

# Roles of Eph/ephrin bidirectional signaling in central nervous system injury and recovery (Review)

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**Abstract.** Multiple cellular components are involved in the complex pathological process following central nervous system (CNS) injury, including neurons, glial cells and endothelial cells. Previous studies and neurotherapeutic clinical trials have assessed the molecular mechanisms that underlie neuronal cell death following CNS injury. However, this approach has largely failed to reduce CNS damage or improve the functional recovery of patients. Erythropoietin-producing human hepatocellular (Eph) receptors and ephrin ligands have attracted considerable attention since their discovery, due to their extensive distribution and unique bidirectional signaling between astrocytes and neurons. Previous studies have investigated the roles of Eph/ephrin bidirectional signaling in the developing central nervous system. It was determined that Eph/ephrin bidirectional signaling is expressed in various CNS regions and cell types, and that it serves diverse roles in the adult CNS. In the present review, the roles of Eph/ephrin bidirectional signaling in CNS injuries are assessed.

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## 1. Introduction

Erythropoietin-producing human hepatocellular (Eph) proteins constitute the largest known receptor tyrosine kinase family, and the first identified was the EphA1 receptor in 1987 (1). The Eph receptor family comprises 14 members in humans and other mammals and is divided into two subfamilies based on sequence conservation and ligand binding affinity: EphA (EphA1-EphA8 and EphA10) and EphB (EphB1-EphB4 and EphB6) (2). Eph receptors are activated when bound to membrane-combined ephrin ligands. A total of nine EphA receptors preferentially bind to five glycosylphosphatidylinositol-anchored ephrin-A ligands (ephrin-A1-A5). In addition, five EphB receptors possess high-affinity binding domains to three transmembrane ephrin-B (ephrin-B1-B3) ligands. EphA4 and EphB2 are exceptions, which can bind to both A-type and most B-type ligands (2). The formation of the Eph/ephrin complex initiates bidirectional signaling, which acts upon Eph-expressing and ephrin-expressing cells. Signaling pathways that are directly initiated by Eph receptors and ephrin ligand activations are termed forward signaling and reverse signaling, respectively (3). Previous studies have reported the diverse roles of Eph/ephrin bidirectional signaling in pathological and physiological processes (4-7).

## 2. Eph and ephrin structure

*Eph structure.* Eph receptors exhibit similar structural characteristics, despite the large number of subtypes. The extracellular region of Eph receptors is primarily comprised of a highly-conserved N-terminal globular domain, which is essential for ephrin identification and binding (8). Following on from the globular domain, the Eph extracellular region also includes one unique cysteine-rich and two fibronectin type III motifs, which affect receptor dimerization (8,9). The intracellular region of Eph contains four structural and functional units: A juxtamembrane region, a conserved kinase domain, a sterile-a-motif (SAM) domain and a PSD95/Dlg/ZO1 (PDZ)-binding motif. The juxtamembrane region is a highly conserved motif containing two tyrosine residues, which are the primary autophosphorylation sites for downstream signal transduction (10,11). The conserved tyrosine kinase domain is involved in the binding and activating of small guanosine 5'-triphosphate (GTP)ases, which are important for the regulation of the cytoskeletal structure (12). The SAM domain

is located in the carboxyl-terminal tail of Eph receptors. It is a conserved region containing 60-70 amino acids, which regulates receptor dimerization and initiates downstream signal transduction (13,14). The postsynaptic density of the protein zona occludens PDZ-binding domain is critical for the assembly and localization of the Eph/ephrin complex (15).

*Ephrin structure.* Ephrin ligands are divided into two subfamilies based on sequence conservation and their respective affinities for Eph receptors; ephrin-A and ephrin-B. Ephrin-A ligands (ephrin-A1-A5) possess a GPI-anchor that attaches ephrin-A to the membrane at the carboxyl terminal. Ephrin-B ligands (ephrin-B1-B3) possess a single transmembrane domain and a highly conserved carboxyl terminal tail, containing five tyrosine phosphorylation sites and a carboxyl-terminal PDZ-domain binding motif that is the structural basis for downstream signal transduction (4,16).

