Journal of Leukemia
ISSN: 2329-6917

Charmsaz and Boyd, J Leuk 2013, 1:1
DOI; 10.4172/2329-6917.1000107

Review Article Open Access

Expression and Function of the Eph Receptor Family in Leukemia and Hematopoietic Malignancies: Prospects for Targeted Therapies

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Abstract

There has been considerable interest in recent years in the development of therapies, which target Eph receptors or their ephrin ligands. The Eph receptor tyrosine kinases and their membrane bound ephrin ligands are cell surface molecules involved in many biological functions and cell behaviors during embryogenesis and in adult life. However, they are also expressed in an aberrant fashion on many tumors and are re-expressed on normal cells in non-malignant pathological states. Some of the eph/ephrins including EphA7 and EphA1 protein are thought to function as tumor suppressors in particular cancers. In tumors where Eph/ephrin proteins are expressed at high levels, being expressed on the cell surface these proteins are readily accessible to antibody-mediated therapies several of which are in advanced pre-clinical or early clinical evaluation, including antibodies specific for EphA2, EphA3, and EphB4 which are expressed on many different tumors. We will review the general features of the Eph/ephrin system and discuss the role of this system in normal hematopoiesis before focusing on the role of Eph proteins in leukemia and other hematological malignancies and possible avenues for therapy.

Keywords: Eph receptor; Ephrin; Receptor Tyrosine Kinase; Hematopoiesis; Hematopoietic Stem Cells; Leukemia

Classification, Structure and Binding of Eph/ephrin

The Eph receptors and their membrane bound ephrin ligands represent the largest family of receptor tyrosine kinases (RTK's). The first member, EphA1, was originally termed Eph, as it was first identified in an Erythropoietin-Producing Hepatocellular (Eph Nomenclature committee, 1997) carcinoma cell line [1]. Fourteen members of the Eph family of RTK have been identified in mammals, which are divided into two groups, the EphA and EphB family. This is based on their sequence homology, ligand specificity and structural features. In mammals there are nine members of the EphA subgroup (EphA1-8 and EphA10) and five EphB receptors (EphB1-4 and EphB6) [2,3].

Eph receptors are type I transmembrane proteins composed of extracellular region including a ligand binding domain with two distinct ligand-binding interfaces (N terminal β jelly roll domain) determining the ephrin binding, and a cysteine-rich region containing an epidermal growth factor (EGF)-like motif that is involved in receptor dimerization followed by two fibronectin type-III domains [4,5]. The intracellular region comprised of a conserved juxtamembrane domain, a tyrosine kinase domain and a sterile alpha motif (SAM) domain, the latter having a potential role in receptor clustering [6,7]. Most of the Eph receptors, possess a C-terminal PDZ (Postsynaptic density protein, Disc large, Zona occludens tight junction protein) binding motif that is involved in signaling at a sub-cellular level and in the assembly of large molecular complexes [3,5,8] (Figure 1a).

The ephrin ligands of Eph receptors are also membrane bound proteins and like the Ephs they are divided into two groups based on their structural features and preferential binding to either EphA or EphB receptors. The two classes of ephrin ligands are the A-class (ephrinA1-5) and B-class (ephrinB1-3) [3]. The two classes of the ephrin ligands have homology at their N-terminus region while their C-terminal amino acid sequence differs, the ephrin-A ligands that are bound to the plasma membrane by a glycosylphosphatidylinositol (GPI)-linker and the ephrin-B ligands, which possess a transmembrane–spanning region and a highly conserved cytoplasmic tail with a number of highly conserved tyrosine residues and a PDZ-binding motif. The conserved tyrosine residues have been shown to serve as a docking site for proteins, which mediate downstream ephrin signaling [2,9] (Figure 1b).

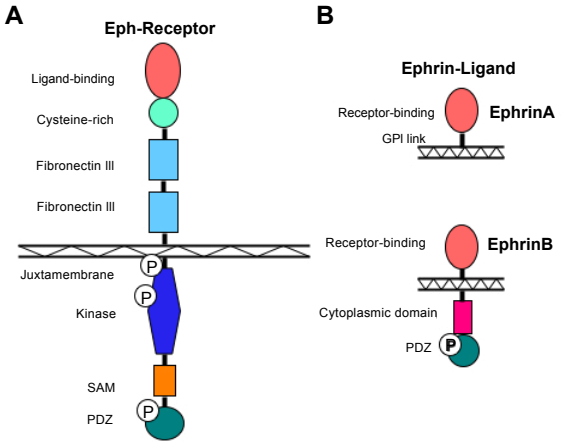

Figure 1: Schematic representation of Eph receptors and ephrin ligand structure. (A) The structure of the Eph receptor. (B) The structure of the ephrin-A and ephrin-B ligand.

