Published in final edited form as:
Adv Cancer Res. 2012 ; 114: 1–20. doi:10.1016/B978-0-12-386503-8.00001-6.

Regulation of tumor initiation and metastatic progression by Eph receptor tyrosine kinases

Jin Chen[1,2,3,4,5]

[1]VA Medical Center, Tennessee Valley Healthcare System, Nashville, TN 37212

[2]Department of Medicine, Vanderbilt University School of Medicine, Nashville, TN 37232

[3]Department of Cancer Biology, Vanderbilt University School of Medicine, Nashville, TN 37232

[4]Department of Cell & Developmental Biology, Vanderbilt University School of Medicine, Nashville, TN 37232

[5]Vanderbilt-Ingram Cancer Center, Vanderbilt University School of Medicine, Nashville, TN 37232

Abstract

In recent years, a growing body of evidence has indicated that signaling molecules previously implicated in axon guidance are important regulators of multi-step tumorigenesis and progression. Eph receptors and ephrins belong to this special class of molecules that play important roles in both axon guidance and cancer. Tremendous progress has been made in the past few years in both understanding the role of Eph receptors and ephrins in cancer and designing therapeutic strategies for cancer therapy. This review will focus on new advances in elucidating the contribution of Eph/ephrin molecules to key processes in tumor initiation and metastatic progression, including cancer cell proliferation, invasion and metastasis, and tumor angiogenesis.

Introduction

Cancer initiation and malignant progression are multi-step processes that involve loss of growth control, evasion of apoptosis, sustained angiogenesis, tissue invasion, and metastasis (Hanahan and Weinberg, 2000, 2011). Emerging evidence has indicated that signaling molecules previously implicated in axon guidance are important regulators of multi-step tumor initiation and progression (Adams and Eichmann, 2010a; Eichmann et al., 2005). These include Eph/ephrin, Semaphorins/ Plexins, VEGF/VEGFR, chemokines/ receptors, Netrins/DCC UN5, Slit/Robo, and Notch/Delta. This review will focus on recent advances on dissecting the role of Eph/ephrin molecules in cancer and tumor angiogenesis. Due to space and scope constraints, we have limited our discussion in the text to the Eph receptors and ephrins for which the most data are available. Tumor and vascular phenotypes of Eph/ephrin knockout and transgenic animals are summarized in Table 1 and Table 2. More comprehensive reviews on Eph-ephrin signaling in physiology and disease can be found elsewhere (Kullander and Klein, 2002; Pasquale, 2005, 2008; Pasquale, 2010).

Dysregulation of Eph receptors in cancer

The Eph family of receptor tyrosine kinases (RTKs) is the largest identified in the vertebrate genome and is subdivided into class A and class B based on sequence homology and binding

Correspondence should be addressed to: Jin Chen, M.D., Ph.D. Associate Professor of Medicine Vanderbilt University School of Medicine, A-4323 MCN, 1161 21st Avenue South Nashville, TN 37232-2363 Phone 00 +1 615 343 3819 FAX 00 + 1 615 322 6248 jin.chen@vanderbilt.edu.

affinity for two distinct types of membrane-anchored ephrin ligands. In general, Class A receptors interact with glycosyl-phosphatidylinositol (GPI)-linked class A ephrins, while class B receptors bind to class B ephrins that are attached to the cell membrane by a transmembrane-spanning domain, although interclass binding does occur among certain family members [reviewed in (Pasquale, 2005, 2008; Pasquale, 2010)]. Binding of ephrin to Eph receptor induces receptor clustering and activation. Recent structural studies of the Eph/ephrin interactions has not only identified ligand-receptor interaction sites, but also discovered receptor-receptor interfaces that function in assembly of higher-order signaling clusters (Himanen et al., 2010; Seiradake et al., 2010). Because both ligand and receptor are membrane-bound, engagement of ephrin with Eph receptor between adjacent cells induces bi-directional signaling through both ligand- and receptor-expressing cells [reviewed in (Kullander and Klein, 2002; Pasquale, 2008)]. Originally characterized as axon guidance regulators, ephrins and Eph RTKs are subsequently recognized to regulate physiologic and pathologic processes during embryonic development, in normal tissue homeostasis, and in disease [reviewed in (Pasquale, 2008; Pasquale, 2010)].

Recent technological advances in analyzing the human cancer genome permit the study of gene copy number, expression level, and mutation status in tumor tissue. These studies demonstrated that Eph receptors are often dysregulated in cancer [reviewed in (Brantley-Sieders, 2011; Brantley-Sieders et al., 2011b; Pasquale, 2010)]. Gene expression studies by microarray analysis and immunohisto-chemistry on tumor tissue microarrays (TMAs) have correlated expression of some Eph receptors in tumor epithelium and/or vasculature with disease stage, metastasis, recurrence, and survival. For example, EphA2 expression is elevated in many types of cancer (Landen et al., 2005b; Wykosky and Debinski, 2008) and high levels of EphA2 correlate with tumor malignancy and poor patient survival in breast cancer (Brantley-Sieders, 2011; Fournier et al., 2006; Martin et al., 2008; Zhuang et al., 2010) and lung cancer (Brannan et al., 2009; Faoro et al., 2010; Kinch et al., 2003). Likewise, levels of several EphB receptors are also elevated in different stages of colon cancer, lung cancer, or breast cancer (Batlle et al., 2002; Brantley-Sieders, 2011; Ji et al., 2011; Kumar et al., 2009; Noren and Pasquale, 2007). However, decreased Eph receptor levels have also been reported in certain types of human cancer (Batlle et al., 2005; Kumar et al., 2009). Dysregulation of Eph receptor expression in tumor has been attributed to multiple mechanisms, including chromosomal abnormality, epigenetic regulation, mRNA stability and transcriptional control [reviewed in (Pasquale, 2010)]. Aside from changes in receptor level, somatic mutations have been found in nearly all Eph receptors. Notably, 11 somatic mutations in EphA3 receptor were identified in 5-10% of lung cancers, placing EphA3 among 27 most frequently mutated genes in human lung adenocarcinoma (Davies et al., 2005; Ding et al., 2008; Greenman et al., 2007; Wood et al., 2006). However, these mutations are scattered throughout the receptor, and it is unclear whether these non-recurrent mutations are “driver” or biologically neutral “passenger” genetic alterations. Elucidating the effects of these mutations will greatly improve our understanding of how Eph receptors function in cancer.

Role of Eph receptors in tumor cell proliferation

Sustained proliferative signals and evasion of growth suppressors are two hallmarkers of cancer. However, Eph receptors were initially thought not to be involved in regulation of cell growth (Brambilla et al., 1995; Bruce et al., 1999; Lhotak and Pawson, 1993). More recent studies found that Eph receptors maintain tissue homeostasis by controlling the proliferation of stem and progenitor cells in adult. For example, in the intestinal stem cell niche, EphB signaling promotes cell-cycle reentry of progenitor cells through Abl and Cyclin D1 and accounts for approximately 50% of the mitogenic activity in the adult mouse small intestine and colon (Genander et al., 2009). Likewise, Eph receptor signaling in neural

progenitor cells in hippocampus also promotes proliferation (Chumley et al., 2007). In contrast, Eph receptors can negatively regulate proliferation of hair follicle and epidermal progenitor cells in the adult mice (Genander et al., 2010), as well as in neural stem/progenitor cells in the lateral ventricle (Conover et al., 2000).

