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Review

Eph/Ephrin Signaling in Injury and Inflammation

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The Eph/ephrin receptor–ligand system plays an important role in embryogenesis and adult life, principally by influencing cell behavior through signaling pathways, resulting in modification of the cell cytoskeleton and cell adhesion. There are 10 EphA receptors, and six EphB receptors, distinguished on sequence difference and binding preferences, that interact with the six glycosylphosphatidylinositol-linked ephrin-A ligands and the three transmembrane ephrin-B ligands, respectively. The Eph/ephrin proteins, originally described as developmental regulators that are expressed at low levels postembryonically, are re-expressed after injury to the optic nerve, spinal cord, and brain in fish, amphibians, rodents, and humans. In rodent spinal cord injury, the upregulation of EphA4 prevents recovery by inhibiting axons from crossing the injury site. Eph/ephrin proteins may be partly responsible for the phenotypic changes to the vascular endothelium in inflammation, which allows fluid and inflammatory cells to pass from the vascular space into the interstitial tissues. Specifically, EphA2/ephrin-A1 signaling in the lung may be responsible for pulmonary inflammation in acute lung injury. A role in T-cell maturation and chronic inflammation (heart failure, inflammatory bowel disease, and rheumatoid arthritis) is also reported. Although there remains much to learn about Eph/ephrin signaling in human disease, and specifically in injury and inflammation, this area of research raises the exciting prospect that novel therapies will be developed that precisely target these pathways. *(Am J Pathol 2012, 181:1493–1503; http://dx.doi.org/10.1016/j.ajpath.2012.06.043)*

Tissue injury or damage is followed by an orderly process that includes the induction of the following: i) an acute inflammatory process, ii) regeneration of cells, iii) migration and proliferation of both parenchymal and connective tissue cells, and iv) tissue remodeling.[1] There has been a parallel drawn between tissue repair and embryo morphogenesis,[2] and the tissue repair gene profile is similar to that expressed during embryological development.[3] This has been confirmed by differential gene expression studies in experimental wounds in model organisms (eg, *Drosophila* and *Caenorhabditis elegans*),[2] *in vitro* gastrointestinal damage,[4] and spinal cord injury.[5] Immediately after tissue injury, there is an acute inflammatory response.[1] Inflammation is characterized by the movement of inflammatory cells to the site of infection or tissue injury.[6] The decreased adhesion between vascular endothelial cells allows the passage of plasma water and proteins into the interstitial space, and vascular endothelial cells become more adhesive to inflammatory cells, which leave the circulation and enter the injured tissue.[7]

The Eph/ephrin receptor–ligand family (which subsequently will be referred to jointly as the Eph/ephrin proteins) is a group of cell surface proteins, and emerging evidence suggests that these proteins play an important role in injury[8] (in particular, wound healing, ischemia-reperfusion injury, and spinal cord injury) and inflammation.[9] The direct implication of the Eph/ephrin proteins in inflammation remains relatively obscure; however, there is evidence to support their role in modulating vascular

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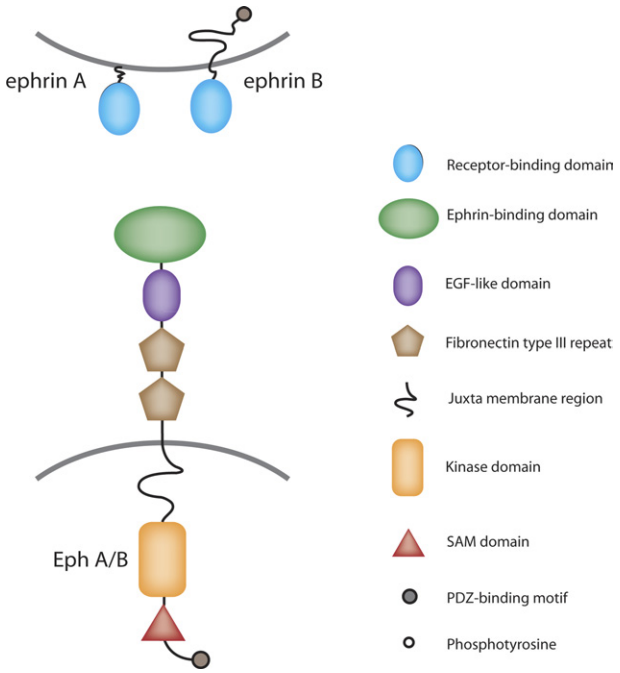

Figure 1. The structure of the Eph receptors and their ephrin ligands. The EphA and EphB receptors have a conserved domain structure. The ephrin-A ligands are attached to the cell membrane by a glycosylphosphatidylinositol anchor. The ephrin-B ligands are transmembrane proteins. PDZ, post-synaptic density protein-95; SAM, sterile alpha motif. Adopted and modified from Murai and Pasquale,[18] with the permission of The Company of Biologists Ltd (copyright 2003).

permeability during inflammation.[10,11] This review will summarize the extensive work on Eph/ephrin proteins in injury and present recent evidence to support the hypothesis that Eph/ephrin proteins play an essential role in inflammation mediated through the vascular endothelium. Finally, the review will anticipate future research that may lead to novel pharmacological approaches for treating injury and inflammation.

The Eph/Ephrin Proteins

Overview

The Eph receptor tyrosine kinases and their ephrin ligands are cell surface molecules with a wide range of biological functions that influence cell behavior during both embryogenesis and adult life.[12] These functions include roles in the following: i) the direction of cell positioning and migration; ii) axon guidance during development; iii) control of tissue morphogenesis and patterning; iv) defining tissue boundary formation during somatogenesis; v) the development of the vascular system; vi) neural plasticity; vii) tumor invasion and metastasis; viii) immune function, hematopoiesis, and blood clotting; ix) the biological characteristics of stem cells; and x) tissue repair and maintenance.[12]

The Eph/ephrin proteins principally modify cytoskeletal organization and cell–cell and cell–substrate adhesion.[13] Cytoskeletal modification regulates the dynamics of cellular protrusions, affects cell-cell adhesion and attachment to the extracellular matrix, triggers cell segregation, and modulates migration.[14] Principle signaling cascades initiated by Eph/ephrin interactions converge on cytoskeletal targets, such as integrins and small Rho family GTPases, although emerging evidence reveals additional direct roles in modulating viability and proliferation, in particular stem and progenitor cells.[15] This is in contrast to other receptor tyrosine kinases, which were first identified as oncogenes, because they activated signaling pathways that target gene transcription and regulate cell proliferation and/or differentiation.[16]

Eph Nomenclature

There are 10 EphA receptors, EphA1–EphA10 (pronounced eff-A), and six EphB receptors, EphB1–EphB4, and EphB6 in vertebrates and an additional EphB receptor, EphB5, which exists in chickens.[17] The ligands of the Eph receptors are known as ephrins (pronounced ef-frins), an abbreviation derived from Eph family receptor-interacting proteins. The initial distinction between EphA and EphB receptors was based on sequence differences within the extracellular ligand binding domain, but also corresponds to the binding preferences for the six glycosylphosphatidylinositol-linked ephrin-A ligands and the three transmembrane ephrin-B ligands, respectively[17,18] (Figure 1). The Eph/ephrin receptor–ligand interactions are promiscuous within each A or B class, with variations in binding affinities, although EphB4 only binds ephrin-B2.[19] There are also exceptions in the binding preferences between A and B classes, because EphA4 binds to ephrin-B ligands (ephrin-B2–ephrin-B3),[19] and EphB2

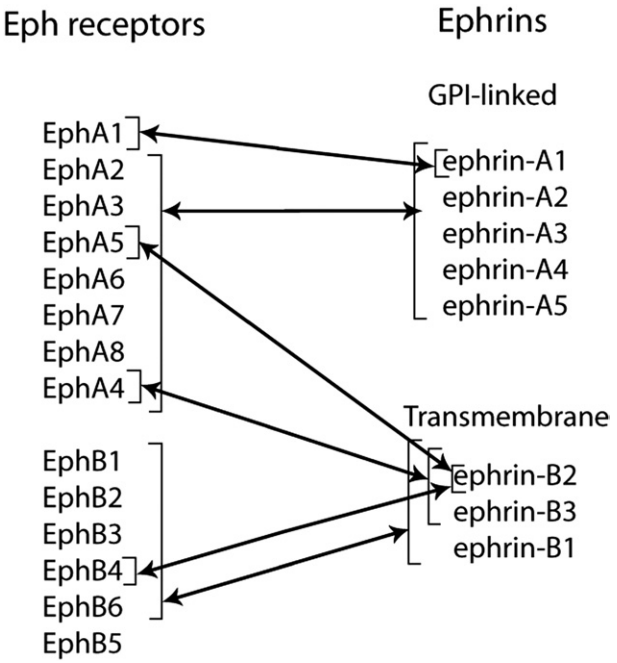

Figure 2. Eph receptor and ephrin ligand binding preferences. **Double-sided arrows**, interactions between the specific Eph receptors and the respective interacting ephrins. GPI, glycosylphosphatidylinositol. Adopted and modified from Wilkinson,[21] with the permission of Elsevier Inc. (2012).

binds to most A-type ephrins, in particular ephrin-A5[20,21] (Figure 2).