*Eph/ephrin complex formation and bidirectional signaling.* A unique feature of the Eph/ephrin complex is that it initiates bidirectional signaling following its formation; the Eph receptor may act as a ligand and the ephrin ligand may act as a receptor (17). Forward and reverse signaling is involved in numerous physiological processes, including cell migration, axonal outgrowth, axonal pathfinding, topographic mapping, axon fasciculation and vascular formation in the developing nervous system (18,19).

The initiation of Eph/ephrin bidirectional signaling requires the formation of highly clustered Eph/ephrin complexes. Previous studies have demonstrated that recombinant soluble ephrin must be pretreated to form clusters and induce Eph receptor phosphorylation and downstream signaling (20,21). Soluble monomeric ephrins act as Eph receptor antagonists instead of Eph receptor agonists (22,23). Similarly, reverse signaling through ephrin ligand requires interactions with clustered Eph receptors (24,25). The blocking functions of soluble monomeric ephrin or Eph extracellular domain (ECD) may therefore provide a potential tool for the manipulation of bidirectional signaling (26).

Eph forward signaling induced by ephrin binding initiates downstream signal transduction following the autophosphorylation of two conserved tyrosine residues in the juxtamembrane region (27). Downstream pathways of Eph forward signaling have been studied extensively (15,17,27-36). Eph receptor-mediated forward signaling modulates the dynamic rearrangement of the cytoskeleton and is involved in cellular remodeling, serving a role in certain regenerative processes, including neurite outgrowth and cell migration (37). Previous studies have demonstrated that Eph receptors are highly specific to Rac, cell division control protein 42 (Cdc42), Rho and small GTPases, which are critical for the regulation of the actin cytoskeleton (37,38). Eph receptor forward signaling inhibits axonal regeneration in neurons by stimulating growth cone collapse through Rac and Cdc42 (37,39-41). In contrast, the blocking of Eph receptors stimulates the activation of downstream Rac and Cdc42, promoting axonal outgrowth (42). Therefore, EphA receptor signaling may also provide repulsive guidance for growing axons via the activation of Rho. It has been demonstrated that Rac and Cdc42 activation promote axonal outgrowth in the absence of Eph forward signaling (31,42,43).

Notably, EphB1/EphB2/EphB3 triple knockout mice had long, thin and immature neural spines compared with wild-type mice, suggesting that ephrin-B/EphB signaling promotes spine formation and maturation (44). Ephexin is a novel member of the diffuse B cell lymphoma-like family of guanine nucleotide exchange factors. It functions to link EphA4 receptors to Rho GTPases, which serve vital roles in axon guidance (31,43). It has been demonstrated that ephrin-A3 acts via EphA4 to suppress Wnt/ $\beta$ -catenin signaling to inhibit the neurogenic potential of retinal stem cells (45). Eph forward signaling may also be involved in the mitogen-activated protein kinase, phosphoinositide 3-kinase (PI3K) and Janus kinase/signal transducer and activator of transcription (STAT) pathways (46-48).

Ephrin signal conduction into its host cell is defined as reverse signaling. Previous studies have revealed that ephrin reverse signaling is involved in neural progenitor proliferation (49), axon guidance (50), neuronal migration (51) and synaptic plasticity (50). However, the intracellular signaling cascades that are initiated following ephrin activation remain unknown. Ephrin-As lack a cytoplasmic tail; however, they are capable of activating downstream Src family kinases (SFKs) and PI3K with the aid of co-receptors (52,53). It was demonstrated that associated transmembrane signaling partners, including topomyosin receptor kinase B and p75 neurotrophin receptor, may act as co-receptors for ephrin-As (54).