In cancer cells, the role of Eph receptors in regulation of tumor cell growth is similarly complex, and the effects of these receptors on cell proliferation are often dependent on ligand stimulation, signaling cross talk, or other contextual factors. This complexity is best illustrated in the case of EphA2 receptor. EphA2 receptor is highly expressed in a number of tumor types, including breast cancer, prostate cancer, ovarian cancer, lung cancer, and glioblastoma multiforme (Brantley-Sieders, 2011; Landen et al., 2005b; Wykosky and Debinski, 2008). High levels of EphA2 are correlated with poor patient survival (Brannan et al., 2009; Brantley-Sieders, 2011; Faoro et al., 2010; Fournier et al., 2006; Kinch et al., 2003; Martin et al., 2008; Zhuang et al., 2010). Silencing of EphA2 in cancer cells by either siRNA-mediated knockdown, anti-sense oligonucleotides, or targeted deletion of EphA2 in knockout mice, inhibited tumor initiation and metastatic progression (Brantley-Sieders et al., 2008; Duxbury et al., 2004; Landen et al., 2005a). Interestingly, the tumor promotion effects of EphA2 appear to be ligand-independent and result from cross talk with HER2 receptor tyrosine kinase (Brantley-Sieders et al., 2008). In support of this notion, overexpression of EphA2 in non-transformed MCF-10A breast epithelial cells induces anchorage-independent colony growth in vitro and tumor formation in vivo (Zelinski et al., 2001). Further investigation revealed that high levels of EphA2 in these tumor cells are minimally phosphorylated, suggesting poor ligand-induced receptor forward signaling. Indeed, exogenous ligand stimulation of EphA2 suppressed colony formation on 2D culture and spheroid growth in 3D Matrigel (Miao et al., 2001; Yang et al., 2011). Treatment of mammary tumors with a ligand-mimetic activating antibody against EphA2 suppressed tumor growth in vivo (Brantley-Sieders et al., 2008; Coffman et al., 2003; Landen et al., 2006), corroborating the notion that EphA2's effects in tumor promotion is ligand-independent. Similarly, EphB4 is often overexpressed in human breast cancer (Wu et al., 2004), and EphB4 knockdown inhibited tumor cell survival, migration and invasion in vitro and tumor growth in vivo (Kumar et al., 2006). However, ephrin-B2-induced EphB4 forward signaling inhibits breast cancer cell viability and proliferation, as well as tumor volume in xenografts (Noren et al., 2006). Taken together, these data suggest a model in which ephrin-induced Eph receptor forward signaling inhibits tumor cell proliferation, whereas in the absence of the ligand, crosstalk between Eph receptors and other oncogenic proteins leads to enhanced cell proliferation and tumorigenesis, presumably independent of ephrin stimulation.

How does Eph receptor forward signaling inhibit tumor cell growth? Ephrin-B2-induced EphB4 receptor forward signaling activates an antioncogenic pathway, leading to Abl kinase activation and inactivation of Crk adaptor function through phosphorylation by Abl (Noren et al., 2006; Noren and Pasquale, 2007). In the case of EphA2, several studies showed that stimulation of tumor cells with ephrin-A1 ligand inhibited the activation of both Ras-MAPK and Akt-mTOR pathways (Macrae et al., 2005; Miao et al., 2001; Yang et al., 2011). However, in PC3 prostate cancer cells, MEK inhibitor does not appears to affect cell proliferation, whereas PI3K inhibitor and Rapamycin efficiently inhibited cell growth, suggesting that Akt-mTOR is the major pathway inhibited by EphA2 forward signaling (Yang et al., 2011). It is interesting to note that EphA2 forward signaling does not change the activity of Akt upstream regulators such as Ras family GTPases, PI3 kinase, integrin, or the SHIP2 lipid phosphatase. Rather, EphA2 inactivates the Akt-mTORC1 oncogenic pathway through Akt dephosphorylation mediated by a PP1-like serine/threonine phosphatase (Yang et al., 2011). Although inhibition of Akt-mTOR pathway by EphA2 forward signaling appears to be a major mechanism in regulation of cell growth in multiple

tumor cell lines, the importance of EphA2-mediated suppression of Akt-mTOR pathway in tumor growth needs to be further validated in animal models in vivo.

Decades of research have demonstrated that “contact inhibition” is an important mechanism used by normal cells to suppress cell proliferation. The mechanism of contact inhibition appears to involve strengthening of cell-cell adhesion and maintaining cell polarity and tissue integrity. In MDCK cells, ligand-activated EphA2 signaling suppresses Arf6 GTPase activity, leading to cell compaction and polarization (Miura et al., 2009), suggesting a role for EphA2 forward signaling in E-cadherin-based cell-cell adhesion and the apical-basal polarization of epithelial cells. However, overexpression of EphA2 receptor in MCF10A mammary epithelial cells destabilizes adherens junctions via a RhoA-dependent mechanism (Fang et al., 2008b). The seemingly conflicting results in these two studies may be due to differences in signaling strength and composition of the signaling complex in the respective cell types, as illustrated in ephrin-B1 regulation of tight junctions (Lee and Daar, 2009; Lee et al., 2008). Either overexpression or loss of ephrin-B1 can disrupt cell-cell contact and tight junctions. Overexpressed or unphosphorylated ephrin-B1 competes with active Cdc42 GTPase for binding to Par-6 and inhibits αPKC activation in the Par polarity complex, leading to tight junction disruption. Tyrosine phosphorylation of ephrin-B1 induces dissociation of ephrin-B1 and Par-6, which is now available to interact with Cdc42 and establish tight junction. However, ephrin-B1 is also associated with Par-6 at adherence junction. Loss of ephrin-B1 entirely may allow Par-6 at adherence junction compete with Par-6 at tight junction for Cdc-42, resulting in disruption of tight junction. Interestingly, phosphorylation of ephrin-B1 can be EphB receptor independent, and can be induced by association with tight junction protein claudin (Tanaka et al., 2005). Together, these studies suggest that de-regulation of ephrin/Eph signaling can affect cell-cell adhesion, but it remains to be determined whether dysregulation of cell-cell adhesion directly leads to changes in cellular proliferation. However, breaking down cell-cell adhesion is often a first step that leads to epithelial-to-mesenchymal transformation, a program that broadly regulates invasion and metastasis.

Role of Eph receptors in invasion and metastasis

High endogenous levels of Eph receptors in cancer cells, or overexpression of Eph molecules, have been implicated in promoting tumor cell invasiveness and motility in vitro and distant metastasis in vivo. Targeted EphA2 receptor knockout significantly inhibited breast cancer lung metastasis in the MMTV-Neu transgenic mammary tumor model (Brantley-Sieders et al., 2008). EphA2-deficient tumor cells isolated from the knockout mice displayed reduced cell invasion and migration in response to serum stimulation, indicating that EphA2 is required for tumor cell motility. High levels of EphA2 in glioblastoma cells were also shown to promote tumor cell invasiveness and motility (Miao et al., 2009; Wykosky et al., 2005). In both cases, EphA2-dependent cell motility appears to be mediated by ligand-independent signaling and cross talk with other oncogenic pathways. In breast cancer cells, EphA2 forms a complex with ErbB2 receptor tyrosine kinase and regulates the activity of Ras-MAPK and RhoA GTPase (Brantley-Sieders et al., 2008). In glioblastoma cells, EphA2-mediated chemotactic cell migration required phosphorylation of EphA2 on Ser897 by Akt (Miao et al., 2009). Ephrin-A1 stimulation of EphA2 negated Akt activation by growth factors and caused EphA2 dephosphorylation on Ser897. As noted above, inhibition of Akt activity by ligand-dependent EphA2 receptor signaling appears to be mediated by a PP1-like phosphatase, resulting in dephosphorylation of Akt at Thr308 and Ser374 sites (Yang et al., 2011).

