HHS Public Access

Author manuscript

Annu Rev Pharmacol Toxicol. Author manuscript; available in PMC 2016 January 01.

Published in final edited form as:

Annu Rev Pharmacol Toxicol. 2015 ; 55: 465–487. doi:10.1146/annurev-pharmtox-011112-140226.

Eph Receptors and Ephrins: Therapeutic Opportunities

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Abstract

The Eph receptor tyrosine kinase family plays important roles in developmental processes, adult tissue homeostasis and various diseases. Interaction with ephrin ligands on the surface of neighboring cells triggers Eph receptor kinase-dependent signaling. The ephrins can also transmit signals, leading to bidirectional cell contact-dependent communication. Moreover, Eph receptors and ephrins can function independently of each other, through interplay with other signaling systems. Given their involvement in many pathological conditions ranging from neurological disorders to cancer and viral infections, Eph receptors and ephrins are increasingly recognized as attractive therapeutic targets and a variety of strategies are being explored to modulate their expression and function. Eph receptor/ephrin upregulation in cancer cells, the angiogenic vasculature, and injured or diseased tissues also offers opportunities for Eph/ephrin-based targeted drug delivery and imaging. Thus, despite the challenges presented by the complex biology of the Eph receptor/ephrin system, exciting possibilities exist for therapies exploiting these molecules.

Keywords

Tyrosine kinase; neurological disease; cancer; angiogenesis; viral infection

INTRODUCTION

Eph (erythropoietin-producing hepatocellular carcinoma) is a large family of receptor tyrosine kinases with important roles in tissue organization and growth during development as well as in adult tissue homeostasis (1–3). Importantly, Eph receptors together with their ligands, the ephrins (Eph receptor interacting proteins), represent key players in many pathological conditions and therefore promising drug targets (2; 4–6). However, a thorough understanding of their involvement in disease processes is needed in order to devise effective Eph/ephrin-based therapeutic strategies that also minimize toxicity.

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DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review. EBP is an inventor in patents on Eph receptor-targeting peptides.

The fourteen human Eph receptors are subdivided into two classes. Nine EphA receptors promiscuously bind five ephrin-A ligands and five EphB receptors promiscuously bind three ephrin-B ligands (Figure 1A), with some exceptions. Combinations of Eph receptors and/or ephrins are present in most, if not all, cell types. Eph receptors bind ephrins on neighboring cells, generating cell contact-dependent bidirectional signals that regulate cell shape, movement, survival and proliferation (1; 3; 4). Eph "forward" signaling depends on ephrin binding, which induces Eph receptor clustering, autophosphorylation and kinase activity (Figure 1A). The tyrosine phosphorylated motifs and other cytoplasmic regions provide binding sites for signaling effectors, including adaptor proteins, kinases, phosphatases, guanine nucleotide exchange factors, and GTPase-activating proteins (1; 4). Eph receptor-ephrin binding can also lead to endocytosis and/or proteolytic cleavage (Figure 2), generating intracellular Eph/ephrin fragments with distinctive signaling abilities (4; 7–9) and often ultimately leading to proteosomal/lysosomal degradation and signal termination (4; 8; 10–12). Ephrin "reverse" signaling is also activated following interaction with Eph receptors (Figure 1A). The glycosyl phosphatidylinositol (GPI)-linked ephrin-As signal by associating with transmembrane partners, such as the p75NTR neurotrophin receptor or the TrkB and Ret receptor tyrosine kinases (8). Signaling by the transmembrane ephrin-Bs involves tyrosine and serine phosphorylation and association with various effector proteins (4; 13).

Bidirectional signal transduction can be further regulated. Ephrin-As can be released from the cell surface by metalloprotease cleavage and activate EphA receptors in a paracrine manner (14; 15) (Figure 2). Lateral "cis" interactions between Eph receptors and ephrins coexpressed in the same cell can attenuate the cell contact-dependent signals induced by "trans" interactions (8; 16). Furthermore, different Eph receptors can cluster together to coordinately generate a signaling output that may depend on the repertoire of coexpressed receptors (17). EphA10 and EphB6 lack kinase activity and thus might serve to functionally modulate the kinase-competent Eph receptors (18).

Eph receptors can also function through "non-canonical" signaling modalities, including interplay with secreted major sperm protein (MSP) domain-containing proteins, the extracellular protein reelin, other receptor tyrosine kinases, the lipoprotein receptor LRP1, or intracellular proteins (4; 19–21). For example, EphA2 is phosphorylated on serine 897 by the AKT kinase (Figure 1B), which leads to increased cell migration/invasiveness (8; 22). This is in stark contrast to the contact-inhibition-of-locomotion mediated by ephrin-induced EphA2 kinase activity (1; 8; 23). Additionally, EphA4 and EphB3 can function as a "dependence" receptor that promote apoptosis following caspase cleavage when not bound to ephrin-B3 (4; 24) (Figure 2). The ephrins can also in some instances signal independently of their association with Eph receptors (13; 25–28) (Figure 1B).

In this review, we discuss Eph/ephrin involvement in various diseases and highlight the associated therapeutic opportunities. Other recent reviews provide more details on Eph/ephrin signaling mechanisms (1; 4; 8; 9; 13; 17; 28; 29), roles in specific diseases (2; 4; 30–35), targeting agents (4; 36; 37) and related patent applications and clinical trials (38; 39).

NERVOUS SYSTEM

Eph receptors and ephrins are highly expressed in the developing nervous system, where they regulate the spatial organization of cell populations, tissue patterning, axon guidance and the formation of synaptic connections (1; 2; 29). Some family members remain substantially expressed in the adult nervous system, where they control the structure and function of synapses and various aspects of neural stem/progenitor cell biology (2; 34; 35; 40). The Eph/ephrin system has also been linked to neuropathologies ranging from inhibition of neural repair after traumatic injury and stroke to neurodegenerative diseases and chronic neuropathic pain. In particular, the highly expressed EphA4 and EphB2 receptors are emerging as key players in several nervous system disorders.

Neural repair

Many Eph receptors and ephrins are upregulated after traumatic or ischemic nervous system injury and negatively affect axon sprouting and other repair processes (2; 30). Indeed, a number of studies have linked pharmacological inhibition of EphA4-ephrin binding and EphA4 gene inactivation with improved functional recovery in rodent spinal cord injury models (41–43). Furthermore, upregulation of EphA4 and its ligand ephrin-A3 can limit the regenerative ability of injured spinal cord axons in mice lacking the axon growth inhibitor Nogo-A (44). These antiregenerative effects probably depend not only on inhibition of axonal growth through interaction of neuronal EphA4 with ephrin-A3/ephrin-B3 present in myelin and ephrin-B2 in astrocytes but also, at least in some models, on EphA4-dependent promotion of reactive gliosis and neuroinflammation (41; 44–47). EphA4 also inhibits muscle reinnervation after motor nerve axotomy, since reinnervation is improved in EphA4-deficient mice (48). Furthermore, ephrin-A5 upregulated in brain reactive astrocytes after stroke likely acts mainly through EphA4 to inhibit sprouting of new axonal connections and motor recovery (49; 50). Thus, inhibition of EphA4, and likely other Eph receptors, could be useful to promote regeneration and functional recovery in the injured nervous system.

