons s6 and s7 encode the membrane proximal region. The transmembrane region is encoded by exon s8, and exons s8–s10 together encode the cytoplasmic tail.

CD44 proteins designated as “CD44v” contain the peptide products of the standard exons and also products of variant exons, each encoded by transcripts inserted between exons s5 and s6 (i.e., exons 5 and 16). There are 10 variant exons expressed in mice (designated v1–v10), whereas humans express only variant exons v2–v10 due to the presence of an in-frame stop codon in exon v1 [2]. The large number of variant exons results in multiple protein isoforms (theoretically, 768 possibilities [3]), of which over 20 have been well-characterized [1]. The largest CD44v protein, called “epican”, contains peptides derived in tandem from variant exons v3–v8 and is typically found on keratinocytes [4,5]. Notably, mammalian HSPCs characteristically express only the CD44s isoform (known also as CD44H, for “Hematopoietic” CD44) [6,7,8]. Mature hematopoietic cells also typically express CD44s, but CD44v isoforms can be found on various native and malignant myeloid cells and lymphoid cells [6,8,9,10,11]. In general, CD44v isoforms are most commonly expressed on epithelial cells and on non-hematopoietic cancer cells (for review, see [1]).

In addition to protein polymorphisms, diversity of CD44 structure is also engendered by a wide variety of post-translational modifications. CD44 can be covalently modified by several glycosaminoglycans (GAG), including chondroitin sulfate (CS), dermatan sulfate (DS), heparan sulfate (HS) and keratan sulfate (KS). All CD44 isoforms bearing covalent

GAG substitutions have m.w. in excess of 110 kDa, and, by definition, are members of the “proteoglycan” family. CD44s displays a GAG linkage consensus amino acid motif (SGXS) in the membrane proximal region of the extracellular portion [12], and CD44s covalently bearing KS, DS, and, more commonly, CS has been reported [13,14,15,16,17,18]. However, typically, chondroitin sulfate and, more specifically, heparan sulfate covalent modifications are found within isoforms containing the v3 region that contains an SGSG sequence together with downstream regulatory amino acids that enable HS substitution [12,19]. As might be expected by its repertoire of variant sequences, epican can be modified by CS, HS and KS [20].

GAG modifications impart distinct ligand specificities on CD44 isoforms. For example, substitution of CD44s and of CD44v isoforms with CS licenses binding to a variety of extracellular matrix molecules, including fibronectin, laminin, and collagen (types I, IV, VI and XIV) [17,21,22,23,24,25,26], though binding to collagen by CD44s has been observed in absence of CS modification [27]. Attachment with either CS or DS promotes CD44 binding to fibrinogen/fibrin [15,18]. Modification with HS mediates CD44 presentation of growth factors such as basic-fibroblast growth factor, heparin-binding epidermal growth factor, and vascular endothelial growth factor [28,29], and modification by HS and/or CS also mediates CD44 binding/presentation of certain chemokines [30,31,32].

CD44 is best known for serving as a receptor for hyaluronic acid (HA), a non-sulfated GAG. The globular head region contains the lectin motif that directs binding of CD44 to HA [33]. Although all CD44 isoforms contain this HA binding pocket, many cells that display high levels of CD44 (especially hematopoietic cells, including human CD34+ HSPCs [34,35,36]) do not constitutively bind HA (for review, see [1,37]). The capacity of CD44 to bind HA is critically regulated by post-translational modifications. Sulfation of CD44 and distinct CD44 carbohydrate modifications can each independently modulate the capacity to engage HA. In addition to GAG substitutions, CD44 is abundantly decorated with both N- and O-linked glycosylations ([38], for review see [1]). Sulfation of CD44 can occur on tyrosines [39], or, more commonly, on attached GAGs (predominantly via chondroitin sulfate [38]) or on glycans attached to the core protein (predominantly N-linked [38,40]). Dynamic sulfation of CD44, induced by inflammatory cytokines such as TNF [40], upregulates adhesion to HA. In contrast, modification of CD44 glycans with terminal sialic acids inhibits binding to HA [41,42]. CD44 glycan modifications on hematopoietic cells can be modulated by cytokines, resulting in either inhibition or stimulation of HA binding depending on the cytokine [35,43,44]. Notably, myeloid cells express a cell surface sialidase, inducible by TNF and by ligation of CD44, that cleaves terminal sialic acids and upregulates CD44 adherence to HA [44,45,46]. Conversely, whereas sialylation inhibits HA binding, it is requisite for function of the HCELL glycoform of CD44 that potently binds selectins (discussed in more detail below).