In colon cancer, EphB-ephrin-B interaction regulates both cell positioning and tumor metastasis (Merlos-Suárez and Batlle, 2008). In normal intestine, Wnt signaling drives

EphB2 and EphB3 expression in crypts (Batlle et al., 2002). EphB2 is expressed at highest levels in intestinal stem cells and its expression decreases in progenitor cells as they differentiate and migrate towards the lumen. EphB3 is localized at the bottom most positions of the crypt. Ephrin-B1 and B2 express complementarily in differentiated cells and their expression is negatively controlled by β-catenine/Tcf activity. In colon cancer, APC mutation activates the Wnt pathway and upregulates the expression of EphB2, B3, and B4 receptors. These tumor-initiating cells repopulate the crypts until they reach the surface epithelium where they encounter normal cells expressing ephrin-Bs. EphB-ephrin-B bidirectional signaling was proposed to restrict tumor spreading (Merlos-Suárez and Batlle, 2008). This model was supported by the observation that low- and medium-grade tumors were enriched in EphB positive tumor cells, whereas high-grade tumors are often EphB negative, suggesting that silencing of EphB expression is associated with more malignant human tumors. Furthermore, loss of EphB3 or expression of a dominant negative cytoplasmic deletion mutant of EphB2 accelerates tumorigenesis in the colon and rectum of $APC^{Min/+}$ mice, and results in the formation of aggressive adenocarcinoma (Batlle et al., 2005). The mechanism by which EphB receptors compartmentalize the expansion of colorectal tumor cells appears to be dependent on E-cadherin-mediated adhesion (Cortina et al., 2007). Thus, constitutive Wnt signaling upregulates EphB expression at early stages of colon cancer. However, EphB silencing is required for malignant tumor expansion at later stages, as clinical studies correlate loss of EphB expression with the transition from adenoma to adenocarcinoma (Batlle et al., 2005).

Interaction between tumor cells and adjacent stromal cells greatly influences tumor cells' ability to move and invade into surrounding tissue. Most studies in the literature have focused on paracrine signaling mediated by secreted factors. Using a tumor cell-fibroblast co-culture system, a recent report showed that a combinatorial code of Eph receptor activation dictates whether a cell moves or stops upon encountering another cell (Astin et al., 2010). Prostate cancer cell line PC3 expresses high levels of ephrin-As and EphA2 and EphA4 receptors. Contact inhibition of locomotion is induced between PC3 cells by EphA forward signaling via RhoA activation and subsequent cell rounding. Knockdown of EphA2 and EphA4 abolished homotypic contact inhibition of motility in PC3 cells. In contrast, fibroblasts express high level of ephrin-B2, which activates EphB3 and EphB4 on PC3 cells to induce Cdc42 activation, lamellipodia formation and cell migration. It is not known if the same combinatorial code of Eph molecules also functions in other tumor cell types. However, it is now clear that signals transduced upon direct cell-cell contact are also crucial for regulating contact inhibition of locomotion and invasiveness, possibly through Eph-ephrin signaling between adjacent cells.

Regulation of tumor angiogenesis by Eph receptors

It is increasingly recognized that the tumor microenvironment plays crucial roles in tumor initiation and malignant progression. Angiogenesis, a process in which capillaries sprout from existing vessels, not only provides nutrients and oxygen for tumor growth, but also allows tumor cells to intravasate and travel to a distant site to form metastatic lesions. Eph receptors have been long known to regulate angiogenesis. Early studies discovered that ephrin-A1, a prototypic ligand for the EphA2 receptor, induces corneal angiogenesis (Pandey et al., 1995). Several years later, ephrin-B2 and EphB4 were shown to regulate angiogenic remodeling and arterial-vein specification during embryonic development (Gerety et al., 1999; Wang et al., 1998). Subsequent studies revealed that ephrin-Eph molecules have diverse functions in regulating angiogenesis, including modulating endothelial cell motility and assembly, recruiting perivascular supporting cells, lymphangiogenesis, and, more recently, angiocrine signaling.

Ephrin-A1 and EphA2 in tumor-endothelium interaction

In addition to dysregulation of Eph receptor in tumor cells, many Eph receptors and ephrins are also upregulated in the tumor vasculature. Elevated EphA2 receptor expression was found in a number of tumor xenografts and human breast cancer clinical specimens (Brantley-Sieders et al., 2011a; Ogawa et al., 2000). Host deficiency of EphA2 receptor tyrosine kinase results in reduced tumor angiogenesis and metastatic progression (Brantley-Sieders et al., 2005). EphA2 deficient endothelial cells display impaired migration and assembly in response to either ephrin-A1 ligand or VEGF stimulation (Brantley-Sieders et al., 2004; Chen et al., 2006). The defective phenotypes resulted from, at least in part, dysregulation of PI3K, Vav guanine nucleotide exchange factor, and Rac1 GTPase signaling in EphA2 knockout endothelial cells (Brantley-Sieders et al., 2004; Hunter et al., 2006). Mapping of phosphorylated tyrosine residues of EphA2 revealed interaction sites between EphA2 and Vav GEF or p85 subunits of PI3K (Fang et al., 2008a), suggesting a critical role for tyrosine phosphorylation in transducing EphA2 forward signaling in vascular endothelial cells. Indeed, knockout of Vav2 and Vav3 in mice resulted in similar vascular defects in vitro (Hunter et al., 2006) and impaired tumor angiogenesis in vivo (Brantley-Sieders et al., 2009), supporting the critical role of Vav-Rac signaling in EphA2-mediated neovascularization.

Expression analysis has correlated ephrin-A1 with tumor neovascularization and progression in several mouse models of cancer and in human samples. Ephrin-A1 expression is induced by TNF-α, VEGF, and HIF (Cheng et al., 2002; Pandey et al., 1995; Yamashita et al., 2008). Targeted gene deletion of ephrin-A1 in mice results in defective heart valve and impaired cardiac function (Frieden et al., 2008). In addition, ephrin-A1-null endothelial cells have reduced migratory and assembly response upon stimulation of VEGF in vitro and impaired angiogenesis in a sponge assay in vivo (Youngblood and Chen, unpublished data), suggesting a critical role of ephrin-A1 in angiogenesis. It is currently unknown whether ephrin-A1 functions in vascular endothelial cells through EphA2 forward signaling, ephrin-A1 reverse signaling, or both. Although ephrins lack a cytoplasmic domain, ephrin-A ligands can complex with transmembrane proteins to transduce their signals. Indeed, reverse signaling by ephrin-As upon binding EphAs controls axon guidance and mapping in the nervous system (Lim YS, 2008), and cell attachment and motility in fibroblasts and tumor cells (Davy et al., 1999; Davy and Robbins, 2000). One recent study showed that p75 neurotrophin receptor (NTR) can form a complex required for Fyn phosphorylation upon binding EphAs, activating a signaling pathway leading to cytoskeletal changes (Lim YS, 2008). It remains to be determined whether ephrin-A1 reverse signaling also plays significant roles in angiogenesis and what key transmembrane proteins act as co-receptors for ephrin-A1 in endothelial cells.

It is well known that tumor-derived proangiogenic factors induce neovascularization to facilitate tumor growth and malignant progression. Conversely, the concept of “angiocrine” signaling, in which signals produced by endothelial cells elicit tumor cell responses distinct from vessel function, has been proposed (Butler JM, 2010). Recently, EphA2 receptor was found to regulate such “angiocrine” signaling (Brantley-Sieders et al., 2011a). Co-transplantation of tumor cells with wild-type, but not EphA2-null, endothelial cells enhanced tumor growth before incorporation of exogenous endothelial cells into blood vessels, suggesting that endothelial-derived factors modulate tumor growth. Interestingly, EphA2 does not appear to up-regulate angiogenic factors. Rather, loss of EphA2 results in elevated Slit2 production from endothelial cells, which inhibits tumor cells growth and motility. In human breast cancer, high levels of EphA2 are associated with low Slit2 expression in tumor endothelium, correlating with poor patient survival. Together, these data suggest that EphA2 RTK not only has a role in tumor-induced angiogenesis, but also functions in angiocrine regulation of tumor growth and motility.