Ephrin-B ligands are composed of a single transmembrane region and a short, highly conserved cytoplasmic domain with a carboxy-terminal PDZ domain-binding motif. Together, these constitute the structural foundation required for reverse signaling (55). The activation of ephrin-B ligands leads to the recruitment of SFKs, which phosphorylate tyrosine residues located in the cytoplasmic domain. Previous research has revealed that Src-homology-2-domain-containing adaptor molecules, such as Grb4, are recruited and phosphorylated by ephrin-B, which further initiates downstream signaling and regulates cytoskeletal dynamics (56-60). The basophil-like protein tyrosine phosphatase is also recruited via its PDZ domain to the carboxy-terminal tail of ephrin-B, leading to its dephosphorylation and the inactivation of SFKs. This inactivation acts as a switch from phosphotyrosine-dependent to PDZ-domain-dependent signaling (61). PDZ regulation of G-protein signaling 3 may inhibit C-X-C chemokine receptor type 4-mediated chemoattraction by inhibiting the  $G\alpha\beta\gamma$ -protein complex, which further regulates the migration of endothelial cells and angiogenesis (62,63) (Fig. 1).

### 3. Eph/ephrin expression in the adult central nervous system (CNS)

Previous studies have assessed the role Eph in the developing CNS. The expression of Eph receptors and ephrin ligands changes markedly during CNS development (19). Ephs and ephrins continue to be expressed in the adult CNS and are distributed in most regions and types of cell (6). Various Eph receptors and ephrins continue to be highly expressed in adult brain regions that possess morphological and physiological plasticity, including the amygdala and hippocampus (64).

Previous studies have elucidated the diverse roles of Eph receptors and their ephrin ligands in the adult CNS. Eph receptors and their ligands serve primary roles in the regulation