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Received February 23, 2013; **Accepted** April 01, 2013; **Published** April 03, 2013

Citation: Charmsaz S, Boyd AW (2013) Expression and Function of the Eph Receptor Family in Leukemia and Hematopoietic Malignancies: Prospects for Targeted Therapies. J Leuk 1: 107. doi:10.4172/2329-6917.1000107

Generally, the EphA receptors will preferentially bind to members of the ephrin-A ligands (ephrinA1-5) while the EphB receptors will preferentially bind to the ephrin-B ligands (ephrinB1-3) [3,10]. There is a high level of binding promiscuity between the Eph receptors and the ephrin ligands, however for an individual Eph receptor there is a distinct order of affinity of ephrin interactions with affinity constants ranging from 5-500 nM [11]. For instance the EphA1 receptor binds with a high affinity to ephrin-A1 and a lower affinity to other members of the ephrin-A family, including ephrin-A3 and ephrin-A4, and shows essentially no binding to ephrin-A5 [12]. Similarly EphA3 and EphB4 have a higher affinity for ephrin-A5 and ephrin-B2 respectively than for other members of the ephrin family [11,13]. High affinity interaction is also possible between the classes, for example EphA4 binds to both ephrin-B and -A ligands and some of its most important functions depend on interaction with ephrin-B3. Another example is EphB2, which binds to ephrin-A5 as well as ephrin-B ligands [14,15] (Figure 2).

Eph/ephrin Activation and Signaling

Eph/ephrin interactions have the capacity to initiate bidirectional signaling. In other words both Ephs and ephrins can act as a ligand as well as a receptor and they both have the ability to initiate signaling. Signaling initiated by the Eph receptor is referred to as forward signaling whereas signaling initiated by the ephrin ligand is termed reverse signaling.

Eph receptor activation occurs following the physical association of the receptor and an ephrin ligand on adjacent cell surfaces mediated by the high affinity binding site (dimerization), this is followed by interaction with a second Eph/ephrin complex, mediated by separate low affinity (tetramerization) binding site to create a hetero-tetrameric complex [16]. The tetramers are then assembled into higher order clusters, which appear to be required for effective forward signaling [17,18]. The clustering of Eph receptors results in ligand-dependent auto-phosphorylation of several tyrosine residues within the cytoplasmic kinase domain and juxtamembrane region of the receptor, which serve as docking sites for downstream signaling proteins

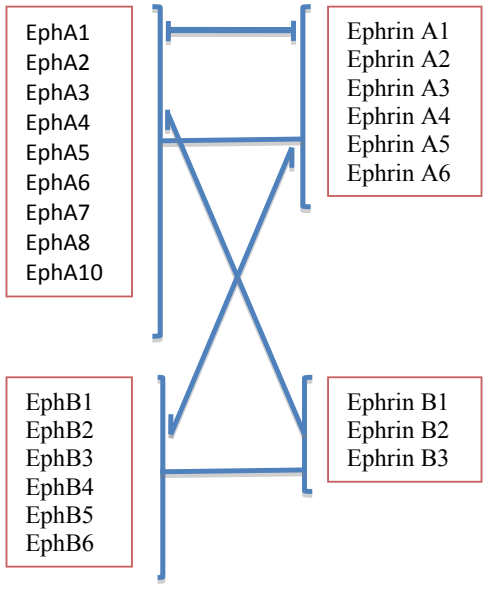

Figure 2: Schematic of Eph receptor and ephrin binding preference.

including the small GTPases of the Rho and Ras family, focal adhesion kinase (FAK), the Janus kinase/signal transducers and activators of transcription (Jak/Stat) and phosphatidylinositol 3-kinase (P13K) pathways [19].