Ephrin-B2 and EphB4 in tumor angiogenesis

Ephrin-B2 and its receptor EphB4 were first discovered to play a role in arterial-vein specification and angiogenic remodeling during embryonic development (Gerety et al., 1999; Wang et al., 1998). Knockout of either ephrin-B2 or EphB4 in mice resulted in similar defects of angiogenic remodeling. Because complete deletion of ephrin-B2 or EphB4 induces embryonic lethality, inducible endothelial specific knockout animals or mice bearing specific mutations in the cytoplasmic tail of ephrin-B2 have been developed. Endothelial cell-specific deletion of ephrin-B2 affected retinal angiogenesis in newborn mice, as well as lymphatic vessel development in dermal skin (Wang et al., 2010). These vascular defects appear to be caused by lack of ephrin-B2 reverse signaling through interaction with PDZ domain-containing proteins (Mäkinen et al., 2005; Sawamiphak et al., 2010). A single amino acid deletion in the PDZ-binding motif of the cytoplasmic tail of the ephrin-B2 leads to similar phenotypes as those in endothelial-specific knockout mice. In contrast, mutations in all 5 tyrosine residues of ephrin-B2 do not appear to affect ephrin-B2 function in angiogenesis, suggesting that ephrin-B2 reverse signaling in angiogenic remodeling is mediated by PDZ interaction (Mäkinen et al., 2005; Sawamiphak et al., 2010).

How does ephrin-B2 reverse signaling affects angiogenesis? Ephrin-B2 is expressed in sprouting capillaries, both in tip cells and stalk cells. One of the characteristics of angiogenic tip cells is that they send numerous fillopodia to sense the microenvironment in response to VEGF released from hypoxic tissues. These tip cells express high levels of VEGF receptor 2 and receptor 3 and guide migration of the angiogenic sprout (Adams and Eichmann, 2010b). Loss of ephrin-B2 or impaired ephrin-B2 reverse signaling significantly decreases the number of fillopodia extensions in angiogenic tip cells without affecting the proliferation of stalk cells (Sawamiphak et al., 2010) (Wang et al., 2010). Because ephrin-B2 and VEGFR are co-localized on fillopodia and the body of the tip cells, the cross talk between these two signaling pathways was investigated. Loss of ephrin-B2 or mutation in the PDZ-binding motif of the ephrin-B2 cytoplasmic tail inhibited VEGF receptor endocytosis and receptor phosphorylation. VEGF receptor endocytosis appears to be critical for its signaling, since inhibition of VEGF receptor internalization by Dynosore, an inhibitor of dynamin GTPase, reduced phosphorylation of VEGFR and Akt in vitro and fillopodia extension in angiogenic spouts. Together, these results elucidate how these two important signaling pathways cross talk in angiogenesis and lymphangiogenesis, although the molecular mechanism underlying how ephrin-B2 PDZ interaction affects VEGFR endocytosis awaits to be further investigated.

Aside from developmental and physiological angiogenesis, ephrin-B2 and EphB4 are also expressed in the tumor blood vessels in a variety of tumors (Erber et al., 2006; Gale et al., 2001; Shin et al., 2001) and are induced under hypoxic conditions (Vihanto et al., 2005), suggesting that this ligand-receptor pair may regulate tumor neovascularization. In support of this hypothesis, A375 melanomas form smaller, less vascularized tumors in the presence of the soluble, monomeric EphB4 extracellular domain *in vivo* (Martiny-Baron et al., 2004). Soluble EphB4 may act, at least in part, by preventing binding of tumor cell EphB receptors to ephrin-B2-positive endothelium, thus disrupting tumor angiogenesis. Further support for this hypothesis is provided from studies in which overexpression of a truncated cytoplasmic deletion EphB4 receptor construct produced increased tumor growth and vascularity in mammary tumors, likely through ephrin-B2 mediated reverse signaling in host endothelium (Noren et al., 2004). Indeed, transplantation of gliomas cells into the brain of mice with ephrin-B2 cytoplasmic mutation results in reduced tumor size and decreased tumor vessel density (Sawamiphak et al., 2010), suggesting ephrin-B2 reverse signaling is required for tumor angiogenesis. Taken together, these studies reveal a critical role for B class receptors and ligands in tumor progression and vascular recruitment for multiple types of human cancer.

Ephrin/Eph in endothelial-mural cell interaction

Perivascular supporting cells or mural cells, called pericytes in capillaries or vascular smooth muscle cells in larger vessels, are essential components of blood vessels and are critical in maintaining vessel stability. Alteration of pericyte density or the stable attachment of pericytes to the endothelium is associated with a number of human diseases including cancer. Paracrine signaling between endothelial and mural cells, such as PDGF-B/PDGFRβ and angiopoietin and Tie2 receptor, has been shown to play essential roles in recruitment of perivascular mesenchymal cells to differentiate into pericytes and maintaining tight interaction between endothelial cells and mural cells [reviewed in (Gaengel K, 2009)]. More recently, ephrin-Eph receptor signaling, ephrin-B2 and EphB4 specifically, has also been implicated in blood-vessel-wall assembly.

During embryonic development, ephrin-B2 is expressed on arterial endothelial cells. However, as development proceeds, ephrin-B2 expression progressively extends through the capillary bed and to pericytes (Erber et al., 2006; Gale et al., 2001; Shin et al., 2001), suggesting a role of ephrin-B2 in vessel wall formation. Mural cell-specific deletion of ephrin-B2 in mice results in perinatal lethality due to vascular defects in multiple organs and abnormal migration of smooth muscle cells to lymphatic capillaries (Foo et al., 2006). Ephrin-B2 deficient pericytes have reduced contacts with endothelial cells and fail to envelope the endothelial tube (Foo et al., 2006), resulting in dysfunction of microvessels and hemorrhage, indicating that ephrin-B2 is required for proper mural cell function. To determine whether the role of ephrin-B2 in mural cells is mediated by reverse signaling, Salvucci et al. mutated the 5 tyrosine residues of the ephrin-B2 cytoplasmic domain (Salvucci et al., 2009). Interestingly, while mutation of all 5 tyrosine residues does not appear to affect angiogenic sprouting in vivo (Sawamiphak et al., 2010) (Wang et al., 2010), the same mutant severely impairs the ability of pericyte and endothelial cell assembly into cordlike structures in a Matrigel plug assay (Salvucci et al., 2009). Thus, ephrin-B2 reverse signaling via PDZ interaction appears to mediate angiogenic sprouting, whereas reverse signaling through phosphorylated tyrosine residues may functions in vascular endothelial-pericyte interaction.

Concluding remarks

Both pre-clinical laboratory studies and data in human cancer clinical specimens provide compelling evidence that members of the Eph family of receptor tyrosine kinases and their ephrin ligands regulate tumor growth, invasion and metastasis, and neovascularization. However, Eph receptor signaling is complex, influenced by differences in ligand-dependent versus ligand-independent signaling, forward versus reverse signaling, and kinase-dependent versus kinase-independent signaling. Some of the paradoxical functions of Eph also appear to be influenced by tissue type and oncogenic context. Given the complexity of signaling regulated by this RTK family, as well as extensive cross-talk with other RTK families involved in cancer, future efforts should be aimed at understanding how Eph receptor expression and function is modulated in the context of relevant cancer pathways.