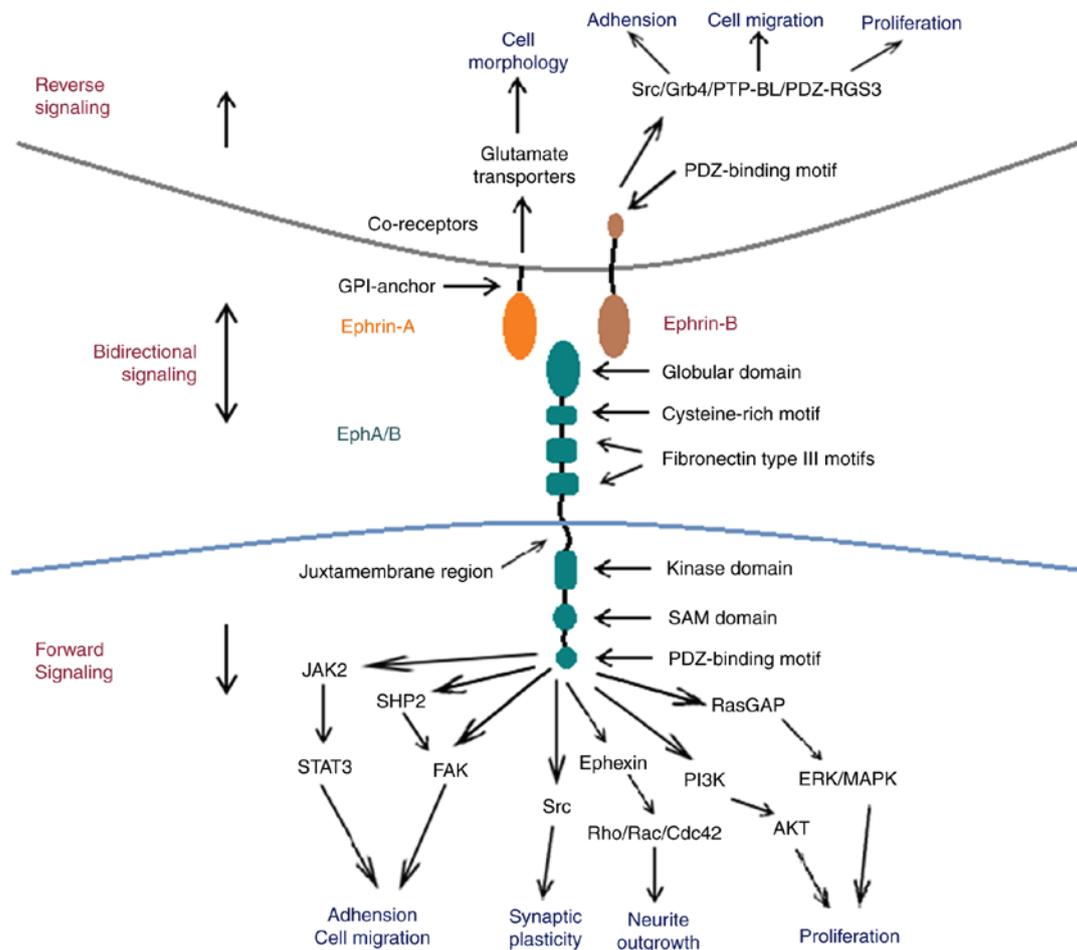


Figure 1. Schematic representation of Eph/ephrin structures and signaling pathways. Eph receptors are comprised of an ephrin-binding globular domain, a cysteine-rich region, two fibronectin type III domains, a juxtamembrane region, a kinase domain, a SAM domain and a PDZ-binding motif. Ephrin-As are attached to the cell membrane via a GPI-anchor, and ephrin-Bs are transmembrane proteins with a cytoplasmic tail and a terminal PDZ-binding motif. Activated Eph/ephrin bidirectional signaling initiates multiple different pathways involved in the regulation of synaptic plasticity, cell morphology, neurite outgrowth, cell migration, adhesion and proliferation. Eph, erythropoietin-producing human hepatocellular receptor; SAM, sterile-a-motif; PDZ, PSD95/Dlg/ZO1; GPI, glycosyl-phosphatidylinositol; RasGAP, Ras GTPase activating protein; ERK, extracellular signal-related kinase; MAPK, mitogen-activated protein kinase; PI3K, phosphoinositide 3-kinase; Cdc42, cell division control protein 42; FAK, focal adhesion kinase; JAK2, Janus kinase 2; STAT3, signal transducer and activator of transcription 3.

of synapse formation, function and plasticity (33,65,66), which is particularly important in the maintenance of hippocampal plasticity (67) and the processing of certain types of pain (68). Previous studies have demonstrated that the activation of EphA4 forward signaling mediates the retraction of dendritic spines and reduces their number and size by remodeling the actin cytoskeleton and modifying the properties of adhesion receptors (35,69,70). It has also been demonstrated that EphA4 blockade leads to significantly longer and overlapping dendritic spines (71). However, contrasting effects were observed in triple EphB (EphB1/EphB2/EphB3) knockout mice. A significant decrease in dendritic spine density and the formation of headless or small-headed spines were observed, suggesting that EphB forward signaling is responsible for dendritic spine formation and synaptic maturation (44,72). Previous studies have also compared Eph/ephrin knockout with wild-type mice and demonstrated that pre- and post-synaptic Eph/ephrins affect memory and learning by controlling synaptic formation (67,73,74). Eph/ephrins may recruit cell surface molecules, such as the N-methyl-D-aspartate receptor (NMDAR), via their PDZ domain (75,76). EphB2

forward signaling and ephrin-B3 reverse signaling also induces the generation of long-term potentiation (LTP) via NMDARs (33,50,77-80).

EphA4/ephrin-A3-mediated bidirectional signaling between neurons and astrocytes was implicated in the alteration of the  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor and spine morphology in the hippocampus (67). In addition, Eph4A forward signaling and glial ephrin-A3 reverse signaling regulates the astrocyte glutamate transporter and the plasticity of synapses within the hippocampus, respectively (81,82). Furthermore, ephrin-Bs/EphB participate in the processing of spinal cord pain via the NMDAR, PI3K and downstream signaling pathways (83-87).