Eph receptor activation influence cell shape and motility through the regulation of the Rho GTPases, including RhoA, Cdc42, Rac and Ras, via interactions with specific Rho GTPase activating (Rho GAP) and exchange (Rho GEF) factors. The interaction with individual Rho GTPases, mediated via direct binding to the cytoplasmic domain of Eph proteins of Rho GAP and Rho GEF or their recruitment via adaptor proteins, thereby mediating different effects on actin dynamics and cell process formation thus regulating cell shape and movement. For example, activation of EphA4 receptor results in recruitment of ephexin (Rho GEF), leading to activation of RhoA, and α-chimaerin (Rho GAP), leading to inactivation of Rac, jointly resulting in actin depolymerization and retraction of cell processes [15]. Activation through EphB plays a role in actin filament extension, morphogenesis and maturation of dendritic spines. Ras family of GTPases (H- and R- Ras) are also activated through Eph receptors however, unlike the Rho GTPases, the majority of Eph receptors negatively regulate the Ras/Mitogen-activated protein kinase (Ras/MAPK) pathway, with activation normally resulting in regulation of proliferation and migration [15].

Eph receptors are also important in mediating a number of other molecules involved in cell migration and adhesion due to their ability to regulate signal transduction molecules including integrin signaling pathway elements paxillin, FAK, P130 (Cas) and integrins themselves. The effect of Eph signaling on integrins is complex as they can mediate either promoting or suppressing effects. FAK is important in mediating integrin signaling and Eph receptors can down-regulate this pathway [20].

The Jak/Stat pathway, involved in cell growth and viability, is regulated by EphA receptor activation [21]. EphB receptors mediate cell migration and proliferation through P13K pathway and the protein kinase B/phosphatidylinositol 3-kinase (Akt/ PI3K) pathway is involved in the regulation of cell proliferation and viability. Eph/ephrin also regulates other signaling pathways including Abl/Arg and p53-family of tumor suppressor proteins [19]. A recent study in glioblastoma multiforme (GBM) shows that loss of EphA3 results in elevated MAPK signaling thereby inducing differentiation and reducing proliferation and self-renewal. This study showed that regulation of extracellular signal-regulated kinases/mitogen-activated protein kinases (ERK/ MAPK) signaling by EphA3 is kinase independent of the upstream activators of MAPK signaling [22]. A similar finding has been made for GBM which express EphA2 at high levels [23].

In the ephrin-expressing cells reverse signaling can be induced after Eph/ephrin interaction [19,24]. Ephrin-B reverse signaling partly depends on the tyrosine phosphorylation of conserved residues in the cytoplasmic region, where phosphorylation is mediated by associated tyrosine kinases, most notably members of the Src family (Src, Fyn, Lyn, Yes) of tyrosine kinases. When the ephrin-B ligand is phosphorylated it can bind to cytosolic adaptor molecules via (Src Homology-2) SH-2 and SH-3 domains or PDZ domains [8]. The protein tyrosine phosphatase basophil-like (PTP-BL) has been identified as a negative regulator of ephrin-B signaling and it binds to ephrin-B through its C-terminal PDZ binding motif. De-phosphorylation of the ephrin-B cytoplasmic domain can inactivate the Src family of kinase and therefore cause termination of reverse signaling [25]. In the activated ephrin-B ligand, the PDZ motif plays an important role in assembly of other signaling

molecules. Ephrin-B binds to cytoplasmic protein PDZ-RGS3, which contains a PDZ domain and a regulator of G-protein signaling (RGS) domain. Activation of the ephrin-B ligand by EphB receptor via the PDZ-RGS interferes with signaling of the stromal derived factor (SDF)-1 via its G-protein coupled receptor and the chemokine receptor-4 (CXCR4). Ephrin-B reverse signaling has thus been implicated with regulation of migration in cerebellar development [26].

The ephrin-A reverse signaling mechanism is not well understood, however it is likely that the signaling response is initiated by activity of the Src family of RTK in which transmembrane adaptor molecules, associating with the lipid anchor of the ephrin-A proteins, transmit the signal across the membrane. For example ephrin-A5 induces signaling within the ephrin-A expressing cell when bound to its cognate Eph receptor [27-29].

Expression and Function of the Eph and Ephrin Genes

Eph signaling controls cell adhesion, migration, invasion and morphology by influencing integrin and intercellular adhesion molecule activity and by modification of actin cytoskeleton organization as described above. Through these mechanisms Eph function effects not only the processes of embryogenesis but also specialized cellular function in adult tissues, including bone remodeling, immune function and synaptic plasticity as well as cell proliferation and survival specific tissue stem cells as described further below [27,30].