Neurodegeneration

The Eph/ephrin system has been implicated in several neurodegenerative diseases. An example is Alzheimer's disease, which is characterized by cognitive decline caused by progressive loss of synapses and neurons. Central to disease pathogenesis is the proteolysis of amyloid precursor protein by the presenilin/γ-secretase intramembrane protease complex, which generates the cytotoxic β amyloid (Aβ) peptides (51). Multiple forms of crosstalk have emerged between the presenilin/γ-secretase/Aβ system and Eph receptors/ephrins known to regulate synapses. Binding of Aβ to EphB2 causes EphB2 proteosomal degradation, leading to reduced N-methyl-D-aspartate (NMDA) receptor-mediated calcium currents and impairment of synaptic transmission (51). Importantly, EphB2 restoration can reverse the cognitive and behavioral defects in an Alzheimer's mouse model. Aβ can also activate EphA4 forward signaling, which has been shown to participate in Aβ synaptotoxicity and can be blocked by EphA4 antagonists (35; 52; 53), and some evidence also suggests that EphA4/ephrin-A1 may in turn be able to enhance Aβ levels (54). In addition, cleavage by presenilin/γ-secretase regulates the signaling ability of EphB2, EphA4 and ephrin-Bs (Figure 2). The intracellular EphB2 fragment generated by the cleavage

phosphorylates the NMDA receptor thus enhancing synaptic calcium currents (8). The intracellular EphA4 fragment, which appears to be decreased in the Alzheimer's brain, can promote dendritic spine formation independently of kinase activity (8; 55) and the intracellular ephrin-B fragment is critical for the downstream activation of Src kinase (8; 13). Thus, defective Eph/ephrin processing may contribute to the neurodegenerative effects of presenilin mutations in the Alzheimer's brain. Other evidence suggests that presenilin might also mediate the neuroprotective activities of ephrin-B/EphB2 by increasing cell surface EphB2 levels independently of γ-secretase (56). Thus, Eph/ephrin-based therapies against Alzheimer's disease could include restoring EphB2 expression/signaling, blocking EphA4-ephrin and EphA4-Aβ interaction, and increasing the EphA4 intracellular fragment.

EphA1 has also been linked to Alzheimer's disease, despite not being detectably expressed in neural cells. Two single nucleotide polymorphisms (SNPs) in EphA1 show significant association with the disease (57). They involve the promoter and an intron, and may thus affect EphA1 expression in a cell population that remains to be identified. The Eph/ephrin system may also be involved in Parkinson's disease, a neurodegenerative disorder characterized by motor and cognitive symptoms (58). A soluble form of the ephrin-A1 ligand (ephrin-A1 Fc) promoted regeneration of the brain dopaminergic neurons that are lost in a rat Parkinson's model (59) and SNPs in several Eph receptors, including EphB1, have been associated with the disease (58). Finally, EphA4 was recently identified as a modifier gene that can worsen the pathology of amyotrophic lateral sclerosis (ALS), a fatal disease involving progressive motorneuron degeneration (48). Low EphA4 mRNA levels and EphA4 loss-of-function mutations in ALS patients were correlated with late disease onset and prolonged survival, while studies in ALS animal models suggest that decreasing EphA4 expression or pharmacological inhibition of EphA4-ephrin interaction could be of therapeutic benefit.

Pain

The EphB/ephrin-B system has been implicated in the induction and persistence of various types of pain, including chronic neuropathic pain caused by peripheral nerve injury, inflammatory pain and cancer pain, as well as in the physical dependence to opiates (31; 34; 60). The mechanism underlying pain involves increased activation of postsynaptic EphB receptors (particularly EphB1) in neurons of the spinal cord by presynaptic ephrin-B ligands (particularly ephrin-B2) expressed in pain sensory neurons as well as hyperexcitability of the sensory neurons. EphB signaling potentiates the efficacy of synapses involved in sensing pain and decreases pain threshold levels by enhancing Src-dependent NMDA receptor phosphorylation and calcium currents. Changes in gene transcription may also play a role in the effects of ephrin-B/EphB signaling on pain. Importantly, reduced EphB1 expression or agents that inhibit EphB-ephrin-B interaction in the spinal cord (such as EphB Fc) can alleviate spontaneous pain, thermal hyperalgesia, mechanical allodynia and opiate-resistant pain in rodent models. This suggests that antagonists targeting EphB receptors, such as EphB1, could represent a novel class of analgesics for the treatment of difficult-to-control chronic pain. Other studies suggest that the EphA/ephrin-A system may also regulate pain. For instance, EphA SNPs have been associated with peripheral sensory neuropathy induced by the chemotherapeutic drug paclitaxel in cancer patients (61). Furthermore, siRNA-

induced EphA4 downregulation (but not blockage of ephrin binding to EphA4) has been associated with increased pain in rodent models of spinal cord injury (41; 62).

CARDIOVASCULAR SYSTEM

The Eph/ephrin system is critical for cardiovascular development, as shown by the heart and blood vessel defects resulting from knockout of Eph receptors or ephrins (63–66). Some family members, such as EphA2/ephrin-A1 and EphB4/ephrin-B2, also play key roles in the adult cardiovascular system. Their involvement in regenerative and pathological forms of angiogenesis has been most studied, but new roles in heart function and repair are also emerging. Additionally, Eph receptors/ephrins expressed in blood vessels and their counterparts in immune cells are involved in inflammatory processes ranging from increased endothelial permeability and inflammatory cell transmigration across the endothelium to atherosclerotic plaque development (32; 33; 67; 68). Furthermore, EphB6 and ephrin-B1 expressed in vascular smooth muscle cells can contribute to blood pressure regulation (69).

Angiogenesis

The Eph/ephrin system controls blood vessel sprouting, assembly, remodeling and stabilization by regulating endothelial cells and their supporting mural cells, including pericytes and vascular smooth muscle cells. EphA2 expression is evident in angiogenic vasculature but low to undetectable in embryonic and postnatal quiescent vasculature (70; 71). EphA2 regulates angiogenesis and vascular permeability mainly in concert with ephrin-A1 also expressed in endothelial cells, through a mechanism that involves interplay with vascular endothelial growth factor (VEGF). Together with evidence that other EphA receptors and ephrin-As are involved in blood vessel regulation, this suggests that the EphA/ephrin-A system represents a promising target for therapeutic inhibition of postnatal angiogenesis and the vascular changes caused by inflammatory cytokines (32; 33).