Ephs and their ephrin ligands are also expressed in the subventricular zone (SVZ) of the lateral ventricle and subgranular zone (SGZ) of the dentate gyrus, where neural stem cells maintain neurogenesis throughout the lifetime of mammals (88). Eph/ephrin bidirectional signaling influences the proliferation and differentiation of neural precursor cells (NPCs) (89). Previous studies have demonstrated that EphB3/ephrin-B3 regulates the proliferation and differentiation of cells in the SVZ

and the rostral migratory stream (RMS) through altering the expression of p53 (90-93). In addition, EphA4 knockout mice exhibit decreased cell proliferation and differentiation disorder in the SVZ and RMS, resulting in a reduced number of neuroblasts (94). It has been demonstrated that EphB1/ephrin-B3 signaling also affects the proliferation and differentiation of NPCs in the SGZ of the dentate gyrus, highlighting a potential therapeutic target for neurodegenerative diseases and brain damage (7). In addition, the interactions between neural stem cells and blood vessels in the SVZ function to regulate quiescence and promote stemness. In particular, ephrin-B2 presented by vascular endothelial cells suppresses the proliferation and differentiation of stem cells through the activation of their respective Eph receptors and downstream notch signaling pathways. Ephrin-B2/Eph and notch signaling is suspended as stem cells detach from blood vessels to differentiate and divide (95).

Eph/ephrin signal transduction is involved in CNS angiogenesis. It has been demonstrated that EphA2 receptor blockade improves the formation of tight junctions between endothelial cells and promotes angiogenesis (96). In addition, an intracerebroventricular injection of ephrin-A1 may promote angiogenesis by stimulating EphA forward signaling (26). Small competitive Eph-ephrin antagonists have also been demonstrated to disrupt the interaction between EphA2 and ephrin-A1, blocking angiogenesis at low micromolar concentrations (97). A previous study also revealed that ephrin-A1 was involved in the modulation of the actin cytoskeleton, demonstrating its vital role in re-endothelialization (98).

#### **4. Expression and regulation of Eph receptors and ephrins in the adult CNS following injury**

Eph/ephrin signaling is also involved in sophisticated pathological processes following CNS injury. Eph receptors and ephrin ligands are upregulated following CNS injury and exhibit diverse changes depending on the location or time at which injuries occur (99-103). Certain types of cell behave differently following CNS damage: Neurons attempt to regenerate damaged connections; astrocytes and microglial cells proliferate, migrate and become activated to maintain homeostasis; and oligodendrocytes initiate remyelination (104). The alteration of Eph and ephrin expression under these situations may reveal the function of Eph/ephrin signaling in the damage response. Ephs and ephrins may regulate axon guidance in the developing CNS and so may serve a similar role during CNS regeneration (105). Eph receptors and their ephrin ligands are also expressed in mature cell types, including neurons and astrocytes. They may therefore exhibit different effects compared with those observed in CNS development, including the mediation of astrocytic gliosis, neural regeneration, vascular remodeling and neuroinflammation (106).

*Eph/ephrin signaling affects glial scar formation and glutamate homeostasis.* Eph receptors and their ephrin ligands may influence the structural and functional reorganization of the CNS during trauma. Ephs and ephrins may respond to CNS injury by promoting the formation of glial scars due to their inhibitory effect on axonal regeneration (105). Previous results have revealed that the sophisticated processes involving gliosis include glial reactivation, extracellular matrix alteration and

