

Cell Adhesion Molecules in Normal and Malignant Hematopoiesis: from Bench to Bedside

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Abstract Hematopoietic stem and progenitor cells (HSPC) express at their surface an array of cell adhesion receptors that enables them to interact with extracellular matrix components and transmembrane cell adhesion molecules expressed within stem cell niches in the bone marrow. Herein, we review the different cell adhesive interactions taking place within the bone marrow between HSPC and their niches (via integrins, CD44, selectin ligands, and Eph receptors) and how they support HSPC anchoring in the bone marrow and regulate HSPC fate during hematopoiesis. We review how malignant hematopoietic cells utilize adhesive interactions to resist chemotherapy treatments. Finally, we review how these adhesive interactions can be therapeutically targeted to increase HSPC mobilization for transplantation and to chemosensitize malignant hematopoietic cells to chemotherapy.

Keywords Integrins · CD44 · Selectin · Eph receptors · Hematopoietic stem cell · Niche · Mobilization · Chemoresistance

Introduction

Hematopoietic stem cells (HSC) are not randomly distributed in the bone marrow (BM) [1] but located in specific functional microenvironmental units called niches [2–5, 6•, 7•]. In adult humans and mice, HSC are mostly dormant in steady-state, dividing approximately once or twice a year, respectively [8, 9]. However in response to stress [9] and infections [10, 11], these HSC can be rapidly stimulated to divide to generate a burst of highly proliferative hematopoietic progenitor cells (HPC) that will expand and differentiate to reconstitute different blood and immune lineages. HPC have very limited self-renewal capacity and HSC with high self-renewal potential must be preserved lifelong. To ensure HSC longevity, HSC return to dormancy once the physiological challenge is resolved and then can serve as a back-up until the next challenge [9]. HSC and HPC are capable of dividing either symmetrically to self-renew, or asymmetrically to generate a lineage committed progenitor cell while preserving a functional original HSC or HPC [12]. Specific HSC niches can exquisitely regulate these different states and functions of HSC and HPC via their specific cellular components, extracellular matrix, cell-cell interactions, soluble mediators, and perfusion gradients. It has also been proposed that several types of HSC niches may be necessary to instruct different fates to HSC [5, 6•, 7•, 13, 14]. Indeed, individual HSC can choose one of several fates, such as to stay quiescent, or to proliferate, to either self-renew or differentiate into committed hematopoietic progenitor cells (HPC). These decisions are thought to be largely controlled by microenvironmental cues from the niches in which HSC reside. Dormant HSC (those HSC in prolonged quiescence) have been reported to associate specifically with small arterioles near the BM—bone interface that are ensheathed by NG2⁺ pericytes (a subset of mesenchymal stromal cell: MSC) [6•, 7•]. In contrast proliferative or

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“active” HSC are found adjacent to sinusoidal endothelium [6•, 7•]. Critical factors regulating HSC survival and self-renewal at these niches are under intense investigation. Cell adhesion molecules such as selectins and integrin-ligand adhesion molecules are involved in maintaining homeostasis at the HSC and HPC BM niche.

In order to find (home to) and stay in their specific niches (retention), HSC and HPC express a range of cell adhesion receptors which interact with extracellular matrix proteins and cell adhesion molecules expressed by niche cells. In addition to their role in ensuring HSC and HPC are retained in their niches, there is mounting evidence that these cell adhesion receptors also signal and influence HSC and HPC regulation. An important aspect of such adhesive interactions is that the affinity of these receptors to their adhesive ligands is regulated by post-translational mechanisms in order to adapt the niche and HSC state to a particular demand. Herein, we will review some of these adhesive interactions between HSC and their niche and how they regulate HSC fate in normal and pathological situations.

Leukocyte Adhesion Receptors Expressed by HSC

HSC express a range of cell adhesion receptors belonging to several structural families with best known examples P-selectin glycoprotein ligand -1 (PSGL-1 of CD162), integrins $\alpha 4\beta 1$ (CD49d/CD29 complex also cells VLA-4) and $\alpha 5\beta 1$ (CD49d/CD29 complex or VLA-5) and CD44 which were identified in the 1990s and more recently the Eph-ephrin axis. We have chosen to limit this review to these well-characterized cell adhesion molecules as summarized in Fig. 1.