With regard to the EphB/ephrin-B system, EphB4 is preferentially expressed in venous endothelial cells and its ligand ephrin-B2 in arterial endothelial cells, and both are involved in arterial-venous specification (63; 66). The EphB/ephrin-B system also regulates interaction of endothelial cells with other endothelial cells and with mural cells. For example, ephrin-B2 in pericytes promotes their association with endothelial cells expressing EphB receptors, which is required for blood vessel stabilization (63; 66). Ephrin-B2 is tyrosine phosphorylated in angiogenic but not in quiescent vasculature, consistent with an active signaling function linked to blood vessel sprouting and remodeling. Ephrin-B2 and EphB4 are also involved in pathological forms of postnatal angiogenesis, such as neovascular disorders of the eye (66). Interestingly, ephrin-B2 is upregulated by VEGF and is in turn required for VEGF receptor endocytosis and angiogenic signaling (66; 72), while ephrin-B2 expressed by inflammatory cells can regulate their interaction with endothelial cells (66). Therefore, agents blocking EphB/ephrin-B2 could complement current drugs targeting the VEGF system (73; 74).

Heart function and repair

Several studies implicate the EphA/ephrin-A system in cardioprotection and heart repair by cardiac progenitor cells. EphA2 is expressed in immature progenitor cells and ephrin-A1 in cardiomyocytes, which act as supporting niche cells (75; 76). In rodent models of myocardial infarction, intramyocardial administration of ephrin-A1 Fc reduced heart damage and promoted the migration of resident progenitor cells to the damaged myocardium (75; 77). Progenitor cells stimulated with ephrin-A1 Fc before their injection into the injured heart also homed more effectively to damaged regions, where they promoted regenerative processes resulting in increased myocyte numbers, decreased infarct size and reduced arrhythmic events (75). On the other hand, EphA2 signaling defects have been linked to the reduced migratory and regenerative capacity of senescent cardiac progenitor cells, which may contribute to the impaired regenerative ability of the aging heart (76). Thus, potentiating EphA2 function could promote tissue salvage and improve cardiac repair by resident or transplanted cardiac progenitors for the treatment of myocardial infarction and heart failure.

Analysis of ephrin-B1 knockout mice also highlighted the importance of ephrin-B1 in maintaining the structural integrity of adult heart tissue by regulating the attachment of the cardiomyocyte lateral membrane to the surrounding extracellular matrix (27). This is a distinctive role for an ephrin, which does not involve Eph receptor binding (Figure 1B). Since ephrin-B1 knockout causes not only heart morphological defects but also impaired electrical conduction and hypersensitivity to pressure overload, it will be important to assess the role of ephrin-B1 downregulation and gene mutations in heart disease as well as the utility of treatments potentiating ephrin-B1 function in the failing heart (27). In addition, intraperitoneal administration of ephrin-B2 Fc was reported to increase capillary density in the infarcted mouse myocardium, although the effects on functional recovery were not assessed (78). Studies in cell culture suggest a role for EphB receptors such as EphB4 in cardiomyocyte gap junctional communication and synchronized contraction (79). These additional EphB/ephrin-B activities should be further investigated in vivo since their modulation could be useful for the treatment of heart disease.

CANCER

Eph receptors and ephrins are aberrantly expressed in tumors and can drastically affect malignancy through both bidirectional signaling and interplay with other signaling systems (4; 68). These different signaling modalities can lead to diverse, even opposite, effects on cancer cells that also depend on the cellular context. Consistent with their ability to either promote or suppress tumorigenicity, Eph/ephrin expression can increase or decrease during cancer progression due to chromosomal amplification or loss, transcriptional regulation by oncogenic signaling pathways, promoter methylation and microRNAs (4; 6; 80).

EphA2 and EphB4 are the Eph receptors most widely overexpressed in tumors and downregulating their expression typically inhibits tumorigenicity, supporting a role in cancer malignancy. Surprisingly, high Eph receptor expression in cancer cells often correlates with low tyrosine phosphorylation, suggesting that Eph oncogenic activities are due to non-conventional signaling mechanisms (Figure 1B) and that Eph forward signaling suppresses

malignancy (4; 68). Indeed, many Eph cancer mutations characterized so far impair ephrin binding or kinase activity (81; 82). However, there are exceptions where Eph receptor activation by ephrins enhances malignancy, which is in some cases due to “oncogene addiction” of cancer cells that have evaded the negative effects of Eph forward signaling (4; 83). Eph/ephrin roles in promoting drug resistance are also beginning to be uncovered. For example, tumor xenograft studies show that EphA2 can promote resistance to the antiestrogen tamoxifen and to human epidermal growth factor receptor 2 (HER2)-targeted therapy in breast cancer (84–86), while ephrin-B2 can promote resistance to anti-VEGF therapy in glioblastoma (74). Moreover, cell culture studies have implicated ephrin-B3 in the resistance of lung cancer cells to ionizing radiation (87).

The Eph/ephrin system clearly has important roles in most cancers through remarkably diverse mechanisms. Here we discuss brain and lung cancer as examples illustrating the complexity of Eph/ephrin activities in cancer cells. Additionally, Eph receptors and ephrins are key regulators of the tumor microenvironment and its communication with cancer cells.

Brain cancer

Glioblastoma is the most prevalent type of primary brain tumor (88). Its highly malignant nature is due to “stem cells” that are highly resistant to chemotherapy/radiation and can regenerate tumors after treatment. The EphA2 and EphA3 receptors were recently found to promote the self-renewal of glioblastoma stem cells and inhibit their differentiation by limiting ERK MAP kinase activity in an ephrin-independent fashion (10; 89; 90). Moreover, EphA2 serine 897 phosphorylation is elevated in the most aggressive tumors, particularly in the stem cell population, suggesting an important role for EphA2 phosphorylation by AKT (Figure 1B) in glioblastoma malignancy (10; 22; 90). Indeed, downregulation of EphA2 or EphA3 expression by RNA interference or administration of high doses of ephrin-A1 Fc drastically reduced glioblastoma xenograft tumorigenicity (10; 89). On the other hand, activation of EphA2 forward signaling can inhibit the AKT-mTORC1 oncogenic pathway and decrease EphA2 serine 897 phosphorylation, consistent with the reported association between ephrin-A1 downregulation and glioblastoma aggressiveness (22; 88; 91).

Another EphA receptor, EphA4, can promote glioblastoma cell proliferation and migration by potentiating fibroblast growth factor (FGF) receptor oncogenic signaling in an ephrin-dependent manner (88). Furthermore EphB2, which can be upregulated in glioblastoma as a consequence of decreased microRNA-204 levels, was shown to promote invasiveness while inhibiting proliferation in glioblastoma-derived neurospheres and mouse orthotopic xenografts (88; 92; 93). Since these effects depend on EphB2 forward signaling, inhibiting EphB2 expression/signaling could help block the infiltration of glioblastoma cells into the brain, but should be accompanied by strategies to inhibit proliferation. The EphB2 gene is also amplified in a subgroup of human ependymomas, and EphB2 overexpression in mouse neural stem cells lacking the Ink4a/Arf tumor suppressor led to the formation of tumors resembling human ependymomas (94).