collagen deposition (107). Multi-cellular components including astrocytes, microglia, oligodendrocyte progenitors and fibroblasts participate in the formation of glial scars (108-110). Ephs and ephrins are expressed in many types of cells associated with gliosis and glial scars and affect their response to damage. Glial cells trigger gliosis in CNS injury as they are highly sensitive to damage (111). Gliosis is a process that begins with glial cell activation and proliferation, and is characterized by morphological and functional changes in astrocytes and microglia. Astrocytic activation results in cellular hypertrophy, proliferation and gliosis (112), which are observed in areas distal to the site of injury (113,114). However, the astro-glial response has positive and negative effects on neuronal cell recovery and degeneration. There are various benefits of glial scar formation, including the separation of the site of injury from surrounding normal tissues, thus reducing the spread of damage and filling of the lesion cavity (115-117). Glial scars help to reconstruct damaged brain areas and re-organize blood vessels following epithelial cell invasion into the scar tissue. Previous studies have demonstrated that glial scars also act as primary barriers to neural regeneration (35,118,119). There is mounting evidence that Eph/ephrin signaling is involved in glial scar formation in CNS disorders. It has been demonstrated in a model of spinal cord injury, that the development of glial scars and the exclusion of meningeal fibroblasts from the site of damage are a result of cell contact-mediated bidirectional signaling cascades, stimulated by the interaction of ephrin-B2 and EphB2 with reactive astrocytes and meningeal fibroblasts, respectively (103). Another previous study demonstrated that ephrin B2 (-/-) mice exhibited a reduction in astrogliosis and an accelerated regeneration of injured corticospinal axons, which resulted in the recovery of murine motor function following spinal cord injury (SCI) (105). It was also demonstrated that astrocytic gliosis and glial scars were greatly reduced in lesioned EphA4<sup>-/-</sup> spinal cords. EphA4<sup>-/-</sup> astrocytes also failed to respond to inflammatory cytokines, including interferon- $\gamma$  and leukemia inhibitory factor *in vitro* (35). In addition, neurons grown in wild-type astrocytes exhibited shorter neurites compared with neurons grown in EphA4<sup>-/-</sup> astrocytes (120). Previous studies have demonstrated that the use of EphA4 inhibitors moderately reduced astrocytic gliosis, promoted axonal regeneration and improved functional outcome following spinal cord hemisection in wild-type mice (35,121).

Glutamate is the primary excitatory neurotransmitter in the CNS; however, it is also a potential neurotoxin as excessive glutamate signaling may lead to excitotoxic cell death (122). The maintenance of extracellular glutamate homeostasis is a supportive function of astrocytes that occurs during brain injury, the function of which may be regulated by Eph/ephrin signaling. The use of clustered EphA4 was demonstrated to decrease the expression of astrocyte glutamate transporters and the glutamate uptake capacity of astrocytes via the activation of ephrin-A3 reverse signaling (123). These results indicated that EphA4-mediated ephrin-A3 reverse signaling is a vital mechanism for astrocytes to control glial glutamate transporters and prevent glutamate excitotoxicity under pathological conditions (123). A novel role of ephrin-B1 was determined in astrocyte-mediated synapse remodeling following traumatic brain injury (TBI). The upregulation of astrocytic ephrin-B1 following injury reduced the vesicular glutamate transporter 1

positive excitatory presynaptic innervation of CA1 neurons via STAT3-mediated signaling in astrocytes (124). Therefore, the regulation of ephrin-B1 signaling in astrocytes may provide novel therapeutic opportunities to aid glutamate homeostasis and functional recovery following TBI (124).

*Eph/ephrin signaling mediates neurogenesis and angiogenesis.* Endogenous NPCs are present in the SGZ of the dentate gyrus and in the rostral SVZ of the lateral ventricles in the mature CNS (125). NPC proliferation in the SVZ and SGZ is triggered under pathophysiological conditions. These neuroblasts may migrate to the lesion area and differentiate into neurons to replace those that are damaged (126,127).