In spite of these challenges, Eph receptors are very attractive therapeutic targets. They are expressed in a broad range of human cancer types in both tumor and the surrounding host tissue. In addition, some of the Eph receptors are known to simultaneously regulate tumor growth and neovascularization, permitting molecularly targeted therapies to potentially disrupt at least two key processes in tumor progression with a single agent. Although the role of Eph receptors in tumor stem cells has not been investigated extensively, Eph receptors/ephrins are expressed in various other types of stem cells, such as in nervous system and the intestine (Genander and Frisén, 2010). Characterizing the Eph/ephrin in cancer stem cells will allow development of targeted therapies to this group of cells,

preventing tumor recurrence and metastasis. Finally, because Eph family is the largest RTK family in the genome and its ligands and receptors often display overlapping expression patterns in both tumor cells and the surrounding host stroma, a key step in translating the biological and mechanistic data from the laboratory into the clinic will be to analyze the expression and mutations of individual Eph receptors in large datasets of human cancer. Together with mechanistic studies in cell lines and animal models, expression profiling and mutation analysis in human tissue will elucidate the mechanisms of tumor initiation and progression and lay foundations for development of new anticancer therapeutics.

Acknowledgments

The author thanks Drs. Daar and Brantley-Sieders for helpful comments on the manuscript. Work in the author's laboratory is supported by a Merit Award from the Veterans Affairs Administration, and grants from National Institute of Health (CA95004 and CA114301).

References

Adams RH, Diella F, Hennig S, Helmbacher F, Deutsch U, Klein R. The cytoplasmic domain of the ligand ephrinB2 is required for vascular morphogenesis but not cranial neural crest migration. Cell. 2001; 104:57–69. [PubMed: 11163240]

Adams RH, Eichmann A. Axon guidance molecules in vascular patterning. Cold Spring Harb Perspect Biol. 2010a; 2:a001875. [PubMed: 20452960]

Adams RH, Eichmann A. Axon guidance molecules in vascular patterning. Cold Spring Harb Perspect Biol. 2010b; 2:a001857.

Adams RH, Wilkinson GA, Weiss C, Diella F, Gale NW, Deutsch U, Risau W, Klein R. Roles of ephrinB ligands and EphB receptors in cardiovascular development: demarcation of arterial/venous domains, vascular morphogenesis, and sprouting angiogenesis. Genes & Development. 1999; 3:295–306. [PubMed: 9990854]

Astin JW, Batson J, Kadir S, Charlet J, Persad RA, Gillatt D, Oxley JD, Nobes CD. Competition amongst Eph receptors regulates contact inhibition of locomotion and invasiveness in prostate cancer cells. Nat Cell Biol. 2010; 12:1194–1204. [PubMed: 21076414]

Batlle E, Bacani J, Begthel H, Jonkeer S, Gregorieff A, van de Born M, Malats N, Sancho E, Boon E, Pawson T, et al. EphB receptor activity suppresses colorectal cancer progression. Nature. 2005; 435:1126–1130. [PubMed: 15973414]

Batlle E, Henderson JT, Beghtel H, van den Born MM, Sancho E, Huls G, Meeldijk J, Robertson J, van de Wetering M, Pawson T, et al. Beta-catenin and TCF mediate cell positioning in the intestinal epithelium by controlling the expression of EphB/ephrinB. Cell. 2002; 111:251–263. [PubMed: 12408869]

Bogan C, Chen J, O'Sullivan MG, Cormier RT. Loss of EphA2 receptor tyrosine kinase reduces ApcMin/+ tumorigenesis. Int J Cancer. 2009; 124:1366–1371. [PubMed: 19089910]

Brambilla R, Schnapp F, Casagranda F, Labrador JP, Bergemann AD, Flanagan JG, Pasquale EB, Klein R. Membrane-bound LERK2 ligand can signal through three different Eph-related receptor tyrosine kinases. EMBO J. 1995; 14:3116–3126. [PubMed: 7621826]

Brannan JM, Dong W, Prudkin L, Behrens C, Lotan R, Bekele BN, Wistuba I, Johnson FM. Expression of the receptor tyrosine kinase EphA2 is increased in smokers and predicts poor survival in non-small cell lung cancer. Clin Cancer Res. 2009; 15:4423–4430. [PubMed: 19531623]

Brantley-Sieders D. Clinical relevance of Ephs and ephrins in cancer: Lessons from breast, colorectal, and lung cancer profiling. Seminars in Cell and Developmental Biology. 2011 (in press).

Brantley-Sieders D, Caughron J, Hicks D, Pozzi A, Ruiz JC, Chen J. EphA2 receptor tyrosine kinase regulates endothelial cell migration and assembly through phosphoinositide 3-kinase-mediated Rac1 GTPase activation. J Cell Sci. 2004; 117:2037–2049. [PubMed: 15054110]

Brantley-Sieders DM, Dunaway CM, Rao M, Short S, Hwang Y, Gao Y, Li D, Jiang A, Shyr Y, Wu JY, et al. Angiocrine factors modulate tumor proliferation and motility through EphA2 repression

of Slit2 tumor suppressor function in endothelium. Cancer Res. 2011a; 71:976–987. [PubMed: 21148069]

Brantley-Sieders DM, Fang WB, Hicks D, Koyama T, Shyr Y, Chen J. Impaired tumor microenvironment in EphA2-deficient mice inhibits tumor angiogenesis and metastatic progression. FASEB J. 2005; 19:1884–1886. [PubMed: 16166198]

Brantley-Sieders DM, Jiang A, Sarma K, Badu-Nkansah A, Walter DL, Shyr Y, Chen J. Eph/ephrin profiling in human breast cancer reveals significant associations between expression level and clinical outcome. PLoS ONE. 2011b; 6:e24426. [PubMed: 21935409]

Brantley-Sieders DM, Zhuang G, Hicks D, Fang WB, Hwang Y, Cates JM, Coffman K, Jackson D, Bruckheimer E, Muraoka-Cook RS, et al. The receptor tyrosine kinase EphA2 promotes mammary adenocarcinoma tumorigenesis and metastatic progression in mice by amplifying ErbB2 signaling. J Clin Invest. 2008; 118:64–78. [PubMed: 18079969]

Brantley-Sieders DM, Zhuang G, Vaught D, Freeman T, Hwang Y, Hicks D, Chen J. Host deficiency in Vav2/3 guanine nucleotide exchange factors impairs tumor growth, survival, and angiogenesis in vivo. Mol Cancer Res. 2009; 7:615–623. [PubMed: 19435813]

Bruce V, Olivieri G, Eickelberg O, Miescher GC. Functional activation of EphA5 receptor does not promote cell proliferation in the aberrant EphA5 expressing human glioblastoma U-118 MG cell line. Brain Res. 1999; 821:169–176. [PubMed: 10064801]

Butler JM, K.H. Rafii S. Instructive role of the vascular niche in promoting tumour growth and tissue repair by angiocrine factors. Nat Rev Cancer. 2010; 10:138–146. [PubMed: 20094048]

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. [PubMed: 16359662]

Cheng N, Brantley DM, Liu H, Lin Q, Enriquez M, Gale NW, Yancopoulos G, Cerretti DP, Daniel TO, Chen J. Blockade of EphA receptor tyrosine kinase activation inhibits VEGF-induced angiogenesis. Mol Cancer Res (formerly Cell Growth and Differentiation). 2002; 1:2–11.