Reverse signaling by ephrins has also been implicated in glioblastoma pathogenesis. For example, ephrin-A5 can act as a tumor suppressor by downregulating the epidermal growth factor (EGF) receptor (88). Ephrin-B2 and ephrin-B3 are, like EphB2, preferentially

expressed in the more invasive glioblastoma cells and their reverse signaling pathways have been linked to invasiveness (88).

Lung cancer

The great majority of lung cancers are caused by somatic mutations due to tobacco carcinogens, and EphA3 is one of the genes most frequently mutated (in 5–15% of the tumors) (81; 82; 95) (cbioportal.org). Most EphA3 mutations are loss-of-function missense mutations that inhibit forward signaling by disrupting ephrin binding, kinase activity, or cell surface localization. Consistent with the heterozygosity of most mutations, several EphA3 mutants were shown to counteract the growth inhibitory effects of coexpressed wild-type EphA3 in tumor xenografts (82). As expected for a tumor suppressor, EphA3 expression is also downregulated in a high proportion of lung cancers due to decreased gene copy number (82). EphA2 and EphA4 are less frequently mutated in lung cancer. High EphA4 expression in patient tumors correlates with improved outcome and EphA4 can inhibit lung cancer cell migration/invasion, suggesting a tumor suppressor role (96). In contrast, EphA2 overexpression correlates with a poor outcome (95) and knockdown/knockout experiments have shown that EphA2 can inhibit lung cancer cell growth in culture and in mouse lung cancer models, possibly through ephrin-independent mechanisms (97; 98). The EphA2 G391R lung cancer mutation was found to prevent ephrin-induced cleavage of EphA2 by the MMP14 (MT1-MMP) metalloprotease (Figure 2), thus increasing EphA2 surface levels (7). Furthermore, the EphA2 G391R mutant introduced in the BEAS-2B bronchial epithelial cell line promoted invasiveness and anchorage-independent growth better than wild-type EphA2 (95). Frequent EphA5 and EphA7 lung cancer mutations that could be pathologically relevant were also identified, but remain to be characterized (99).

EphB3 is also overexpressed in lung cancers, whereas the ephrin-B1 and ephrin-B2 ligands are downregulated (100). This enhances lung cancer cell migration/invasiveness as well as growth through an EphB3 ligand- and kinase-independent mechanism that remains to be characterized. Consistent with a selective advantage of low EphB3 forward signaling, ephrin-B-induced activation of this receptor suppresses lung cancer metastasis through a pathway involving inactivation of the oncogenic AKT kinase by the PP2A phosphatase (101). EphB4 overexpression in lung cancer can also contribute to malignancy (102). In contrast, the kinase-inactive EphB6 is downregulated in aggressive lung cancers due to promoter hypermethylation and represents a prognostic marker indicating low metastatic potential (101). Consistent with a tumor suppressor role, EphB6 expression reduced lung cancer cell migration in culture and metastasis in a xenograft model, and these activities were impaired by EphB6 lung cancer mutations (103).

Tumor microenvironment

The activities of the Eph/ephrin system in tumors are not limited to the cancer cells but also impact the microenvironment. Angiogenesis is critical for tumor growth and metastasis and critically depends on the Eph/ephrin system (4; 63; 66; 104). Additionally, EphA2 expressed in tumor cells has been implicated in vasculogenic mimicry (4; 32; 66; 104). Pharmacological inhibition of EphA2/ephrin-A1 and EphB/ephrin-B2 in tumor blood vessels was indeed shown to reduce tumor growth (4; 66; 70; 71). Eph/ephrin-dependent

regulation of immune/inflammatory cells and their interplay with cancer cells likely also plays a role in cancer progression, including the extravasation of metastatic cells (4; 32; 66; 105). Another form of cell-cell communication orchestrated by the Eph/ephrin system in tumors involves repulsion of cancer cells expressing Eph receptors by surrounding cells of the tumor microenvironment expressing ephrin ligands (3; 23). This can confine tumor cells, thus repressing both invasiveness and tumor expansion. In contrast, repulsive interactions between tumor cells expressing both Eph receptors and ephrins can promote cancer cell dispersion from the tumor (7; 68). Thus, the benefits of inhibiting Eph receptor-ephrin binding can vary depending on the Eph/ephrin expression patterns in both cancer cells and the tumor microenvironment.

VIRAL INFECTIONS

The involvement of certain Eph receptors/ephrins in viral infections suggests new avenues for antiviral therapies. The best characterized role involves ephrin-B2 and ephrin-B3 as entry receptors for henipaviruses, an emerging zoonotic group of deadly viruses (106). Henipaviruses bind to the same ephrin region that also interacts with Eph receptors to infect blood vessels and the nervous system, causing respiratory and encephalitic illness. Henipaviruses are particularly dangerous because of their broad species tropism, which is due to the high conservation of the ephrin-Bs. The ephrin-B2 extracellular domain (ECD) and an antibody to the viral glycoprotein that binds ephrin-Bs have antiviral activity, supporting the value of blocking ephrin-viral interaction to prevent viral spread during outbreaks or possible biological warfare. On the other hand, the ephrin-As were identified as entry receptors for IAPE (intracisternal A-type particles elements with an envelope), a mouse endogenous retrovirus that can infect human cells (107).

EphA2 is required for endothelial cell infection by Kaposi's sarcoma-associated herpesvirus (KSHV), a causative agent of Kaposi's sarcoma and B cell malignancies (108; 109). KSHV binding activates EphA2, which leads to viral entry through macropinocytosis and productive infection. A siRNA screen also identified EphA2 as a host factor enabling infection by the hepatitis C virus through an indirect mechanism not involving direct interaction of EphA2 with viral glycoproteins (110). Importantly, EphA2/ephrin-A ECDs and EphA2-targeting antibodies have been successfully used to inhibit KSHV and hepatitis C infection of cultured cells (108–110). Moreover, the efficacy of the wide spectrum kinase inhibitor dasatinib against KSHV and hepatitis C may involve inhibition of EphA2 signaling, while heat-shock protein HSP90 inhibitors may function against KSHV at least in part by decreasing EphA2 expression (111).

Roles of Eph receptors/ephrins in other viral infections likely remain to be discovered. For example, a siRNA screen of A549 lung cancer cells identified EphB6 as a host factor important for H1N1 influenza virus replication (112).

OTHER DISEASES

The Eph/ephrin system participates in a number of other highly prevalent diseases and aging-associated conditions. An area of particular interest is regenerative medicine, given the potential to therapeutically control stem cells through modulation of the Eph receptors

and ephrins (39; 40; 59; 113; 114). In addition, Eph/ephrin family members are upregulated in injured tissues, where their activities can hinder certain wound healing processes such as cell growth and movement, but facilitate others such as inflammatory cell trafficking, angiogenesis and reestablishment of tissue organization (2; 3; 32; 115).