Eph/Ephrin Receptor–Ligand Interaction

The interaction of an ephrin ligand with its cognate Eph receptor involves both forward (Eph-mediated) and/or reverse (ephrin-mediated) signaling, which can result in either cell–cell adhesion or de-adhesion.[12,22] The interaction between Eph receptors and ephrin ligands occurs between receptor–ligand pairs expressed on two opposing cells (*trans*),[23] whereas the relevance of reported interactions on the same cell (*cis*) is disputed.[16,24,25] Some reports imply that *cis* binding does not lead to active signaling but interferes with receptor activation by the ephrin-A presented on the surrounding cells.[25] The downstream effects of Eph/ephrin protein signaling are ultimately mediated through changes in cytoskeletal proteins (responsible for cell shape and motility) and cell surface receptors for extracellular matrix proteins (responsible for cell adhesion).[26] The specificity of the cellular response for both forward and reverse signaling events and the final outcome are determined by the type and abundance of Ephs and ephrins on the interacting cell surface and the sum of the Eph receptors on a cell surface, which are competing for available ephrin targets on the interacting cells.[27]

Molecular Mechanisms of Eph/Ephrin Protein Signaling

The interaction of an Eph receptor with its ephrin ligand results in the formation of an Eph/ephrin tetramer and juxtaposition of two catalytically autoinhibited Eph receptor monomers in a ring-like complex, juxtaposing two Ephs for potential cross phosphorylation.[28] Once formed, the heterotetrameric Eph/ephrin complex promotes ephrin-independent Eph/Eph interactions between neighboring Ephs that promote the assembly of higher-order oligomers, which are required for Eph phosphorylation and activation of downstream signaling.[27] Recent findings suggest that this oligomerization via Eph/Eph protein interfaces in the globular (ephrin-binding) and cysteine-rich domains occurs independent of Eph signaling capacity and subclass specificity, so that the composition of the signaling cluster reflects the expression profile of Ephs on a cell surface.[28] Once activated, tyrosine phosphorylation–induced changes in the conformations of Eph/ephrin cytoplasmic domains, in particular release of the Eph kinase domain from the inhibitory interaction with the juxtamembrane domain, allow the specific binding of Src homology (domains) 2 and 3, phosphotyrosine domain, or post-synaptic density protein-95 domain–containing downstream signaling molecules and activate corresponding signaling pathways[22,27] (Figure 3).

Eph/ephrin protein signaling results in the activation of several cytoplasmic downstream signaling pathways, including the following: i) Src family kinases, ii) mitogen-activated protein kinase, iii) p-21 activated kinase, iv) post-synaptic density protein-95–dependent pathways,

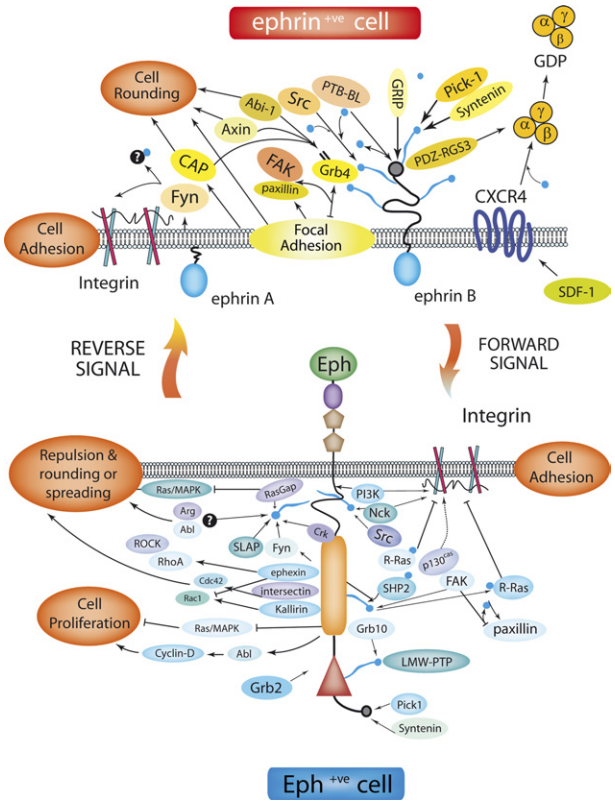

Figure 3. Eph/ephrin signaling pathways. Structurally and functionally significant domains within Eph and ephrin proteins and signaling pathways of activated Eph receptors and ephrins that modulate cell shape and attachment. **Arrows**, positive outcome; **blue circle**, phosphorylation; **flat end lines**, inhibition. Adopted and modified from Himanen et al,[20] with the permission of Taylor and Francis Group Ltd (copyright 2010).

v) chemokine pathways, vi) heterotrimeric G-protein pathways, and vii) integrin-mediated pathways.[12,13,26] The activity of each of these pathways is dependent on the activity of the Rho family GTPases, including RhoA, Ras-related C3 botulinum toxin substrate (Rac) 1, and cell division control protein 42 (Cdc42); the Rac–GTPase-activating protein (GAP); α-chimerin[13]; and a variety of guanine nucleotide exchange factors, including ephexins.[29] The guanine nucleotide exchange factors, which mediate the downstream signaling, are specific for the cell type[29]; this may, in part, account for the different cell responses, either cell adhesion or cell–cell repulsion (de-adhesion), after Eph/ephrin receptor activation.[23] The attenuation and termination of Eph/ephrin protein signaling involves proteolytic cleavage by a disintegrin and metalloproteinase 10[30] and γ-secretase,[26] receptor-mediated endocytosis,[26] and tyrosine phosphatase activity.[31]

The Role of the Eph/Ephrin Proteins in Tissue Injury

Wound Healing

The Eph/ephrin proteins play a role in tissue repair and maintenance.[32] The pathological features that follow traumatic injury and tissue damage include formation of a

platelet plug and coagulation of extravasated blood, which initiates a complex signaling cascade to recruit inflammatory cells, stimulate fibroblast and epithelial cell proliferation, direct cell migration, and induce angiogenesis to restore tissue integrity.[1,33] The classic histological observation that many of the features of normal wound healing are similar to the tumor microenvironment suggested that the tumor stroma is normal wound healing gone awry.[34] Fibroblasts exposed to serum express many of the genes involved in wound healing.[34] The fibroblast serum response was investigated with a cDNA microarray genome-wide survey and confirmed a gene expression signature similar to metastatic cancer.[34] However, although this approach of measuring gene expression has thus far not revealed Eph/ephrin proteins as candidates at the time points considered,[34] the Eph/ephrin proteins are involved in angiogenesis[26] and cell migration,[13] both critical aspects of wound healing.[1,6,33]

Ischemia-Reperfusion Injury

Tissue injury can also result from vascular disease in which the blood and nutrient supply is interrupted with severe consequences to major organs (eg, acute myocardial infarction and cerebrovascular stroke).[6] The ischemic damage is then followed by reperfusion injury when blood flow is restored, either as part of the natural history of the disease or as a result of therapeutic measures.[35] The ischemia-reperfusion injury is characterized by the following: i) an inflammatory response regulated by the pro-inflammatory cytokines, tumor necrosis factor (TNF)-α, IL-1, and IL-6; ii) up-regulation of endothelial adhesion molecules; and iii) recruitment of inflammatory cells to the damaged tissue.[35]