Chumley MJ, Catchpole T, Silvany RE, Kernie SG, Henkemeyer M. EphB receptors regulate stem/progenitor cell proliferation, migration, and polarity during hippocampal neurogenesis. J Neurosci. 2007; 27:13481–13490. [PubMed: 18057206]

Coffman KT, Hu M, Carles-Kinch K, Tice D, Donacki N, Munyon K, Kifle G, Woods R, Langermann S, Kiener PA, et al. Differential EphA2 epitope display on normal versus malignant cells. Cancer Res. 2003; 63:7907–7912. [PubMed: 14633720]

Conover JC, Doetsch F, Garcia-Verdugo JM, Gale NW, Yancopoulos GD, Alvarez-Buylla A. Disruption of Eph/ephrin signaling affects migration and proliferation in the adult subventricular zone. Nat Neurosci. 2000; 3:1091–1097. [PubMed: 11036265]

Cortina C, Palomo-Ponce S, Iglesias M, Fernandez-Masip JL, Vivancos A, Whissell G, Huma M, Peiro N, Gallego L, Jonkheer S, et al. EphB-ephrin-B interactions suppress colorectal cancer progression by compartmentalizing tumor cells. Nat Genet. 2007; 39:1376–1383. [PubMed: 17906625]

Davies H, Hunter C, Smith R, Stephens P, Greenman C, Bignell G, Teague J, Butler A, Edkins S, Stevens C, et al. Somatic mutations of the protein kinase gene family in human lung cancer. Cancer Res. 2005; 65:7591–7595. [PubMed: 16140923]

Davy A, Nicholas WG, Murray EW, Klinghoffer RA, Soriano P, Feuerstein C, Robbins SM. Compartmentalized signaling by GPI-anchored ephrin-A5 requires the Fyn tyrosine kinase to regulate cellular adhesion. Genes & Development. 1999; 13:3125–3135. [PubMed: 10601038]

Davy A, Robbins SM. Ephrin-A5 modulates cell adhesion and morphology in an integrin-dependent manner. Embo J. 2000; 19:5396–5405. [PubMed: 11032807]

Ding L, Getz G, Wheeler DA, Mardis ER, McLellan MD, Cibulskis K, Sougnez C, Greulich H, Muzny DM, Morgan MB, et al. Somatic mutations affect key pathways in lung adenocarcinoma. Nature. 2008; 455:1069–1075. [PubMed: 18948947]

Duxbury MS, Ito H, Zinner MJ, Ashley SW, Whang EE. EphA2: a determinant of malignant cellular behavior and a potential therapeutic target in pancreatic adenocarcinoma. Oncogene. 2004; 23:1448–1456. [PubMed: 14973554]

Eichmann A, Makinen T, Alitalo K. Neural guidance molecules regulate vascular remodeling and vessel navigation. Genes Dev. 2005; 19:1013–1021. [PubMed: 15879551]

Erber R, Eichelsbacher U, Powajbo V, Korn T, Djonov V, Lin J, Hammes HP, Grobholz R, Ullrich A, Vajkoczy P. EphB4 controls blood vascular morphogenesis during postnatal angiogenesis. Embo J. 2006; 25:628–641. [PubMed: 16424904]

Fang WB, Brantley-Sieders DM, Hwang Y, Ham AJ, Chen J. Identification and functional analysis of phosphorylated tyrosine residues within EphA2 receptor tyrosine kinase. J Biol Chem. 2008a; 283:16017–16026. [PubMed: 18387945]

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. 2008b; 121:358–368. [PubMed: 18198190]

Faoro L, Singleton PA, Cervantes GM, Lennon FE, Choong NW, Kanteti R, Ferguson BD, Husain AN, Tretiakova MS, Ramnath N, et al. EphA2 mutation in lung squamous cell carcinoma promotes increased cell survival, cell invasion, focal adhesions, and mTOR activation. J Biol Chem. 2010 (in press).

Foo SS, Turner CJ, Adams S, Compagni A, Aubyn D, Kogata N, Lindblom P, Shani M, Zicha D, Adams RH. Ephrin-B2 controls cell motility and adhesion during blood-vessel-wall assembly. Cell. 2006; 124:161–173. [PubMed: 16413489]

Fournier MV, Martin KJ, Kenny PA, Xhaja K, Bosch I, Yaswen P, Bissell MJ. Gene expression signature in organized and growth-arrested mammary acini predicts good outcome in breast cancer. Cancer Res. 2006; 66:7095–7102. [PubMed: 16849555]

Frieden L, Townsend TA, Vaught DB, Hwang Y, Barnett JV, Chen C. Regulation of heart valve morphogenesis by ephrin-A1 in cardiac development in vivo. Circ Res. 2008; 239:3226–3234.

Frieden L, Townsend TA, Vaught DB, Hwang Y, Barnett JV, Chen C. Regulation of heart valve morphogenesis by Eph receptor ligand, ephrin-A1. Dev Dyn. 2010; 239:3226–3234. [PubMed: 20960543]

Gaengel K, G.G. Armulik A, Betsholtz C. Endothelial-mural cell signaling in vascular development and angiogenesis. Arterioscler Thromb Vasc Biol. 2009; 29:630–638. [PubMed: 19164813]

Gale NW, Baluk P, Pan L, Kwan M, Holash J, DeChiara TM, McDonald DM, Yancopoulos GD. Ephrin-B2 selectively marks arterial vessels and neovascularization sites in the adult, with expression in both endothelial and smooth-muscle cells. Dev Biol. 2001; 230:151–160. [PubMed: 11161569]

Genander M, Frisén J. Ephrins and Eph receptors in stem cells and cancer. Curr Opin Cell Biol. 2010; 22:611–616. [PubMed: 20810264]

Genander M, Halford MM, Xu NJ, Eriksson M, Yu Z, Qiu Z, Martling A, Greicius G, Thakar S, Catchpole T, et al. Dissociation of EphB2 signaling pathways mediating progenitor cell proliferation and tumor suppression. Cell. 2009; 139:679–692. [PubMed: 19914164]

Genander M, Holmberg J, Frisén J. Ephrins negatively regulate cell proliferation in the epidermis and hair follicle. Stem Cells. 2010; 28:1196–1205. [PubMed: 20506314]

Gerety SS, Wang HU, Chen ZF, Anderson DJ. Symmetrical mutant phenotypes of the receptor EphB4 and its specific transmembrane ligand ephrin-B2 in cardiovascular development. Molecular Cell. 1999; 4:403–414. [PubMed: 10518221]

Greenman C, Stephens P, Smith R, Dalgliesh GL, Hunter C, Bignell G, Davies H, Teague J, Butler A, Stevens C, et al. Patterns of somatic mutation in human cancer genomes. Nature. 2007; 446:153–158. [PubMed: 17344846]

Guo H, Miao H, Gerber L, Singh J, Denning MF, Gilliam AC, Wang B. Disruption of EphA2 receptor tyrosine kinase leads to increased susceptibility to carcinogenesis in mouse skin. Cancer Res. 2006; 66:7050–7058. [PubMed: 16849550]

Hanahan D, Weinberg RA. The hallmarks of cancer. Cell. 2000; 100:57–70. [PubMed: 10647931]

Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011; 144:646–674. [PubMed: 21376230]

Himanen JP, Yermekbayeva L, Janes PW, Walker JR, Xu K, Atapattu L, Rajashankar KR, Mensinga A, Lackmann M, Nikolov DB, et al. Architecture of Eph receptor clusters. Proc Natl Acad Sci U S A. 2010; 107:10860–10865. [PubMed: 20505120]

Hunter SG, Zhuang G, Brantley-Sieders DM, Swatt W, Cowan CW, Chen J. Essential role of Vav family guanine nucleotide exchange factors in EphA receptor-mmediated angiogenesis. Mol Cell Biol. 2006; 26:4830–4842. [PubMed: 16782872]