Several Eph receptors and ephrins are present in various skin compartments, including the epidermis and hair follicles. EphA2 expressed in keratinocytes controls epidermal differentiation and homeostasis, and its deregulation has been associated with skin diseases such as psoriasis and cancer (115; 116). Various Eph/ephrin family members regulate hair growth, with evidence suggesting that loss of ephrin-A3 contributes to androgenic alopecia (115; 117). Thus, the Eph/ephrin system could represent a new target for counteracting the effects of aging on hair loss.

Another emerging role of the EphA/ephrin-A system is the control of glucose homeostasis. EphA forward signaling inhibits insulin secretion in pancreatic β cells when glucose levels are low, while ephrin-A5 reverse signaling promotes insulin secretion in response to elevated glucose (2). Interestingly, EphA forward signaling induced by ephrin-A5 also plays a complementary role in the glucose-sensing hypothalamic region of the brain, where it promotes the release of hormones that correct hypoglycemia (118). Consistent with these findings, mouse preclinical studies show that pharmacological Eph kinase inhibition can enhance glucose-stimulated insulin secretion, suggesting its potential use for diabetes treatment (119).

Eph receptors and ephrins also regulate bone homeostasis and remodeling. The bone anabolic effects of EphB4 expressed in osteoblasts likely depend on coordinately promoting osteoblast differentiation and restraining osteoclast precursor differentiation through interactions with ephrin-B2/ephrin-B1 expressed by the two cell types (114). In contrast, EphA2/ephrin-A2 signaling in osteoblasts/osteoclasts can negatively regulate bone formation. Thus, Eph/ephrin family members could represent therapeutic targets to treat bone disorders such as arthritis, osteolytic lesions in multiple myeloma, bone-associated metastases and osteoporosis.

Finally, Eph/ephrin genetic mutations and polymorphisms are beginning to be associated with a number of other illnesses besides cancer. For example, EphA2 mutations cause age-related cataracts (8), Eph receptor SNPs are linked to Alzheimer's and Parkinson's diseases (57; 58) and ephrin-B2 SNPs to schizophrenia (120).

STRATEGIES FOR TARGETING THE EPH/EPHRIN SYSTEM

The altered expression and functional involvement of Eph receptors and ephrins in many diseases offers the opportunity for therapeutic strategies based on modulating the activities of the relevant family members. Different agents can be used to increase or inhibit activities of a single Eph receptor/ephrin or multiple family members, and for targeted delivery of drugs and imaging agents to diseased tissues (Table 1).

Types of molecules that can be used to interfere with Eph/ephrin signaling

Recombinant Eph/ephrin ECDs are widely used as soluble surrogates for their membrane-bound counterparts to activate as well as inhibit forward and/or reverse signaling (1; 4; 36). These ECDs bind with high affinity and can have long in vivo half-life, particularly when coupled to an Fc domain or albumin. They affect the activities of multiple family members, which could enhance efficacy but also increase unwanted side effects. Monomeric ephrin-A ECDs can weakly activate at least some EphA receptors through an unknown mechanism (14; 15), whereas monomeric ephrin-B and Eph ECDs inhibit both forward and reverse signaling (4). For example, the monomeric EphB4 ECD shows promise in animal models as an anti-cancer and anti-angiogenic agent (4; 74; 121–124). Interestingly, an N-terminal fragment of the EphA7 ECD that mimics an endogenous alternatively spliced form can act as a tumor suppressor against follicular lymphoma xenografts (125). Other Eph ECD fragments generated by alternatively splicing or cancer mutations (cbioportal.org) likely also exert biological effects that could be harnessed for therapeutic applications.

Ephrin ECDs fused to Fc are dimeric and can activate Eph forward signaling, but in some cases they promote Eph degradation and thus loss of signaling activities (4; 10). Furthermore, in some systems ephrin Fc proteins seem to function as Eph receptor inhibitors unless they are oligomerized with anti-Fc antibodies (117), perhaps because monomeric/dimeric ephrins are weaker activators than the endogenous membrane-bound ephrins they displace. On the other hand, Eph Fc proteins can promote ephrin reverse signaling. In addition, Eph/ephrin Fc proteins can also compete with their endogenous counterparts and reduce their signaling ability. EphA2 and EphA3 Fc, for example, can function as anti-cancer agents in mouse models by inhibiting EphA2 forward signaling in the tumor vasculature (70; 104). Applications of ephrin ECDs include attachment of ephrin-A1 Fc or the ephrin-B2 ECD to biomimetic hydrogels for therapeutic angiogenesis (126; 127) and of ephrin-A1 Fc to albumin microspheres to inhibit cancer cell growth/migration (128). In recent developments, multivalent ephrin ECD bioconjugates with precise geometrical configurations were generated by incorporation into nanostructured biomaterials (40; 129; 130). These nanoparticles can accurately modulate Eph/ephrin oligomerization in target cells, and thus signaling output, which could lead to new fine-tuned nanotherapeutics.

Antibodies are particularly suitable for modulating the Eph/ephrin system, given their high binding affinity and specificity coupled with long in vivo half-life. Both activating and inhibitory monoclonal antibodies recognizing Eph/ephrin ECDs have been developed for applications against cancer and angiogenesis, with particular focus on EphA2, EphA3, EphB4 and ephrin-B2 (4). EphA2 antibodies used as single agents to activate EphA2 antioncogenic signaling/degradation have shown efficacy in some mouse preclinical models but not others (104; 131–135). In combination treatments, these antibodies can also enhance the effects of established chemotherapeutic drugs such as tamoxifen, paclitaxel and docetaxel (85; 104; 133). Additionally, EphB4 and ephrin-B2 inhibitory antibodies have shown efficacy in mouse tumor xenografts as antiangiogenic/anticancer agents, in some cases combined with anti-VEGF therapy (73; 74; 136; 137).

Peptides have also proven their potential for modulating Eph/ephrin signaling with high selectivity and binding affinity. A series of dodecapeptides that can selectively target the

ephrin-binding pocket of individual Eph receptors, or subsets of receptors, and antagonize ephrin binding were identified by phage display (36; 138). The affinities of these peptides are in the low micromolar range, but can be dramatically improved to low nanomolar by optimization (139; 140). Other modifications can increase peptide resistance to proteolytic degradation and increase in vivo half-life (141–143). Most of the identified peptides act as antagonists except for peptides targeting EphA2, which function as agonists that promote receptor activation and internalization through an unknown mechanism (144; 145). Preclinical studies have been carried out so far only with the KYL peptide targeting EphA4 in the nervous system (42; 48).