CD44 functions as a critical signaling receptor in a wide variety of hematopoietic and non-hematopoietic cells, both native and malignant. Though details are beyond the scope of this review, it is well-recognized that ligation of CD44, either by natural ligands or by anti-CD44 antibody, is associated with activation of a variety of cell signaling pathways that regulate cell biology, including cell migration, survival and proliferation, cytokine production, and cytolytic activities (for review see [47,48,49]). CD44 is found in lipid rafts of the plasma membrane, coupled to a variety of protein tyrosine kinases and other signaling effectors. The intracytoplasmic tail of CD44 can be phosphorylated, and though phosphorylation may not be necessary for HA binding [50], it appears to regulate HA-dependent cell migration [51]. The tail is also complexed with ankyrin [27,52] and with other cytoskeletal proteins (i.e., ezrin-moesin-radixin) that link to spectrin and actin, respectively [53,54]. In this manner, CD44 forms an important sensor of the microenvironment, connecting the extracellular

milieu with the cytoskeleton, and vice-versa. The surface distribution of CD44 can markedly change with cell migration and with changes in the milieu, and there is evidence that palmitoylation of CD44 regulates partitioning into lipid rafts and binding to ankyrin [55,56].

HCELL: MUCH MORE THAN 'SWEET' CD44

Studies over several decades have shown that HA is a critical structural and regulatory component of hematopoietic microenvironments, with its various effects mediated via CD44 ligation [57, 58, 59, 60, 61]. In addition to its function as an HA receptor, CD44 in human HSPCs executes a unique role exercised by a specialized glycoform, “Hematopoietic Cell E-/L-selectin Ligand” (HCELL), that contains sialofucosylated lactosamines (reviewed in [62]). These sialofucosylated glycans, displayed as the terminal tetrasaccharide known as sLex, are the prototypical binding determinants for selectins, a family of three C-type lectins: L-selectin (expressed on HSPCs, and mature myeloid and lymphoid cells), E-selectin (expressed on endothelial cells), and P-selectin (expressed on platelets and endothelial cells) [63]. HCELL was first defined functionally by its unique selectin ligand properties well before it was discovered to be a novel glycovariant of CD44 [64, 65, 66]. Though CD44 serves as the scaffold to display the pertinent selectin-binding glycans, it is inaccurate to state that “CD44 is a selectin ligand” for several reasons. To begin, the working end of HCELL is not the CD44 protein, it is the carbohydrates: indeed, HCELL activity is retained in the presence of complete denaturation of the CD44 protein core [66, 67, 68]. Thus, the glycan modifications alone confer the new biology on CD44. Importantly, whereas CD44 is uniformly recognized as a lectin (i.e., it binds the carbohydrate HA), HCELL is a ligand for lectins. Moreover, only HCELL, among all CD44 isoforms and glycovariants, mediates cell-cell interactions via adhesion to integral membrane glycoproteins (i.e., E-selectin and L-selectin). Apart from these abundant structural and operational features that clearly distinguish HCELL among all CD44 structures, there is etymologic precedent for designating this structure by proper label, just as the E-selectin-binding glycoform of P-selectin glycoprotein ligand-1 (PSGL-1) is conventionally known as “Cutaneous Lymphocyte Antigen” (“CLA”, reviewed in [62]) and certain CD44 isoforms have specific titles (e.g., “Epican”) to differentiate them from other moieties.

HCELL is the most potent E- and L-selectin ligand found on human cells. It is widely expressed on human HSPCs (CD34+ cells), but not on mouse HSPCs (Lin-/c-Kit+/Sca-1+ cells), and its expression is profoundly upregulated by G-CSF administration [69]. HCELL is also characteristically expressed on human leukemic blasts, but is not present on normal myeloid, erythroid, lymphoid, or megakaryocytic progenitors, nor on mature blood cells [66, 67, 68, 69]. On all human HSPCs and leukemic blasts that express HCELL, the relevant sLex determinants are exclusively displayed on N-glycans located in the globular head of CD44s [66]. Importantly, the fact that HCELL is a distinctly human HSPC glycoprotein indicates that findings derived exclusively from mouse models actually underrepresent the breadth of CD44 biology in human hematopoiesis.