Both Eph and ephrin proteins are important in regulating cell-cell interactions and their interaction can initiate either cell adhesion or repulsion. Cell repulsion occurs when bidirectional signaling triggers cytoskeletal contraction, loss of focal adhesions, cell rounding and cell detachment, whereas cell attachment occurs when signals are in favor of cell adhesion and migration [31]. This interaction influence many different cell behaviors during embryogenesis and in adult life [27]. Eph/ephrin interactions mediate formation of tissue boundaries (e.g. hindbrain rhombomeres) [32], control axon guidance during development and also tissue morphogenesis and patterning [33,34]. Eph/ephrin interactions are also involved in development of vascular system [35], stem cell biology [36], hematopoiesis, erythropoiesis, immune function and in tumor invasion and metastasis [27,30,37-39].

Many members of the Eph/ephrin family are expressed at high levels in some cancer cells and also elements of the tumor microenvironment, where they influence tumor growth and spread. Specific examples include lung, breast and prostate cancer, as well as melanoma, sarcomas and leukemias [40,41]. There is evidence that the Eph receptors can have either tumor suppressing or tumor promoting activity, depending on the tissue and their expression pattern [27]. Thus, the role and function of Eph/ephrins in cancer is not yet fully elucidated as some tumors show an elevated level of Eph expression while others show a decrease in Eph expression and as yet no single model of their function encompasses all cancers. For example EphA2 is up regulated in many cancers including breast and prostate and its expression is linked to an increase in malignancy [42,43] but it is down regulated in colon cancer [44]. Similarly, EphA1 expression is up-regulated in ovarian cancer [40] but down-regulated in advanced skin and colorectal cancers [45,46]. Studies have also shown the role of EphA7 as a tumor suppressor in follicular lymphoma [47], tumor suppressor function has also been reported for EphB receptors, including EphB2 and EphB3 in colorectal cancer [30,48]. EphB4 is another important example as this gene can act as either a tumor suppressor or an oncogene in different facets of breast cancer progression [49,50]. Table 1 represents the expression of Eph receptors in normal and malignant tissues [51-82].

The Eph/ephrin in HSC and Leukemia

Eph/ephrin expression in HSC and progenitors

The expression of Eph/ephrin has been detected on purified population of hematopoietic stem cells (HSCs) in both human and mouse. Gene expression analysis of HSC showed expression of ephrin-B2, indicating that it may be involved in signaling between HSC and their microenvironment. Other array based studies on primary human HSC ($CD34^+$ hematopoietic cells) shows expression of the EphA1 protein and its ligands ephrin-A3 and ephrin-A4 suggesting that their interaction may play a role in hematopoietic stem and progenitor cell positioning and function [83,84]. Further analysis of $CD133^+$ and $CD34^+$ hematopoietic stem cells in peripheral blood showed expression of EphA2 in all $CD34^+$ cells and the majority of $CD133^+$ cells however EphB2 was expressed in all $CD133^+$ cells and fifty percent of $CD34^+$ cells, these data suggest that a number of elements of the Eph/ephrin system may have a role in HSC function through regulatory effects on cell adhesion, migration and differentiation but also that there may be a degree of functional redundancy between several Eph proteins [85].

Real-time quantitative PCR of mouse $Lin^-ckit^+sca1^+$ (KLS) showed detectable expression of all EphA receptors except EphA6 and EphA8, along with ephrin-A ligands, with ephrin-A4 and ephrin-A5 being the most highly expressed ligands on purified HSCs in the mouse bone marrow [86]. Flow cytometric analysis of EphA2, A3, A4 and A5 along with ephrinA1-5 showed that EphA2 and EphA3 were the highest expressing EphA receptors. Expression of EphA2, A3, A4 and A5 was also detected in the mouse stromal cell lines however human stromal cell line showed only EphA2 expression at moderate levels [86]. Whilst the function has not been fully investigated, a role in HSC trafficking was demonstrated by treatment of mice with an Eph/ephrin inhibitor, EphA3-Fc, which resulted in mobilization of bone marrow progenitor cells into peripheral blood [86].