Small molecule agonists and antagonists targeting Eph receptors so far display modest binding affinities in the micromolar range (36; 37; 53), likely because of the large size and flexibility of the ephrin-binding pocket. Efforts to improve affinity have resulted in more promising but larger (>500 kDa) compounds (146–148). Small molecules are better-suited to bind the ATP-binding pocket in the Eph kinase domain (36; 39). Advantages of small molecule kinase inhibitors are their extensive track record as drugs, potential for oral bioavailability, and in many cases ease of synthesis. However, most kinase inhibitors exhibit poor selectivity and target multiple kinases. Indeed, several small molecules identified as inhibitors of other kinase families, such as dasatinib, can also potently inhibit Eph receptors. Surprisingly, dasatinib was recently reported to also inhibit kinase-independent EphA2 oncogenic signaling in cells through an indirect mechanism (149). Various types of screens to identify Eph kinase inhibitors have also yielded some promising compounds, with mouse preclinical studies showing the potential usefulness of several of them for inhibition of angiogenesis (150; 151) or the treatment of diabetes (119).

Antisense oligonucleotides or siRNAs can also be used to downregulate Eph/ephrin expression (4). These agents are highly selective and eliminate all activities of the targeted Eph receptor/ephrin, but their in vivo delivery can be inefficient. The most promising results were obtained with EphA2 knockdown in gynecologic cancer xenografts, which were treated using siRNA delivered using neutral liposomes or nanoparticles (104; 152), and with EphB4 knockdown in a series of tumor xenografts treated with siRNA or oligonucleotides (4; 137). These siRNAs can also enhance the effects of other treatments, including microRNAs (80; 137; 153).

Targeting molecules for delivery of therapeutics and imaging agents

Antibodies and peptides can be conjugated to drugs, toxins, radioisotopes, imaging agents and nanoparticles to enhance their targeted delivery to tumors and other diseased tissues overexpressing the relevant Eph receptor or ephrin. The roles of Eph receptors and ephrins as entry receptors for viruses also highlight their potential value for intracellular delivery of drug conjugates. For example, the 1C1 EphA2 agonistic antibody conjugated to a microtubule-disrupting drug showed efficacy against tumor xenografts (135; 154; 155). This antibody also shows promise for imaging of EphA2-overexpressing tumors (156). Additionally, the EphA2-specific YSA agonistic peptide and its improved derivatives were conjugated to paclitaxel, increasing the efficacy of this widely-used but poorly bioavailable chemotherapeutic drug in mouse xenografts (143; 145). YSA was also used to enhance the

effectiveness of doxycyclin-containing liposomes in a rat choroidal neovascularization model (157) and to target siRNAs and nanoparticles to cancer cells in culture (12; 158). A radiolabeled EphA3-activating antibody also shows promise for imaging and therapy, based on mouse xenograft studies (89; 159). Ephrin-As conjugated to a radioisotope or a toxin can also target tumor cells overexpressing EphA receptors, although rapid clearance from the circulation will have to be overcome for in vivo use, particularly against solid tumors (14; 159). Additionally, ephrin-A1-targeted gold-coated nanoshells were used for in vitro photothermal ablation of cancer cells (160). With regard to the EphB/ephrin-B system, an EphB2 agonistic antibody coupled to a microtubule-disrupting drug showed activity against colorectal cancer xenografts (161). Finally, EphB4 antibodies and the high-affinity EphB4-targeting peptide TNYL-RAW have been used to deliver imaging agents and therapeutic nanoparticles to mouse tumor xenografts (36; 162–166).

Cancer immunotherapy

Harnessing the power of the immune system is a particularly promising strategy against cancer. Given their preferential expression in tumors, Eph receptors represent possible targets for anticancer vaccines. For example, raising an immune response against EphA2- or EphB6-derived peptides can promote the attack of glioblastomas by cytotoxic T-lymphocytes (4; 167). In the case of EphB6, a receptor variant preferentially expressed in the tumors allows especially selective targeting. Additionally, EphA2 agonistic antibodies have been engineered to promote T-lymphocyte cytotoxicity against various cancer cells, which enhanced the antitumor efficacy of the antibodies in xenograft models (134; 168).

Potential toxicities associated with targeting the Eph/ephrin system

Therapies targeting Eph receptors/ephrins could conceivably give rise to on-target toxicities associated with abnormalities in physiological processes regulated by the affected Eph/ephrin, including changes in glucose tolerance, bone homeostasis, immune function, pain sensation, memory and cognition. Although information is still very limited, preclinical animal studies have in general not revealed obvious toxicities for the Eph/ephrin-targeting agents examined so far. Several factors could contribute to the favorable therapeutic index observed in most cases.

First, Eph/ephrin upregulation in diseased tissues provides the opportunity for more selective therapeutic targeting, and distinctive characteristics of certain Eph receptors in diseased tissue allow further selectivity. For example, an EphA2 antibody recognizes epitopes accessible in cancer cells but not normal epithelia (132) while a distinctive EphB6 variant preferentially expressed in glioblastoma enables selective immunotherapy (167). Furthermore, the ephrin-binding pocket of Eph receptors should be more accessible to targeting agents in tumors, where occupancy by ephrins is often low compared to normal cells (4; 115).

Second, compensatory activities of other Eph family members may prevent severe disruption of physiological processes. Additionally, homeostasis of critical physiological processes is typically ensured through multiple feedback mechanisms involving different signaling systems that can override the need for individual components. For example,

although EphB6 and ephrin-B1 expressed in vascular smooth muscle cells have been implicated in blood pressure regulation, loss-of-function of either molecule is insufficient to increase blood pressure in rodents unless other abnormalities/stressors are also present (69).

More in-depth analyses, including on the effects of chronic treatments, and clinical studies may nevertheless uncover liabilities and toxicities for some of the molecules in the increasing arsenal of Eph/ephrin-targeting agents. A notable example is the EphA2-targeting antibody 1C1 conjugated with a highly toxic microtubule-disrupting drug (MEDI-547). When tested in a phase I clinical trial at a subtherapeutic dose, this conjugate caused adverse events, including bleeding and coagulation events, resulting in premature termination of the trial (169). However, this does not necessarily invalidate EphA2 as a potential drug target, since the extremely high cytotoxicity of the conjugated drug (an auristatin derivative) can cause much greater side effects than other EphA2-targeted therapeutics. It also cannot be excluded that the toxicity of MEDI-547 may be due to the targeting of another protein besides EphA2 (169).

Personalized treatments based on assessment of which Eph/ephrin family members are functionally relevant in a particular tumor could help achieve more effective and less toxic therapies. Less selective agents, such as Eph/ephrin ECDs (Table 1), might have increased potential for toxicity. Knockdown approaches, which are less readily reversible and affect all functions of an Eph receptor/ephrin (Table 1), also have increased potential for toxicity unless they are targeted to the diseased tissue. Finally, it should be noted that an Eph/ephrin-targeting agent could exhibit some desirable “side-effects”, ranging from increasing glucose tolerance or bone mass to decreasing inflammation, atherosclerosis or pain.