UNIFYING PERSPECTIVES IN HEMATOPOIESIS: HCELL AND CD44, AND THEIR “SIGNIFICANT OTHERS”

The absence of HCELL on murine HSPCs has hindered studies on its function in vivo, but a critical role for HCELL in early hematopoietic events can be inferred from the fact that it is expressed only on primitive human hematopoietic progenitors. In general, selectins and their ligands are best known for mediating cell-cell adhesive interactions under fluid shear conditions, thereby promoting initial attachment of blood-borne cells onto endothelial surfaces (reviewed in [70]). E-selectin expression on endothelial cells is tightly regulated by inflammatory cytokines that induce transient transcription of its mRNA, followed by translation of mature protein. However, intravital microscopy in mice has shown that

migration of HSPCs to marrow is mediated by constitutive expression of E-selectin on marrow microvessels, colocalized uniquely with the chemokine CXCL12 (SDF-1), precisely at endothelial beds that are the sites of cellular recruitment [71]. These and other observations have led to a multistep model of cell migration to marrow, wherein expression of E-selectin ligands and of CXCR4 (the receptor for chemokine CXCL12) on blood-borne HSPCs triggers marrow homing (reviewed in [63]). According to the model, E-selectin receptor/ligand interactions mediate initial tethering/rolling-type interactions of circulating cells onto the endothelial surface (Step 1), whereby the cells can engage CXCL12 via CXCR4, resulting in G-protein-coupled activation of the β 1 integrin VLA-4 (which is expressed on HSPCs) (Step 2). Activated VLA-4 then mediates firm adherence by binding to its ligand VCAM-1 (which is also constitutively expressed on marrow endothelial beds) (Step 3), followed by transendothelial migration (Step 4) (see Figure 2). Due to its potency in binding E-selectin, we reasoned that HCELL could serve as a key Step 1 effector in this process. To examine the effect(s) of HCELL expression on osteotropism of circulating cells, we developed a platform technology called “Glycosyltransferase-Programmed Stereosubstitution” (GPS) for custom-modifying glycans on the surface of living cells and utilized a target cell devoid of Step 1 effectors, mesenchymal stem cells (MSCs) [72]. Using GPS, the native CD44 on human MSCs was converted into HCELL, without effects on cell viability or multipotency; enforced HCELL expression was transient (lost within 72 hours), likely due to natural turnover of cell surface CD44. Notably, the human MSCs used in these studies expressed VLA-4, but did not express CXCR4. Intravital microscopy in immunocompromised mice showed that intravenously administered HCELL+ human MSCs homed to marrow, with infiltrates evident within one hour post-injection. These data thus provide direct evidence that HCELL is a “bone marrow homing receptor” and, furthermore, that CXCR4 signaling is dispensable for marrow infiltration by HCELL+ MSC.

The observation that HCELL+ human MSC infiltrated murine marrow *in vivo* without CXCR4 signaling raised the possibility that MSC transendothelial migration may be encoded by chemokine-independent pathways. Recent investigations using human MSC show that this is the case: engagement of HCELL (via E-selectin) or of CD44 (via HA) in each case triggers VLA-4 activation via G-protein-dependent signal transduction [73]; activated VLA-4 thus binds to its ligands VCAM-1 and fibronectin, and, on endothelial cells stimulated to express both E-selectin and VCAM-1, MSC transmigration occurs without chemokine input [73]. These findings establish an expanded role for HCELL and for CD44 as effectors of cell migration, unveiling a previously unrecognized dimension of mechanosignaling that mediates a “step 2-bypass pathway” of the canonical multistep paradigm (see Figure 3). A formal modification of the “address” concept of cell migration proposed in the original description [74] of the multistep cascade is now required: although Step 2 integrin activation is retained, it is best to refer to this novel pathway as a “step 2-bypass” since the chemokine receptor/ligand combinatorial diversity inherent to conventional Step 2 is by-passed by mechanosignaling-triggered integrin activation, and, hence, chemokine-dependent selectivity in cell recruitment is obviated. Since marrow endothelial cells express HA in addition to E-selectin [61, 75], this step 2-bypass pathway could program trafficking of HSPCs to marrow independently of CXCR4, and could thus account for the finding that CXCR4-deficient HSPCs can migrate to marrow [76]. Such CD44/VLA-4 crosstalk could also profoundly affect localization of extravasated cells via engagement with respective extracellular matrix ligands, HA and fibronectin, programming lodgment of HSPCs, and of MSCs, at discrete hematopoietic microenvironments within the marrow parenchyma. Moreover, since MSCs express VCAM-1 [77] and also produce copious amounts of HA [78] [79], CD44/VLA-4 crosstalk could firmly anchor HSPCs to HA/VCAM-1-laden MSCs in the hematopoietic “niche” [80]. Indeed, this notion is supported by studies performed decades ago [81, 82, 83].