In both an *in vivo* and *in vitro* mouse model of renal ischemia-reperfusion injury, EphA2 was up-regulated through an Src kinase–dependent pathway.[36] A mouse skin flap model was used to determine the response of Eph/ephrin proteins to hypoxia. Partial cutaneous oxygen tension and tissue lactate/pyruvate measurements monitored by microdialysis confirmed tissue hypoxia, and quantitative PCR confirmed induction of hypoxia-inducible factor-1α and vascular endothelial growth factor (VEGF).[37] The expression levels of EphB4, ephrin-B2, EphA2, and ephrin-A1 were up-regulated in hypoxic skin, and the temporal expression pattern was determined, which supports the hypothesis that Eph/ephrin proteins are involved in revascularization after hypoxic injury.[37] Also, hypoxia-inducible factor-2α (but not hypoxia-inducible factor-1α) binds the hypoxia response element in the ephrin-A1 promoter and plays a role in tumor vascularization by inducing ephrin-A1 expression.[38] Furthermore, ephrin-B2 is required during angiogenesis and expressed specifically in arteries, which have a higher oxygen tension than veins. In fact, chromatin immunoprecipitation, mutagenesis, and small-interfering RNA knockdown experiments indicate that hypoxia drives arterial differentiation by increasing ephrin-B2 expression in endothelial cells through stimulating protein 1 activation.[39]

Optic Nerve and Spinal Cord Injury in Lower Vertebrates

The function of the Eph/ephrin proteins was first characterized in axon guidance.[17] Reciprocal gradients of Eph/ephrins were responsible for the precise projection of the retinal ganglion cells onto the optic tectum/superior colliculus.[40] In fish and amphibians, damage to the optic nerve or spinal cord is followed by infiltration of microglial cells and macrophages and subsequent axon regrowth and functional recovery.[41] In contrast, at the injury site in mammals, there is expression of chondroitin sulfate proteoglycan (lecticans and neuroglycan 2) in the extracellular matrix and inhibitory factors, including axon guidance molecules (semaphorins, ephrins, and netrins) and prototypic myelin inhibitors (Nogo, myelin-associated glycoprotein, and oligodendrocyte myelin glycoprotein), that actively inhibit axonal regeneration, resulting in poor functional recovery.[42] In fish, the neurons that undergo successful axonal regeneration have a similar, but not identical, molecular profile to neurons in the embryonic state.[43] In adult goldfish with optic nerve injury, immunohistochemical (IHC) studies indicated that there was transient up-regulation of EphA3 and EphA5 in the retinal ganglion cells (RGCs), coincident with up-regulated tectal ephrin-A2 expression, both of which were required for restoration of the normal retinotectal topographic map.[44]

Optic Nerve and Spinal Cord Injury in Rodents

In mice with optic nerve de-afferentation, the graded expression patterns of ephrin-A2 and ephrin-A5 in the superior colliculus were similar to those found during development.[45] In rat optic nerve injury, there was up-regulation of ephrin-A2 in the superior colliculus and EphA5 in the retina.[46] A strain of mutant mice that expressed the yellow fluorescent protein in a small, fixed proportion of RGC axons was bred with EphB3-null mice.[47] After optic nerve crush injury, macrophages expressing EphB3 accumulated at the injury site, and ephrin-B3 was expressed on RGC axons at the injury site.[47] In mice with reduced EphB3 function, there was decreased axon sprouting after optic nerve crush injury.[47] This suggests a role for EphB3-expressing macrophages interacting with ephrin-B3–expressing RGC axons in the remodeling events that follow optic nerve injury.[47]

Spinal cord injury in rats resulted in a marked increase in EphB3 mRNA at day 7 after injury, and was confirmed by immunolocalization of EphB3 expression in white matter astrocytes and gray matter neurons.[48] In another study using semiquantitative PCR of the injured adult rat spinal cord, EphA3, EphA4, and EphA7 mRNAs were up-regulated. Furthermore, EphA3, EphA4, EphA6, and EphA8 immunoreactivity was increased in the ventrolateral white matter. The EphA receptor expression localized in the white matter to glial cells, both astrocytes and oligodendrocytes, and localized to neurons in the gray matter. The expression of EphA3 mRNA and protein after spinal cord injury was elevated from day 2 to day 28, and EphA3 immunoreactivity was observed in reactive astro-

cytes.[49] However, in a contusive model of rat spinal cord injury, ephrin-A1 was the only ephrin-A ligand up-regulated.[50] EphB3 expression detected by *in situ* hybridization was up-regulated in rats subjected to complete thoracic spinal cord transection and was confirmed by IHC.[51] IHC data suggested that ephrin-B2 was expressed on reactive central nervous system astrocytes and that EphB2 was present on fibroblasts invading the injury site from the adjacent meninges.[52]

After spinal cord hemisection, EphA4 was up-regulated in wild-type mice on astrocytes associated with the glial scar at the injury site, whereas EphA4-null mice showed markedly reduced astrocytic gliosis and scar formation.[53] The EphA4-null mice exhibited axonal regeneration, characterized by axons growing across the injury site, associated with significant functional recovery 1 to 3 months after the injury.[53] EphA4 up-regulation after spinal cord contusion injury in rats was blocked by infusing EphA4 antisense oligonucleotides; however, although this did not result in enhanced locomotor recovery, it did improve chronic pain scores.[54] In nonhuman primates, cortical injury resulted in up-regulation of EphA4 on reactive astrocytes at the lesion.[55] A more complete understanding of the molecular basis of recovering axons in the fish, amphibian, and rodent central nervous systems will provide valuable insight into potential therapeutic advances after brain and spinal cord injury in humans.[56]

Signaling Mechanisms in Spinal Cord Injury

The precise downstream intracellular signaling mechanisms that mediate the inhibitory effect of ephrins after spinal cord injury remain unknown. However, in the days after contusive spinal cord injury in rats, Western blot analysis studies identified an increased expression profile of the Rho guanine exchange factor, ephexin, in reactive astrocytes, activated macrophages, and neurons at the lesion site, which colocalized with EphA3, EphA4, and EphA7.[57] *In vitro* studies determined that up-regulation of VAV-2 in Schwann cells mediated the inhibitory signal.[58] The interactions of EphB receptors with ephrin-B ligands modulate spinal cord synaptic efficiency in an N-methyl-D-aspartate receptor–dependent manner and contribute to neuropathic and inflammatory pain states mediated via a mitogen-activated protein kinase–dependent mechanism.[59]

Adult Brain Disorders

The Eph/ephrin proteins are important in brain development and synapse function in the adult brain and have been implicated in brain disorders.[44] In this regard, single-nucleotide polymorphism and haplotype analyses suggest that the *EFBN2* (ephrin-B2) locus is associated with schizophrenia in the Han Chinese population.[60] Furthermore, the Eph protein expression profiles in both active and inactive central nervous system lesions of multiple sclerosis, normal adjacent white matter, and control tissues have been characterized by IHC.[61] Inflammatory cells in active multiple sclerosis lesions expressed ephrin-A1 to A4 and EphA1, A3, A4, A6, A7, and not EphA2, A5, and A8. In axons adjacent to active multiple sclerosis lesions, EphA3, A4, and A7 and ephrin-A1 expression was increased.[61]

The Role of the Eph/Ephrin Proteins in Inflammation

The Development of the Vascular Endothelium

The normal development of the cardiovascular and lymphatic system requires the coordinated function of several important transcription factors, receptor–ligand pairs, growth factors, and guidance molecules.[62] These molecules include the Eph/ephrin proteins, VEGF and receptors 1 and 2, angiopoietins (Ang-1 and Ang-2) and their receptors (Tie-1 and Tie-2), netrins, slits and their receptors (Robo), semaphorins and plexins (the receptors for the semaphorins), and neuropilins.[63] The expression of ephrin-B2 and its receptor, EphB4, in a complementary pattern on embryonic arteries and veins, respectively, suggested a reciprocal interaction in the vascular remodeling process.[63]

The Vascular Endothelium in Inflammation

The initial evidence for the role of Eph/ephrin proteins in vascular biology was the identification of ephrin-A1 (previously B61) as a TNF-α–responsive gene in endothelial cells highlighting a potential role in inflammatory responses.[64] The vascular endothelium controls the passage of fluid, proteins, and inflammatory cells from the blood into the interstitium via the paracellular spaces between endothelial cells.[65] The endothelial cell–cell junctional structures, which include the gap, adherens, and tight junctions (zona occludens), play an important role in determining and regulating this endothelial barrier function.[65] The endothelial cell–cell junctional structures are a complex of transmembrane proteins, and barrier function is influenced by several external factors acting through signaling pathways that regulate the paracellular space.[65] The gap junctions facilitate the movement of ions and second messengers between adjacent endothelial cells.[66] The adherens junctions are particularly important in the post-capillary venule, which also expresses the receptors for inflammatory mediators, including TNF-α, IL-1β, and VEGF.[67] The predominant structural protein of the adherens junction is VE-cadherin, which interacts with the p120 catenin and β-catenin proteins.[65] The tight junctions (zona occludens-1) are located at the most apical part of the cell membrane, and the claudins, occludins, and JAM-A are major constituent proteins (Figure 4).[65]