Clinical trials

Several clinical trials with Eph receptor-targeting agents are in progress (39). The more advanced trials are evaluating multi-targeted kinase inhibitors that target Eph receptors among other kinases, including XL647 (which inhibits EphB4) against non-small-cell lung cancer and dasatinib (which inhibits EphA2) against various solid tumors. Other clinical trials are evaluating the EphB4 ECD fused to albumin in patients with recurrent/metastatic solid tumors, a humanized EphA3 antibody in patients with hematological malignancies, liposomes delivering EphA2 siRNA in patients with advanced solid tumors, and an EphA2 peptide vaccine in patients with late stage melanoma.

PERSPECTIVES

The involvement of the Eph/ephrin cell communication system in so many biological processes offers rich therapeutic opportunities, but also raises the possibility that intervention against an Eph/ephrin-driven pathology could cause toxicities due to alterations in physiological processes. Although numerous preclinical animal studies have not revealed particular toxicity problems for a variety of Eph/ephrin-targeting agents, information from clinical studies is still limited. Emerging evidence also shows that Eph/ephrin expression levels can vary substantially in different individuals and affect the course of a disease (48). Therefore, systems biology data could be mined to characterize the relationship of Eph/ephrin expression, post-translational modifications and SNPs with specific disease

processes. This will uncover Eph/ephrin family members representing key drug targets as well as biomarkers for diagnosis, prognosis, response to therapy, and drug resistance. Indeed, areas where Eph/ephrin-related medical applications are likely to dramatically expand include the involvement of the Eph/ephrin system in drug resistance and the use of Eph/ephrin-targeting agents in combination therapies to potentiate the effects of established drugs and for non-invasive imaging of diseased tissues. Eph/ephrin downstream effectors and signaling networks could also be targeted for therapy once better characterized, in some cases with already approved drugs. A comprehensive understanding of Eph/ephrin signaling mechanisms will help choose the best mode of intervention to selectively inhibit a pathological activity while avoiding toxic effects due to disruption of normal functions.

Acknowledgments

The authors thank members of the Pasquale lab for helpful comments on the manuscript and acknowledge support from the NIH and California TRDRP (EBP) and the Spanish Ministry of Science and Education (for a postdoctoral fellowship to AB).

ACRONYMS AND DEFINITIONS

Reactive gliosis	hypertrophy and proliferation of glial cells as a consequence of central nervous system injury
Dendritic spines	structures associated with excitatory synapses and important for cognitive functions. Dendritic spine numbers are reduced in Alzheimer's disease patients
Neuropathic pain	pain caused by damage or disease affecting sensory neurons that process pain sensation
Thermal hyperalgesia	increased sensitivity to pain induced by thermal stimuli
Mechanical allodynia	sensitivity to normally not-painful stimuli
Peripheral sensory neuropathy	damage or disease affecting peripheral sensory neurons
Niche cells	cells that are in contact with stem/progenitor cells and support their development and maintenance
Arrhythmic events	periods of irregular heartbeats
Electrical conduction	propagation of an electrical impulse throughout the heart stimulating contraction
Pressure overload	occurs when heart chambers have to overcome abnormally high pressure while contracting. This can be caused, for example, by hypertension or by restricted blood outflow and when cronic can lead to various heart pathologies
Oncogene addiction	dependence of cancer cells on the expression of one/several oncogenes for their growth and survival

Neurosphere	clusters of neural stem/progenitor cells grown in suspension in culture medium
Ependymoma	tumor that arises from a thin layer of tissue called ependyma, which lines the ventricles of the brain and spinal cord
Vasculogenic mimicry	phenomenon whereby tumor cells contribute to the formation of functional blood vessel-like structures
Macropinocytosis	endocytic process involving formation of plasma membrane invaginations that give rise to large vesicles carrying extracellular fluid and the macromolecules it contains into the cell interior
Androgenic alopecia	baldness due to the susceptibility of aging hair follicles in the scalp to damage by androgens such as dihydrotestosterone
Choroidal neovascularization	pathological formation of new blood vessels in the choroid layer of the eye, which is the vascular layer near the retina

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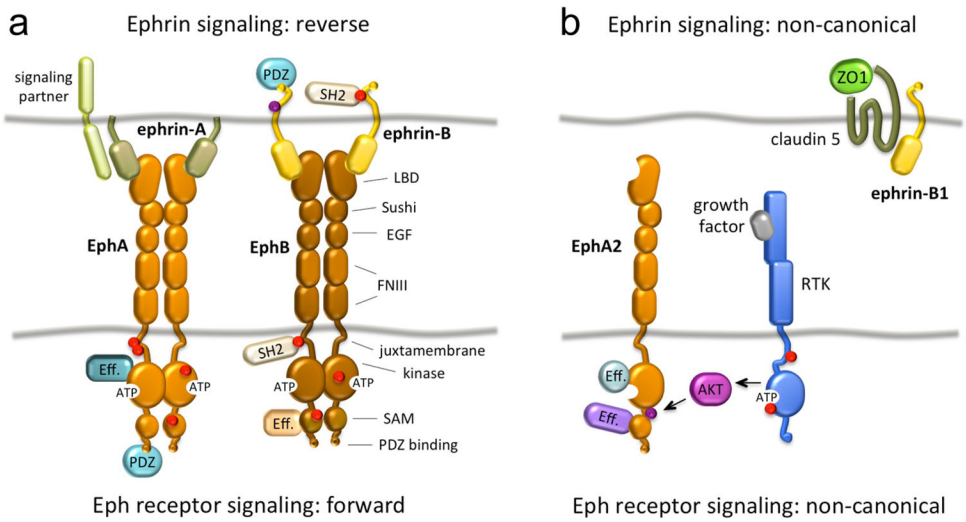

Figure 1.

Eph receptor/ephrin domain structure and signal transduction. (a) Bidirectional signaling at cell-cell contact sites. Red circles indicate tyrosine phosphorylation sites; purple circles indicate serine phosphorylation sites; SH2 indicates signaling proteins that interact with phosphorylated motifs through their SH2 domain; PDZ indicates binding partners containing a PDZ domain; other binding partners/signaling effectors (Eff.) are also shown schematically. Ephrin-As mediate reverse signals through association with a transmembrane signaling partner such as p75NTR or the TrkB and Ret receptor tyrosine kinases. LBD, ligand-binding domain; FNIII, fibronectin type III domain. (b) Examples of Eph receptor/ephrin non-canonical signaling modalities occurring through interplay with other signaling systems and independently of Eph receptor-ephrin interaction. For example, EphA2 can be phosphorylated by the serine/threonine kinase AKT activated downstream of receptor tyrosine kinases (RTKs), such as members of the EGF receptor family, or as a consequence of cancer mutations. EphA2 phosphorylated on serine 897 has unique signaling activities. Ephrin-B1 can control the interaction of cardiomocytes with the extracellular matrix by interacting with the claudin 5/ZO1 complex.