Ji XD, Li G, Feng YX, Zhao JS, Li JJ, Sun ZJ, Shi S, Deng YZ, Xu JF, Zhu YQ, et al. EphB3 is overexpressed in non-small-cell lung cancer and promotes tumor metastasis by enhancing cell survival and migration. Cancer Res. 2011; 71:1156–1166. [PubMed: 21266352]

Kinch MS, Moore MB, Harpole DHJ. Predictive value of the EphA2 receptor tyrosine kinase in lung cancer recurrence and survival. Clin Cancer Res. 2003; 9:613–618. [PubMed: 12576426]

Kullander K, Klein R. Mechanisms and functions of Eph and ephrin signaling. Nat Rev Mol Cell Biol. 2002; 3:475. [PubMed: 12094214]

Kumar SR, Scehnet JS, Ley EJ, Singh J, Krasnoperov V, Liu R, Manchanda PK, Ladner RD, Hawes D, Weaver FA, et al. Preferential induction of EphB4 over EphB2 and its implication in colorectal cancer progression. Cancer Res. 2009; 69:3736–3745. [PubMed: 19366806]

Kumar SR, Singh J, Xia G, Krasnoperov V, Hassanieh L, Ley EJ, Scehnet J, Kumar NG, Hawes D, Press MF, et al. Receptor tyrosine kinase EphB4 is a survival factor in breast cancer. Am J Pathol. 2006; 169:279–293. [PubMed: 16816380]

Landen CN Jr. Chavez-Reyes A, Bucana C, Schmandt R, Deavers MT, Lopez-Berestein G, Sood AK. Therapeutic EphA2 gene targeting in vivo using neutral liposomal small interfering RNA delivery. Cancer Res. 2005a; 65:6910–6918. [PubMed: 16061675]

Landen CN, Kinch MS, Sood AK. EphA2 as a target for ovarian cancer therapy. Expert Opin Ther Targets. 2005b; 9:1179–1187. [PubMed: 16300469]

Landen CN, Lu C, Han LY, Coffman KT, Bruckheimer E, Halder J, Mangala LS, Merritt WM, Lin YG, Gao C, et al. Efficacy and antivascular effects of EphA2 reduction with an agonistic antibody in ovarian cancer. J Natl Cancer Inst. 2006; 98

Lee H, Daar IO. EphrinB reverse signaling in cell-cell adhesion. Cell Adhesion & Migration. 2009; 3:250–255. [PubMed: 19276658]

Lee HS, Nishanian TG, Mood K, Bong YS, Daar IO. EphrinB1 controls cell-cell junctions through the Par polarity complex. Nat Cell Biol. 2008 (in press).

Lhotak V, Pawson T. Biological and biochemical activities of a chimeric epidermal growth factor–Elk receptor tyrosine kinase. Mol Cell Biol. 1993; 13:7071–7079. [PubMed: 8413296]

Lim YS, M.T. Sung TC, Santiago A, Lee KF, O'Leary DD. p75(NTR) mediates ephrin-A reverse signaling required for axon repulsion and mapping. Neuron. 2008; 59:746–758. [PubMed: 18786358]

Macrae M, Neve RM, Rodriguez-Viciana P, Haqq C, Yeh J, Chen C, Gray JW, McCormick F. A conditional feedback loop regulates Ras activity through EphA2. Cancer Cell. 2005; 8:111–118. [PubMed: 16098464]

Mäkinen T, Adams RH, Bailey J, Lu Q, Ziemiecki A, Alitalo K, Klein R, Wilkinson GA. PDZ interaction site in ephrinB2 is required for the remodeling of lymphatic vasculature. Genes Dev. 2005; 19:397–410. [PubMed: 15687262]

Martin KJ, Patrick DR, Bissell MJ, Fournier MV. Prognostic breast cancer signature identified from 3D culture model accurately predicts clinical outcome across independent datasets. PLoS ONE. 2008; 3:e2994. [PubMed: 18714348]

Martiny-Baron G, Korff T, Schaffner F, Esser N, Eggstein S, Marme D, Augustin HG. Inhibition of tumor growth and angiogenesis by soluble EphB4. Neoplasia. 2004; 6:248–257. [PubMed: 15153337]

Merlos-Suárez A, Batlle E. Eph-ephrin signalling in adult tissues and cancer. Curr Opin Cell Biol. 2008; 20:194–200. [PubMed: 18353626]

Miao H, Li DQ, Mukherjee A, Guo H, Petty A, Cutter J, Basilion JP, Sedor J, Wu J, Danielpour D, et al. EphA2 mediates ligand-dependent inhibition and ligand-independent promotion of cell migration and invasion via a reciprocal regulatory loop with Akt. Cancer Cell. 2009; 16:9–20. [PubMed: 19573808]

Miao H, Wei B-R, Peehl DM, Li Q, Alexandrou T, Schelling JR, Rhim JS, Sedor JR, Burnett E, Wang B. Activation of EphA receptor tyrosine kinase inhibits the Ras/MAPK pathway. Nature Cell Biol. 2001; 13:527–530. [PubMed: 11331884]

Miura K, Nam JM, Kojima C, Mochizuki N, Sabe H. EphA2 engages Git1 to suppress Arf6 activity modulating epithelial cell-cell contacts. Mol Biol Cell. 2009; 20:1949–1959. [PubMed: 19193766]

Munarini N, Jager R, Abderhalden S, Zuercher G, Rohrbach V, Loercher S, Pfanner-Meyer B, Andres AC, Ziemiecki A. Altered mammary epithelial development, pattern formation and involution in transgenic mice expressing the EphB4 receptor tyrosine kinase. J Cell Sci. 2002; 115:25–37. [PubMed: 11801721]

Noren NK, Foos G, Hauser CA, Pasquale EB. The EphB4 receptor suppresses breast cancer cell tumorigenicity through an Abl-Crk pathway. Nat Cell Biol. 2006; 8:815–825. [PubMed: 16862147]

Noren NK, Lu M, Freeman AL, Koolpe M, Pasquale EB. Interplay between EphB4 on tumor cells and vascular ephrin-B2 regulates tumor growth. Proc Natl Acad Sci U S A. 2004; 101:5583–5588. [PubMed: 15067119]

Noren NK, Pasquale EB. Paradoxes of the EphB4 receptor in cancer. Cancer Res. 2007; 67:3994–3997. [PubMed: 17483308]

Ogawa K, Pasqualini R, Lindberg RA, Kain R, Freeman AL, Pasquale EB. The ephrin-A1 ligand and its receptor, EphA2, are expressed during tumor neovascularization. Oncogene. 2000; 19:6043–6052. [PubMed: 11146556]

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. [PubMed: 7536959]

Pasquale EB. Developmental Cell Biology: Eph receptor signalling casts a wide net on cell behaviour. Nat Rev Mol Cell Biol. 2005; 6:462–475. [PubMed: 15928710]

Pasquale EB. Eph-ephrin bidirectional signaling in physiology and disease. Cell. 2008; 133:38–52. [PubMed: 18394988]

Pasquale EB. Eph receptors and ephrins in cancer: bidirectional signaling and beyond. Nat Rev Cancer. 2010; 10:165–180. [PubMed: 20179713]

Salvucci O, Maric D, Economopoulou M, Sakakibara S, Merlin S, Follenzi A, Tosato G. EphrinB reverse signaling contributes to endothelial and mural cell assembly into vascular structures. Blood. 2009; 114:1707–1716. [PubMed: 19411631]

Sawamiphak S, Seidel S, Essmann CL, Wilkinson GA, Pitulescu ME, Acker T, Acker-Palmer A. Ephrin-B2 regulates VEGFR2 function in developmental and tumour angiogenesis. Nature. 2010; 465:487–491. [PubMed: 20445540]