Some of the members of the Eph/ephrin family are also involved in development and regulation of mature hematopoietic cells. For example, EphA4 and EphB1 receptors along with ephrin-B1 ligand are expressed on human platelets [87], these studies also shows that EphA4 is involved in regulation of platelet aggregation and adhesion to fibrinogen, a process dependent on integrin αIIbβ3 engagement [39].

EphB4 was originally identified on human bone marrow $CD34^+$ cells and its expression has been reported on erythroid progenitor cells in early stages of red blood cell development. Significantly, the EphB4 ligand, ephrin-B2, is expressed on bone marrow stromal cells [88] where it has been reported to be involved with regulating erythropoiesis via interaction with EphB4 [89]. Studies by Suenobu et al. [89] showed co-culturing hematopoietic progenitor cells expressing EphB4 with stromal cells expressing ephrin-B2 results in hematopoietic progenitor cells detachment from stromal layer and differentiation into a mature erythroid cells accompanied by EphB4 down regulation, however co-culturing these cell with ephrin-B2-negative stromal cells resulted in less maturation of erythroid cells and no change to EphB4 expression [89]. Ephrin-B2 ectopic expression in stromal cells increased adhesion of hematopoietic cells to stromal calls and decreased transmigration of hematopoietic cells beneath a stromal cell monolayer. These findings strongly support a role for the EphB4/ephrin-B2 interaction in migration and colonization of stem/progenitors cells in the bone marrow microenvironment [90].

Some of the Eph/ephrin molecules are also involved in lymphoid development. The expression of Eph/ephrin has been studied

Eph	Tissue expression	Expression in cancer	References
EphA1	Widely expressed in mouse epithelial tissues evidence of expression in hematopoietic progenitors	Over expressed in many different cancers including hepatocellular, prostate, lung, gastric and colon cancer Down regulated in non-melanoma skin cancer, colorectal cancer	1,12,24,45,46,51-55
EphA2	Expressed highly in adult human epithelial cells and endothelium	Over expressed in prostate, breast, melanoma, lung and ovarian cancers Up-regulated in glioblastoma Down-regulated in colon cancer	23,24,42,43,54-61
EphA3	Expressed in various stages of embryonic development and in adult central nervous system	Expressed in neural cancers, leukemia, lymphomas and sarcomas Up-regulated in lung, brain, liver and kidney Over expressed in melanoma	24,54,55,62-66
EphA4	Expressed in development, final stages of embryogenesis and central nervous system	Expressed in prostate, pancreatic cancer Up-regulated in lung cancer Down-regulated kidney Over expressed in gastric cancer	24,54,55,62,67-70
EphA5	Expressed in nervous system	Expressed in neuroblastomas and neural cancer Down-regulated in breast cancer	24,54,55,70-72
EphA6	Expressed more prominently in adult tissues than in embryonic tissues	Down-regulated in colon cancer and renal carcinoma Up-regulated in lung and liver cancer	24,54,55
EphA7	Expressed in developing neural tubes, thymus, lymphoid tissues and fetal bone marrow	Expressed in colorectal cancer, lung and follicular lymphoma Up-regulated in ALL1 leukemia	24,44,47,54,55,73-75
EphA8	Expressed in spinal cord and neuronal cells	Expressed in colon cancer Down-regulated in glioblastoma	24,54,55
EphA10	Expressed in testis	Over expressed in breast cancer	76,77
EphB1	Expressed in Brain and colon	Expressed in lung cancer Down-regulated in colon carcinoma and in kidney cancer	24,46,54
EphB2	Expressed in epithelial cells, thymus, lymphoid, osteoblastic and osteoclastic cells	Over expressed in gastrointestinal Expressed in colon, ovarian and lung cancer Up-regulated in colorectal, kidney and hepatocellular cancer	24,54,78
EphB3	Expressed in various tissues	Expressed in prostate, lung and melanoma	24,54
EphB4	Expressed in placenta and in range of primary tissues including brain, endothelium, hematopoietic cells	Expressed in colon, endometrial, breast, neuroblastoma, glioblastoma and leukemia and lymphoma cancer	24,54,79-81
EphB6	Expressed in various tissues including brain, pancreas, thymus and T-cells	Expressed in T cell tumors and leukemia Up-regulated in colon cancer Down-regulated in breast, lung and kidney cancer	24,54,82

Table 1: Eph expression in normal and malignant tissues.

extensively in T-lymphocytes and expression of some of the members of this family including EphA1, EphA2, EphA3, EphA4, EphA7, EphB2, EphB6, ephrin-A1 ephrin-A3, ephrin-A5 and ephrin-B1 has been reported in the thymus, both on thymic stroma and lymphoid cells suggesting a role in T-cell development [91-93]. Interestingly, there are no reports of defective T lineage development in knockout mice, perhaps a result of there being multiple Eph receptors with over-lapping functions in the T cell compartment.