Selectins

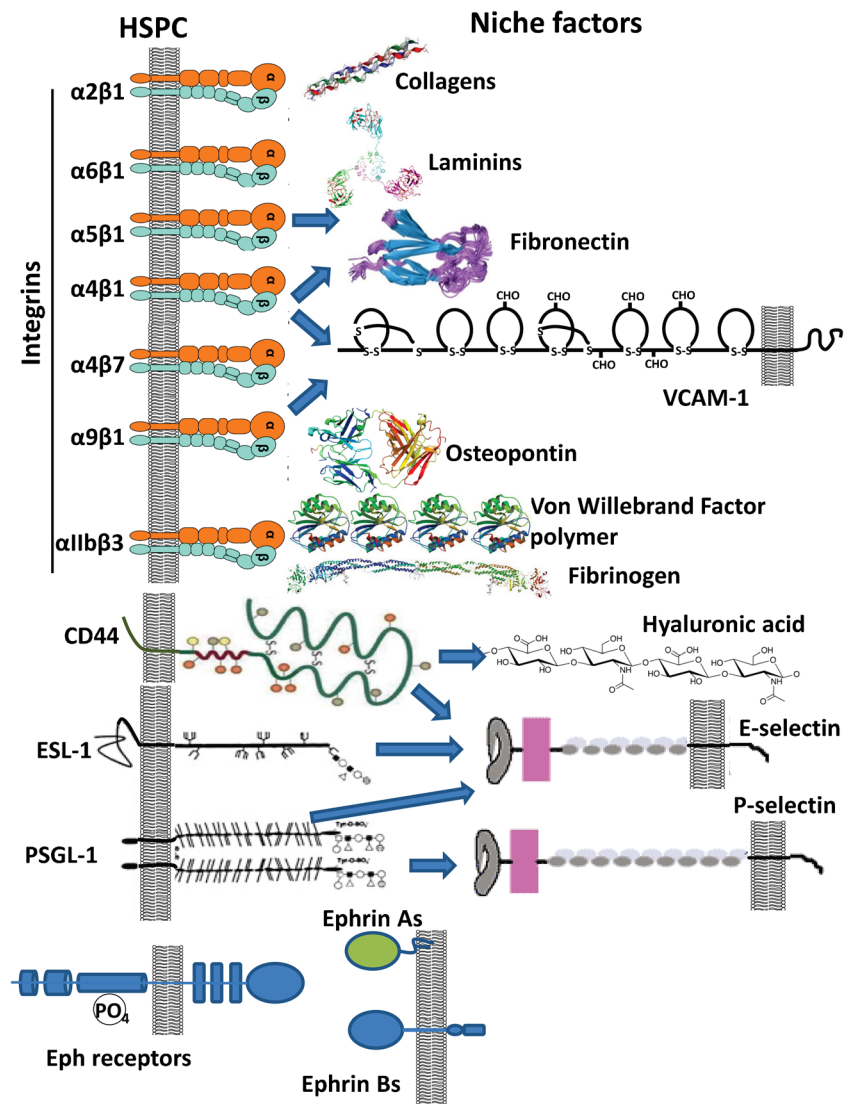
Selectins are a family of three transmembrane calcium-dependent lectins (L-selectin, E-selectin, and P-selectin) mediating cell-cell adhesion. Selectins are responsible for recruitment and homing of leukocytes to sites of inflammation, lymphoid organs, and the BM [15]. They specifically bind to fucosylated and sialylated tetrasaccharide motifs called sialyl Lewis^x (sLe^x) that decorates glycoproteins and glycolipids on the surface of many leukocytes. The three selectins mediate rolling and tethering of fast passing leukocytes onto activated (as in the case of inflammation) or specialized endothelial cells (such as in lymph nodes or BM). This step of rolling is mediated by a weak adhesive interaction, enabling leukocytes in the circulation to slow down and tether to these endothelial cells, a preliminary step to further strong attachment mediated by integrins. These steps of rolling and attachment are necessary for leukocyte extravasation across the endothelium and migration into the extravascular compartment of the tissue.

L-selectin (CD62L) is expressed by most leukocytes and best known to mediate the recruitment of leukocytes (lymphocytes and monocytes) to the high endothelial venules in lymph nodes via its interaction with glycosylation-dependent cell adhesion molecule (GlyCAM)-1 and to the endothelium of gut-associated lymphoid organs via mucosal vascular address in cell adhesion molecule (MadCAM)-1. Although expressed on HSC and HPC, the role of L-selectin on these cells remains unclear. *Sel^{-/-}* mice in which the L-selectin gene has been deleted have defective lymphocyte migration into the lymph nodes, Peyer’s patches and spleen, and defective granulocyte recruitment to inflamed peritoneum [16].

P-selectin (CD62P), expressed by megakaryocytes, platelets, inflamed endothelial cells, and a subset of BM endothelial cells, mediates the rolling of leukocytes expressing its transmembrane ligand PSGL-1. P-selectin in concert with E-selectin plays an important role in leucocyte recruitment to inflamed tissues and homing of HSPC to the BM. P-selectin knock-out mice (*Selp^{-/-}*) display a subtle phenotype. Megakaryocytes have recently been identified as critical HSC niche component [17] that secrete transforming growth factor (TGF)- β which maintain HSC quiescence [18•, 19•]. As megakaryocyte express P-selectin and human/mouse HSC express a functional P-selectin ligand PSGL-1 [20, 21], P-selectin may play an important role in anchoring HSC in close association with megakaryocytes in the BM. This remains, however, to demonstrate.