Seiradake E, Harlos K, Sutton G, Aricescu AR, Jones EY. An extracellular steric seeding mechanism for Eph-ephrin signaling platform assembly. Nat Struct Mol Biol. 2010; 17:398–402. [PubMed: 20228801]

Shi L, Itoh F, Itoh S, Takahashi S, Yamamoto M, Kato M. Ephrin-A1 promotes the malignant progression of intestinal tumors in Apc(min/+) mice. Oncogene. 2008; 27:3265–3273. [PubMed: 18246128]

Shin D, Garcia-Cardena G, Hayashi S, Gerety S, Asahara T, Stavrakis G, Isner J, Folkman J, Gimbrone MA Jr. Anderson DJ. Expression of ephrinB2 identifies a stable genetic difference between arterial and venous vascular smooth muscle as well as endothelial cells, and marks subsets of microvessels at sites of adult neovascularization. Dev Biol. 2001; 230:139–150. [PubMed: 11161568]

Stephen LJ, Fawkes AL, Verhoeve A, Lemke G, Brown A. A critical role for the EphA3 receptor tyrosine kinase in heart development. Dev Biol. 2006

Tanaka M, Kamata R, Sakai R. Phosphorylation of ephrin-B1 via the interaction with claudin following cell-cell contact formation. Embo J. 2005; 24:3700–3711. [PubMed: 16211011]

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. [PubMed: 16081502]

Wang HU, Chen ZF, Anderson DJ. Molecular distinction and angiogenic interaction between embryonic arteries and veins revealed by ephrin-B2 and its receptor Eph-B4. Cell. 1998; 93:741–753. [PubMed: 9630219]

Wang Y, Nakayama M, Pitulescu ME, Schmidt TS, Bochenek ML, Sakakibara A, Adams S, Davy A, Deutsch U, Lüthi U, et al. Ephrin-B2 controls VEGF-induced angiogenesis and lymphangiogenesis. Nature. 2010; 465:483–486. [PubMed: 20445537]

Wood LD, Calhoun ES, Silliman N, Ptak J, Szabo S, Powell SM, Riggins GJ, Wang TL, Yan H, Gazdar A, et al. Somatic mutations of GUCY2F, EPHA3, and NTRK3 in human cancers. Hum Mutat. 2006; 27:1060–1061. [PubMed: 16941478]

Wu Q, Suo Z, Risberg B, Karlsson MG, Villman K, Nesland JM. Expression of Ephb2 and Ephb4 in breast carcinoma. Pathol Oncol Res. 2004; 10:26–33. [PubMed: 15029258]

Wykosky J, Debinski W. The EphA2 receptor and ephrinA1 ligand in solid tumors: function and therapeutic targeting. Mol Cancer Res. 2008; 6:1795–1806. [PubMed: 19074825]

Wykosky J, Gibo DM, Stanton C, Debinski W. EphA2 as a novel molecular marker and target in glioblastoma multiforme. Mol Cancer Res. 2005; 3:541–551. [PubMed: 16254188]

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. [PubMed: 18434321]

Yang NY, Fernandez C, Richter M, Xiao Z, Valencia F, Tice DA, Pasquale EB. Crosstalk of the EphA2 receptor with a serine/threonine phosphatase suppresses the Akt-mTORC1 pathway in cancer cells. Cell Signal. 2011; 23:201–212. [PubMed: 20837138]

Zelinski DP, Zantek ND, Stewart JC, Irizarry AR, Kinch MS. EphA2 overexpression causes tumorigenesis of mammary epithelial cells. Cancer Res. 2001; 61:2301–2306. [PubMed: 11280802]

Zhuang G, Brantley-Sieders D, Vaught D, Yu J, Xie L, Wells S, Jackson D, Muroaka-Cook R, Arteaga C, Chen J. Elevation of receptor tyrosine kinase EphA2 mediates resistance to trastuzumab therapy. Cancer Res. 2010; 70:299–308. [PubMed: 20028874]

Table 1

Tumor phenotypes of Eph/ephrin mutant animals

Mouse Strain	Tumor Model	Phenotype	Reference
EphA2$^{-/-}$ knockout mice	MMTV-Neu Breast cancer	Decreased tumor incidence, tumor burden, and metastasis	(Brantley-Sieders et al., 2008)
EphA2 $^{i/i}$ gene trap mice	DMBA/TPA Skin cancer	Increased tumor number, tumor burden, and invasiveness	(Guo et al., 2006)
EphA2$^{i/i}$ gene trap mice	APC $^{Min/+}$ Colon cancer	Decreased tumor number and size in small and large intestine	(Bogan et al., 2009)
Villin-*ephrinA1* Transgenic mice	APC $^{Min/+}$ Colon cancer	Increased tumor numbers and invasiveness	(Shi et al., 2008)
MMTV-*EphB4* Transgenic mice	MMTV-Neu Breast cancer	Accelerated tumor onset and increased metastasis	(Munarini et al., 2002)
Villin-*EphB2*$^{\Delta C}$ *EphB3*$^{-/-}$ Vil-*Cre/ephrinB1*$^{\Delta/\Delta}$	APC $^{Min/+}$ Colon cancer	Increased tumor number and tumor invasion	(Batlle et al., 2005; Cortina et al., 2007)

Table 2

Vascular phenotypes of Eph/ephrin mutant animals

Gene knock out/knock in	Phenotype	Reference
ephrinA1$^{-/-}$	Defects in heart valves and impaired cardiac function	(Frieden et al., 2010)
EphA3$^{-/-}$	Perinatal lethality; hypoplastic heart valve	(Stephen et al., 2006)
EphA2$^{-/-}$	Defects in tumor angiogenesis	(Brantley-Sieders et al., 2005; Brantley-Sieders et al., 2008)
EphB2$^{-/-}$ *EphB3*$^{-/-}$	Embryonic lethal; die at E10.5 (~30%); Defective vessel remodeling, similar to those observed in *ephrinB2*$^{-/-}$	(Adams et al., 1999)
EphB4$^{-/-}$	Embryonic lethal, die at E10.5; Defective vessel remodeling, similar to those observed in *ephrinB2*$^{-/-}$	(Gerety et al., 1999)
ephrinB2$^{-/-}$	Embryonic lethal, die at E10.5; Defective vessel remodeling and sprouting	(Wang et al., 1998)
ephrinB2$^{\Delta C/\Delta C}$ (deletion of cytoplasmic domain)	Defects in angiogenic remodeling similar to those observed in *ephrinB2*$^{-/-}$	(Adams et al., 2001)
ephrinB2$^{\Delta V/\Delta V}$ (deletion of Val. residue in PDZ-binding motif)	Defects in angiogenic remodeling similar to those observed in *ephrinB2*$^{-/-}$ Defects in tumor angiogenesis	(Mäkinen et al., 2005; Sawamiphak et al., 2010)
ephrinB2$^{5Y/5Y}$ (knockin mutation in 5 tyrosine residues)	Mild lymphatic phenotype	(Mäkinen et al., 2005; Sawamiphak et al., 2010)
ephrinB2$^{i\Delta EC}$ (VE-cadherin promoter driven CreERT2, EC-specific knockout)	Defects in angiogenic sprouting in both blood vessels and lymphatic vessels.	(Wang et al., 2010)
ephrinB2^{iGOF} (Tie2-rtTA driven tetO-ephrinB2 transgenic)	Increased angiogenic sprouting.	(Wang et al., 2010)
ephrinB2$^{\Delta PC/vSMC}$ (PDGFRβ promoter driven Cre, mural cell-specific knockout)	Perinatal lethality; vascular defects in multiple organs; abnormal migration of smooth muscle cells to lymphatic capillaries.	(Foo et al., 2006)