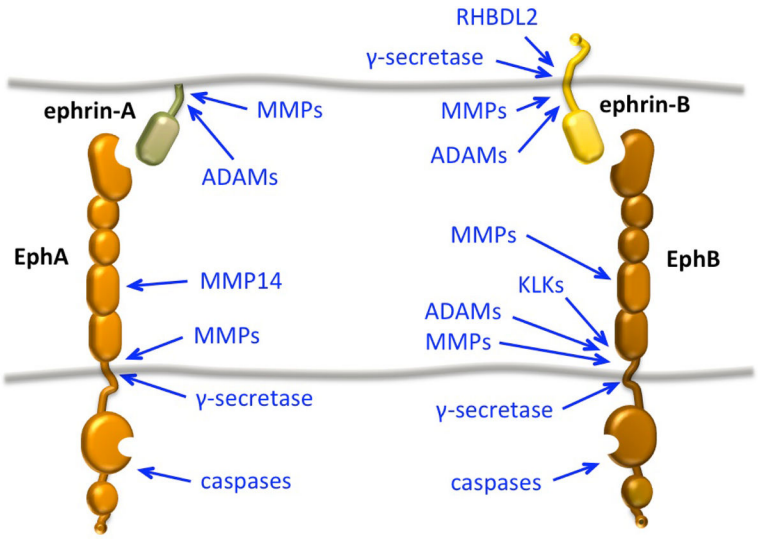

Figure 2.

Modulation of Eph receptor/ephrin signaling by proteases. Proteases can cleave Eph receptors and ephrins in their extracellular, transmembrane and intracellular regions in a manner that can be dependent or independent of Eph receptor-ephrin interaction (although for simplicity only unbound Eph receptors and ephrins are shown). These cleavages are important for biological effects that involve separation of Eph receptor and ephrin expressing cells, including neuronal growth cone collapse, cell-cell repulsion and cell segregation. The Eph/ephrin proteolytic fragments generated can also have distinctive signaling functions in the extracellular space, cytoplasm and nucleus. Ephrin-As are cleaved near their C terminus by matrix metalloproteases (MMPs) such as MMP1, MMP2, MMP9 and MMP13, which release soluble monomeric ephrin-As that can activate EphA receptors in a paracrine manner (15). Proteases of the ADAM (A disintegrin and metalloproteinase) family, such as ADAM10 and ADAM12, can associate with EphA receptors and cleave *in trans* ephrin-As expressed in neighboring cells, allowing EphA/ephrin-A endocytosis and cell separation or weakening intercellular junctions (8; 170). Ephrin-Bs can also be cleaved extracellularly by ADAM8, ADAM10 and ADAM13 to regulate angiogenesis, neural tube morphogenesis, and induction of cranial neural crest (8; 171; 172). Interaction with EphB receptors can enhance ephrin-B extracellular cleavage by MMPs, such as MMP8 in the case of ephrin-B1 (171), followed by intramembrane cleavage by γ-secretase (8). The ephrin-B cytoplasmic fragment generated can enhance phosphorylation of uncleaved ephrin-Bs by SRC (8) and translocate to the nucleus to regulate transcription (8; 171). RHBDL2, a rhomboid transmembrane serine protease, cleaves the transmembrane segment of ephrin-Bs, with a preference for ephrin-B3 (171). EphA receptors such as EphA4 can be cleaved in the second fibronectin domain by MMPs activated by calcium influx independently of ephrin binding, followed by intramembrane cleavage by γ-secretase (8). The EphA4 cytoplasmic fragment generated promotes dendritic spine formation in neurons by activating the RHO family GTPase RAC1. EphA4 can also be cleaved in the kinase domain by caspases such as caspase-3 to promote apoptosis, an effect that can be reversed by ephrin-B3 binding (4). EphA2 is cleaved in the first fibronectin domain by the transmembrane metalloprotease MMP14 (MT1-MMP), which enables receptor internalization, RHOA activation and cell-

cell separation (7). EphB receptors such as EphB4 can be cleaved near the transmembrane segment by ADAM8, ADAM9 and ADAM17 (171). EphB2 can be cleaved near the transmembrane segment by a metalloprotease activated by calcium influx, such as ADAM10, or by a distinct yet to be identified metalloprotease activated by ephrin binding (8). Ephrin-B binding also induces MMP7/MMP9-dependent EphB2 cleavage at two sites in the first fibronectin domain (one of which is conserved within the Eph family), which prolongs receptor activation and promotes RHOA signaling and cell-cell repulsion (171). KLK8 (Kallikrein 8 or Neuropsin) cleaves EphB2 in the brain in a stress-dependent manner leading to anxiety (8) and other kallikreins (KLKs), cleave the EphB4 extracellular domain at least in vitro (171). These extracellular EphB cleavages are typically followed by intramembrane cleavage by γ-secretase, which generates an EphB2 cytoplasmic fragment that phosphorylates the NMDA receptor and promotes its cell surface localization, thus modulating synaptic function (8). Caspases can cleave the kinase domain of EphB3 not bound to ephrins, leading to neuronal apoptosis after adult brain injury (24).

Annu Rev Pharmacol Toxicol. Author manuscript; available in PMC 2016 January 01.

Table 1

Agents that can be used to target Eph receptors and ephrins

Targeting agent	Target		Functional effects			
	Eph/ephrin	Other targets	Eph signaling: forward	Ephrin signaling: reverse	Eph signaling: non-canonical	Ephrin signaling: non-canonical
Eph ECD[a]	Ephrin, A or B class[b]	Unlikely	↓	↓	↓/×	×
Ephrin ECD	Eph, A or B class	Unlikely	↑[c]/↓	↓	×	↓/×
Multimeric[d] Eph ECD	Ephrin, A or B class	Unlikely	↓	↑	↓/×	×
Multimeric ephrin ECD	Eph, A or B class	Unlikely	↑/↓[e]	↓	×	↓/×
Eph inhibitory antibody	Single Eph	Unlikely	↓	↓[f]/×	↓/×	×
Ephrin inhibitory antibody	Single ephrin	Unlikely	↓[f]/×	↓	×	↓/×
Eph activating antibody	Single Eph	Unlikely	↑	↓[f]/×	↓/×	×
Eph peptide antagonist	Single Eph	Unlikely	↓	↓	×	×
Eph peptide agonist	Single Eph	Unlikely	↑	↓	↓/×	×
Eph small mol. antagonist	Multiple Ephs	Possible	↓	↓	×	×
Eph small mol. agonist	Multiple Ephs	Possible	↑	↓[f]	↓/×	×
Eph kinase inhibitor	Multiple Ephs	Yes	↓	×	×	×
Eph siRNA/antisense	Single Eph	Unlikely	↓	↓	↓	×
Ephrin siRNA/antisense	Single ephrin	Unlikely	↓	↓	×	↓

Symbols: ↑, increased; ↓, decreased; ×, unchanged.

[a] ECD, extracellular domain.

[b] Depending on Eph receptor-ephrin binding specificity.

[c] Monomeric ephrin-As can weakly activate EphA receptors.

[d] Includes dimeric Fc fusion proteins.

[e] Dimeric ephrin Fc fusion proteins can inhibit Eph receptors in some cases.

[f] If the agent inhibits Eph-ephrin interaction.