E-selectin (CD62E) is expressed by endothelial cells during inflammation as well as in specialized domains of the BM endothelium [22]. Unlike P-selectin, E-selectin has many potential ligands [23] such as PSGL-1 [24], a particular glycoform of CD44 called HCELL [25], E-selectin ligand (ESL)-1 [26•], and poorly defined fucosylated cell surface glycosphingolipids [27]. The combination of both E-selectin and P-selectin is critical to granulocyte recruitment into inflamed tissue and HSPC homing to the BM. Indeed, P- and E-selectin are not normally expressed by naive endothelial cells; however, following inflammation, endothelial selectins become rapidly expressed, and leukocytes with counter-receptors begin to roll on the activated endothelium. P-selectin is stored as a pre-form protein in endothelial cell Weibel-Palade bodies and translocated to cell surfaces within minutes of endothelial cell activation while expression of E-selectin begins hours later following de novo synthesis. Initial “fast” rolling (shear-resistant adhesion) of leukocytes on the endothelium is mediated by P-selectin, and rolling begins to “slow” when E-selectin is also expressed. Final immobilization of leukocytes occurs with the activation of integrins followed by extravasation to the site of tissue damage/inflammation (Fig. 1). Consistent with this role, leukocytes in mice deficient for both E- and P-selectins (*Selp^{-/-} Sele^{-/-}*) are unable to migrate properly to sites of tissue injury or inflammation [28]. Although these mice are more susceptible to

Fig. 1 Schematic representation of main cell adhesion receptors and molecules expressed by HSC and niche cells or in the niche extracellular matrix. Ribbon structure from X-ray crystallography data has been shown when available



bacterial infections, they are also less likely to develop hypersensitivity (allergic) or other inflammatory reactions. HSC homing and engraftment upon transplantation of wild-type HSC is defective in lethally irradiated *Selp^{-/-} Sele^{-/-}* mice [29]. This function of E- and P-selectin in HSC homing and engraftment is somewhat redundant as HSC homing and engraftment can still occur in recipient mice in which either selectin gene [29].

Unique Role of E-Selectin in Regulating HSC

Unlike P- and L-selectin, it has recently emerged that E-selectin plays a unique role in HSC regulation. E-selectin, which is expressed on the surface of specialized endothelial cells in HSC perivascular niches, promotes HSC proliferation. In steady-state, only ~3–5 % of BM vascular endothelial cells express E-selectin,

which this rises dramatically following hematopoietic stress, such as the recovery phase following irradiation, where >90 % of BM endothelial cells express E-selectin at a time when the surviving HSC pool must cycle to replenish the depleted blood and immune system [30••]. When E-selectin is therapeutically blocked with the small molecule antagonist GMI-1070 or is absent in *Sele^{-/-}* E-selectin gene-deleted mice, a large proportion of HSC returns to quiescence with heightened self-renewal potential as shown by increased potential for serial-transplantation in mice [30••]. Another consequence of slower HSC cycling is their increased resistance to cytotoxic chemotherapy or radiation in the absence of E-selectin or following administration of the E-selectin antagonist [30••]. Surprisingly, these effects are mediated solely by E-selectin, not P-selectin as *Selp^{-/-}* mice do not display increase in HSC dormancy.

Selectin Ligands on HSC

Most of the physiological selectin ligands are mucin-type glycoproteins decorated with sialylated, fucosylated glycan chains which are presumed to interact with the C-type lectin domain of the selectins (for example PSGL-1, CD34, CD44, GlyCAM-1, MADCAM-1) [15]. Mucins are large rod-like proteins with extensive O- and N-linked glycosylations that extend away from the cells outer surface. Selectins appear to recognize the “sialyl Lewis^x” (sLe^x) or similar tetrasaccharide structures, displayed at the ends of these long glycan chains or glycolipids. However, purified sLe^x tetrasaccharide only partially blocks selectin-mediated cell adhesion [31]. Therefore, although sLe^x is part of the recognition element for selectins, it must be correctly presented on the appropriate polypeptide or glycolipid backbone to mediate the shear-resistant adhesion that occurs in vivo. PSGL-1 (CD162) is a well-characterized selectin receptor on leukocytes and binds to all three selectins with varying affinities depending on adequate glycosylations and tyrosine sulfation. Although most hematopoietic cells express PSGL-1, they cannot adhere to selectins without the co-expression of five different glycosyl-transferases required to generate the sLe^x tetrasaccharide. In particular, fucosyl-transferase (FucT)-VII is absolutely essential to generate functional selectin receptors. Leukocytes from *Fut7*^{-/-} mice can no longer adhere to P- and E-selectin even though they express PSGL-1 [32].