As well as T-lymphocytes the expression of Eph/ephrin gene has been also reported in B-lymphocytes [38,94,95]. EphA7 and EphA4 transcripts were found in human fetal bone marrow pro-B and pre-B cells. EphA4 expression is found in both adult and fetal pro-B and pre-B lineage cells with high levels of expression in peripheral blood. The full length EphA7 transcript, however, was not found in mature fetal B-lineage and adult B-lineage cells. This suggests that EphA7 may be involved in expansion and /or differentiation of pre/pro B-cell but is lost on mature B cells [95]. Further studies show that there are different types of EphA7 mRNA, one of which encodes the full length EphA7 and another splice variant encodes a truncated, soluble protein that lacks the cytoplasmic domain. Studies by Dawson et al. [74] showed that normal lymphocytes express and secrete the truncated form of EphA7 [74], which have been shown to have tumor suppressive effects in lymphoma [47].

Eph/Ephrin in vascular development

In the context of hematopoiesis, the vascular system is crucial in development of blood cells within the bone marrow and also in the function and migration of mature hematopoietic cells. The vascular system arises from two distinct processes known as vasculogenesis and angiogenesis. Vasculogenesis is an early event in embryonic development involving mesodermal cells differentiation to form a vascular plexus in embryonic tissues and angiogenesis is a process in which new blood vessels are formed from existing blood vessels. Angiogenesis has a role both in early development and in the adult hematopoietic system, it also promotes sprouting of new blood vessels in embryonic and postnatal vasculature and it has been shown to be important in development and metastasis of solid tumors [96]. Some members of Eph/ephrin family have established roles in vasculogenesis and angiogenesis. The expression analysis of Eph/ephrin using real-time polymerase chain reaction (RT-PCR) has shown expression of EphB2, EphB3, EphB4, ephrin-B1 and ephrin-B2 in the yolk sac [97]. Ephrin-B1 expression has been detected on both arteries and veins while the high affinity ligand for EphB4, ephrin-B2, is only detected on arteries and EphB4 expression is only detected on veins. Knock out ephrin-B2 mice and some of the EphB2 and EphB3 double mutants mice have defects in embryonic vasculature and therefore these mice are embryonically lethal. EphB2 and ephrin-B2 expression in mesenchyme adjacent to vessels and the vascular defects in EphB2/EphB3 double mutants indicate a requirement for Eph/ephrin signaling between endothelial cells and surrounding mesenchymal cells [97]. EphA2 has also been reported to have a role in angiogenesis and the expression of EphA2 and its ligand ephrin-A1 has been reported in both human and mouse tumor vasculature [61].

Eph/Ephrin on bone remodeling and formation

The bone marrow is the principal site of hematopoiesis in adult animals and requires both vascular and other stromal cell types to create the hematopoietic niche. Important amongst these are the osteoblasts and osteoclasts, which mediate bone formation and remodeling. Bone is constantly remodeled through resorption of mineralized bone by osteoclast and formation of new bone by osteoblast. Coupling of bone resorption and formation is critical during normal bone remodeling and it is necessary for bone growth, any deregulation in this process will result in pathological bone disease [19,78].