Furthermore, the junctional structures are linked to the actin and myosin filament cytoskeleton of the endothelial cell.[65] The cortical actin filaments are critical components of the cellular cytoskeleton and interact with myosin filaments through myosin light chain kinase, resulting in changes to endothelial cell shape.[72] The distortion of endothelial cell shape allows the development of gaps in the monolayer, permitting the passage of fluid, proteins,

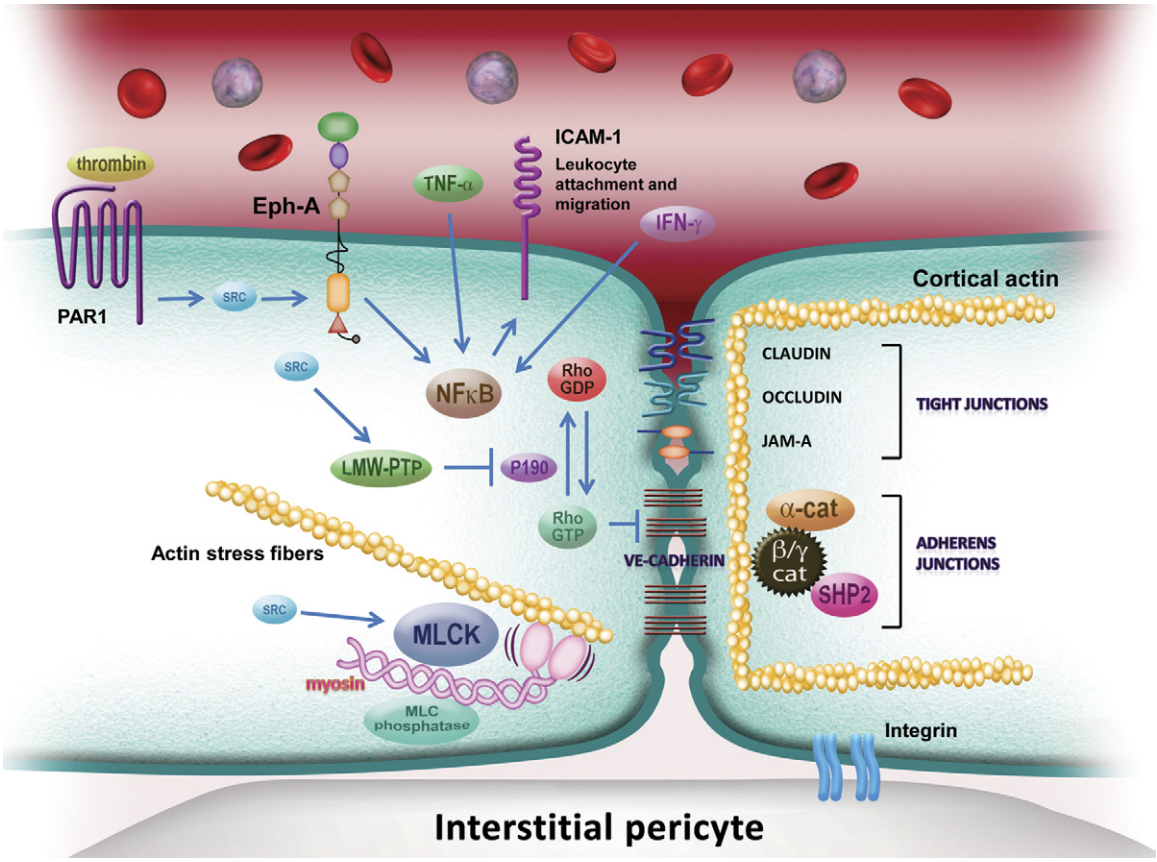

Figure 4. Mechanism of EphA2 signaling in the endothelium. The passage of fluid and inflammatory cells across the endothelium is regulated by both the shape of the endothelial cell and the permeability of the endothelial gap junctions.[68] The actinomyosin contractile elements that control cell shape are regulated by signaling pathways acting through the myosin light chain kinase (MLCK).[69] Thrombin binding to the proteinase-activated receptor-1 increases Src kinase activity and influences cell shape through MLCK.[70] EphA2 signaling increases recruitment of both Src kinase and low-molecular-weight phosphotyrosine phosphatase.[70] Increased low-molecular-weight phosphotyrosine phosphatase dephosphorylates the p190 RhoGAP that inhibits p190 Rho-GAP activity and up-regulates Rho-GTP, which destabilizes adherens junctions.[71] The inflammatory mediators, TNF-α and interferon (IFN)-γ, up-regulate NF-κB, which increases intercellular adhesion molecule 1 expression, facilitating leukocyte migration and attachment.[70] Furthermore, NF-κB increases MLCK activity, thus altering endothelial cell shape.[69] Ephrin-A1, the ligand for EphA2, is up-regulated by TNF-α,[64] and EphA2 up-regulates NF-κB.[70] Thus, EphA2 may have a central role in endothelial cell permeability in inflammation. SHP, Src homology region 2 domain–containing phosphatase.

and inflammatory cells from the blood into the interstitial tissues.[72] The actin filaments are tethered to membrane proteins, including VE-cadherin, and are dynamically regulated by the Rho family guanosine triphosphatases (Rho-GTPases), specifically RhoA, Rac1, and Cdc42, which are known targets of Eph/ephrin signaling. In general, Rac1 and Cdc42 activation stabilizes actin, whereas RhoA activation, in response to inflammatory stimuli, including thrombin and VEGF, disrupts the actin cytoskeleton[73] (Figure 4).

The Role of Specific Eph/Ephrin Proteins in Inflammation

The up-regulation of various Eph/ephrin proteins in response to the pro-inflammatory cytokines suggests a role in inflammation.[9] Rats administered lipopolysaccharide (LPS) respond with a biphasic or polyphasic (phase 1 to 3) fever, dependent on dose.[74] When rats were administered LPS, there was altered regulation of several Eph/ephrin proteins in the LPS processing organs (liver and lung) determined by differential mRNA display and RT-PCR.[74] There was increased EphA2 expression in the hypothalamus, with no change in ephrin-A1; however, there was a counterchange in corresponding receptor–ligand pair expression in the liver and lung.[74] There was a biphasic change in ephrin-A1 expression with early (phase 2) down-regulation (threefold) and up-regulation of EphA2 (16-fold) in the liver (similar changes in the lung), followed by later (phase 3) up-regulation (fourfold) of ephrin-A1 and normalization of EphA2.[74] Furthermore, there was up-regulation of ephrin-B2 and down-regulation of EphB3 (liver and similar in lung), with up-regulation of ephrin-A1/ephrin-A3 and down-regulation of EphA1/EphA3 (EphA3 was 21-fold underexpressed in the lung during phase 3), and these findings were confirmed by immunoblotting.[74]

The vasculature is central to the pathogenesis of inflammation, and the vascular endothelium plays a key role in orchestrating the response to injury or infection.[6] Changes in the phenotype of the endothelium allow the passage of fluid into the interstitium and enable inflammatory cells to localize and migrate into an injured or infected tissue.[6] The up-regulation of ephrin-A1 by

TNF-α, IL-1β, and LPS in the endothelium is mediated by a p38 mitogen-activated protein kinase and a stress-activated protein kinase/c-Jun NH2-terminal kinase–dependent mechanism.[75] Ephrin-A1 is chemoattractive for migrating endothelial cells *in vitro*, induces tubule formation in assays with human umbilical vein endothelial cells, and has previously induced sprouting blood vessels in a corneal pocket assay.[76]

EphA2/Ephrin-A1 Signaling in Vascular Endothelial Injury

Both EphA2 and ephrin-A1 are expressed in distal normal lung tissue, and *in vitro* studies of pulmonary vascular endothelial cells determined that ephrin-A1 increases monolayer permeability with evidence of tight and adherens junction disruption.[10] Intravenous injection of ephrin-A1 in rats induces leakage of labeled albumin, with histological evidence of endothelial disruption, and the EphA2 receptor was markedly up-regulated in the lungs of hypoxic infected rats.[10] Furthermore, when rats were exposed to viral infection, EphA2 was up-regulated by an endothelin-dependent effect in lung endothelial cells, and when EphA2 signaling was blocked with a soluble ligand competitor (ephrin-A1-Fc), there was markedly reduced extravasation of albumin and reduced lung edema formation.[11] The pattern of distribution of vascular EphA2 receptors in the lung is predominantly in the alveolar microvasculature, indicating localization to the capillary bed as the source of fluid and protein extravasation.[11] Surprisingly, EphA2-deficient mice treated with *Mycoplasma pulmonis* infection or sensitized to ovalbumin to cause airway inflammation displayed increased cytokine production and greater leukocyte infiltration at the site of inflammation,[77] in contrast with a bleomycin model of lung injury, in which EphA2 knockout mice were protected from lung injury.[78]