Although mouse and human HSC express active selectin ligand PSGL-1 [20, 21], the effect of E-selectin on HSC cycling is not mediated by PSGL-1 nor CD44, the alternative canonical E-selectin ligand [25], as the anti-proliferative effect of E-selectin gene deletion is not replicated in mice lacking these two genes (*Selplg*^{-/-} *Cd44*^{-/-}). Furthermore, HSC from these mice still bind to E-selectin [30••]. Candidate receptors for this proliferative effect of E-selectin are not definitively identified but may include membrane glycosphingolipids [30••]. ESL-1 has also been recently shown to promote HSC quiescence; however, this effect was independent of E-selectin involving instead decreased release of anti-proliferative TGF-β [33••].

Integrins

Integrins form a vast and diverse array of cell adhesion receptors. They are formed by a heterodimeric complex between one out of 18 possible integrin α subunits and one out of 8 possible integrin β subunits all encoded by a unique gene. Not all combination of αβ complexes exist but the numbers of existing integrins remains mind boggling. They are classified according to the β subunit involved (e.g., β1 integrins, β2 integrins, etc). Each type of integrin αβ dimer has a unique repertoire of ligands that can be either extracellular matrix molecules or transmembrane cell adhesion molecules. Each

integrin has unique function. For the sake of simplicity, we will review those known to be expressed on HSC which include several β1, β2 [34•], β3, and β7 integrins [35].

The β1 integrin chain (CD29) dimerizes with integrins α1 to α11 or integrin αv. Mouse and human HSC are known to express only integrin α2 (CD49b), integrin α4 (CD49d), integrin α5 (CD49e), integrin α6 (CD49f), and integrin α9 [36–39]. Each of these integrins binds to several extracellular matrix proteins such as collagens (integrin α2β1), fibronectin (α4β1 and α5β1), laminin (α6β1), cleaved osteopontin (α4β1, α9β1) [39], and a key transmembrane cell adhesion molecule called vascular cell adhesion molecule-1 (VCAM-1 or CD106) that binds to integrins α4β1, α9β1, and α4β7 all present on HSC [35, 39]. The distribution of these extracellular matrix proteins in the BM can thus act as an address code. For instance collagens I and IV are abundant at the endosteum [40]. Fibronectin is distributed throughout the BM and particularly abundant at the endosteum and in and around megakaryocytes [40], which are both associated with HSC niches. Laminin is abundant in the wall of arteries and sinusoids in the BM [40], again two sites where niches for more primitive HSC have been described [6••, 7••]. Osteopontin has been reported at the endosteum [41] where it is cleaved by thrombin that has been activated by megakaryocytes [42] to become a ligand for integrins α4β1 and α9β1 [39]. Osteopontin after cleavage by thrombin is an important regulator of HSC within their niche as it inhibits HSC proliferation in vitro and mice knocked out for the osteopontin gene (*Opn*^{-/-}) have increased number of HSC in the BM. Furthermore, HSC lodgment into their *Opn*^{-/-} endosteal niche is perturbed after transplantation [41]. Finally, the integrin-ligand VCAM-1 is expressed by most cells known to participate in HSC niches, particularly BM endothelial cells [43], mesenchymal stem cells and osteoprogenitors [44], osteoblasts, and a subset of BM-resident macrophages while it is absent from lymphocytes, HSPC, and monocytes [45]. Conditional deletion of the VCAM-1 gene in hematopoietic and BM stromal cells, or conditional deletion of integrin α4 gene in hematopoietic cells both results in spontaneous HSPC mobilization into the blood and spleen with dramatic expansion of splenic hematopoiesis [45, 46] proving that the adhesive interaction between VCAM-1 and α4 integrins (which include α4β1 and α4β7 both expressed by HSPC [35]) is key to anchor HSC in their niche.

The β2 integrin subunit (CD18) can dimerize with four integrin α subunits, namely αL (CD11a), αM (CD11b), αX (CD11c), and αD (CD11d). Only β2 integrin αLβ2 (previously called LFA-1) has been reported on primitive HSPC. It binds to intercellular adhesion molecules (ICAM)-1, -2 and -3, transmembrane adhesion molecules expressed by endothelial cells. Integrin αLβ2 has been found expressed on short-term reconstituting multipotent progenitors but absent from long-term reconstituting HSC in adult mice [47•]

and in mouse embryos [48]. Therefore, integrin $\alpha\text{L}\beta\text{2}$ and its ligands are not regulator of HSC but could play an important role in regulating multipotent progenitors in their respective niches. This would be consistent with the recent observation that mice expressing an hypomorphic variant of integrin β2 have increased numbers of multipotent progenitors in the BM [34•].