Eph receptors and ephrin ligands are important in bone remodeling and homeostasis during this process Eph/ephrin bidirectional signaling regulates differentiation and function of the bone cells. Real time PCR (RT-PCR) analysis of the Eph/ephrin showed mRNA expression of ephrin ligands, ephrin-B1, ephrin-B2 and ephrin-A1, A2, A4 and A5, as well as Eph receptors including EphB2-4, EphB6, EphA2-4 and EphA7 receptors on osteoblastic and osteoclastic cells [98-100]. Expression of EphB4 is observed on the osteoblasts and forward signaling through EphB4 results in bone formation and reverse signaling through ephrin-B2 inhibit bone resorption therefore ephrin-B2/EphB4 act as coupling stimulator [98]. Expression of ephrin-A2 was observed during early osteoclastogenesis and unlike the ephrin-B2 it acts as coupling inhibitor as reverse signaling through ephrin-A2 result osteoclastogenesis and EphA2 forward signaling into osteoblast inhibit osteoblastic bone formation and mineralization [99]. Ephrin-A2/EphA2 bidirectional signaling facilitates bone remodeling at initiation phase, forward signaling through EphA2 receptor on osteoblast inhibit osteoblastic differentiation and bone formation and reverse signaling into osteoclast through ephrin-A2 promote osteoclast differentiation [78,99]. Ephrin-B1 full knockout mice are prenatally lethal and they have skeletal defects. Studies on disruption of ephrin-B1 on collagen I producing cells result in reduced bone formation and skull defect and studies on ephrin-B1 conditional knockout mice shows defects in osteoblastic mediated bone formation with no increase in osteoclastic bone resorption and this condition results in reduction in bone size and density [101,102].

The importance of Eph/ephrin interactions has also been shown in various stem cell niches, including neural, dental and intestinal stem cell compartments [103,104]. More recent studies show their involvement in bone homeostasis and mesenchymal stem cell (MSC) regulation. Arthur et al. [104] showed increase in osteogenic differentiation upon ephrin-B1 and/or ephrin-B2 expression by MSC. They also showed that ephrin-B1 activation promoted chondrogenic differentiation; therefore EphB/ephrin-B interactions may be involved in recruitment, migration and differentiation of MSC during bone repair [104]. Studies by Ting et al. [86] shows that ephrin-A signaling interact with stem/progenitor cells in the bone marrow niche as it's signaling mediates the release of progenitor cells from hematopoietic niche [86].

Interestingly, Eph/ephrin interactions are also involved in bone malignancies and tumors, osteocarcinoma is a malignant bone tumor in adolescence and microarray analysis studies show increased expression of EphA2, EphA4, ephrin-B1 and ephrin-A1 in osteosarcoma cells [105,106].

Eph/Ephrin expression in leukemia and other hematopoietic tumors

Both chronic and acute myeloid leukemia are malignant diseases of the hematopoietic system which in most cases are believed to arise through the abnormal proliferation of either uncommitted or partially committed HSC [107]. The origin of other types of leukemia such as promyelocytic leukemia, pre-B acute lymphoblastic leukemia (ALL) and T-ALL and chronic lymphoblastic leukemia (CLL) are more likely due to malignant transformation of more mature progenitor cells. Expression of elements of the Eph/ephrin system has been detected on many types of human leukemia. One of the best studied is EphA3, which was originally identified in the LK63 pre-B ALL cell line and further investigations revealed its expression in T-cell leukemia cell lines such as Jurkat, JM and HSB-2 [65,108]. It has been shown that EphA3 can induce both adhesive and cell repulsive responses in different cell types [109]. In analyzing ephrin induced cell adhesion in LK63 cells, a critical role was identified for protein phosphatase activity, which prevented EphA3 phosphorylation and hence maintained the Eph/ephrin adhesive bond and prevented initiation of the signaling mechanisms leading to cell repulsion [31]. In leukemia, EphA3 is expressed at significantly higher levels compared to normal blood cells, elevated expression of EphA3 being detected in a proportion of clinical samples from cases of lymphoid and myeloid leukemias [111]. Elevated EphA3 expression has also been detected on other cancers such as lung cancer, melanoma and brain tumors [22,64,65,109], whereas expression was found to be absent or low in corresponding normal tissues [54,65,110]. Recent array based studies also showed EphA3 as one of the genes with copy number alteration (CNA) in the genome of acute myeloid leukemia (AML) patients [111]. Further studies by Guan et al. [112] showed copy number variation (CNV) of EphA3 to be associated with various types hematological malignancies and therefore CNV of EphA3 could be used as a diagnostic indicator for different types of leukemia [112]. Many cancers, including leukemia, require multiple cooperative oncogene mutations for malignant cell transformation. Specific sets of synergistically dysregulated by cooperative oncogenes are known as cooperative response gene (CRGs), which regulate leukemia stem cell (LSC) growth and survival. Studies by Ashton et al. [113], where stem cells were retrovirally transduced with two fusion genes found in human myeloid leukemias. NUP98-HOXA9 and BCR-ABL, have identified EphA3 as a common CRG. They showed that shRNA knock down of EphA3 in leukemic stem cells reduced leukemic cell engraftment, concluding that this gene may be responsible for leukemia stem cell growth and survival in bone marrow microenvironment [113]. With the involvement of EphA3 in many different types of leukemia a high affinity monoclonal antibody to EphA3 (IIIA4) [65] has been fully humaneered by Kalabios and the resulting antibody, KB004, is now in phase I clinical trial in leukemia and other hematological cancers [114].