Eph/ephrin bidirectional signaling influences the proliferation and differentiation of NPCs, affecting their response to CNS injury. EphB3/ephrin-B3 regulates the proliferation and differentiation of cells in the SVZ and the RMS by controlling p53 levels (90-93). Post-ischemic neurogenesis in ephrin-B3 (-/-) mice was strongly enhanced and associated with the caspase-3-dependent activation of STAT1 (128). EphB2 has been demonstrated to control the migration of dentate progenitor cells into the dorsal half of the developing dentate gyms (129). A previous study revealed that blockade of EphB2 enhanced neurogenesis in the SVZ and improved neurological function following cerebral cortical infarction in hypertensive rats (130). Neurons adapt their structure and function to microenvironmental changes by controlling neural plasticity. Previous studies have demonstrated that Eph/ephrin signaling exhibits an inhibitory effect on neurite outgrowth in CNS damage (131-133). For example, ephrin-A5 reverse signaling induces growth cone collapse and inhibits axonal regeneration by activating RhoA or dependent protein kinases (131). Ephrin-A5-mediated EphA4 forward signaling also triggers axonal growth cone collapse via the downstream Rac GTPase-activating protein  $\alpha$ 2-chimera-independent signaling pathway (132). The intervention of ephrin-A5/EphA4 communication may therefore serve a vital role in the suppression of neuron generation through the phosphorylated (p)-Akt and p-extracellular signal-related kinase (ERK) pathways (133). EphA4 targeting using miR-93 was demonstrated to promote neurite outgrowth in spinal cord injury in rats following a reduction in p-Ephexin and active RhoA levels (134).

Eph/ephrin bidirectional signaling regulates oligodendrocyte precursor cells (OPCs) and oligodendrocytes. Eph-ephrin interactions between axons and OPCs may control the distribution of OPCs in the optic axonal tracts and the cessation of their migration (135). It was revealed that ephrin-B3 is expressed in postnatal myelinating oligodendrocytes and acts as myelin-based inhibitor through a combined p75 neurotrophin receptor (136). A previous study demonstrated that EphB3 functions as a dependence receptor that mediates oligodendrocyte cell death following SCI, which further supports the development of ephrin-B3 based therapies to promote recovery (137).

It is now relatively well accepted that neurogenesis and angiogenesis are coupled processes. Eph receptors and their ephrin ligands are also involved in angiogenesis, which is critical for the remodeling of vasculature following CNS injury (138). EphA2 is an essential regulator of post-natal angiogenesis. The stimulation of ephrin-A1 induces the PI3K-dependent activation of Ras-related C3 botulinum toxin I

(Rac1) in wild-type endothelial cells, and EphA2-deficient cells fail to activate Rac1 upon stimulation. EphA2-deficient endothelial cells fail to undergo vascular assembly and migration in response to ephrin-A1 *in vitro* (139). The competitive small molecule UniPR129 acts as an Eph/ephrin antagonist and blocks angiogenesis at low concentrations *in vitro* (140). It has been suggested that increasing ephrin-B2 levels may promote vascular endothelial growth factor (VEGF)-induced VEGF receptor 2 endocytosis and the angiogenic function of endothelial cells (141). Previous studies have demonstrated that the ephrin-A5/EphA4 interaction mediates the ERK and Akt signaling pathways in pilocarpine-induced epilepsy, and that the intervention of ephrin/Eph interactions suppresses newborn neuron generation and microvessel remodeling in a mouse model of temporal lobe epilepsy (142,143).