The β3 integrin subunit (CD61) can associate with either integrin αIIb (CD41) or αV (CD51). Integrin $\alpha\text{IIb}\beta\text{3}$ is expressed by quiescent HSC in aged mice [49•] and has been known to be expressed by embryonic HSC in the yolk sac [50]. Integrin $\alpha\text{IIb}\beta\text{3}$ is the classic platelet glycoprotein IIb-IIIa complex that mediates platelet aggregation during blood clotting. It binds fibrinogen, fibronectin, vitronectin, and von Willebrand factor (vWF or CD146) following its activation by ADP or thrombin [51]. Integrin $\alpha\text{IIb}\beta\text{3}$ plays an important role in adult hematopoiesis as integrin αIIb deficient mice (*Itga2b*^{-/-}) display a pancytopenia while their HSC display increased apoptosis and proliferation, with a transplantation defect [49•]. As vWF, an integrin $\alpha\text{IIb}\beta\text{3}$ ligand, is produced by megakaryocytes and endothelial cells which are necessary to maintain HSC quiescent [18•, 19•, 52•], it may be that vWF plays a key role in maintaining HSC in contact with megakaryocytes and endothelial cells at their niche. Interestingly, only a small proportion of HSC express $\alpha\text{IIb}\beta\text{3}$ in the young adult mouse suggesting that the state of HSC quiescence may vary with age. The function of $\alpha\text{IIb}\beta\text{3}$ in yolk sac HSC is probably different as these cells need to expand to populate the growing embryo with primitive hematopoietic cells (mostly fetal erythrocytes and macrophages).

Integrin Activation States—Key to Find the Good Address

The spectrum of integrins expressed by HSC suggests that they play a key role in HSC anchoring in the BM, as well as homing to the BM during development or following transplantation. A key aspect of integrin biology is that unlike other cell adhesion receptors, integrins require prior conformational activation by extracellular soluble mediators to become capable of binding their ligands. This is because integrin $\alpha\beta$ complexes can adopt several conformational states with different levels of affinity or avidity for their ligands. As many integrin ligands are expressed in many different tissues, this enables them to lock-in cells at the right time at the right place provided that the integrin activating molecule is present. Integrins require metallic divalent cations such as Mg^{2+} and Ca^{2+} to complex together in the plasma membrane. On hematopoietic cells, β1 integrins can be activated by inside-out signaling by cytokine receptors such as the interleukin-3 receptor or c-Kit [36, 53]. Integrins have also divalent cation binding sites near the ligand binding site. Mg^{2+} at concentrations over 1 mM is sufficient to initiate β1 integrin activation without cytokine. In contrast, Ca^{2+} in excess of 10 mM (concentrations found

physiologically in the periphery of osteoclasts resorbing bones) reduces β1 integrin activation by cytokines [54]. Interestingly, Mn^{2+} is a “super-activator” of β1 integrins as between 0.03 and 0.1 mM it locks β1 integrins in an activated state [54]. The recent finding that hematopoietic cells in the endosteal region of the BM have high level of Mn^{2+} associated with their plasma membrane [55•] opens new perspectives. Indeed, Mn^{2+} is necessary to proper bone development and at 1.4 ppm is relatively abundant in the compact bone [56] compared to the blood (1.5–2.0 $\mu\text{g/L}$) [57]. Mn^{2+} release under the effect of osteoclasts remodeling the bone, could play a major role in maintaining β1 integrins activated at the surface of HSC to lock them in at their niches.

CD44

CD44 is a highly polymorphic and multifunctional transmembrane proteoglycan expressed on most leukocytes and HSPC. CD44 is expressed with many splicing variants, some of them characteristic of leukemic transformation. Appropriately decorated with sLe^x fucosylated polysaccharides, CD44 can be a receptor for L-selectin and E-selectin [25, 58] as mentioned above. CD44 is also a receptor for high molecular weight hyaluronic acid. Hyaluronic acid is a highly hydrophilic polysaccharide that is produced in many tissues to form a gel once hydrated. Hyaluronic acid is present in the BM at the endosteum and around blood vessels [59]. It is also synthesized by HSPC themselves via the expression of hyaluronate synthase 3 [60]. CD44 expressed by human and mouse HSPC binds to hyaluronic acid [60, 61]. Similar to β1 integrins, the binding of CD44 to hyaluronic acid is activated by interleukin-3 receptor and c-Kit by an undefined inside-out signaling mechanism [61]. HSC from mice knocked out for the hyaluronate synthase-3 gene (*Has3*^{-/-}) have reduced lodgment in the metaphyseal endosteal region of the BM following transplantation [59]. Therefore, hyaluronic acid is likely to guide HSC to their niche via CD44.