Biochemical studies now show that CD44 and VLA-4 form a bimolecular complex on MSC membranes [73] similar to that reported previously on lymphoid cells [84]. Prior studies indicated that binding of CD44v isoforms to osteopontin, a chemoattractant and regulatory constituent of the hematopoietic niche, is also dependent on CD44 coupling with $\beta 1$ integrins [85]. These findings highlight yet another remarkable feature of CD44: its propensity to physically and functionally partner with heterologous glycoproteins on the cell surface (see Table 1). Cross-immunoprecipitation studies in various cells have revealed that, in addition to serving to anchor growth factors such as HBEGF and bFGF (discussed above), CD44 can form cooperating complexes with an array of membrane growth factor receptors, including VEGFR2 (KDR) [86], c-Met [87], TGF β R1 [88] and HER2 [89] (and, possibly, other ErbB receptor family members [49]), as well as the receptor for migration inhibitory factor (and invariant polypeptide of MHC II), CD74 [90]. For each CD44-receptor complex, it is important to define if/how CD44 mediates observed hematopoietic effects currently attributed to the receptor: for example, whether engagement of CD44 impacts the observed myelosuppressive effects of TGF β R1 signaling in myelodysplastic syndrome [91]. It is of interest that HER2 is expressed on subsets of human hematopoietic cells, and its expression is upregulated on cycling HSPCs [92]. Importantly, both c-Met and VEGFR2 are expressed on early HSPCs [93, 94], and each contributes in various ways to both normal and malignant hematopoiesis [95, 96, 97]. For the case of c-Met, activation by its ligand hepatocyte growth factor (HGF) requires CD44 cooperation to organize cytoskeletal association(s) with Met-specific signaling components [87]. During G-CSF mobilization, CXCL12 induces HGF release from marrow neutrophils, and HGF induces HSPC mobilization [98, 99] in part due to upregulation of the matrix metalloproteases MMP9 and MT1-MMP [98]. It is well-recognized that CD44 anchors cell surface presentation of MMP9 [100, 101] and of MT1-MMP [102], the latter also serving to cleave CD44 from the cell surface [103]. Recent studies in mice have further clarified this biology, showing that G-CSF-induced HSPC mobilization is dependent on MT1-MMP-mediated cleavage of membrane CD44 [104]. Consistent with these findings in mice, following G-CSF mobilization in humans, there is evidence that circulating CD34⁺ cells show diminished CD44 expression compared to steady-state marrow counterparts [105, 106], but a more informative study would be to compare CD44 levels and HCELL expression in blood and marrow CD34⁺ cells, concomitantly, during G-CSF treatment [107].

Studies in knock-out mice show that CD44 is not necessary for hematopoiesis, but, compared to wild-type, affected mice show markedly increased numbers of HSPC in marrow relative to spleen, and defective HSPC mobilization following G-CSF administration [108]. The fact that CD44-deficient mice have relatively normal hematopoiesis and no overt developmental deficits is consistent with the reported compensation in HA binding by other HSPC HA receptors such as RHAMM [109], which is known to be upregulated in CD44-deficient mice [110]. More specifically, in adult wild-type animals, interference with CD44 biology (e.g., by use of function-blocking mAb) has yielded significant effect(s) on hematopoiesis. Importantly, despite absence of HCELL expression in mouse HSPCs, studies in wild-type models have shown that CD44 is critical for both marrow homing and lodgement of HSPCs [61,111], and that engagement of CXCR4 (by CXCL12) upregulates CD44 adhesion to HA [61]. Conversely, administration of anti-CD44 mAb induces mobilization of HSPCs [112,113].

CONCLUSION: EMERGING PERSPECTIVES ON CD44 BIOLOGY IN HEMATOPOIESIS

Clearly, the fact that CD44 co-associates with a variety of surface molecules, including growth factors, proteases, and other adhesion molecules, indicates a broader and more significant role for CD44 in both normal and malignant hematopoiesis than previously perceived, well beyond its well-recognized role as a receptor for HA and selectins. At

present, we cannot be confident that we know the identity of all cell membrane molecules that can partner with CD44 (see Table 1 for existing list). However, with current information, it is possible to offer several novel perspectives on CD44 biology in hematopoiesis.