*Eph/ephrin signaling and neuroinflammation.* Post-injury inflammation is implicated in most types of CNS injury. Neurodegeneration, trauma and ischemia stimulate an inflammatory response that causes microglial activation and circulating immune cell infiltration in the brain (144). Inflammation is generally considered to be beneficial for the clearance of debris formed by necrotic cells. However, severe inflammation causes cerebral swelling and vascular dysfunction, which exaggerates neuronal damage (144). Previous studies have indicated that Eph/ephrin proteins are involved in the inflammatory process following CNS injury. EphA2 and ephrin-A1 serve roles in the maintenance of endothelial junction integrity and cytoskeletal structure, potentially in response to the upregulation of inflammatory mediators, resulting in vascular leakage (145,146). EphA2 inactivation promotes the formation of tight junctions in the endothelial cells of the brain (96). There is also considerable interest in ephrin-B2/EphB4 signaling. Ephrin-B2 is a marker of arterial endothelial cells in the vasculature and EphB4, one of its cognate receptors, is predominantly expressed in the venous endothelium. Endothelial ephrin-B2 primarily functions via the VEGF receptor to mediate vascular responses during inflammation (147). Therefore, therapies that inhibit the function of ephrin-B2/EphB4 may suppress the inflammatory response following injury (148). EphB receptor inhibition using EphB1-fragment crystallizable reduced formalin-induced inflammation and chronic constrictive injury-induced neuropathic pain behaviors via the control of PI3K and PI3K crosstalk to ERK signaling (87). Ephrin-B/EphB signaling also serves a primary role in the regulation of inflammatory pain via NMDAR subunit NR2B and PKC $\gamma$  regulation (76,149).

## 5. Therapeutic implications in neurological disorders

The underlying mechanisms of Eph/ephrin signaling remain poorly understood. The key roles of Eph/ephrin signaling in the progression of a large range of neurological disorders suggest that Ephs and ephrins may be potential therapeutic targets. Previous studies have indicated that Eph/ephrin signaling may be a suitable therapeutic target for the treatment of neurological diseases.

*Alzheimer's disease (AD).* AD is the most common type of neurodegenerative disorder that manifests as a progressive decline in

cognitive function. It has been demonstrated that EphB2 and EphA4 are downregulated in AD (74). Soluble amyloid- $\beta$  ( $A\beta$ ) peptide oligomers are derived from amyloid precursor proteins (APPs) and are a major causative agent of synaptic impairment in AD. Previous studies have suggested that  $A\beta$  oligomers indirectly affect the NMDAR NR1 subunit and induce NMDAR depletion by forming a complex with EphB2 (150,151). In addition, increased EphB2 expression reverses deficits in NMDAR-dependent LTP and memory impairments in murine models of AD (152). EphA4 may also be a potential therapeutic target of AD. It has been demonstrated that EphA4 mediates the  $A\beta$ -induced impairment of synaptic plasticity; the depletion or blockade of postsynaptic EphA4 activity reverses synaptic deficits in murine models of AD. Rhy is a small-molecule inhibitor of EphA4 that rescues  $A\beta$ -induced impairments in neurotransmission and LTP in murine models of AD (153).

*Amyotrophic lateral sclerosis (ALS)*. ALS is a neurodegenerative disease that is caused by the progressive degeneration of the upper and lower motor neurons in the anterior horn of the spinal cord, brainstem and cerebral cortex (154). The ALS8 gene leads to the development of familial ALS and accounts for 10-15% of all ALS cases (155). The ALS8 protein vesicle-associated membrane protein-associated B (VAPB) is a ligand for EphA4. Mutations of VAPB may enhance EphA4/ephrin-A3 signaling and lead to the dysfunction of glial glutamate transporters, as observed in ALS (81,156). However, the specific role of EphA4 in the pathology of ALS requires further investigation (81,155).

## 6. Conclusions

The observations described in the present review provide evidence that Ephs and ephrins serve a vital role in determining the regenerative outcomes of CNS disorders. Signaling through Eph/ephrin complexes directly regulates neural regeneration by stimulating growth cone collapse, promoting glial scar formation, regulating homeostasis, reducing neurogenesis, inhibiting myelination and exaggerating inflammation together with injury-induced neuropathic pain. In addition, the interaction between Ephs and ephrin ligands is essential for angiogenesis. Therefore, the regulation of these molecules following CNS injury may serve as therapeutic targets for the treatment of various neurological diseases.

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