EphA2/Ephrin-A1 Signaling in Retinovascular Disease

In retinopathy of prematurity, diabetic retinopathy, neovascular glaucoma, and age-related macular degeneration, vision loss results from abnormal retinal angiogenesis.[79] The VEGF-induced angiogenic responses of cultured retinal endothelial cells are inhibited by EphA2-Fc.[79] In a rat model of retinopathy of prematurity, an intraocular injection of a soluble EphA2-Fc receptor resulted in a significant reduction in abnormal retinal revascularization without affecting normal retinal vessels.[79] In a mouse model of proliferative retinopathy, an intraocular injection of ephrin-A1-Fc suppressed ischemic retinal revascularization in a dose-dependent manner by inhibiting VEGF-induced angiogenesis and vasopermeability.[80]

Eph/Ephrin Signaling and Inflammatory Cells

The regulation and maturation of hematopoietic stem cells are complex and partly modulated by EphA/ephrin-A interactions.[81] The migration of T lymphocytes between the peripheral blood and organized lymphoid tissue is tightly regulated by an array of chemokines, and the migration of T cells to inflammatory sites is also chemokine dependent.[82] Similarly, the organization of the thymus and trafficking and maturation of T lymphocytes within thymic microenvironments is, in part, regulated by Eph/ephrin protein interactions, with the balance between Eph and ephrin-B signaling important for T-cell development, which is under the influence of thymic epithelial cells.[82] The blockade of Eph/ephrin signaling with EphA-Fc fusion proteins reduces $CD4^+CD8^+$ thymocytes. EphA4 knockout mice have a block in T-cell maturation because of changes in the nonlymphoid thymic microenvironment. Ephrin-B1 is critical for T-cell development, whereas EphB6 overexpression results in breakdown of the thymic cortex-medulla limits.[82] The chemokine response of T cells can be modulated by costimulation with ephrin-A and ephrin-B.[83] In chronic lymphocytic leukemia, EphA2/ephrin-A4 interactions mediate trafficking of malignant B lymphocytes into tissues through the high endothelial venules.[84]

Recently, both ephrin-A2 and ephrin-B2 expression has been documented on human polymorphonuclear neutrophils, and gene microarray confirmed ephrin-A2 and ephrin-B2 expression in polymorphonuclear neutrophil–induced inflammation-mediated angiogenesis in a CD18-deficient mouse model.[85] However, the precise role of Eph/ephrins in human polymorphonuclear neutrophils remains obscure. Peripheral blood leukocytes stimulated with TNF-α led to an increased expression of ephrin-B2, which has the potential to activate the endothelium in inflammation.[86]

Eph/Ephrin Signaling and Chronic Inflammation

There is evidence for the involvement of Eph/ephrin proteins in a range of chronic inflammatory diseases. The levels of circulating cytokines, TNF-α and IL-1β, are elevated in chronic heart failure, and differential display identified EphA3 as a cytokine-responsive gene in cultured rat cardiomyocytes; however, a link between EphA3 and cardiac failure has not been determined.[87] The Eph/ephrin proteins are expressed during small intestine development.[88] EphA2 (formerly Eck) and ephrin-A1 (formerly B61) have been described in the maintenance of the intestinal barrier, and EphB/ephrin-B expression gradients direct intestinal epithelial cell positioning within the crypts.[89] Real-time PCR and cDNA microarray analysis determined that EphA2, ephrin-A1, EphB2, and ephrin-B1/B2 expression was up-regulated in the intestinal epithelial cells of mucosal lesions in patients with inflammatory bowel disease.[4] EphA2, ephrin-A1, EphB2, and ephrin-B1/2 had increased expression in the intestinal cells of patients with Crohn's disease.[90] Stimulation of the ephrin-B2 reverse signaling pathway induced the expression of wound healing–associated genes in an intestinal epithelial cell line-6.[90] Stimulation of ephrin-B1/2 with EphB1-Fc in intestinal epithelial cells induced pro-inflammatory genes (cyclooxygenase-2 and monocyte chemotactic protein-1) and genes involved in

wound healing (FAK and ERK 1/2 mitogen-activated protein kinase pathway), resulting in faster wound healing.[4]

Ephrin-B1 expression was significantly increased in patients with rheumatoid arthritis in the synovial fibroblast cells and invading CD3-positive lymphocytes compared with patients with osteoarthritis.[91] An increase in ephrin-B1 expression was also seen in peripheral blood lymphocytes of patients with rheumatoid arthritis compared with healthy people.[91] In an animal model of rheumatoid arthritis, animals treated with an ephrin-B1-Fc fusion protein that activates the EphB1 receptor resulted in an increase in TNF-α and IL-6 production and increased the number of peripheral blood lymphocytes migrating into the joint.[91] Higher levels of ephrin-B1 expression may be associated with increased inflammation in rheumatoid arthritis.[91] In human bone samples from patients with osteoarthritis, the EphB4 receptor was up-regulated.[92] In subchondral bone tissue cultures from patients with osteoarthritis, ephrin-B2 and its receptor, EphB4, inhibited bone resorption factors.[92] The activation of EphB4 with ephrin-2B resulted in decreased IL-1β, IL-6, and matrix metalloproteinase (1, 9, and 13) production.[92]

The Mechanism of Eph/Ephrin Receptor–Ligand Signaling and Vascular Leak

The principal effect of Eph/ephrin receptor–ligand interaction is cell repulsion or de-adhesion mediated through a complex signaling cascade converging on a final common pathway, which regulates the activity of the Rho family GTPases (RhoA, Rac1, and Cdc42), which mediate changes to cytoskeletal proteins[13] (Figure 3). Vascular leak in inflammation is well described, but the precise mechanism linking inflammatory mediators with increased endothelial paracellular permeability is still not well understood.[69] Ephrin-A1 is a TNF-α–responsive gene that potentially links systemic inflammation and the release of inflammatory mediators with EphA2-ephrin-A1 signaling events.[64] However, EphA2 stimulation by ephrin-A1-Fc in cultured bovine retinal endothelial cells resulted in suppression of VEGF receptor 2 phosphorylation and VEGF-mediated increased vasopermeability[80]; these findings were confirmed in a rodent model.[80]

Although the Eph/ephrin signaling system may be partly responsible for vascular leak, recently, other signaling systems (eg, the Slit-Robo system) have also been implicated.[68] In MDCK cells, EphA2 activation by ephrin-A1-Fc phosphorylates claudin-4 in tight junctions and attenuates claudin-4 association with zona occludens-1, increasing paracellular permeability.[93] In a brain microvascular endothelial cell line, EphA2 associated with the tight junction and stimulation by recombinant ephrin-A1-Fc increased monolayer permeability, whereas EphA2 inactivation by RNA interference or a kinase-inactive mutant promoted tight junction formation[94] (Figure 4). EphA2 co-associates with and regulates cadherin expression in the adherens junction, and activation of EphA2 suppresses cell proliferation and cell adhesion in a range of cells, including endothelial cells.[95] In human mammary epithelial cells (MCF10A), overexpression of EphA2 destabilizes the adherens junction by weakening E-cadherin–mediated cell–cell adhesion through activation of a Rho-GTPase signaling pathway, which involves Src-kinase–enhanced low-molecular-weight phosphotyrosine phosphatase activity and inhibition of Rho-GAP.[71]

EphA2 mediates thrombin-induced up-regulation of intercellular adhesion molecule 1; therefore, EphA2 may be responsible for changes to the endothelial cell surface in addition to the change in permeability of the endothelial cell–cell junctions.[70] In addition, monocyte migration is partly mediated through interaction of monocyte-expressed EphB receptors with endothelial ephrin-B2, and endothelial cells overexpressing ephrin-B2 displayed stronger adhesions with monocytes than endothelial cells expressing truncated ephrin-B2 or no ligand.[96] The evidence suggests that EphA2/ephrin-A1 signaling on the vascular endothelial cell, possibly in response to inflammation-induced up-regulation of TNF-α, thrombin, and other inflammatory mediators, influences both the integrity of endothelial junctions and cytoskeleton structure, resulting in a vascular leak.[70,71,95] In summary, Eph/ephrin receptor–ligand interactions may be (partly) responsible for vascular endothelial cell layer leakiness (to fluid and proteins)[70,71,95] and stickiness (to inflammatory cells).[96] Both of these changes to the phenotype of vascular endothelium are fundamental to the response to injury and the pathogenesis of systemic inflammation (Figure 4).