The capacity to enforce HCELL expression, together with the fact that CD44 forms a functional bimolecular complex with VLA-4, offers exciting opportunities to augment cell migration to marrow for all clinical indications, especially hematopoietic stem cell transplantation in settings of limited numbers of donor cells (e.g., cord blood transplants). Similarly, because both E-selectin and VCAM-1 are upregulated in endothelial beds at all sites of tissue injury/inflammation, enforced HCELL expression could be exploited to enable stem cell-based regenerative medicine and other adoptive cell therapeutics (e.g., immunotherapy for malignancy) for a variety of conditions. For at least two cell surface receptors, c-Met and CD74, the CD44 molecule is the obligatory signaling component of the receptor [87,90]. Thus, targeting CD44 may offer novel ways of modulating c-Met and CD74 activity. Indeed, the finding that antibody to CD74 induces cell death of malignant lymphoid cells may say more about CD44 than about CD74 itself [114]. In this regard, it is important to emphasize that a large body of evidence has shown that, depending on leukemic subtype and the specific antibody employed, anti-CD44 mAbs are effective in directly killing and/or forcing differentiation of human leukemic blasts in vitro ([44,115,116], reviewed in [117]), and are capable of selectively eliminating human leukemic stem cells in vivo [118]. The capacity of different CD44-specific antibodies to induce varying biologic reactions despite targeting the same molecule suggests that such variable effects are secondary to interruption of functional CD44 cooperating complexes and/or variable signaling induced by ligation of specific CD44 epitopes. Importantly, induced leukemia models in mice have shown a role for CD44 in homing and engraftment of leukemic stem cells [119], and have also shown that high level expression of cell surface CD44 maintains the leukemic phenotype following withdrawal of the instigating transformation event [120]. These data indicate that the effect(s) of CD44 in normal and malignant hematopoiesis reflect not only CD44-mediated cell-cell and cell-matrix adhesive interactions, but also a combination and balance of CD44-dependent signals, via CD44 itself and/or its cooperating partners. Thus, despite decades of study of CD44, there is much more yet to learn regarding the complex multistructural/multifunctional tapestry of this remarkable molecule.

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KEY POINTS

- (1) CD44 is a multistructural and multifunctional molecule that serves as the principal receptor for hyaluronic acid, and is a key mediator of both normal and malignant hematopoiesis.
- (2) A specialized glycoform of CD44 known as “HCELL” is expressed on human hematopoietic stem/progenitor cells (HSPCs) and serves as the most potent E-selectin and L-selectin ligand on human cells.
- (3) CD44 (and/or HCELL) can form a functional biomolecular complex with integrin VLA-4, whereby engagement of CD44/HCELL triggers G-protein-dependent upregulation of VLA-4 adhesiveness and transendothelial migration of cells without chemokine input (“Step 2- bypass Pathway”).
- (4) Besides VLA-4, CD44 forms bimolecular complexes with various growth factor and chemokine receptors, and mediates intracellular signaling for receptors c-Met and CD74.
- (5) Monoclonal antibodies directed to CD44 are capable of inducing differentiation and/or death of human leukemic blasts, depending on the subtype of leukemia and the epitope targeted by the mAb.

FIGURE 1. Schematic representation of CD44 gene structure in mouse and man

The CD44 gene is located on chromosome 2 in humans and 11 in mice. The red X shown in variant exon 1 (V1) designates the absence of a functional V1 product in humans due to the presence of an in-frame stop codon. Parentheses below blocks show usual numbering of CD44 exons; for clarity, exons here are depicted as members of two groups: Standard (S) and Variant (V). See text for details.

FIGURE 2. The Canonical Multistep Paradigm of Cell Migration

Schematic representation of the multiple steps in cell migration from vascular to extravascular compartments. Step 2 integrin activation via chemokine receptor/ligand interactions confers selectivity in cell recruitment, depending on the relevant chemokine(s) present in the target endothelial bed and the chemokine receptor(s) expressed by circulating cell(s). Depicted here is the conventional view of cellular homing to marrow: Following initial tethering/rolling contact of blood-borne cell on the endothelium (Step 1), chemokine receptor CXCR4 binds to its cognate ligand CXCL1 (SDF-1), thereby triggering G-protein-coupled VLA-4 activation (Step 2), with subsequent firm adhesion (Step 3) and transmigration (Step 4).

FIGURE 3. The Step 2-bypass Pathway of Cell Migration

Schematic representation of steps in cell migration for blood-borne cells expressing HCELL together with VLA-4, encountering endothelial beds expressing E-selectin and VCAM-1.

Note that compared to canonical pathway (see Figure 2), VLA-4 activation (Step 2) occurs via G-protein-mediated mechanosignalling primed by HCELL binding to E-selectin (and/or activated CD44 binding to endothelial HA), bypassing the need for chemokine input.

TABLE 1

CD44 Bimolecular Complexes: “Significant Other” Cell Membrane Molecules

Growth Factor Receptors
c-Met
HER2 (?other ErbB family members)
TGF β 1 receptor
VEGFR2
Chemokine Receptors
CD74
Adhesion Molecules
VLA-4