Interestingly, the monoclonal anti-human CD44 antibody H90 activates CD44 on CD34⁺ HSPC to bind to hyaluronic acid [61]. Most interestingly, this antibody reverses the differentiation blockage in acute myeloid leukemia (AML) cells in vitro which then progress towards a more mature monocytic morphology [62]. Likewise in immune-deficient mice transplanted with primary human AML cells, administration of H90 antibody to the leukemic mice reduced leukemia progression, induced AML cell differentiation with induction the monocytic antigen CD14 expression and inhibition of engraftment in secondary transplants. This suggests that this anti-CD44 antibody directly targets leukemia stem cells [63]. A6 peptide is a capped octopeptide with the sequence Ac-KPSSPPEE-NH₂ derived from urokinase plasminogen activator [64]. A6 peptide has been shown to bind and activate CD44 with downstream phosphorylation of focal adhesion

kinase (FAK) and increase adhesion to hyaluronic acid [65]. A6-peptide selectively kills primary chronic lymphoblastic leukemia cells in vitro and in vivo in xenograft in immunodeficient mice [66].

Eph and Ephrins

Transmembrane cell-cell adhesion receptors Eph and their ligands ephrins form a unique family of cell adhesion molecules. Eph receptors are integral tyrosine kinases belonging to two different classes A and B with 10 different EphA and 6 different EphB all encoded by different genes. In general, EphA receptors can bind to 5 different glycoposphoinositide-anchored ephrin A, while EphB receptors can bind to 3 different transmembrane ephrin B, with the exception of EphB2, ephrin B2, and EphA4 which can bind across both A and B classes of Eph and ephrins [67]. Binding of ephrins to Eph receptors initiates both forward signaling via the tyrosine kinase domain of Eph receptors and reverse signaling in the cell presenting ephrins [68]. Eph and ephrins are best known for their role in axonal guidance and remodeling of the nervous system during development [68]. Their expression is also induced in a number of tissues following systemic inflammation or injury [67].

In relation to the hematopoietic system, EphA2 and EphB2 were found expressed on mobilized human CD34⁺ or CD133⁺ HSPC [69] while EphA3 has been reported on erythroid progenitors [70]. Human BM stromal cells express EphB1 and EphB2, EphB4, ephrin-B1 and ephrin-B2 [71]. In the mouse EphA 1–5, receptors were found expressed at the surface of mouse HPSC [72]. Mouse HSPC were also found to express ephrins ligand using EhpA1-Fc and EhpA3-Fc probes; however, their precise identity could not be confirmed unambiguously as monoclonal antibodies specific for mouse ephrins were not available. Likewise, primary mouse BM stromal cells were also found to express EphA2 and EphA3 as well as ephrins binding to the EphA3-Fc and EphA1-Fc probes [72]. In other reports, EphA2, EphB4, ephrin B1, and ephrin B2 were detected on mouse BM stromal cells [71]. Interestingly, stimulation of the mouse myeloid cell line FDCP1 with EphA3-Fc construct increased cell adhesion to VCAM-1 while in vivo administration of EphA3-Fc into mice caused a rapid and transient mobilization of colony-forming units (CFU) and more primitive CFU-S₁₂ and cobblestone area forming cells (CAFC) into the peripheral blood [72]. Therefore, both HSPC and stromal cells express an array of EphA receptors and ephrin ligands suggesting that this system may establish a cross-talk between HSC and their niche stromal components. However, this may be difficult to demonstrate genetically as Eph and ephrins are redundant with promiscuity in their receptor/ligand preference. To illustrate this, EphA2^{-/-} mice did not show any obvious hematopoietic abnormality [73]. Eph receptors have been found expressed on AML [74], chronic lymphoblastic leukemia [75] and a variety

of B [76] and T leukemia cell lines [77] and may have a role in the biology of these hematopoietic malignancies.

Clinical Application: HSC Mobilization and Transplantation

Until the early 1990s, HSPC harvested for use in transplantation were collected by multiple BM aspirations under general anesthesia. This, however, changed rapidly when it was discovered that daily injection of the cytokine granulocyte colony-stimulating factor (G-CSF) promoted the egress of HSPC from the BM into the blood in mice [78], a phenomenon called mobilization. Subsequent studies rapidly confirmed G-CSF-induced mobilization in humans [79] leading to progressive replacement of BM aspirates by peripheral blood CD34⁺ cells as the source of HSC for transplantation [80]. Owing to this success, over one million HSC transplantations have been performed with mobilized blood CD34⁺ cells worldwide to date.