The Future

The role of the Eph/ephrin proteins in human disease is an emerging field. Although much remains to be explored, the evidence raises hopes for the development of novel therapies that precisely modulate the molecular mechanisms of disease through the administration of specifically targeted molecules, such as Eph-Fc or ephrin-Fc, which disrupt Eph/ephrin signaling interactions.[97] The intramyocardial administration of ephrin-A1-Fc promoted tissue salvage in a model of myocardial infarction in mice.[98] Furthermore, in another mouse model of myocardial infarction, EphA2/ephrin-A1 signaling promoted cardiac stem cells to migrate into the injured tissue.[99] The ability to influence the migration of stem cells to restore tissue integrity after ischemia-reperfusion events, such as myocardial infarction and stroke, is a particularly exciting prospect.[99] In rodent models of spinal cord injury, the administration of EphA4 antagonists reduces astrocytic glial scarring and encourages spinal cord axons to regenerate across an area of spinal cord injury and promote functional recovery.[56] The postmortem analysis of human brains (n = 19) after traumatic brain injury revealed up-regulation of EphA4 expression, again suggesting that blocking EphA4 activation may represent a therapeutic approach to improving recovery after brain trauma.[100] The release of soluble ephrin-A1 from tumor cells may contribute to the vascular leak in cancer syndromes, which could respond to specific inhibitors.[101] The increased vascular permeability that results in fluid leakage plays a significant role in the pathogenesis of the circulatory failure (shock) that complicates sepsis/sys-

temic inflammatory response syndrome[68] and, in combination with neutrophil infiltration, directly contributes to impaired organ function and multiple organ dysfunction syndrome.[68] In fact, the restoration of endothelial barrier function by treatment with a recombinant Slit receptor fragment improved mortality in a mouse model of systemic inflammation, suggesting that endothelial barrier function is a potential therapeutic strategy.[102] A further understanding of the role of the Eph/ephrin proteins in the trafficking and maturation of lymphocytes may allow us to modify the natural history of chronic inflammatory and degenerative disorders.[84]

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References

1. Diegelmann RF, Evans MC: Wound healing: an overview of acute, fibrotic and delayed healing. Front Biosci 2004, 9:283–289
2. Martin P, Parkhurst SM: Parallels between tissue repair and embryo morphogenesis. Development 2004, 131:3021–3034
3. Carmichael ST: Gene expression changes after focal stroke, traumatic brain and spinal cord injuries. Curr Opin Neurol 2003, 16:699–704
4. Hafner C, Meyer S, Hagen I, Becker B, Roesch A, Landthaler M, Vogt T: Ephrin-B reverse signaling induces expression of wound healing associated genes in IEC-6 intestinal epithelial cells. World J Gastroenterol 2005, 11:4511–4518
5. Ahn YH, Lee G, Kang SK: Molecular insights of the injured lesions of rat spinal cords: inflammation, apoptosis, and cell survival. Biochem Biophys Res Commun 2006, 348:560–570
6. Robbins SL, Cotran RS, Collins T, Kumar V: Robbins Pathologic Basis of Disease. Philadelphia, Saunders, 1999, pp 52–65
7. Ait-Oufella H, Maury E, Lehoux S, Guidet B, Offenstadt G: The endothelium: physiological functions and role in microcirculatory failure during severe sepsis. Intensive Care Med 2010, 36:1286–1298
8. Fabes J, Anderson P, Brennan C, Bolsover S: Regeneration-enhancing effects of EphA4 blocking peptide following corticospinal tract injury in adult rat spinal cord. Eur J Neurosci 2007, 26:2496–2505
9. Ivanov AI, Romanovsky AA: Putative dual role of ephrin-Eph receptor interactions in inflammation. IUBMB Life 2006, 58:389–394
10. Larson J, Schomberg S, Schroeder W, Carpenter TC: Endothelial EphA receptor stimulation increases lung vascular permeability. Am J Physiol Lung Cell Mol Physiol 2008, 295:L431–L439
11. Cercone MA, Schroeder W, Schomberg S, Carpenter TC: EphA2 receptor mediates increased vascular permeability in lung injury due to viral infection and hypoxia. Am J Physiol Lung Cell Mol Physiol 2009, 297:L856–L863
12. Pasquale EB: Eph-ephrin bidirectional signaling in physiology and disease. Cell 2008, 133:38–52
13. Lackmann M, Boyd AW: Eph, a protein family coming of age: more confusion, insight, or complexity? Sci Signal 2008, 1:re2
14. Nievergall E, Lackmann M, Janes PW: Eph-dependent cell-cell adhesion and segregation in development and cancer. Cell Mol Life Sci 2012, 69:1813–1842
15. Genander M, Halford MM, Xu NJ, Eriksson M, Yu Z, Qiu Z, Martling A, Greicius G, Thakar S, Catchpole T, Chumley MJ, Zdunek S, Wang C, Holm T, Goff SP, Pettersson S, Pestell RG, Henkemeyer M, Frisen J: Dissociation of EphB2 signaling pathways mediating progenitor cell proliferation and tumor suppression. Cell 2009, 139:679–692
16. Himanen JP, Saha N, Nikolov DB: Cell-cell signaling via Eph receptors and ephrins. Curr Opin Cell Biol 2007, 19:534–542
17. Eph Nomenclature Committee: Unified nomenclature for Eph family receptors and their ligands, the ephrins. Cell 1997, 90:403–404
18. Murai KK, Pasquale EB: "Eph"ective signaling: forward, reverse and crosstalk. J Cell Sci 2003, 116:2823–2832
19. Blits-Huizinga CT, Nelersa CM, Malhotra A, Liebl DJ: Ephrins and their receptors: binding versus biology. IUBMB Life 2004, 56:257–265
20. Himanen JP, Chumley MJ, Lackmann M, Li C, Barton WA, Jeffrey PD, Vearing C, Geleick D, Feldheim DA, Boyd AW, Henkemeyer M, Nikolov DB: Repelling class discrimination: ephrin-A5 binds to and activates EphB2 receptor signaling. Nat Neurosci 2004, 7:501–509
21. Wilkinson DG: Eph receptors and ephrins: regulators of guidance and assembly. Int Rev Cytol 2000, 196:177–244
22. Vearing CJ, Lackmann M: "Eph receptor signalling: dimerisation just isn't enough." Growth Factors 2005, 23:67–76
23. Arvanitis D, Davy A: Eph/ephrin signaling: networks. Genes Dev 2008, 22:416–429
24. Marquardt T, Shirasaki R, Ghosh S, Andrews SE, Carter N, Hunter T, Pfaff SL: Coexpressed EphA receptors and ephrin-A ligands mediate opposing actions on growth cone navigation from distinct membrane domains. Cell 2005, 121:127–139
25. Himanen JP, Yermekbayeva L, Janes PW, Walker JR, Xu K, Atapattu L, Rajashankar KR, Mensinga A, Lackmann M, Nikolov DB, Dhe-Paganon S: Architecture of Eph receptor clusters. Proc Natl Acad Sci U S A 2010, 107:10860–10865
26. Pitulescu ME, Adams RH: Eph/ephrin molecules: a hub for signaling and endocytosis. Genes Dev 2010, 24:2480–2492
27. Janes PW, Nievergall E, Lackmann M: Concepts and consequences of Eph receptor clustering. Semin Cell Dev Biol 2012, 23:43–50
28. Janes PW, Griesshaber B, Atapattu L, Nievergall E, Hii LL, Mensinga A, Chheang C, Day BW, Boyd AW, Bastiaens PI, Jorgensen C, Pawson T, Lackmann M: Eph receptor function is modulated by heterooligomerization of A and B type Eph receptors. J Cell Biol 2011, 195:1033–1045
29. Cowan CW, Shao YR, Sahin M, Shamah SM, Lin MZ, Greer PL, Gao S, Griffith EC, Brugge JS, Greenberg ME: Vav family GEFs link activated Ephs to endocytosis and axon guidance. Neuron 2005, 46:205–217
30. Janes PW, Wimmer-Kleikamp SH, Frangakis AS, Treble K, Griesshaber B, Sabet O, Grabenbauer M, Ting AY, Saftig P, Bastiaens PI, Lackmann M: Cytoplasmic relaxation of active Eph controls ephrin shedding by ADAM10. PLoS Biol 2009, 7:e1000215
31. Miao H, Wang B: EphA receptor signaling: complexity and emerging themes. Semin Cell Dev Biol 2012, 23:16–25
32. Hafner C, Schmitz G, Meyer S, Bataille F, Hau P, Langmann T, Dietmaier W, Landthaler M, Vogt T: Differential gene expression of Eph receptors and ephrins in benign human tissues and cancers. Clin Chem 2004, 50:490–499
33. Baum CL, Arpey CJ: Normal cutaneous wound healing: clinical correlation with cellular and molecular events. Dermatol Surg 2005, 31:674–686; discussion 686
34. Chang HY, Sneddon JB, Alizadeh AA, Sood R, West RB, Montgomery K, Chi JT, van de Rijn M, Botstein D, Brown PO: Gene expression signature of fibroblast serum response predicts human cancer progression: similarities between tumors and wounds. PLoS Biol 2004, 2:E7
35. Cannon RO: Mechanisms, management and future directions for reperfusion injury after acute myocardial infarction. Nat Clin Pract Cardiovasc Med 2005, 2:88–94
36. Baldwin C, Chen ZW, Bedirian A, Yokota N, Nasr SH, Rabb H, Lemay S: Upregulation of EphA2 during in vivo and in vitro renal ischemia-reperfusion injury: role of Src kinases. Am J Physiol Renal Physiol 2006, 291:F960–F971
37. Vihanto MM, Plock J, Erni D, Frey BM, Frey FJ, Huynh-Do U: Hypoxia up-regulates expression of Eph receptors and ephrins in mouse skin. FASEB J 2005, 19:1689–1691
38. Yamashita T, Ohneda K, Nagano M, Miyoshi C, Kaneko N, Miwa Y, Yamamoto M, Ohneda O, Fujii-Kuriyama Y: Hypoxia-inducible transcription factor-2alpha in endothelial cells regulates tumor neovascularization through activation of ephrin A1. J Biol Chem 2008, 283:18926–18936
39. Sohl M, Lanner F, Farnebo F: Sp1 mediate hypoxia induced ephrinB2 expression via a hypoxia-inducible factor independent mechanism. Biochem Biophys Res Commun 2010, 391:24–27
40. Scicolone G, Ortalli AL, Carri NG: Key roles of Ephs and ephrins in retinotectal topographic map formation. Brain Res Bull 2009, 79: 227–247