As mentioned previously EphB4 was originally identified in human bone marrow $CD34^+$ cells and its expression been reported in erythroid progenitor cells, however it's ligand ephrin-B2 is expressed in bone marrow stromal cells [88]. Co-expression of EphB4 and ephrin-B2 is found in the yolk sac, which is the first site of hematopoiesis and vascular development during embryogenesis. EphB4/ephrin-B2 expression has been shown in the majority of leukemia and lymphoma cell lines although expression in clinical samples appears less prominent [81]. Antibodies to EphB4 have undergone extensive pre-clinical evaluation and shown good anti-tumor effects in solid tumors, which over-express EphB4 and by inhibition of angiogenesis, although no efficacy has been shown in hematopoietic tumors to date [115]. Nevertheless, these antibodies may have the potential to be developed to target EphB4 in leukemia and related blood cancers.

Studies by Nakanishi et al. [75] shows EphA7 up regulation in the ALL1 associated leukemia (ALL1/AF4 and ALL1/AF9). They also showed that EphA7 up-regulation was associated with phosphorylation

Eph	Targeted therapy	References
EphA1	Tumor suppressor in colorectal cancer	44
EphA2	EphA2 targeting reagents in ovarian cancer therapy	119
EphA3	Therapeutic target in leukemia and glioblastoma	22,110
EphA7	Tumor suppressor in T-LBL and follicular lymphoma	47,120
EphB4	EphB4 antibody to inhibit solid tumor growth	115

Table 2: Eph receptors as a therapy target for cancer.

of ERK and treatment with a phosphorylated ERK blocking drug resulted in apoptotic cell death in ALL1/AF4 leukemic blast cells [75]. Thus, anti-EphA7 antibodies or other inhibitors may well have a role in leukemia associated with this translocation. In contra-distinction to this positive role in leukemia, EphA7 is lost in lymphomas, where the gene is hypermethylated and repressed in germinal center B-cell non-Hodgkins lymphomas and this has a potential to influence tumor progression and spread [74]. In this study a soluble form of EphA7 was shown to inhibit lymphoma in a mouse model, a chimeric protein consisting of soluble EphA7 and CD20 antibody had still greater therapeutic effect. Further studies show EphA7 as targeted tumor suppressor gene in T-cell lymphoblastic leukemia and lymphoma (T-LBL) and follicular B cell lymphoma [47,116].

EphB6 expression has been observed in normal human tissue and over-expression of EphB6 has also been reported in both myeloid [117] and lymphoid leukemias [82,118]. The expression level of EphB6 decreases with maturation of the cells in T-cell derived leukemia-cells, therefore suggesting that EphB6 expression regulates T-cell development but has less significant role in mature T cells [82]. To date there are no reports of experimental therapies targeting EphB6. Table 2 [110,119,120] represents the summary of Eph receptors used as therapy target in various malignancies.

Summary

In summary, the aberrant expression of Eph receptors in hematopoietic tumors reflects the spectrum of functions of these receptors in all cancers. In some cancers these genes act as tumor suppressor, examples being EphA1 in colorectal cancer and EphA7 in follicular lymphomas. On the other hand these proteins can also have oncogenic effects, examples being the expression of EphA2 and EphA3 in glioma and the over-expression of EphA3 in leukemia. In terms of therapy, the over-expression in certain tumors, taken together with the surface expression of these proteins, makes a strong case for targeted therapies. This is particularly the case when expression on normal tissues is minimal; this is exemplified by EphA3 and EphB4 where no toxicity was evident in pre-clinical testing of potential therapeutic antibodies. These studies reveal the therapeutic potential of targeting components of the Eph/ephrin system in leukemia and other cancers. These results should prompt further research into the specific roles of these proteins in different cancers as a prelude to designing and optimizing the therapeutic targeting of these proteins.

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