For many years, the mechanisms by which G-CSF promotes HSPC mobilization remained obscure. However, intense research since the early 2000s has identified several mechanisms working in parallel. G-CSF causes a complete remodeling of the BM landscape and HSPC niches [81]. The expression of VCAM-1 cell adhesion protein and the chemokine CXCL12 (also called stromal-derived factor-1; SDF-1) which both work in concert to retain HSPC within their niches are both strikingly down-regulated in response to G-CSF in vivo [82, 83]. This is due to a combination of mechanisms such as (1) proteolytic cleavage by neutrophil proteases released in the BM as a consequence of neutrophil expansion [82–84] and (2) direct down-regulation of *Vcam1* and *Cxcl12* transcripts in BM stromal cells [85, 86]. This latter effect is in part mediated by BM-resident macrophages which express the G-CSF receptor [86–88].

While in healthy donors G-CSF treatment is sufficient to elicit mobilization of sufficient numbers of CD34⁺ HSPC to ensure safe engraftment in an allogeneic recipient, G-CSF fails to mobilize adequate numbers of CD34⁺ HSPC in a proportion of heavily pre-treated multiple myeloma and non-Hodgkin's lymphoma patients eligible for autologous HSC transplantation which adversely affect their prognosis of survival [89]. A number of therapeutic strategies have been devised to overcome HSC mobilization failure in these patients. The only drug which is currently approved to rescue mobilization in patients who previously failed to mobilize in response to G-CSF is the small synthetic CXCR4 antagonist Plerixafor (previously called AMD3100). By inhibiting chemotaxis in response to CXCL12, Plerixafor synergizes with G-CSF in eliciting HSPC mobilization [90] enabling mobilization rescue in an important proportion of these poor mobilizers [91, 92]. Alternative CXCR4 antagonists are being

developed to elicit mobilization in autologous transplantation patients [93]. An alternative strategy to CXCR4 antagonism is to directly target integrins (particularly $\alpha4\beta1$ and $\alpha9\beta1$) responsible for HSPC anchoring to VCAM-1 within their niche. The humanized anti-integrin $\alpha4$ antibody natalizumab is used to treat relapsing multiple sclerosis and Crohn's disease by blocking lymphocyte recruitment in chronically inflamed nervous system and gut. Natalizumab is a potent HSC mobilizing agent [94, 95]. Interestingly, CD34⁺ HSPC mobilized with natalizumab have higher level of CXCR4 expression and enhanced chemotaxis to CXCL12 compared G-CSF-mobilized HSPC [95]. BIO5192, a small synthetic antagonist of integrin $\alpha4$ also causes weak mobilization of HSPC alone and synergizes with G-CSF to levels similar to Plerixafor [96]. As VCAM-1 binds to both integrins $\alpha4\beta1$ and $\alpha9\beta1$ on HSPC, *N*-(benzenesulfonyl)-L-prolyl-L-*O*-(1-pyrrolidinylcarbonyl)tyrosine (BOP) a small synthetic molecules that antagonizes both integrins has been developed. Interestingly, a single dose of BOP rapidly mobilized HSC within 30–60 min in mice and showed strong synergism with G-CSF [55•]. Most interestingly, transplantation of blood mobilized with the combination of BOP and Plerixafor led to higher reconstitution and engraftment than blood mobilized with G-CSF alone [55•]. Therefore, targeting both the CXCL12/CXCR4 axis and the VCAM-1/integrin $\alpha4\beta1$ $\alpha9\beta1$ axis by combining CXCR4 antagonist and BOP may represent a superior alternative to mobilize HSC in patients that mobilize poorly in response to G-CSF. However, at present only Plerixafor has been approved by the FDA to enhance HSC mobilization.

Clinical Application: Blocking Cell Adhesion to Overcome Chemoresistance

It has known for nearly two decades that adhesion of multiple myeloma [97], B-lymphoma [98], B cell acute lymphoblastic leukemia (B-ALL) [99], B-CLL [100], AML [101] and more

recently chronic myeloblastic leukemia (CML) cells [102] to BM stroma cells or immobilized cell adhesion molecules increases malignant cell survival to cytotoxic drugs or in some cases to tyrosine-kinase inhibitors and immunomodulators such as lenalidomide. This phenomenon called cell adhesion-mediated drug resistance (CAM-DR) has been shown in many cases to involve in part adhesive interaction between $\alpha4$ integrins and VCAM-1 [97, 98, 103–107, 108•].