41. Hui SP, Dutta A, Ghosh S: Cellular response after crush injury in adult zebrafish spinal cord. Dev Dyn 2010, 239:2962–2979
42. Giger RJ, Hollis ER 2nd, Tuszynski MH: Guidance molecules in axon regeneration. Cold Spring Harb Perspect Biol 2010, 2:a001867
43. Bernhardt RR: Cellular and molecular bases of axonal regeneration in the fish central nervous system. Exp Neurol 1999, 157:223–240
44. Goldshmit Y, McLenachan S, Turnley A: Roles of Eph receptors and ephrins in the normal and damaged adult CNS. Brain Res Rev 2006, 52:327–345
45. Knoll B, Isenmann S, Kilic E, Walkenhorst J, Engel S, Wehinger J, Bahr M, Drescher U: Graded expression patterns of ephrin-As in the superior colliculus after lesion of the adult mouse optic nerve. Mech Dev 2001, 106:119–127
46. Rodger J, Lindsey KA, Leaver SG, King CE, Dunlop SA, Beazley LD: Expression of ephrin-A2 in the superior colliculus and EphA5 in the retina following optic nerve section in adult rat. Eur J Neurosci 2001, 14:1929–1936
47. Liu X, Hawkes E, Ishimaru T, Tran T, Sretavan DW: EphB3: an endogenous mediator of adult axonal plasticity and regrowth after CNS injury. J Neurosci 2006, 26:3087–3101
48. Miranda JD, White LA, Marcillo AE, Willson CA, Jagid J, Whittemore SR: Induction of Eph B3 after spinal cord injury. Exp Neurol 1999, 156:218–222
49. Irizarry-Ramirez M, Willson CA, Cruz-Orengo L, Figueroa J, Velazquez I, Jones H, Foster RD, Whittemore SR, Miranda JD: Upregulation of EphA3 receptor after spinal cord injury. J Neurotrauma 2005, 22:929–935
50. Arocho LC, Figueroa JD, Torrado AI, Santiago JM, Vera AE, Miranda JD: Expression profile and role of EphrinA1 ligand after spinal cord injury. Cell Mol Neurobiol 2011, 31:1057–1069
51. Willson CA, Foster RD, Onifer SM, Whittemore SR, Miranda JD: EphB3 receptor and ligand expression in the adult rat brain. J Mol Histol 2006, 37:369–380
52. Bundesen LQ, Scheel TA, Bregman BS, Kromer LF: Ephrin-B2 and EphB2 regulation of astrocyte-meningeal fibroblast interactions in response to spinal cord lesions in adult rats. J Neurosci 2003, 23:7789–7800
53. Goldshmit Y, Galea MP, Wise G, Bartlett PF, Turnley AM: Axonal regeneration and lack of astrocytic gliosis in EphA4-deficient mice. J Neurosci 2004, 24:10064–10073
54. Cruz-Orengo L, Figueroa JD, Velázquez I, Torrado A, Ortiz C, Hernández C, Puig A, Segarra AC, Whittemore SR, Miranda JD: Blocking EphA4 upregulation after spinal cord injury results in enhanced chronic pain. Exp Neurol 2006, 202:421–433
55. Goldshmit Y, Bourne J: Upregulation of EphA4 on astrocytes potentially mediates astrocytic gliosis after cortical lesion in the marmoset monkey. J Neurotrauma 2010, 27:1321–1332
56. Goldshmit Y, Spanevello MD, Tajouri S, Li L, Rogers F, Pearse M, Galea M, Bartlett PF, Boyd AW, Turnley AM: EphA4 blockers promote axonal regeneration and functional recovery following spinal cord injury in mice. PLoS One 2011, 6:e24636
57. Rosas OR, Figueroa JD, Torrado AI, Rivera M, Santiago JM, Konig-Toro F, Miranda JD: Expression and activation of ephexin is altered after spinal cord injury. Dev Neurobiol 2011, 71:595–607
58. Afshari FT, Kwok JC, Fawcett JW: Astrocyte-produced Ephrins inhibit Schwann cell migration via VAV2 signaling. J Neurosci 2010, 30:4246–4255
59. Ruan J-P, Zhang H-X, Lu X-F, Liu Y-P, Cao J-L: EphrinBs/EphBs signaling is involved in modulation of spinal nociceptive processing through a mitogen-activated protein kinases-dependent mechanism. Anesthesiology 2010, 112:1234–1249
60. Zhang R, Zhong NN, Liu XG, Yan H, Qiu C, Han Y, Wang W, Hou WK, Liu Y, Gao CG, Guo TW, Lu SM, Deng HW, Ma J: Is the EFNB2 locus associated with schizophrenia? single nucleotide polymorphisms and haplotypes analysis. Psychiatry Res 2010, 180:5–9
61. Sobel RA: Ephrin A receptors and ligands in lesions and normal-appearing white matter in multiple sclerosis. Brain Pathol 2005, 15:35–45
62. Kume T: Specification of arterial, venous, and lymphatic endothelial cells during embryonic development. Histol Histopathol 2010, 25: 637–646
63. Adams RH, Eichmann A: Axon guidance molecules in vascular patterning. Cold Spring Harb Perspect Biol 2010, 2:a001875
64. Dixit VM, Green S, Sarma V, Holzman LB, Wolf FW, O'Rourke K, Ward PA, Prochownik EV, Marks RM: Tumor necrosis factor-alpha induction of novel gene products in human endothelial cells including a macrophage-specific chemotaxin. J Biol Chem 1990, 265: 2973–2978
65. Dejana E: Endothelial cell-cell junctions: happy together. Nat Rev Mol Cell Biol 2004, 5:261–270
66. Maeda S, Tsukihara T: Structure of the gap junction channel and its implications for its biological functions. Cell Mol Life Sci 2011, 68: 1115–1129
67. Aird WC: Phenotypic heterogeneity of the endothelium, II: representative vascular beds. Circ Res 2007, 100:174–190
68. Goldenberg NM, Steinberg BE, Slutsky AS, Lee WL: Broken barriers: a new take on sepsis pathogenesis. Sci Transl Med 2011, 88ps253:
69. Capaldo CT, Nusrat A: Cytokine regulation of tight junctions. Biochim Biophys Acta 2009, 1788:864–871
70. Chan B, Sukhatme VP: Receptor tyrosine kinase EphA2 mediates thrombin-induced upregulation of ICAM-1 in endothelial cells in vitro. Thromb Res 2009, 123:745–752
71. Fang WB, Ireton RC, Zhuang G, Takahashi T, Reynolds A, Chen J: Overexpression of EPHA2 receptor destabilizes adherens junctions via a RhoA-dependent mechanism. J Cell Sci 2008, 121:358–368
72. Mehta D, Malik AB: Signaling mechanisms regulating endothelial permeability. Physiol Rev 2006, 86:279–367
73. Beckers CM, van Hinsbergh VW, van Nieuw Amerongen GP: Driving Rho GTPase activity in endothelial cells regulates barrier integrity. Thromb Haemost 2010, 103:40–55
74. Ivanov AI, Steiner AA, Scheck AC, Romanovsky AA: Expression of Eph receptors and their ligands, ephrins, during lipopolysaccharide fever in rats. Physiol Genomics 2005, 21:152–160
75. Cheng N, Chen J: Tumor necrosis factor-alpha induction of endothelial ephrin A1 expression is mediated by a p38 MAPK- and SAPK/JNK-dependent but nuclear factor-kappa B-independent mechanism. J Biol Chem 2001, 276:13771–13777
76. Pandey A, Shao H, Marks RM, Polverini PJ, Dixit VM: Role of B61, the ligand for the Eck receptor tyrosine kinase, in TNF-alpha-induced angiogenesis. Science 1995, 268:567–569
77. Okazaki T, Ni A, Baluk P, Ayeni OA, Kearley J, Coyle AJ, Humbles A, McDonald DM: Capillary defects and exaggerated inflammatory response in the airways of EphA2-deficient mice. Am J Pathol 2009, 174:2388–2399
78. Carpenter TC, Schroeder W, Stenmark KR, Schmidt EP: Eph-A2 promotes permeability and inflammatory responses to bleomycin-induced lung injury. Am J Respir Cell Mol Biol 2012, 46:40–47
79. Chen J, Hicks D, Brantley-Sieders D, Cheng N, McCollum GW, Qi-Werdich X, Penn J: Inhibition of retinal neovascularization by soluble EphA2 receptor. Exp Eye Res 2006, 82:664–673
80. Ojima T, Takagi H, Suzuma K, Oh H, Suzuma I, Ohashi H, Watanabe D, Suganami E, Murakami T, Kurimoto M, Honda Y, Yoshimura N: EphrinA1 inhibits vascular endothelial growth factor-induced intracellular signaling and suppresses retinal neovascularization and blood-retinal barrier breakdown. Am J Pathol 2006, 168:331–339
81. Ting MJ, Day BW, Spanevello MD, Boyd AW: Activation of ephrin A proteins influences hematopoietic stem cell adhesion and trafficking patterns. Exp Hematol 2010, 38:1087–1098
82. Muñoz JJ, Cejalvo T, Alonso-Colmenar LM, Alfaro D, Garcia-Ceca J, Zapata A: Eph/Ephrin-mediated interactions in the thymus. Neuroimmunomodulation 2011, 18:271–280
83. Sharfe N, Nikolic M, Cimpeon L, Van De Kratts A, Freywald A, Roifman CM: EphA and ephrin-A proteins regulate integrin-mediated T lymphocyte interactions. Mol Immunol 2008, 45:1208–1220
84. Trinidad EM, Zapata AG, Alonso-Colmenar LM: Eph-ephrin bidirectional signaling comes into the context of lymphocyte transendothelial migration. Cell Adh Migr 2010, 4:363–367
85. Schruefer R, Sulyok S, Schymeinsky J, Peters T, Scharffetter-Kochanek K, Walzog B: The proangiogenic capacity of polymorphonuclear neutrophils delineated by microarray technique and by measurement of neovascularization in wounded skin of CD18-deficient mice. J Vasc Res 2006, 43:1–11
86. Zamora DO, Babra B, Pan Y, Planck SR, Rosenbaum JT: Human leukocytes express ephrinB2 which activates microvascular endothelial cells. Cell Immunol 2006, 242:99–109

87. Li YY, McTiernan CF, Feldman AM: IL-1 beta alters the expression of the receptor tyrosine kinase gene r-EphA3 in neonatal rat cardiomyocytes. Am J Physiol 1998, 274:H331–H341
88. Islam S, Loizides AM, Fialkovich JJ, Grand RJ, Montgomery RK: Developmental expression of Eph and ephrin family genes in mammalian small intestine. Dig Dis Sci 2010, 55:2478–2488
89. Wong SY, Chiam K-H, Lim CT, Matsudaira P: Computational model of cell positioning: directed and collective migration in the intestinal crypt epithelium. J R Soc Interface 2010, 7(Suppl 3):S351–S363
90. Hafner C, Meyer S, Langmann T, Schmitz G, Bataille F, Hagen I, Becker B, Roesch A, Rogler G, Landthaler M, Vogt T: Ephrin-B2 is differentially expressed in the intestinal epithelium in Crohn's disease and contributes to accelerated epithelial wound healing in vitro. World J Gastroenterol 2005, 11:4024–4031
91. Kitamura T, Kabuyama Y, Kamataki A, Homma MK, Kobayashi H, Aota S, Kikuchi S, Homma Y: Enhancement of lymphocyte migration and cytokine production by ephrinB1 system in rheumatoid arthritis. Am J Physiol Cell Physiol 2008, 294:C189–C196
92. Kwan Tat S, Pelletier JP, Amiable N, Boileau C, Lajeunesse D, Duval N, Martel-Pelletier J: Activation of the receptor EphB4 by its specific ligand ephrin B2 in human osteoarthritic subchondral bone osteoblasts. Arthritis Rheum 2008, 58:3820–3830
93. Tanaka M, Kamata R, Sakai R: EphA2 phosphorylates the cytoplasmic tail of Claudin-4 and mediates paracellular permeability. J Biol Chem 2005, 280:42375–42382
94. Zhou N, Zhao WD, Liu DX, Liang Y, Fang WG, Li B, Chen YH: Inactivation of EphA2 promotes tight junction formation and impairs angiogenesis in brain endothelial cells. Microvasc Res 2011, 82: 113–121
95. Orsulic S, Kemler R: Expression of Eph receptors and ephrins is differentially regulated by E-cadherin. J Cell Sci 2000, 113(Pt 10): 1793–1802
96. Pfaff D, Heroult M, Riedel M, Reiss Y, Kirmse R, Ludwig T, Korff T, Hecker M, Augustin HG: Involvement of endothelial ephrin-B2 in adhesion and transmigration of EphB-receptor-expressing monocytes. J Cell Sci 2008, 121:3842–3850
97. Krause DS, Van Etten RA: Tyrosine kinases as targets for cancer therapy. N Engl J Med 2005, 353:172–187
98. Dries JL, Kent SD, Virag JA: Intramyocardial administration of chimeric ephrinA1-Fc promotes tissue salvage following myocardial infarction in mice. J Physiol 2011, 589:1725–1740
99. Goichberg P, Bai Y, D'Amario D, Ferreira-Martins J, Fiorini C, Zheng H, Signore S, del Monte F, Ottolenghi S, D'Alessandro DA, Michler RE, Hosoda T, Anversa P, Kajstura J, Rota M, Leri A: The ephrin A1-EphA2 system promotes cardiac stem cell migration after infarction. Circ Res 2011, 108:1071–1083
100. Frugier T, Conquest A, McLean C, Currie P, Moses D, Goldshmit Y: Expression and activation of EphA4 in the human brain after traumatic injury. J Neuropathol Exp Neurol 2012, 71:242–250
101. Wykosky J, Palma E, Gibo DM, Ringler S, Turner CP, Debinski W: Soluble monomeric EphrinA1 is released from tumor cells and is a functional ligand for the EphA2 receptor. Oncogene 2008, 27:7260–7273
102. London NR, Zhu W, Bozza FA, Smith MC, Greif DM, Sorensen LK, Chen L, Kaminoh Y, Chan AC, Passi SF, Day CW, Barnard DL, Zimmerman GA, Krasnow MA, Li DY: Targeting Robo4-dependent Slit signaling to survive the cytokine storm in sepsis and influenza. Sci Transl Med 2010, 2:23ra19