In addition to integrin-mediated CAM-DR, the interaction between CD44 and hyaluronic acid has also been reported to mediate CAM-DR in multiple myeloma [109]. L-selectin has also recently been reported to mediate CAM-DR in B-CLL cells by promoting B-CLL cell escape in lymph nodes and protection once adhered to BM stromal cells [110, 111•]. Finally, we have recently found in an immune competent mouse model of human AML, that E-selectin but not P-selectin promotes potent CAM-DR in vitro and in vivo as deletion of the E-selectin receptor in mice or administration of the small E-selectin antagonist GMI-1271 sensitized leukemia stem cells to chemotherapy [112]. Likewise, E-selectin has been found to induce CAM-DR to the tyrosine-kinase inhibitor imatinib in an immune competent mouse model of human CML an effect reversed in vivo by GMI-1271 [113].

The mechanisms of CAM-DR have not been fully elucidated and may be disease dependent. Activation of focal adhesion kinase [101], which is activated by integrin clustering, NF- κ B/RelB [108•, 114], Wnt/ β -catenin signaling [115], or by activating drug efflux by ATP-binding cassette transporters [106] may play a role.

These findings have prompted a few clinical trials to test safety and efficacy of disrupting cell adhesion as an adjuvant therapy to conventional therapy (Table 1). The most common approach has been to mobilize hematopoietic malignant cells from their protective niches to sensitize them to chemotherapy. This approach has been successful in

Table 1 Clinical trials testing drugs targeting CAM-DR in hematological malignancies

Targeted molecules	Tested molecules	Disease	Clinical trial number	Phase	Status
CXCR4	Plerixafor	AML	NCT02605460	II	Recruiting
CXCR4	BL-8040	CML	NCT02115672	I/II	Not yet recruiting
CXCR4	BL-8040	T-ALL, T lymphoma	NCT02763384	IIa	Not yet recruiting
CXCR4	Plerixafor	AML, ALL	NCT01319864	I	Ongoing
CXCR4; GCSF receptor	Plerixafor and G-CSF	AML	NCT00906945	I/II	Ongoing
$\alpha4$ integrins	Natalizumab	Multiple myeloma	NCT00675428	I/II	Terminated
CD44	A6-peptide	CLL, Small lymphocytic lymphoma	NCT02046928	II	Recruiting
E-selectin	GMI-1271	AML	NCT02306291	I/II	Recruiting
EphA3 receptor	KB004	Myelofibrosis, myelodysplasia	NCT01211691	I/II	Suspended

mouse models of AML [116, 117]. There are currently five clinical trials registered in the USA to test chemosensitizing effect of various CXCR4 antagonists. Of note a number of phase I/II trials to test Plerixafor safety and efficacy in treatment of AML have been completed with only one with published results [118].

While the anti- $\alpha 4$ integrin natalizumab is in many clinical trials to test efficacy in treating multiple sclerosis, there was only one trial on its effect on multiple myeloma, a trial that was terminated in 2014 due to low enrolment and technical problems with measurements.

There is currently one phase II clinical trial recruiting to test efficacy and tolerability of CD44-activating A6-peptide in CLL and small lymphocytic lymphoma patients.

The efficacy of small E-selectin antagonist GMI-1271 in combination with conventional chemotherapy is being tested in a phase I/II trial on refractory and relapsed AML patients. In view of promising initial results, the FDA granted Fast Track status to this clinical trial in June 2016 to progress to phase II trial on newly diagnosed AML patients.

Finally, the efficacy of blocking EphA3 receptor with the humanized antibody KB004 was trialed on myelodysplasia and myelofibrosis patients; however, the trial was suspended in November 2015 after the company Kalobios making KB004 filed for bankruptcy.

Conclusions

Recent studies with genetically modified mice in which genes encoding cell adhesion molecules are deleted have clearly demonstrated that some of these cell adhesion molecules are key not only to HSPC homing and anchoring within their specific niches in the BM but also regulate HSPC fate within the BM in steady-state such as the balance between quiescence and proliferation. Another essential aspect is how malignant cells utilize these adhesive interactions to resist cytotoxic chemotherapies and targeted therapies, which ultimately causes relapse from resistant clones that accumulate additional mutations during the process causing multi-drug resistance. Understanding these interactions has enabled the design of drugs targeting specific adhesive interactions which can be used to treat chronic inflammatory diseases but also to enhance HSC mobilization for transplantation. Targeting cell adhesive interactions to chemosensitize hematological malignancies is a new direction for treatments. While it is too soon to conclude on the efficacy of this approach, this is an exciting development in the treatment of incurable hematological malignancies such as multiple myeloma and CLL or diseases with very poor prognosis in the absence of allogeneic HSC transplantation such as AML.

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Compliance with Ethical Standards

Conflict of Interest Jean-Pierre Levesque and Ingrid G. Winkler declare that GlycoMimetics Inc. has supported a portion of their research involving their E-selectin antagonist.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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